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Foreword: Guidelines 3

Infections represent a major cause of morbidity and mortality in transplant candidates and recipients. In order for the transplant community to provide comprehensive care to its patients, it is critical that transplant providers have an understanding of the complexity of the field, both with regards to general principles and specific disease entities. Given the constant changes in clinical practice in both transplantation and infectious diseases, periodic critical evaluation and updating of the guidelines is essential to maintain their relevance. This new version of the Infectious Diseases guidelines reflects the evolution of the discipline and is designed to inform current clinical practice.

This updated edition of the Infectious Diseases guidelines represents the collaboration of members of the Infectious Diseases Community of Practice (ID COP) of the American Society of Transplantation (AST) and is supported jointly by the American Society of Transplantation and the Canadian Society of Transplantation. This update provides some unique features. The growth of the AST ID COP has been enormous since the last version of these guidelines and the current community is notable for the diversity of its expertise. Consequently all of the authors of the guidelines as well as nearly all of the reviewers are members of the ID COP. Many new authors were added to the current guidelines; in some cases the previous authors developed the new guideline but other sections were written in collaboration with a new co-author or by new authors. The efforts of the previous authors of the guideline have been acknowledged in each section. In addition to providing significant updates to each of the chapters, we have added three new sections: Ventricular Assist Devices, West Nile Virus and Arenaviruses, and Human T-lymphotropic Virus 1/2, as we felt these were important areas deserving of their own consideration. Finally, for the first time, we have paired these guidelines with both educational activities and "Apps," to improve reader accessibility and utilization of the document.

Prior versions of these guidelines have been immensely popular and frequently quoted and this version was informed significantly by the past guidelines. In order to meet the current needs of the transplant community, the development of the new version began with a survey sent to all members of AST soliciting feedback about the second edition. After consideration of these comments, we enlisted the ID Community of Practice participants, requesting volunteers for section authors and reviewers. Individuals were asked to note their areas of expertise and previous authors were requested to note if they wanted to update their previously written section. Because we had a large number of volunteers, two authors were assigned to each individual chapter and no author was allowed to write more than one chapter. Authors were required to adhere to a specific format for chapter development. Individual chapters were reviewed by two experts in the field; the vast majority were also members of the ID COP. In a few cases, additional external expert opinion was solicited. Additionally each section was reviewed by at least one editor, although many sections were reviewed by more than one editor. The three new chapters were reviewed by each of the five editors. Following review, revisions were made and these were then reviewed by at least one of the editors prior to finalization. The final document was then reviewed and approved by the Executive Committee of the American Society of Transplantation. The Canadian Society of Transplantation also reviewed the process by which the guidelines were developed. Of note, neither the AST nor the CST dictated the contents of the guidelines; although the AST provided another level of critical review prior to the submission of the document in its entirety to the American Journal of Transplantation. The AJT editors and staff were given full access to all review documents and all versions of each chapter to ensure that a critical review process had occurred.

Given the varying degrees of evidence in the field of transplantation and transplant infectious diseases, the editors felt that it was critical that all recommendations be graded according to the level of evidence, so that the readers can be informed on the strength of each recommendation. We considered several different grading systems and ultimately, after some discussion within the ID COP, decided to use the system from the second edition of the guidelines as the authors felt that this most clearly reflected the strength behind the individual recommendations (Table 1). Given the nature of the current level of evidence, the majority of the recommendations were either level II or III. The absence of significant randomized prospective clinical trials is notable and hopefully future research will provide more robust support for guideline development in the future.

The editors would like to gratefully acknowledge the tremendous efforts of the many authors and reviewers involved in this document. Their contributions were enormous as they provided not only their expertise but their valuable time, complying with very tight timelines in order to complete the document in record time. We would also

Table 1: Quality of evidence upon which recommendation is based

Grade	Definition
I	Randomized controlled trials
II-1	Controlled trials without randomization
II-2	Cohort or case-control analytic studies
II-3	Multiple time series, dramatic uncontrolled experiments
III	Opinions of respected authorities, descriptive epidemiology

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like to thank both the AST and CST for their support, both from a financial as well as administrative standpoint. We are especially grateful to Libby McDannell (Executive Director of the AST), Jason Polinsky, Deanna Bright, and Roz Mannon for all of their help. Finally, we would like to recognize the support of our many colleagues both in the ID COP and the transplant community at large; without their suggestions it would have been impossible to move these guidelines forward. We hope that you read these guidelines as they were intended, to help guide your thoughts as you care for your patients. They are not meant to replace the valuable consultation of your local experts, but rather to enhance your understanding of the complex nature of caring for transplant candidates and recipients with infections.

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Special Article

Introduction: Infections in Solid Organ Transplantation

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Key words: donor-derived infection, immunosuppression, opportunistic infection, transplant infectious disease

Abbreviations: BOS, bronchoiolitis obliterans syndrome; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; PCP, *Pneumocystis jirovecii* pneumonia; PTLD, posttransplant lymphoproliferative disorder; RSV, respiratory syncytial virus; SOT, solid organ transplantation; WNV, West Nile virus.

The use of solid organ transplantation (SOT) has been established as accepted therapy for end-stage disease of the kidneys, liver, heart and lungs for nearly 30 years. Intestinal and pancreas transplantation are also generally available but are provided on a more limited basis. While surgical procedures are well established, the field of transplantation continues to explore and experience innovations in immunosuppressive therapy with goals of improving outcomes and in pursuit of tolerance. The potential for surgical and technical complications combined with the impact of immune suppression predisposes recipients of SOT to clinically important infectious sequelae. The diversity and consequences of infectious complications of SOT have led a growing numbers of infectious disease specialists to focus their career interests on the pursuit of clinical expertise with this population resulting in the acquisition of a growing body of clinical evidence in support of optimal management of these patients. The availability of this evidence (or in some cases the development of clinical consensus where definitive evidence is lacking) serves as the basis for this 3rd edition of the AST Guidelines for the Prevention and Treatment of Infectious Complications of Solid Organ Transplantation. While individual sections within the 3rd edition of the Guidelines focus on specific pathogens or disease categories, risk factors for and timing of presentation of infectious complications in this population tend to apply to recipients of all types of organs and to most pathogens and their associated clinical syndromes. Accordingly, an understanding of these general principles provides a strong foundation for the care and prevention of infections in this population.

Predisposing Factors for Infection After SOT

Risk factors that predispose to infections in recipients of organ transplantation can be categorized as being present before transplant within the recipient or donor and those secondary to intraoperative and posttransplant events.

Pretransplant factors—recipients

For all recipients of SOT, the organ being transplanted is the critical determinant of the location of infection in these patients, especially during the first 3 postoperative months (1). The chest, abdomen and urinary tract are the most common sites of infection experienced by recipients of thoracic, liver and kidney transplantation, respectively. The likely explanation for predilection to these sites include the presence of local ischemic injury and bleeding, as well as potential contamination (2).

The underlying illnesses causing organ failure may also be associated with an increased risk for developing infection after organ transplantation. For example, patients with cystic fibrosis who undergo lung or less commonly liver transplantation are predisposed to pseudomonal and fungal infections. Similarly, adult liver recipients undergoing transplantation for HCV-associated cirrhosis are at an increased risk for recurrent infection in the new hepatic allograft although strategies to protect against recurrence are increasingly being evaluated. More generally, a history of palliative surgery before transplant as part of the management of the underlying illness increases the technical difficulty of the transplant procedure, enhancing the risk of developing a posttransplant infection (3). In general, the severity of the underlying disease leading to end organ failure and its impact on other organs systems at the time of transplantation correlates with risk of postoperative morbidity and mortality (4). Similarly, chronic malnutrition predisposes to infections before and after transplantation. Attempts to correct nutritional deficits with intravenous TPN increases the likelihood of catheter-associated blood stream infections. Finally, mechanical ventilation prior to SOT increases the likelihood of developing infection with multidrug-resistant nosocomial pathogens.

The age of the recipient at the time of transplant significantly impacts on susceptibility to and severity of infection in organ recipients. Transplantation at a young age has

been associated with higher rates of infection during the first few years after transplantation (5). Infants and toddlers undergoing SOT experience greater morbidity and mortality with community-acquired viruses (e.g. respiratory syncytial virus [RSV], parainfluenza) compared to older children or adult recipients. They are more likely to develop primary infection with cytomegalovirus (CMV) and Epstein–Barr virus (EBV), predisposing them to worse outcomes compared to patients experiencing viral reactivation or reinfection with a new strain of these pathogens (6,7). By contrast, other pathogens, such as *Cryptococcus neoformans,* are rarely seen in children but are important opportunistic pathogen in adult organ recipients. Age also potentially impacts on risk of infection for older (>65 year old) organ transplant recipients. Preliminary evidence suggests that older organ recipients may experience exaggerated effects of immune senescence compared to age matched controls (8). Accordingly, they may be more prone to infectious risks after transplant than younger adult recipients. However, evidence confirming this risk is limited (9).

Finally, younger children frequently undergo SOT before they are fully immunized, increasing their risk for vaccine-preventable infections. When vaccines are given after transplant, they may not provide full protection. Accordingly, at least some younger recipients are at increased risk for infection with vaccine preventable diseases despite being immunized after transplant (10). Similarly, adult patients undergoing organ transplantation in their 60s may also be at increased risk for vaccine preventable disease. Although not carefully studied, booster immunizations of these older recipients may be missed due to the presence of end-stage organ disease or lack of attention to updating vaccinations by the transplant specialists who have often assumed primary responsibility for candidates care.

Pretransplant factors—donors

Organ transplant recipients are at risk of acquiring pathogens from donors with active or latent infections at the time of procurement. While many potential infectious exposures from the donor can be anticipated or identified, some donor-derived infections occur unexpectedly, defying efforts to effectively recognize the presence of risk within a given donor. Examples of pathogens associated with expected donor-derived infections include CMV (11–13), EBV and *Toxoplasma.* Knowledge of the serologic status of the donor and recipient informs the use of preventive strategies mitigating infectious risk from these pathogens. Of greater concern is the development of unexpected donor-derived infection from a growing number of pathogens, including, *Mycobacterium tuberculosis, Histoplasma* spp., West Nile virus (WNV), hepatitis B (HBV) and C viruses (HCV) and human immunodeficiency virus (HIV) (14). The unexpected transmission of these agents can lead to infection in one or more recipients and cause significant morbidity and occasional mortality. Strategies have been developed in an effort to reduce the incidence and impact of unexpected transmission of potential pathogens from the donor. In considering the use of any donor, there is clearly a potential risk to the recipient who experiences unexpected transmission of a pathogen but there is also a consequence to potential recipients on the waiting list when potentially viable donors are turned down. Issues related to donor-derived transmission of infectious pathogens are discussed in detail in chapter 3 of the guidelines.

Another donor-related concern is the presence of bacteria or fungi colonizing the respiratory tract of a lung donor or infecting organs or vessels from other allografts; such organisms can cause infection in the postoperative period (15). Similarly, unrecognized acute bacteremia or viremia at the time of organ recovery is an additional risk to the recipient. The presence of potential pathogens may be identified by culture or by histopathology. The true identity of pathogens identified by pathologic methods may never be known or proven. However, recognition of the presence of some marker of potential risk in the donor can allow for the recipient's transplant team to develop a rationale response. Early recognition of potential risk might allow the implementation of a serial monitoring of the recipients and in some circumstances may warrant the use of antimicrobial therapy as prophylaxis or treatment of subclinical infection. As increasing attention focuses on the problem of donor-derived infection, our recognition of specific risk factors for and the potential to screen for and implement prophylaxis against these infections will likely increase leading to improved clinical outcomes.

Intraoperative factors

The choice of surgical reconstruction used for a given transplant recipient can predispose to infectious complications. For example, the risk of infection is different in liver transplant recipients undergoing duct-to-duct biliary anastomosis compared to those whose biliary drainage is accomplished via Roux-en-Y anastomosis (16). Unexpected events occurring during surgery also predispose to infection. Injury to the phrenic, vagal, or recurrent laryngeal nerves during surgery affect pulmonary toilet, predisposing a lung transplant recipient to pneumonia (17). Ischemic injury to the allograft during the transplant procedure reduces its viability and increases the risk of infection. Additional factors, including prolonged operative time, contamination of the operative field, and bleeding at or near surgical sites have been associated with an increased risk of postoperative infections in these patients.

Posttransplant factors

Immunosuppression is the major risk factor for infection following transplantation. The immunosuppressive regimens used in SOT recipients continue to evolve with a goal of minimizing toxicity and side effects while optimizing organ function. Unfortunately, while the level of infectious risk may vary by individual agent or specific combination,

all such combinations appear to place organs recipients at some risk for opportunistic infections. However, it is difficult to quantify how immunosuppressed an individual organ recipient is. Although there are nonspecific assays that measure immunity, these are not always predictive of infection. Requirement for augmented immune suppression to treat rejection further increases the risk of infection after SOT. This risk associated with the use of immune suppression continues throughout the entire posttransplant course. The use of antilymphocyte preparations and many of an increasingly diverse list of biologic agents used in these patients have been associated with an enhanced risk of infection (13,16,18). As newer immunosuppressive agents are introduced, clinicians must be aware of and alert for changes in infectious manifestations and profiles seen in these patients (19).

Technical problems affecting the vascular supply and functional integrity of the allograft are major risk factors for infectious complications that manifest after the transplantation. Examples of specific technical problems associated with infection include thrombosis of the hepatic artery after liver transplantation (20); vesicoureteral reflux after renal transplantation (21) and mediastinal bleeding requiring re-exploration in thoracic transplantation. These complications have been associated with hepatic abscesses and blood stream infection (20), graft pyelonephritis (21) and mediastinitis, respectively (11). The ongoing presence of uncorrected technical problems can predispose to multiple episodes of recurrent infections until these issues are corrected. Efforts should be made to identify and potentially correct these technical problems in patients presenting with infectious syndromes associated with their presence.

The prolonged use of indwelling cannulas is another significant risk factor for infection after transplantation. The use of central venous catheters is associated with bloodstream infections; urethral catheters predispose to urinary tract infection; the use of a cannula in an obstructed biliary tract predisposes to cholangitis; and prolonged endotracheal intubation is associated with pneumonia. The risk for these catheter-associated infectious syndromes persists until the catheter is removed. Accordingly, active efforts should be undertaken to review the ongoing requirements for these cannulas with removal undertaken as soon as practical.

Nosocomial exposures constitute the final group of posttransplant risk factors. All transplant recipients are at risk for developing infection with transfusion-associated pathogens. Patients undergoing transplantation during the winter months are often exposed nosocomially to viruses associated with annual community based outbreaks (e.g. RSV, influenza, rotavirus). While this is particularly true in pediatric patients, adult recipients can also experience clinically significant infections secondary to these pathogens through exposure to affected hospital staff, family and other visitors. The presence in the hospital of areas of heavy contamination with pathogenic fungi, such as *Aspergillus* spp. increases the risk of invasive fungal disease in these patients. And finally, there is increasing concern for nosocomial exposure to and development of infection with multiple-drug resistant bacteria after transplantation.

Finally, community exposure is an important potential source of later infection after organ recipients are discharged from hospital. These exposures may vary from common community-acquired viral infections to less commonly seen pathogens that might be related to occupational or travel exposures. These are discussed in more detail in Chapter 30, which focuses on 'safe living' after transplantation.

Timing of Infections After SOT

The timing of specific infections developing after SOT is generally predictable regardless of which organ is transplanted. The majority of clinically important infections occur within the first 180 days; individual pathogens typically present at stereotypical times after transplantation. However, the time of onset for certain pathogens can be affected by the use of prophylactic strategies, alterations in immune suppression or need for additional surgery. In considering potential causes of infection in SOT recipients, it is useful to divide risk periods into three major intervals in order to consider which pathogens are most likely: (1) early (0–30 days after transplantation); (2) intermediate (30–180 days) and (3) late (beyond 180 days). However, this assessment by time is not absolute. Some infections can occur throughout the posttransplant course and others may occur outside of their usual risk period. Nevertheless, consideration of these time intervals provides a useful framework for the approach to a patient with fever after transplantation, guiding the initial differential diagnosis (Table 1).

Early infections

Early infections (0–30 days after transplant) are usually associated with the presence of preexisting conditions or complications of surgery. Bacteria and yeast are the most frequent pathogens recovered during in the first 30 days after transplant (11,22). Fifty percent or more of all bacterial infections that develop after transplantation occur during the early posttransplant period (11,22). Superficial and deep surgical site infections are among the most common infectious complications seen during this period. Technical difficulties, particularly those resulting in anastomotic stenosis, leaks or other complications, are important risk factors for the development of invasive infection in the first month after most types of organ transplantation. Finally, donor-derived bacterial and/or fungal infections may present during this time period and when donor derivation is suspected, notification of the appropriate local and national organizations/agencies should be performed to minimize the risk to other recipients.

Table 1: Timing of infectious complications following transplantation[1]

Early period (0–1 months)	Middle period (1–6 months)	Late period (> 6 months)
Bacterial infections	Viral infections	Viral infections
Gram-negative enteric bacilli	Cytomegalovirus	Epstein–Barr virus
Small bowel, liver, neonatal heart	All transplant types	All transplant types, but less than middle period
Pseudomonas/Burkholderia spp.	Seronegative recipient of seropositive donor	Varicella-zoster virus
Cystic fibrosis: lung	Epstein–Barr virus	All transplant types
Gram-positive organisms	All transplant types	Community-acquired viral infections
All transplant types	Seronegative recipient	All transplant types
Fungal infections	Small bowel highest-risk group	Bacterial infections
All transplant types	Varicella-zoster virus	*Pseudomonas/Burkholderia* spp.
Viral infections	All transplant types	Cystic fibrosis: lung
Herpes simplex virus	Opportunistic infections	Lung recipients with chronic rejection
All transplant types	*Pneumocystis jirovecii*	Gram-negative bacillary bacteremia
Nosocomial respiratory viruses	All transplant types	Small bowel
All transplant types	*Toxoplasma gondii*	Fungal infections
	Seronegative recipient of a heart from a seropositive donor	*Aspergillus* spp.
	Bacterial infections	Lung transplants with chronic rejection
	Pseudomonas/Burkholderia spp.	
	Pneumonia	
	Cystic fibrosis: lung	
	Gram-negative enteric bacilli	
	Small bowel	

[1]Listed in decreasing order of relative importance.

Intermediate period

The intermediate period (31–180 days after transplant) is the typical time of onset of infections attributable to latent pathogens transmitted from donor organs and blood products and those reactivated within the recipient. This is also the period where classical 'opportunistic infections' will present. In the absence of prophylaxis, CMV infection peaks during this time period (11–13). Similarly, in the absence of the use of preventive strategies, EBV-associated posttransplant lymphoproliferative disorders (PTLD) (6,23,24), *Pneumocystis jirovecii* pneumonia (PCP) (25–27) and toxoplasmosis (28), could also occur during this period. A review of autopsies found infections to be the most common cause of death during this period after lung or heart-lung transplantation; disseminated adenovirus and *Aspergillus* infection predominated, followed by CMV and EBV disease (29,30).

Late infections

In the later period (beyond 180 days following transplantation), infection risks vary with immunosuppression and exposures. There are some differences in adults and children. In general, rates and severity of infection in children more than 6 months after transplantation are similar to those observed in otherwise healthy children (7). This is most likely attributable to the fact that pediatric transplant recipients are usually maintained on lower levels of immunosuppression at that time. This may not be the case for adults in whom underlying comorbidities, such as diabetes mellitus and malignancies, may increase the risk for infections during this later period. Those individuals who require increased immunosuppression, either related to rejection or underlying disease, will be at greater risk for late opportunistic infections. CMV can manifest late, particularly in children and adults who receive prolonged prophylaxis (31) and PTLD continues to manifest in the late period (23,24). In addition, recurrent infections with stereotypical pathogens may occur late after transplant in certain recipients with specific conditions as demonstrated in recipients of lung transplantation with chronic lung rejection manifested as bronchiolitis obliterans syndrome (BOS). These patients frequently become infected with *Pseudomonas, Stenotrophomonas* and Aspergillus (15,29,30). In both pediatric and adult organ recipients, chronic or recurrent infections continue to occur in the subset who have uncorrected anatomic or functional abnormalities (e.g. vesicoureteral reflux, biliary stricture). During this period, children are more likely to be at risk for primary infection with certain community-acquired viral pathogens, such as the herpesviruses (Varicella, EBV and CMV) (32). Finally, both adult and pediatric patients continue to be at risk of being exposed to community-acquired respiratory and gastrointestinal viral pathogens. In general, in the absence of ongoing requirements for higher levels of immune suppression or graft dysfunction, these infections are fairly well tolerated by transplant recipients late after SOT.

Infections occurring throughout the postoperative course

Some infections occur irrespective of time. These may reflect nosocomial acquisition which is seen more commonly in the presence of invasive devices (e.g.

intravenous catheters, urinary cathteters, endoctracheal intubation and surgical procedures). Community and nosocomial exposures to diverse bacteria, viruses, fungi and parasites/protozoa may also result in new infections in this population at any time. In some cases, these may be seasonal (e.g. influenza, RSV, rotavirus) or related to unique outbreak situations. Diagnostic studies should be modified to address these possibilities. Specific pathogens are addressed throughout these guidelines.

AST infectious disease guidelines: use and applications

The third edition of the AST Infectious Disease Guidelines updates and expands the content and recommendations provided in the first two editions. As a comprehensive set of clinical practice guidelines, they were developed to assist in clinical decision making. They are based upon the highest level of scientific evidence available. The content of the guidelines includes salient background, clinical and pathophysiologic data as well as specific statements and recommendations relevant to the diagnosis, management and prevention of specific pathogens and disease entities. Given the unique circumstances associated with individual transplant candidates and recipients, these guidelines are not proscriptive; rather they provide preferred approaches for management of these very complex patients. It is hoped that through the application of the general principles outlined in this introduction and the more specific recommendations included throughout the guidelines, practitioners will acquire useful knowledge that will enhance the outcome and care of recipients of SOT.

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Disclosure

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Special Article

Screening of Donor and Recipient in Solid Organ Transplantation

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Key words: Donor infection, donor-to-host transmission, latent infection, prevention, screening, testing

Abbreviations: HBV, hepatitis B virus; HIV, human immunodeficiency virus; PPD, purified protein derivative (TB screening test).

Background

Pretransplant screening of potential organ donors and recipients is essential to the success of solid organ transplantation (1–4). The goals of pretransplant infectious disease screening are to identify conditions which may disqualify either donor or recipient; identify and treat active infection pretransplant; recognize and (if possible) define the risk of infection and develop strategies for preventing and mitigating posttransplant infection; and implement preventative measures, including immunizations (5). While there is general agreement on the major infections for which routine screening is performed, centers vary in the extent of infectious diseases investigation and the actions taken as a result.

Potential recipients should be evaluated for infection risk by obtaining a thorough medical history, including details of prior infections, places of travel and residence, and exposures to animal and environmental pathogens. While all potential recipients undergo screening for the presence of infections such as HIV, hepatitis C (HCV) and cytomegalovirus (CMV), the detailed history can focus additional testing if necessary to mitigate and prevent the reactivation of latent infections posttransplant. Pretransplant recipient screening also helps determine immunity to vaccine-preventable illnesses and may help with allocation of infected donor organs to recipients with known immunity to certain pathogens (6). The pretransplant period is an ideal time for detailed counseling of the recipient and his/her family about safe food handling and the risk of infection associated with pets, travel and hobbies such as gardening and woodworking. Infection prevention approaches including hand hygiene, prophylactic antimicrobials, postexposure prophylaxis and updating of immunizations should be addressed as well.

A variety of pathogens may be transmitted by transplantation (Table 1) (7–10). Previous guidelines for pretransplant screening have been developed by a number of national and international multidisciplinary transplant groups (6,10–15). The Centers for Disease Control and Prevention (CDC) have published guidelines for the prevention of HIV transmission through transplantation (16). These are in the process of revision in order to address updated knowledge of transmission of HIV and other bloodborne pathogens. In addition, the work of the *ad hoc* United Network of Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) Disease Transmission Advisory Committee (DTAC) has helped define the risk of infection and disease transmission in organ donation in the United States and shape the discussion of screening and preventive measures (17,18).

While conventional screening strategies are very effective in most cases, they are not a guarantee against donor-derived infections. There have been a number of high-profile incidents of donor-transmitted infection reported in recent years, including rabies (19), lymphocytic choriomeningitis virus (20), West Nile virus (21), HIV (22–24) and HCV (23,24), which have renewed discussion of the process of organ donor screening. In addition to DTAC, other transplant and public health community initiatives have helped guide practice in the hope of developing a more robust sentinel network to detect and respond to donor transmission events in a more timely manner (20,25–28).

This guideline summarizes current opinions on screening for bacterial, mycobacterial, fungal, parasitic and viral infections in the donor and recipient (Table (2) (5). More detailed discussions of these infections, including posttransplant monitoring, prophylaxis and treatment are found in other sections of these Guidelines.

Due to the lack of expansion in the available organ pool despite steady increases in the need for organ replacement for end-stage diseases, it has become necessary to consider marginal donors, including those with active infection at the time of donation, higher risk serologic profiles, or a

Table 1: Pathogens reported to be transmitted with solid organ transplantation

Bacteria	Mycobacteria
Staphylococcus aureus	*Mycobacterium tuberculosis*
Klebsiella species	Nontuberculous mycobacteria
Bacteroides fragilis	Parasites/protozoa
Pseudomonas aeruginosa	*Toxoplasma gondii*
Escherichia coli	*Strongyloides stercoralis*
Salmonella species	*Plasmodium* species
Yersinia enterocolitica	*Trypanosoma cruzi*
Treponema pallidum	*Pneumocystis jiroveci*
Brucella species	Viruses
Enterobacter species	Cytomegalovirus
Acinetobacter species	Epstein–Barr virus
Legionella species	Herpes simplex virus
Nocardia species	Varicella-zoster virus
Listeria monocytogenes	Human herpesvirus-6
Fungi	Human herpesvirus-7
Aspergillus species	Human herpesvirus-8
Candida species	Hepatitis B, D
Coccidioides immitis	Hepatitis C
Cryptococcus neoformans	Human immunodeficiency virus
Histoplasma capsulatum	Parvovirus B19
Scedosporium apiospermum	Rabies
Prototheca species	Lymphocytic choriomeningitis virus
Zygomycetes	West Nile virus
	BK virus
	Human T cell lymphotropic virus (HTLV)-1/2

Table 2: Frequency utilized serologic tests for screening of donor and recipient prior to transplantation

Tests commonly obtained in both donor and recipient
Human immunodeficiency virus (HIV) antibody
HSV (herpes simplex) IgG antibody (at some centers)
Cytomegalovirus (CMV) IgG antibody
Hepatitis C (HCV) antibody
Hepatitis B (HBV) surface antigen (HBsAg)
Hepatitis B core antibody (HBcAb IgM and IgG, or total core antibody)
Hepatitis B surface antibody (HBsAb)
Rapid plasma reagin (RPR)
Toxoplasma antibody (especially in heart recipients)
Epstein–Barr virus (EBV) antibody (EBV VCA IgG, IgM)
Varicella-zoster virus (VZV) antibody
Other screening measures for infectious diseases
Purified Protein Derivative (PPD) or interferon gamma release assay (IGRA) for latent TB infection in recipients
Strongyloides serology (for recipients from endemic areas)
Coccidioides serology (for recipients from endemic areas)
Trypanosoma cruzi serology (for donors and recipients from endemic areas)
Serologies for tetanus, diphtheria, measles, mumps and pneumococcal titers as an aid to pretransplant immunization (at some centers)
Optional screening measures
West Nile virus serology or NAT
HHV-8 serology
BK serology (kidney donor and recipients)
Nucleic acid amplification testing (NAT) for HIV, HCV, HBV, particularly in donors with high-risk social histories

social history indicating potential exposure to bloodborne pathogens such as HIV or HCV. The natural history and treatment options for donor infection, the urgency of transplantation of a vital organ into a recipient and the likelihood (or lack thereof) of another organ offer for the patient on the transplant waiting list must all be weighed in determining the acceptability of the potentially infected donor.

Donor Screening

Living donors

The differences in screening of the living donor and the deceased donor are largely based on the different time constraints during which the evaluation must take place. For the living donor, it is often possible to treat active infection and delay transplantation until the infection resolves. If there is a significant delay between donor evaluation and transplantation, interim evaluation may be indicated to rule out recently acquired infection. Clinical reassessment of the prospective living donor is indicated if clinical signs or symptoms of possible infection occur, particularly any unexplained febrile illness between the time of initial screening and the planned date of transplantation. The CDC has recommended that all living donors be rescreened with HIV serology and HIV nucleic acid amplification testing (NAT) prior to organ donation, to look for evidence of recently acquired infection (29). Similarly, consideration should be given to repeating serologic HBV testing and HCV NAT in the potential living donor with risk factors for these infections.

The screening of a prospective living donor includes a thorough medical and social history, physical examination, laboratory studies including serologic testing (Table 2) and radiographic workup as indicated by the donor's history and the procedure to be performed. The medical history should include an assessment of previous infections, vaccinations, travel and occupational exposures, as well as the presence of behaviors posing risk for bloodborne or sexual pathogen exposure (e.g. drug use, sexual practices, incarceration). Living donors should be screened for syphilis, HIV, hepatitis B and C, and tuberculosis via a tuberculin PPD skin test or interferon-gamma release assay (IGRA) (II-2). If there is any suspicious donor history, additional testing may be warranted. Consultation with a transplant infectious disease specialist may help with determining additional workup, counseling and management while awaiting transplantation, should another living donor not be available.

Deceased donors

By contrast, the time frame for deceased donor evaluation is typically hours. Serologic workup is performed in laboratories associated with organ procurement organizations

or similar screening agencies (hereafter referred to as OPOs) which operate on a 24-h basis to generate the data needed to determine donor suitability. Because of time constraints and the extensive geographic areas covered by some OPOs, testing is often limited to serologic methods that are rapid and routinely available. Because more sensitive testing may not be available, some infections, such as HIV and HCV, may be difficult to diagnose at an early stage, before the development of specific antibody (23–25,30). Thus, a comprehensive social and medical history on the donor is required to identify risk for infections that might not be detected by serologic testing. Furthermore, certain infections may come to light only after the transplant has been performed, when results of routine procurement cultures of blood, urine and sputum become available. Increasingly, some OPOs are utilizing rapid molecular testing, particularly in high-risk potential donors, including NAT testing for HCV, HBV and HIV. A recent consensus conference on the utility of routine NAT testing was, however, inconclusive, largely due to concerns that testing is not feasible within the deceased donor timeframe in some areas, as well as concern that false positive test results in potential donors with no identified risk factors for infection might result in wastage of viable organs (30). Testing for certain pathogens with particular geographic significance such as *Trypanosoma cruzi* (Chagas' disease), endemic mycoses and West Nile virus may be performed by some OPOs. If a deceased donor with uncertain risk is to be used, informed consent of the recipient should include the risk for infection transmission.

Donor screening: bacterial infections

The goal of evaluation of the potential living or decreased donor is to diagnose any infection with the risk of transmission to the recipient(s). Bacterial infections of the respiratory tract, urinary tract or the organ to be transplanted should be treated with documentation of resolution of infection prior to donation. The potential kidney donor with urinary tract infection should be investigated to rule out upper tract involvement. In the potential donor with a history or suspicion of prior bloodstream infection, a thorough investigation should be performed to insure that infection is not present in the target organ.

Syphilis may be latent and asymptomatic in the donor and requires therapy if time permits. Syphilis has rarely been transmitted by transplantation, but it is not a contraindication to organ donation if each recipient is treated posttransplant with an appropriate course of penicillin (31) (II-3).

Deceased donors may harbor known or unsuspected bacterial infections (6,30–35). Attempts to rule out the presence of active infection should include obtaining a detailed history from the donor's family, recent contacts and (if possible) primary care physician, as well as a complete review of medical records, vital signs, physical exam, radiographic studies and any available microbiologic studies. Blood cultures should be obtained to rule out occult donor bacteremia. Bacteremia with virulent organisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* may result in early posttransplant sepsis or mycotic aneurysm formation at the site of allograft vascular anastomoses (32–36). Although a review of 95 bacteremic donors found no evidence of transmission when recipients were treated with antimicrobial therapy for a mean of 3.8 days posttransplant (36), the standard of care is to administer longer courses of therapy in the recipient (e.g. 2 weeks) if the donor is known to have been bacteremic with a virulent organism (II-2).

In general, there is no reason to treat the recipient of an allograft from a deceased donor with nonbacteremic, localized infection not involving the transplanted organ, with the exception of meningitis, in which occult bacteremia frequently occurs (III). Organs have been successfully transplanted from donors with bacterial meningitis due to pathogens such as *Streptococcus pneumoniae* when appropriate antimicrobial therapy was administered to both the donor and recipients (37).

Lung transplantation deserves special attention (38). Donor bacterial colonization is common, as the lungs are in contact with the external environment, and the airways are colonized with multiple organisms, with increasing resistance noted in the hospitalized, critically ill potential organ donor. Donor bronchoscopy with cultures performed at the time of evaluation and/or procurement allows for the administration of antibiotics directed at these colonizing organisms, and can prevent invasive infection in the recipient (III) (7,8,38).

Allograft contamination may occur during organ procurement or processing (39). Interpretation of organ preservation solution cultures is challenging, as contamination can occur (39–42); however, infection transmission from contaminated solutions appears to be uncommon (39,40,42). A report of kidney preservation fluid contamination with *Candida* species in eight recipients demonstrated that the risk of mycotic aneurysm rupture can be mitigated with appropriate antifungal therapy (41).

If a donor is determined to have active bacterial infection at the time of procurement, antibiotics should be administered to each recipient for at least 14 days for infections with Gram-negative bacilli, *Staphylococcus aureus*, or *Candida* species (II-3). A shorter course of therapy may be considered for less virulent organisms (III).

Donor screening: mycobacterial infections

Mycobacterium tuberculosis (TB) has been transmitted by transplantation; in the largest study to date (511 recipients), donor transmission accounted for approximately 4% of reported posttransplant TB cases (43). Potential living donors should have PPD testing performed (a two-stage tuberculin

skin test if from an endemic area) or TB interferon-gamma release assay (IGRA) testing (43–45); if either test is positive, additional testing should be performed to rule out the presence of active infection (III). Any donor with active tuberculosis should be excluded from donating until therapy has been completed and all signs of infection have resolved. A positive PPD is defined as the presence (at 48–72 h) of 5 mm or more of induration in immunosuppressed patients or those with contact with a person with active TB; 10 mm or more in injection drug users, employees or residents of hospitals, nursing homes or other group settings, and children under the age of 4; and 15 mm or more for all others. All potential donors with evidence of latent TB infection (i.e. a positive PPD or a positive IGRA test) should have a chest radiograph to look for evidence of active pulmonary infection. If there are symptoms of infection or radiographic findings suggestive of active disease, acid fast bacilli (AFB) cultures of sputum and/or other appropriate specimens should be performed. In the potential kidney donor with evidence of latent TB infection (LTBI), this could include urine AFB cultures and abdominal CT scanning. If there are no signs or symptoms of active disease and the chest radiograph is normal, sputum AFB cultures are not indicated due to their low yield.

Management of the prospective living donor with LTBI varies with the degree of local endemicity. Delay of transplant until the living donor is treated (with isoniazid for 9 months, rifampin for 4 months, or isoniazid and rifapentine for 3 months) is appropriate, should another suitable donor not be available. In TB endemic areas, where as many as 30–40% of donors have LTBI, it may be difficult to avoid the use of infected donors. Isoniazid prophylaxis of the recipient of an organ from a living donor with LTBI is an option but controlled studies are needed to determine the efficacy of this practice (III).

In deceased donors, time does not allow for tuberculin skin testing, and the IGRA is not logistically practical in most cases. Donors in whom active tuberculosis is a clinical possibility should not be utilized (II-2). In cases where a potential donor is known to have recent PPD skin test conversion, suggesting recent acquisition of infection with the potential for a high organism burden, transplantation should be approached with caution due to the risk of dissemination in the recipient. Donors with a history of an untreated positive PPD but without evidence of active disease are acceptable, but warrant consideration of treatment of the recipient(s) with isoniazid (III) (43,45,46). New guidelines for the prevention and management of *Mycobacterium tuberculosis* in organ transplantation have been published in the *American Journal of Transplantation* (47).

Donor screening: fungal infections

Active systemic fungal infection in the donor is a contraindication to transplantation. The endemic mycoses may be difficult to diagnose, as infection may be dormant. Transmission of histoplasmosis by transplantation has been described (48), but most cases appear to be the result of reactivation of past infection in the recipient. In many individuals from the Midwestern United States, calcified pulmonary, hilar and splenic granulomata are the radiographic residua of old *Histoplasma* infection, but such signs have not traditionally been considered a contraindication to donation (III). Transmission of coccidioidomycosis by lung transplantation has been reported in the Southwestern United States (49), although reactivation of coccidioidomycosis in the previously infected recipient appears to be far more common (50). There are no uniform recommendations for donor screening for endemic mycoses.

Donor screening: parasitic infections

Toxoplasmosis is a significant issue in heart transplantation, where the *Toxoplasma*-seronegative recipient of a *Toxoplasma*-seropositive heart is at highest risk for developing active toxoplasmosis posttransplant (51–53). Toxoplasmosis has also rarely been transmitted to liver and kidney recipients (52,53). Donor seropositivity is not a contraindication to heart donation but allows for appropriate prophylaxis to be administered to the recipient; routine trimethoprim-sulfamethoxazole prophylaxis against *Pneumocystis jiroveci* is effective in preventing toxoplasmosis and may negate the need for serologic testing in areas of low prevalence (53). Screening of donors for *Toxoplasma* is not routinely performed for noncardiac donors but is part of the screening panel at some transplant centers and OPOs.

Transmission of Chagas' disease (*Trypanosoma cruzi*) by transplantation is a significant problem in endemic areas (Mexico, Central and South America) but has increasingly been reported in the United States (54). A recent consensus conference resulted in recommendations including avoidance of transplantation of the hearts from infected donors and monitoring other recipients with PCR and microscopy of buffy coat to detect early infection and initiate therapy (55).

Donor and recipient screening: viral infections

As the serologic status of both donor and recipient is crucial in determining the risk of infection, screening for viral infections in both the donor and recipient will be discussed together, and is detailed in Table 3. Caution should be used in interpreting antibody status in infants, due to the role of maternal antibody. More detailed information on the clinical presentation and treatment of these infections is found elsewhere in these Guidelines.

Cytomegalovirus (CMV)

The CMV serologic status of donor and recipient is an important predictor of posttransplant infection, with the CMV seronegative recipient of a CMV seropositive donor organ (D+/R−) being at highest risk for development of tissue-invasive CMV, recurrent CMV and

Table 3: Interventions related to donor and recipient screening results

Pathogen	Donor antibody status	Recipient antibody status	Recommendations regarding transplantation	Comment
HIV	Positive	Negative	Reject donor	HIV + donors must be excluded in the United States by law
	Negative	Positive	Proceed if HIV well controlled; be cautious about major drug interactions between antiretrovirals and CNIs	
HTLV-1/2	Positive		Generally exclude HTLV 1 + donors for organ donation (may be used in life-threatening situations, with informed consent)	Lack of a rapid assay distinguishing HTLV-1 and 2 is a significant concern; if HTLV-2 is confirmed, proceed with transplant. If confirmed HTLV-1+ would reject donor.
CMV	+ or −	Positive	Proceed	D/R status used to determine prevention strategy (preemptive therapy versus prophylaxis)
	Positive	Negative	Accept; high risk for CMV infection	See CMV guideline for approach to management of the CMV D+R- recipient
EBV	+ or −	Positive	Proceed	
	Positive	Negative	Accept; higher risk for primary EBV infection and PTLD	Consider posttransplant NAT monitoring to guide immunosuppression
Toxoplasma gondii	+ or −	Positive	Proceed	TMP/SMX prophylaxis effective in prevention
	Positive	Negative	Accept	Heart transplant donors should receive prophylaxis with TMP/SMX. If intolerant or allergic, use atovaquone or dapsone with pyrimethamine and folinic acid.
HCV	Positive	Positive	? Accept	If used, reserve HCV + organs for recipients with Ab to HCV or severely ill recipient
	Positive	Negative	Decision depends on urgency of transplantation	Some centers accept in severely ill recipient and/or elderly recipient; controversial in kidney transplantation
HBV	HBsAb+	+ or −	Accept	
	HBsAg+	− HBsAb	Reject	
		+ HBsAb	Reject	Some centers use in life-saving situations with preemptive antiviral treatment of the recipient
	HBcAb	− HBsAb	Reject	
	IgM+	+ HBsAb	Reject	Some centers use in life-saving situations with preemptive antiviral treatment of the recipient
	HBcAb IgG+ (with concurrent negative HBsAg and negative HBcAb IgM)	− HBsAb	Reject unless for liver transplant in life-saving situation	Risk of transmission high, some centers use with intensive prophylaxis (HBIg +/- antivirals)
		+HBsAb	? Accept	Some centers accept for extrahepatic transplants, in immune recipient, with antiviral prophylaxis
RPR (syphilis)	Positive	+ or −	Accept	Recipients should be treated for presumed transmission with penicillin
CNS viral pathogens (e.g. LCMV, rabies, WNV)	Clinical suspicion of infection		Reject	

CNIs = calcineurin inhibitors; D+/R− = donor seropositive, recipient seronegative; PTLD = posttransplant lymphoproliferative disease; RPR = rapid plasma reagin; TMP-SMX = trimethoprim sulfamethoxazole.

ganciclovir-resistant CMV (56–58). Consequently, all donors and recipients should be tested for CMV infection using commonly available serologic techniques. While not a contraindication to transplantation, D+/R− status is an indication for more intensive monitoring and prevention strategies posttransplant than in donor/recipient pairs with a lower risk of CMV infection (II-2). The seropositive recipient, regardless of donor status, is at risk for CMV reactivation and usually receives either prophylaxis or preemptive monitoring and therapy. There are many different protocols in use; a full discussion of CMV prevention and treatment is found elsewhere in the Guidelines.

Epstein–Barr virus (EBV)

While primary EBV infection can be severe and disseminated in the posttransplant setting, the development of posttransplant lymphoproliferative disease (PTLD) is the most feared EBV-associated complication. The highest PTLD risk is in the EBV seronegative recipient of an EBV seropositive graft, which most commonly occurs in pediatric recipients (59–61). The risk of PTLD can also be increased in the seropositive recipient, especially under the influence of potent immunosuppressants such as antithymocyte globulin (ATG) and belatacept. Awareness of pretransplant serologies helps target the highest risk group for close monitoring by EBV-PCR and preemptive interventions such as decreasing immunosuppression (II-2) (59–61). EBV serology should be performed on all donors and recipients in order to define the risk of posttransplant lymphoma (II-2). The British Transplantation Society and British Committee for Standards in Haematology recently published extensive guidelines on the pretransplant screening and diagnosis of PTLD in organ transplant recipients (62).

Other herpesviruses

Other herpesviruses of clinical importance in the transplant recipient include herpes simplex virus (HSV-1 and HSV-2), varicella-zoster virus (VZV), human herpesvirus-6 and 7 (HHV-6 and -7), and HHV-8. HSV screening is performed by some centers, whereas other centers administer universal antiviral prophylaxis for at least the first month posttransplant. As primary varicella infection posttransplant can be fatal, VZV screening of the recipient is important, with vaccination of the seronegative recipient pretransplant if at all possible (III).

Recent awareness of the possible roles of HHV-6 and HHV-7 as cofactors for CMV effects, fungal infections and possibly allograft dysfunction has led to increasing interest in these viruses (63). Since almost all adults are seropositive, however, donor and recipient screening for these viruses has not generally been recommended. Whether such screening would be helpful in pediatric transplant programs is unknown. HHV-8, the agent of Kaposi's sarcoma, can reactivate after transplantation and may be transmitted by transplantation (64–66). Seroprevalence varies widely according to the population studied. Optimal strategies for prevention of reactivation have not been defined; thus definitive recommendations for pretransplant screening cannot be made at this time.

Hepatitis B (HBV)

All donors and recipients should be tested for hepatitis B using standard serologic techniques. The complex issues surrounding HBV and transplantation are discussed in more detail in the hepatitis section of these Guidelines. Donor screening should include at least hepatitis B surface antigen (HBsAg) and HBV core antibody (HBcAb, which should be performed as separate IgG and IgM to be most useful). Donor HBsAg positivity or HBcAb-IgM positivity indicates active HBV infection. HBsAg negative, HBcAb-IgM positive persons may be in the 'window period'; such donors have generally not been utilized, although some centers have used these donors in recipients with evidence of immunity to hepatitis B (those with a positive hepatitis B surface antibody, HBsAb) and/or with intensive posttransplant prophylaxis and monitoring. Isolated HBsAb positivity usually indicates prior vaccination or resolved infection and is not generally considered a risk for HBV transmission.

The most complex question is the use of the HBsAg negative, HBcAb-IgG positive donor ('core-positive donor') (67–69). This may represent either a false-positive test (if isolated HBcAb positive) or the presence of chronic HBV infection. In the latter, there is a significant risk of transmission of HBV to a liver transplant recipient, and therefore these livers were often not utilized in the past (II-2); however, it has now become more common to transplant livers from HBcAb positive donors utilizing intensive posttransplant prophylaxis (68). The risk for transmission to extrahepatic recipients appears to be low, but has occurred (68,70–72); this risk can be decreased by pretransplant HBV vaccination of the recipient. Some centers restrict the use of organs from the core-positive donor to life-threatening situations and/or vaccinated recipients, or would utilize posttransplant prophylaxis with hepatitis B immune globulin (HBIG) and/or lamivudine if transplanted into a nonimmune recipient (II-3) (12,13,72). Because of the possibility of being offered such an organ, it is prudent to vaccinate all seronegative transplant candidates with HBV vaccine, although the response to this vaccine in patients with end-stage organ disease may be suboptimal, requiring higher doses and repeated injections to attain immunity (III). A donor HBV-DNA level provides helpful information for designing prophylactic strategies, even if the result is received after transplant (14). Additional information on prophylactic strategies may be found in the hepatitis section of these Guidelines (see chapter 16).

Recipient screening for HBV is helpful in posttransplant management. In patients undergoing a liver transplant because of end-stage liver disease due to HBV, there are a variety of posttransplant protocols for prevention of reactivation of HBV, many utilizing HBIg and/or antiviral agents. Extrahepatic transplantation in HBsAg positive recipients has been controversial. In the early days of kidney transplantation, such transplants were performed, resulting in early fulminant hepatitis in some recipients and chronic liver disease in many. Some have maintained asymptomatic status after many years despite evidence of active viral replication (70). With effective antiviral therapies such as lamivudine, adefovir and tenofovir being available, it appears theoretically possible to transplant such recipients more safely (72) although antiviral resistance may become an issue (III).

Hepatitis C (HCV)

HCV infection is frequently chronic, and donors and recipients should be tested for the presence of HCV via standard serologic techniques. HCV is a major indication for liver transplantation, and although HCV recurrence is common posttransplant, patient and allograft survival are not significantly worse than with other pretransplant diagnoses. HCV seropositive renal transplant candidates are at higher risk for liver disease and sepsis after transplant than are their HCV seronegative counterparts, but compared with no transplantation as the alternative, the risk is outweighed by the benefit in most cases (73,74). The role of pretransplant treatment of HCV viremia remains under study. Strategies for management of HCV in the recipient are discussed in detail elsewhere in the Guidelines (see chapter 16).

Utilization of hepatitis C antibody-positive donors remains controversial, due to the high risk of transmission of HCV through transplantation of any organ. A positive donor HCV NAT (HCV-RNA), indicative of active viral replication, has been associated with a higher risk of transmission, but results of this testing may not be available prior to transplantation from a deceased donor (30). The risk of transmission from NAT negative, HCV antibody positive donors has not yet been fully defined. As recent transmission events have proven, HCV can be transmitted to multiple organ and tissue transplant recipients from a seronegative donor (23,24). The time between infection and antibody production can vary in HCV-infected individuals, although viral RNA is present much earlier than antibody after acute infection. More rapid molecular tests are in development in the hope of clarifying risk from deceased donors prior to a decision to accept an organ. Whenever an HCV seropositive donor is utilized, stringent informed consent is advisable.

Human immunodeficiency virus (HIV)

HIV-seropositive donors have not been utilized in transplantation, due to the known risk of transmission to the recipient; in the United States, use of HIV seropositive donors is illegal. HIV-1 and HIV-2 serologies are required for all potential donors and recipients; while HIV-2 is rare in the United States and HIV-2 screening serologies are frequently falsely positive, specific testing for this virus should be performed on those donors or recipients from western Africa, where HIV-2 is endemic. Western blot testing should be obtained for confirmation of any positive screening test for either HIV-1 or -2. In the potential living donor with risk factors for HIV exposure but negative HIV serology, NAT testing should be obtained, as these tests become positive prior to the development of a positive antibody test. Due to the efficacy of highly active antiretroviral therapy (HAART), HIV infection in the recipient is no longer a contraindication to solid organ transplantation. Multiple studies worldwide, including a multicenter prospective trial in the United States, have evaluated transplantation in the stable HIV-infected patient (75,76). One- and 3-year graft and patient survival data are comparable to non-HIV infected patients undergoing transplantation, but meticulous clinical care and careful attention to pharmacokinetics in the setting of significant drug interactions between immunosuppressive agents and HAART are paramount to success (75,76). While a higher than expected acute rejection rate was noted in 150 HIV-positive kidney transplant recipients, HIV infection remained well controlled and patient and graft survival was comparable to the non-HIV population (76). The complex issues involved in transplanting this population are more fully discussed in the HIV section of these Guidelines (see chapter 17).

Human T-Lymphotropic virus (HTLV-1/2)

HTLV-1 is endemic in certain parts of the world including the Caribbean, Japan and parts of Africa, and is often asymptomatic. However, infection with HTLV-1 can progress after years or even decades to HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) or to adult T cell leukemia/lymphoma (ATL); progression occurs in <1% and 2–4% of seropositive individuals in endemic regions, respectively (77–81). HTLV-2 is a virus which is likely more widespread geographically and is serologically difficult to distinguish from HTLV-1, although its association with disease processes is less certain.

Screening for HTLV-1/2 in deceased donors (but not recipients) was standard in US practice until 2009, when UNOS/OPTN discontinued the requirement to perform prospective deceased donor screening, largely as a result of the lack of a serologic test to distinguish HTLV-1 from HTLV-2 (77). Despite its low prevalence in the United States, cases of donor-transmitted infection have occurred, some with significant neurologic and malignant complications (82–85). Graft and patient survival in recipients of HTLV-1/2 seropositive donor organs has been noted to be similar to that of recipients of HTLV-1/2 seronegative organs (79). Western blot testing or NAT may be used to distinguish HTLV-1 from HTLV-2, and may prevent unnecessary wastage of organs from donors with false positive test results or HTLV-2 infection, neither of which should preclude donation (79,80). However, reports from Spain of donor-derived transmission of HTLV-I with rapid development of myelopathy in the recipients suggest that caution should be exercised in the use of HTLV-1 infected donors (82–84). In endemic areas, recipients are often tested for HTLV-1/2 antibodies, although little is known about the course of infection following solid organ transplantation. No cases of HTLV-1 reactivation were observed in a series of Japanese HTLV-1 seropositive recipients undergoing renal transplantation (85).

Emerging or unusual viral infections—West Nile virus, lymphocytic choriomeningitis virus, rabies and SARS

It has been increasingly recognized that emerging donor-derived viral infections can have an impact on transplant outcomes, with unusually severe presentations in recipients (19–21,86,87). In most cases, an effective screening

test is not available, so that familiarity with the risks for acquisition and the natural history of these infections is important to assessing offers for deceased donor organs.

West Nile virus (WNV) is a flavivirus which can cause meningoencephalitis, and which has recently appeared in the United States. First reported in 2002, WNV has been transmitted via blood transfusion and solid organ transplantation (21,87,88). It is unclear as yet what the magnitude of the risk of such transmission is, and any risk assessment is complicated by the fluctuating levels and geographic distribution of WNV infection in mosquitoes and humans each year. Serology and PCR for WNV are available but are time consuming. It is prudent to avoid any donor who has had an unexplained febrile illness, mental status changes, or meningitis or encephalitis. Transplant centers should be especially concerned about the use of such donors during times of high prevalence of infection in the region. Since July 2002, all US blood bank products have been tested for WNV using a NAT assay. In the fall of 2003, the US Health Resources and Service Administration (HRSA) issued a Guidance statement regarding organ donors and West Nile virus, which recommended testing all prospective live donors with NAT close to the time of transplant; avoiding donors with any form of unexplained or confirmed WNV encephalitis; and heightened clinical suspicion on the part of the treating clinician for any febrile illness occurring shortly after transplant. NAT poses logistical challenges in some UNOS regions, and is not currently mandated for donor screening. There is also concern that false positive NAT results may lead to a loss of noninfected organs and net loss of life, particularly for liver and heart candidates on the waiting list (89).

Lymphocytic choriomeningitis virus (LCMV): A rodent-associated arenavirus has been reported in several clusters of donor-derived transmission to multiple organ recipients, all but one of which had fatal infection (20,90–92). In one cluster, the outbreak originated from a new pet hamster in the donor's home (20,90,93). To date, despite several similar outbreaks, an effective screening test to rule out infection with LCMV in potential organ donors has not been developed (20,94). The CDC has issued guidelines for minimizing the risk of LCMV related to pet rodents (95). Transplant centers should consider the possibility of LCMV infection in the donor with aseptic meningitis, as well as in the seemingly asymptomatic donor with contact with wild or pet rodents (10,91).

Rabies is another potentially fatal donor-derived infection. In the most well-described outbreak, recipients of transplants from a donor who died of subarachnoid hemorrhage developed rapidly progressive encephalitis; all succumbed to infection (19). Retrospectively, the donor was determined to have had a recent bat bite and was seropositive for rabies virus (19,96). In the United States, rabies is transmitted most commonly by bites, scratches or other saliva exposure from bats, raccoons, skunks or foxes. The rabies and LCMV cases raise the question of whether all donor evaluations should include information about exposure to animals, bites and other environmental exposures to supplement the already detailed information obtained. Because of the highly fatal nature of rabies infection, clinicians are encouraged to avoid donors where even a small possibility of rabies is present.

In 2003, a new respiratory pathogen was reported to cause severe disease with rapid international spread. *SARS (Severe Acute Respiratory Syndrome)* was found to be due to a previously undescribed coronavirus (SARS-CoV), with nosocomial and household transmission. At least 10% of affected patients required mechanical ventilation; at least one transplant recipient died of SARS (97). While transmission by transplantation is theoretically possible, the extent of this risk is unknown. Current principles of donor and recipient selection would likely exclude patients with recent acute illnesses meeting SARS criteria; however the consequences of a more remote history of SARS, or a subclinical infection, are unknown. Screening tools for potential adult and pediatric donors were proposed by experts in Toronto (one of the major centers of the 2003 outbreak) which took into account the risk of SARS transmission at the donor's hospital as well as donor's symptoms, travel and contact history (97). If another SARS or a similarly transmitted emerging virus outbreak should occur, this donor-screening algorithm would be useful.

Influenza A

In 2009, a novel influenza virus A(H1N1pdm09) caused a worldwide pandemic. Infection was most common in younger patients with severe disease and secondary bacterial infections in pregnant women and those with underlying chronic lung disease, many of whom required intensive care support. The impact on pediatric transplantation was considerable, with prolonged hospitalizations, secondary infections, yet few reported deaths in those who received early antiviral therapy (98). Guidelines for pretransplant screening of potential donors and recipients were published (99). These recommended screening of donors with symptoms consistent with influenza infection; routine screening was not recommended. Due to concern for possible donor transmission, it was recommended that donors who had received adequate antiviral therapy be considered safe for nonlung or small bowel donation. Empiric treatment of the recipients of organs from infected donors with incomplete treatment was recommended (100–102). The pandemic emphasized the need for transplant centers to be vigilant about vaccination of recipients and staff, and to be alert for local outbreaks of disease with the possibility of transmission through transplantation.

Other new and emerging, potentially communicable agents may arise which may affect donor acceptability or recipient activation on the transplant list (86,87). It is advisable to avoid transplantation involving individuals with

potentially communicable infections for which inadequate information exists to provide appropriate recommendations regarding precautionary measures.

Ancillary screening tests for emerging pathogens, or more sensitive testing for known pathogens, may be proposed by guidelines committees in the future (8,30,103). Such groups will have to consider the feasibility of testing within the limited deceased donor timeframe as well as the risk of false-positive test results which could lead to wastage of otherwise life-saving organs (88,103).

Recipient screening: pretransplant detection of active infection in the recipient

Transplant recipients are at risk for infections related to complications of end organ failure. Patients awaiting kidney transplantation may have infected hemodialysis or peritoneal dialysis access sites or catheters, or complicated upper- and/or lower-tract urinary infections. Candidates awaiting liver transplants are at risk for aspiration pneumonia, spontaneous bacterial peritonitis, urinary tract infection and infections associated with intravenous catheters. Pancreas transplant candidates can develop diabetic foot infections and associated osteomyelitis. Those awaiting heart transplants may have infections related either to indwelling intravenous catheters, or to ventricular assist devices (VADs) utilized as a bridge to transplantation (104,105). In addition, heart candidates are also at risk for pneumonia in the setting of congestive heart failure and debilitation.

VAD (ventricular assist-device)-associated infections are not a contraindication to transplantation, as complete removal of the VAD at the time of transplant, combined with appropriate posttransplant antibiotic therapy, is often curative (104,105).

Screening of lung transplant recipients includes an assessment of colonizing airway flora, and careful review of their previous pulmonary infections (106). Cystic fibrosis patients may be colonized with multi-resistant strains of *Pseudomonas* and/or *Burkholderia cepacia* as well as other organisms such as *Staphylococcus aureus*, *Alcaligenes*, *Klebsiella, Acinetobacter, Stenotrophomonas*, *Aspergillus* and *Scedosporium*. Knowledge of the pretransplant colonizing flora can assist in developing an individualized peri-transplant prophylactic antimicrobial regimen. There is controversy as to whether patients colonized with *Burkholderia* should be excluded from receiving lung transplants; molecular typing of *Burkholderia* isolates may be used to define risk, as genomovar III (*B. cenocepacia*) is associated with the highest risk of poor outcomes after transplantation (107–109).

Recipient screening: mycobacterial infections

All patients should have a PPD (tuberculin skin test) performed prior to transplant, and those who have a positive skin test, or a history of active tuberculosis, should undergo additional screening to rule out active disease (II-2) (43). Interferon-gamma release assays (IGRAs) may be particularly useful in assessing patients who received Bacillus Calmette–Guerin (BCG) vaccination, as the IGRA assay has the potential to distinguish PPD positivity related to BCG from that related to latent TB infection in those above the age of 5 (44,110).

Patients with LTBI should be given prophylaxis to prevent reactivation of disease in the setting of immunosuppression (I). Details on the treatment of LTBI are found in the Tuberculosis section of these Guidelines (chapter 8) (43, (46).

In transplant candidates with a clinical history, radiographs and/or cultures suggesting infection with TB or nontuberculous mycobacteria, a thorough evaluation for active disease should be performed, which may include CT scans, bronchoscopy or other tests as deemed clinically necessary. Any mycobacterial infection should optimally be treated with documented microbiologic and radiographic resolution before transplantation is considered.

Recipient screening: fungal infections

Pretransplant colonization with fungi such as *Aspergillus* is common in lung transplant recipients, particularly in cystic fibrosis patients. Such colonization should prompt a rigorous evaluation to exclude active infection. Although posttransplant aspergillosis is a feared complication, transplant clinicians have generally relied more on posttransplant preemptive and prophylactic strategies rather than pretransplant antifungal therapy for colonized patients. A pretransplant candidate with invasive fungal infection (rather than colonization) should be treated at least until there is radiographic, clinical and microbiologic resolution in order to minimize the risk of this high-mortality infection posttransplant (III). Additional information on the diagnosis, prevention and treatment of infection with *Aspergillus* is found in other parts of these Guidelines.

Pretransplant screening for endemic mycoses is most useful in areas endemic for coccidioidomycosis, where a pretransplant history of active disease and/or seropositivity may prompt lifelong azole prophylaxis (II-2) (50). Pretransplant screening for histoplasmosis is of limited value since latent histoplasmosis may be present with negative serology (III); instead, heightened awareness of the possibility of histoplasmosis is important when investigating a posttransplant febrile illness in a patient from an endemic area.

Recipient screening: parasitic infections

Patients from (or with prolonged travel history to) endemic areas for strongyloidiasis, including most tropical countries and parts of the southeastern United States, are at risk for development of disseminated strongyloidiasis after transplant. Screening with serology for *Strongyloides* is much more sensitive than stool exams, and is recommended for

those at epidemiologic risk (III). For seropositive patients, a short course of ivermectin or thiabendazole is indicated pretransplant, although randomized data are not available. As discussed above, *Toxoplasma* serology should be performed in heart transplant candidates, and seronegative heart recipients with seropositive donors should receive prophylaxis (II-2) (51–53). Chagas' disease and other parasitic infections are more fully discussed elsewhere in these Guidelines (see chapter 29).

Recipient screening: viral infections

Active primary infection with viruses such as CMV, EBV, or HBV at the time of transplant is uncommon. Nonetheless, if active viral infection is detected in a potential recipient, transplantation should likely be delayed until the infection resolves in order to allow for development of natural immunity prior to transplant immunosuppression (III). This recommendation also extends to candidates who present for transplantation with clinical symptoms suggestive of an acute community-acquired viral infection. If there is any chance of exposure to HIV pretransplant, the potential recipient should have an HIV NAT and HIV antibody test performed (III). Viral screening of both donor and recipient are discussed in more detail above.

Pretransplant immunizations

The pretransplant evaluation presents an important opportunity to update the potential recipient's immunizations, since most vaccinations are more effective when administered prior to the onset of transplant immunosuppression (I). More detailed immunization recommendations are summarized in another section of these Guidelines (see chapter 31).

All potential recipients should be screened for vaccine-preventable infections and vaccinated as possible prior to transplant. The VZV-seronegative candidate should ideally be immunized against varicella prior to transplantation (II-3). However, if transplantation is expected imminently, it may be best to withhold vaccination with this live attenuated vaccine (III). The zoster vaccine, also a live vaccine, is currently licensed for older adults who are not immunocompromised. Further data are awaited regarding whether pretransplant zoster vaccine prevents posttransplant zoster reactivation, but at the present time it would appear reasonable to administer the zoster vaccine if the transplant candidate meets current criteria for the vaccine and if transplant is not expected within 4 weeks.

A hepatitis B vaccine series should ideally be administered pretransplant to seronegative individuals (II-2); especially as a potential donor may be found who is HBsAg negative but HBcAb positive; in dialysis patients, the higher-dose formulation should be given. Patients with advanced liver disease are at particularly high risk for fulminant hepatitis A and should receive hepatitis A vaccination (II-2). This vaccine is likely more effective when administered early on in liver disease (II-2). The combined hepatitis A and B vaccine is immunogenic but data are awaited in transplant candidates and recipients.

Measles–mumps–rubella (MMR) vaccine contains live virus. Patients born in or before 1956 are presumed to have natural immunity. Patients born after 1956 who have not received a second dose of the MMR vaccine should receive a second dose, given pre- rather than posttransplant (III).

Pneumococcal vaccine should also be administered to transplant candidates over the age of 2 who have not received it within the past 5 years (III). The Tdap (tetanus–diphtheria–acellular pertussis) vaccine should be administered if the potential adult recipient has not had a tetanus–diphtheria toxoid (Td) booster within 5–10 years, and should be considered in all potential recipients in light of the increase in pertussis cases in recent years (III).

Pretransplant counseling

Preventive strategies for infection should not be confined to medications and vaccinations. Extensive education of the transplant recipient and his or her family is a very important preventive tool. Pretransplant classes and printed materials are helpful and should include information on handwashing/hand hygiene, environmental exposures, activities to avoid, food safety and handling, foodborne pathogens, pets and travel. It is also helpful for patients to have a general idea of the infections to which transplant patients are susceptible and the preventive strategies in use at their particular center. It is fundamental that patients know what to expect, what can go wrong and what is expected of them.

Conclusion/future directions

Pretransplant screening of the potential organ donor and recipient affords an opportunity to assess the feasibility and safety of transplantation, to determine the prophylaxis and preventive strategies utilized posttransplant, to detect and fully treat active infection in the potential recipient prior to transplant, to update the vaccination status of the potential recipient, and to sufficiently educate the patient and family about preventive measures. Future advances will incorporate the increasing use of rapid molecular diagnostic testing, and possibly ancillary testing for emerging pathogens in clinical practice.

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Special Article

Donor-Derived Infections in Solid Organ Transplantation

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Key words: Donor infection, donor-to-host transmission, donor screening, testing, latent infection

Abbreviations: CMV, cytomegalovirus; CNT, Centro Nazionale Trapinti; CSF, cerebrospinal fluid; D.R.IN, donor risk infection; DTAC, Disease Transmission Advisory Committee; ELISA, enzyme-linked immunosorbent assay; FDA, US Food and Drug Administration; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IWDT, intervention without disease transmission; LCMV, lymphocytic choriomeningitis virus; MSM, man who has sex with another man; MRSA, methicillin-resistant *S. aureus*; NAT, nucleic acid test; OPTN, Organ Procurement and Transplantation Network; PCR, polymerase chain reaction; REDS, Retrovirus Epidemiology Donor Study; UNOS, United Network for Organ Sharing; VRE, vancomycin-resistant *Enterococcus*; WNV, West Nile virus.

Introduction and Definitions

Advances in surgical technique, immunosuppression and antimicrobial prophylaxis have resulted in significantly reduced morbidity and mortality following organ transplantation. As a result, transplantation is currently considered the definitive therapy for individuals with end-organ failure. Despite these advances, unexpected transmission of infections from the donor to the recipient remains a rare complication of transplantation; when it does occur, the event is frequently associated with significant morbidity and mortality (1,2). In this chapter, the epidemiology of unexpected donor-derived infectious diseases transmissions, risk mitigation strategies and general approach to a patient with possible donor-derived infection will be reviewed.

Definitions

Most donor-derived disease transmissions are expected. Such expected transmissions, including cytomegalovirus (CMV) and hepatitis B virus (HBV), result with the knowedge that the transmission will occur; the donor is known to be infected with the pathogen and virological monitoring with preemptive therapy and/or universal prophylaxis are utilized to minimize the impact of the disease transmissions (I) (1,2). This guideline will not discuss such expected disease transmissions as they are reviewed elsewhere in this supplement. Instead, this guideline will focus exclusively on unexpected transmissions, such as Chagas, HIV, HCV, lymphocytic choriomeningitis virus (LCVM), *Mycobacterium tuberculosis*, multidrug-resistant (MDR) bacteria, rabies and West Nile virus (WNV), which may occur despite current screening strategies and are not expected in the donor at the time of organ placement (3–16). In some of these transmission events, clinical disease in the donor was not recognized at the time of donor death (14,16), while in other cases, screening, although available, was not performed for the pathogen of interest (4–6). Although most disease transmissions have involved deceased donors, recent transmissions of HIV and HCV showed that recipients of living donors may also be at risk (7,17).

Recently, international consensus definitions of donor-derived infections agreed upon (Table 1) (18). These definitions should optimally be utilized to faciliate comparison of data between published studies and reports collected globally.

Epidemiology of Donor-Derived Infectious Disease Transmissions

There are currently few robust systems to assess the epidemiology of donor-derived infectious disease transmissions. Currently, systems are well established in France (Agence de la Biomédecine) and the United States (Organ Procurement and Transplantation Network (OPTN)'s *Ad Hoc* Disease Transmission Advisory Committee) with a more recently established system in Italy (DRIN) (2,19). Additionally, there was a research infrastructure that tracked disease transmission for a finite period in Spain (RESITRA) (20). The French, Italian and US systems require recognition that the disease in the recipient is potentially of donor origin and then the disease must be reported to the national registry. As such, underrecognition and

Table 1: Definitions of imputability for donor origin infectious diseases transmissions (18)

Term	Definition
Proven	Clear evidence of the same infection disease in the donor and at least one of the recipients
Probable	Strong evidence suggesting but not proving a disease transmission
Possible	Used for all situations where data suggest a possible transmission but are insufficient to fulfill criteria for confirmed transmission (proven and/or probable) and transmission cannot be formally excluded
Unlikely	Used for situations where it is possible that the disease in question could have been transmitted from the donor to at least one of the recipients but the available data suggests that donor origin is unlikely
Excluded	Clear evidence of an alternative, nondonor origin of disease
Intervention without Documented Transmission (IWDT)	All or some of the recipients received an intervention (i.e. antimicrobial therapy, specific immunoglobulins or organ removal) and no disease was recognized in any of the recipients
Positive assay without apparent disease transmission	Used for instances in which a donor assay is positive for infection (i.e. coagulase negative *Staphylococcus* in perfusate culture) that is felt by the clinicans not to be clinically significant, is not treated and not associated with disease transmission
Not assessable	When there are insufficient data available to assess imputability of the disease transmission (either from insufficient data being provided in a published document or sufficient donor and/or recipient testing)

Table 2: Summary of potential donor-derived infectious disease transmissions reported to the United States organ procurement and transplantation network 2005–2011 (2)

Infection type	Number of donor reports	Number of recipients with confirmed transmission	Number of DDI-attributable recipient deaths
Viruses[1]	166	48	16
Bacteria[2]	118	34	9
Fungi[3]	75	31	10
Mycobacteria[4]	53	10	3
Parasites[5]	35	22	7

[1]Viruses: adenovirus, HBV, HCV, HEV, HIV, HTLV, herpes simplex, influenza, LCMV, parainfluenza (PIV)-3, parvovirus B19, rabies, West Nile virus.
[2]Bacteria: *Acinetobacter, Brucella, Enterococcus* (including VRE), *Ehrlichia* spp, *E. coli*, Gram-positive bacteria, *Klebsiella, Legionella, Listeria, Borrelia burgdorferi, Nocardia, Pseudomonas,* Rocky Mountain Spotted Fever, *Serratia, S. aureus* (MRSA), *Streptococcus* spp, *Treponema pallidum, Veillonella*; bacterial meningitis & bacterial emboli.
[3]Fungi: Aspergillus spp, Candida spp, Coccidioides imitis, Cryptococcus neoformans, Histoplasma capsulatum, Scopulariopsis, zygomyces.
[4]Mycobacteria: tuberculosis, non-TB mycobacteria.
[5]Parasites: Babesia, *Balamuthia mandrillaris*, Chagas (*Trypanosoma cruzi*), *Naegleria fowleri*, schistosomiasis, strongyloides.

underreporting of cases is likely and limits current data; Italian system (DRIN) is collecting reports of all recipient infections.

Despite these limitations, it is possible to draw several generalizations. It appears that donor-derived infectious diseases complicate approximately 0.2% of deceased organ donor transplants (details from the OPTN data are in Table 2) (2,19); it should be noted that a slightly higher rate (1.7%) was noted during the RESITRA study period (20). When an infection is transmitted, it is typically associated with significant morbidity and mortality (2,19,20); there is likely underrecognition and therefore underreporting of cases that are associated with less severe disease (i.e. transient bacteremia that responds quickly to therapy but was likely of donor origin). Further, there are variable rates of transmission likely related to inoculum of pathogen, organ transplanted and type of immune suppression used (i.e. lymphocyte depletion) (2,19,20).

Risk Mitigation

Although it is impossible to completely remove the risk of disease transmission through solid organ transplantation, there are a number of ways to mitigate against disease transmission (2). Basically, these can be classified as follows:

(1) Risk stratification from the donor medical and social history.
(2) Careful physical assessement of the donor and the donor organs.
(3) Laboratory screening of the donor for infection.

The limitations and benefits of each risk mitigation strategy must be understood by the accepting center to properly inform the risk of donor-derived infectious disease transmission. Lastly, care must be taken to find the appropriate balance between minimizing the risk of disease transmission and organ wastage in making decisions utilizing these risk mitigation strategies (2,21). Currently, there are many more individuals who could benefit from organ transplantation than there are available organs. As such, discarding organs from donors with risk factors needs to be minimized when utilizing these risk mitigation strategies.

Table 3: Behavioral risk factors for a donor to be at increased risk of transmitting human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV)

- High risk sexual contacts:
  - Persons who have had sex with a person known or suspected to have HIV, HBV or HCV infection in the preceeding 12 months
  - Men who have had sex with another man (MSM) in the preceeding 12 months
  - Women who have had sex with a man with a history of MSM behavior in the preceeding 12 months
  - Persons who have had sex in exchange for money or drugs in the preceeding 12 months
  - Persons who have had sex with a person who injected drugs by intravenous, intramuscular or subcutaneous route for nonmedical reasons in the preceeding 12 months.
- Birth to a mother infected with HIV, HBV or HCV (for infant donors $\leq$ 2 years of age)
- Persons who have injected drugs by intravenous, intramuscular, or subcutaneous routes for nonmedical reasons in the preceeding 12 months
- Inmates of a correctional facility (e.g. jail, prison, or juvenile detention) for > 3 days in the preceeding 12 months
- Persons who have or have been treated for syphilis, gonorrhea, chlamydia, or genital ulcers in the preceeding 12 months
- Persons who have been on hemodialyalsis in the preceeding 12 months

Based on proposed US Public Health Services Guideline which are currently under revision. Consult current US Public Health Service Guideline for current criteria.

Table 4: Residual risk of undiagnosed human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection per 10 000 donors at increased risk of infection (24,25)

	HIV		HCV	
Risk factor	Serology alone	Serology + NAT	Serology alone	Serology + NAT
Men who have sex with men	8.3	3.4	36.0	3.8
Nonmedical intravenous, intramuscular or subcutaneous drug use	12.9	5.3	350.0	37.8
Hemophilia	0.05	0.02	0.46	0.05
Persons who have had sex in exchange for money or drugs	2.9	1.2	107.8	11.5
Partners with any of the above risk factors	2.7	1.1	126.2	13.5
Individuals who have been exposed to blood or blood products from someone with HIV or HCV	1.3	0.5	22.0	2.3
Incarceration	1.5	0.6	68.6	7.3

As a point of reference, in the United States there is a 0.34% (34/10 000) risk of developing hepatitis C per year while on dialysis.

Donor risk assessment

Risk stratification is commonly achieved through careful review of the donor's medical and social history (22). The donors chart should be screened carefully to identify cultures and other assays (e.g. serology and nucleic acid testing (NAT; sometimes also referred to as PCR or viral load testing) that were ordered by the team caring for the patient to diagnose infections (22). Positive results should be interpreted by the accepting teams to match the risk of disease transmission with the risk tolerance and medical status of the recipient. Most importantly, some cultures or other assays may yield results well after the organs have been placed (i.e. mycobacteria cultures frequently take up to 8 weeks) (2,22). The organ procurement organization and recipient center should be aware of the pending results and have a plan for information transmission and recipient management (III). Additionally, the social history is optimally obtained from an individual who knows the patient well (2,22). Attention to travel history is critical to identify donors at risk of endemic infections (such as histoplasmosis, blastomycosis, coccidiomycosis, Chagas disease, strongyloides and tuberculosis, to name just a few). If risk factors for exposure to endemic infections are identified, consideration of additional screening or use of recipient preventative strategies should be considered (III). These will be discussed in further details in later sections. A uniform donor health questionaire is currently being developed by the American Association of Tissue Banks with the goal of standardizing the acquisition of the medical and social history from the next-of-kin or friends who are available. It is important to recognize that the historian may not be aware of all of the donor's risk behaviors and attempts to assess how well the historian knows the donor should be undertaken. Results of the review of the medical history and collection of the social history can be used to identify patients at increased risk of transmitting HIV, HBV and HCV (see Table 3) (2,23). Recipients of organs from donors at increased risk of transmitting HIV, HBV and HCV should be informed of the risk and alternatives to use of organs from the increased risk donors, and should be screened posttransplant for acquisition of these infections. (residual risk of infection despite serologic and/or NAT screening associated with specific behaviors is listed in Table 4 and below) (III) (24,25).

Currently, there are two ways in which organ donors are risk stratified: In the United States, donors are dichotomized as being either at increased risk or without identified risk for transmission of infectious diseases; while

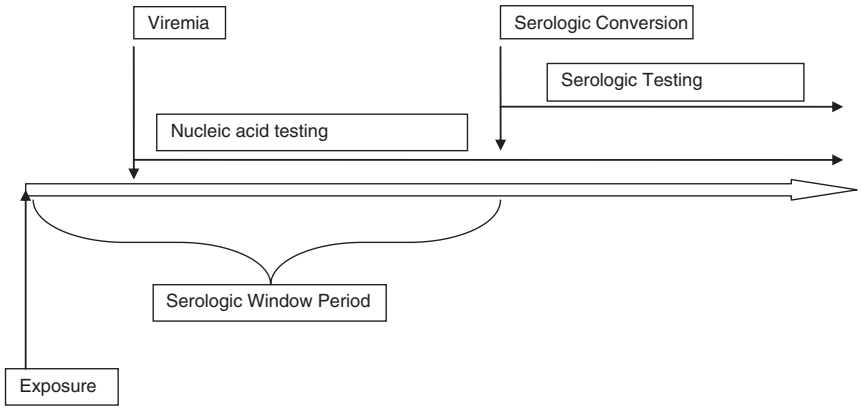

Figure 1: Schematic of viral infection and detection by serology and nucleic acid testing.

in Europe, a more graded risk assessment is utilized. In the US system, which has traditionally focused on HIV, HBV and HCV, behavioral risk factors (see Table 3), hemodilution and lack of donor social history have been utilized to classify a donor as increased risk of transmitting blood-borne infections while all other recipients are not further classified (2,22,23). The European classification system was initially developed in 2002 by Italian National Center for Transplantation (CNT) but has been more broadly applied throughout Europe to evaluate the safety and acceptability of donors (26). The CNT/European risk classification system (http://www.edqm.eu/en/Search-519.html) defines donors as follows:

(1) *Unacceptable risk* includes absolute contraindication, with the exception of some life-saving transplantation procedures in the absence of other therapeutic options on a case-by-case basis.
(2) *Increased but acceptable risk* includes cases where transmissible organisms or diseases are identified during the evaluation process of the donor, but organ utilization is justified by the specific health situation of the recipient or the severity of their clinical condition.
(3) *Calculated risk* (criteria referring to protocols for elective transplants) includes all cases where, even in the presence of transmissible diseases, transplantation is allowed for recipients with the same disease or with a protective serological status; this risk applies also to donors with documented bacteremia and/or bacterial meningitis provided that the donor was on targeted antimicrobial treatment for a minimum duration of 24–48 h.
(4) *Not assessable risk (RL 4)* includes cases where the evaluation process does not allow an appropriate risk assessment for transmissible diseases.
(5) *Standard risk (RL 5)* includes cases where the evaluation process did not identify a transmissible disease.

With both systems, it is recommended that a specific informed consent is obtained from every recipient if there is defined risk identified in the donor.

Physical asssessment

Careful physical assessment of the donor's body should be conducted by both the organ procurement team and the procuring surgeon, who should evaluate the explanted organs and vessels. The body should be assessed for evidence of infections, including abscesses, ulcers, genital or anal trauma, lymphadenopathy, in addition to looking for evidence of recent drug use, such as the presence of track marks. The examination should also assess for evidence of other underlying disease, such as cirrhosis or other surface manifestations of infections or malignancies. The explanting surgeon should make sure that there is no free spillage of intestinal contents and that there is no obvious pus or infection of the organ or vessel, including lymphadenopathy.

Donor serologic and nucleic acid testing

Following viral infection, the virus may initially be detected in the blood prior to the infected individual developing antibodies; this is termed the serologic window (see Figure 1). Once the patient develops antibodies directed against the infecting virus, serologic testing will detect the infection in the donor. Several donor-derived infection transmissions have resulted from window period infections missed by serologic screening of donors only (7,14). The period from HIV exposure to the development of HIV antibodies is approximately 22 days, but can be up to 6 months. Thus the donor may be seronegative while potentially infectious. The use of individual donor NAT would reduce the window period for HIV to between 5.6 and 10.2 days (i.e. 4–15 days in which infection is detected by NAT but not ELISA (27–31). A fourth-generation HIV antibody-antigen combination serology diagnostic test was recently approved in the United States and may reduce the window period to 1–2 weeks; it should be noted that the assay is not approved for screening blood or plasma donors and there are limited data on its efficacy in deceased organ donor screening. Recent data estimated incidence of undetected HIV infection by serologic screening was 1 in 50 000 for normal risk potential donors and 1 in 11 000 for OPTN-defined increased risk potential donors (32). HBV surface antigen (HBsAg) ELISA assays have a window period of 38.3–49.7 days,

with NAT in the 20.4–25.7 day range (27,33–36). The use of HBV NAT testing may detect viral replication in hepatitis B core antigen positive who are HBsAg negative. HCV ELISAs have a window period of between 38 and 94 days which is reduced significantly to 6.1–8.7 days by the use of NAT (24,31,32). Recent data estimated incidence of undetected HCV infection by serologic screening was 1 in 5000 for normal risk potential donors and 1 in 1000 for OPTN-defined increased risk potential donors (32). There is a fourth-generation HCV antibody screening assay that is available outside the United States but is not yet approved for use in the United States; it has a reduced window period compared to currently approved assays in the United States.

While these data suggest that NAT will detect infections missed by routine serologic screening of organ donors, many in the transplant community have only advocated for the use of NAT for OPTN-defined increased risk donors because of concern of loss of uninfected organs from false-positive testing (III) (21). More recently, data suggested that organs may be successfully placed from donors with proven or suspected false-positive NAT results (37,38). Further, another group demonstrated that there was a substantial proportion of donors who were seropositive but negative by NAT for HIV, HBV and HCV (38). Such donors could be used in selected transplant candidates (i.e. HBV infected or vaccinated candidates) or in appropriately consented candidates. It should be noted that current US law does not allow use of donors who are known to be infected with HIV. If there is clear evidence suggesting that results are likely false positive (i.e. + serology but negative NAT in donor without risk factors for HIV infection), use of organs can be considered as long as all details of these testing results are clearly disclosed to the recipient and recipient center (III).

There has been recent attention on screening donors for other transmissible infections, such as tuberculosis, Chagas Disease and West Nile virus; these will be discussed in detail in later sections, but key features will be summarized here. Screening of donors for tuberculosis is challenging and supported by limited data. Use of the PPD is not currently an option because there is typically insufficient time to place the antigen and await a response; additionally donors may be rendered anergic by the underlying cause of brain death and/or steroids used for donor stabilization. Use of interferon-gamma release assays is currently under study and therefore cannot be advocated for wide use in screening donors. Donors with risk factors for tuberculosis (exposure to a moderate to high endemicity nation, homelessness, drug abuse, or incarceration) should be screened for active tuberculosis; donors with active tuberculosis should not be used (III). Further details can be found in a recent consensus paper (39). Targeted *T. cruzi* screening of potential donors born in Mexico, Central America and South America has been advocated by a recent group of experts (10). It should be noted that most currently available donor screening assays have a high rate of false-positive results and confirmatory testing is recommended for all positive results. Such confirmatory testing is typically not available in time for the donor offer but can direct posttransplant interventions. Given the relative low rate of transmission, kidneys and livers from *T. cruzi*-infected donors or donors with positive initial screening results should be considered for use with informed consent from recipients (II 3). Hearts from infected or screen-positive donors should not be utilized because of the high rate of disease transmission (10). West Nile virus also represents an infection that can be transmitted from donor to recipient for which screening assays are currently available. Existing data suggest that if donors are to be screened, serum WNV NAT should be utilized; screening of urine by NAT or serum for serology is not recommended at this time. Since WNV NAT will generally yield false-positive results when there is limited WNV in the donor service area, screening is only recommended when there is active disease in the region where that donor has come from; collaboration with local blood banks to determine when screening should be considered has been recommended (III).

Special circumstances

Hemodilution of donor blood samples: Massive blood loss followed by intravascular volume replacement with blood products or infusions of colloids and crystalloids can cause hemodilution and result in unreliable donor test results for infectious diseases (40). The US Food and Drug Administration (FDA) has guidelines for how to assess hemodilution for tissue donors and these can be used to estimate the degree of hemodilution in organ donors (40,41). Hemodilution currently classifies donors as increased risk for disease transmission by the current OPTN definition. As such, care should be utilized in interpreting serologic screening results and recipients of organs from donors with significant hemodilution should be informed about the risk of false-negative testing in the setting of hemodilution (III).

Testing of newborns: In general, maternal antibodies may pass from the mother to the child and last anywhere from 6–15 months of age. Interpretation of antibody results should take this into consideration. Some advocate for testing of infant urine for CMV to confirm infection.

Live donors: A recent transmission of HIV from a live donor to his recipient highlighted the need for testing of live donors close to the time of organ procurement (7). Current guidance suggests that all live donors should be tested for HIV, HBV and HCV (7). Additional testing, within 28 days of procurement but optimally within 14 days, has been recommended for all live organ donors (AHRQ-funded consensus conference available at http://www.feinberg.northwestern.edu/transplant/Increased%20Risk%20Consensus%20Conference/index.html). This additional late testing should include HIV and HCV NAT and hepatitis

B surface antigen (HBsAg) to directly detect the presence of the virus in the donor (III) (7). Lastly, donors should be educated about ways in which they can avoid acquisition of infections between the time of screening and donation.

Donors With Documented Infections at the Time of Procurement

Decisions regarding the use of organs from donors with active or suspected infection should be based upon the urgency of transplantation for the recipient, the availability of alternative organs and recipient informed consent. Care should be taken in carefully assessing all available data about the donor and the infection present in the donor, including susceptibility testing, antimicrobial therapy utilized and evidence of clinical response to therapy in the donor (III) (22). Consultation of specific guidance documents may help in determining donor suitability and risk mitigation strategies posttransplant (9,22,39,42,43). In general, any active bacterial or fungal infection in the donor or recipient should be treated and, ideally, resolved prior to transplantation *(II-3)*; organs known to be infected with pathogens likely to be transmitted to the recipient should not be transplanted *(II-3)*.

Bacteremic donors

It has been estimated that 5% of organ donors have bacteremia at the time of organ procurement (2,44,45). Transmission has been described, typically involving bacteria that were not susceptible to typically utilized perioperative antibiotics. When transmissions occur, there is frequently significant graft loss, morbidity and mortality (2,44–46). Although bacteremia and bacterial infections in the donor pose a potential risk for the transmission of infection to the recipient, discarding organs from such donors could further compromise the already limited donor pool and aggravate the organ donor shortage. The risk of donor-transmitted infection varies with the type of bacteria causing the infection. Among Gram-positive bacteria, there is a low risk of transmission with relatively avirulent bacteria, like coagulase-negative staphylococci. Gram-negative bacilli in the donor appear to pose a greater risk for transmission and is associated with poorer outcomes than that caused by Gram-positive bacteria (47–54).

Of greatest concern is the ever-increasing challenge of multiresistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococcus (VRE) and multidrug-resistant Gram-negative rods. The problem is particularly serious with Gram-negatives producing carbapenemases, which usually exhibit extended-drug resistant phenotypes and remain susceptible to only a few antibiotics. There have been only a few reports related the optimal evaluation and risk mitigation management related to these highly resistant bacteria (51,54–57). Open and rapid interinstitutional and -agency communication, antibiotic prophylaxis based on *in vitro* susceptibility testing and careful infection control practices are rational approaches to minimize the impact of donor transmitted bacteria following organ transplantation (57). Further work is needed to identify when organs can be safely used from potential donors with MDR Gram-negative infections, how to prospectively identify donors that may harbor subclinical infection and how to best manage recipients at risk for donor derived infections following transplantation (57).

Emerging data suggest that bacteremic donors may be utilized in certain circumstances (II-2) (44,45,47,51,58,59). Generally, it is recommended that the infected donor receives targeted antimicrobial treatment for at least 24–48 h, optimally with some degree of clinical response (improved white blood cell count, improved hemodynamics, defervescence) (22). In addition, it is recommended that the recipient is treated with a 7- to 14-day course of antibiotics targeted to the organism isolated from the donor (III) (22).

Donors with bacterial meningitis

There are significant data suggesting that donors with proven bacterial meningitis can be safely used for organ donation (II-2). Documentation of bacterial meningitis is essential since transmission of infections and malignancies have been documented from donors with presumed, but not proven bacterial meningitis. Kidneys, livers and thoracic organs from donors with bacterial meningitis due to *Neisseria meningitidis*, *Streptococcus pneumoniae, Haemophilus influenzae* and *Escherichia coli* have been successfully transplanted (60–67). Generally, donors are treated for 24–48 h with antibiotics directed at the identified bacteria prior to procurement, optimally with evidence of clinical improvement. The recipient is typically treated for 7–14 days posttransplant with antibiotics directed at the cultured bacteria (II-2) (22). Meningitis caused by highly virulent or intracellular organisms such as Listeria species are still considered a contraindication by many transplant centers.

Donors With Proven or Presumed Infectious Encephalitis

It is important to note that encephalitis, particularly with fever, without a documented source is frequently associated with disease transmission. Transmission of rabies, parasitic infections, lymphomas and leukemias have occurred when donors with encephalitis without a proven cause were accepted as organ donors (2,16). As such, donors dying of encephalitis without a proven cause should likely be avoided (II-3). The two exceptions to this general caution include donors with proven bacterial meningitis (see above) and donors with proven *Naegleria fowlerii* meningoencephalitis. *Naegleria* infection is generally limited to the CNS; even when there is molecular evidence of

the parasite outside the CNS, transmission has not been documented. If the donor has proven *N. fowlerii* meningoencephalitis, the organs can be utilized with a low risk of transmission, as long as the recipients are informed of the risk and monitored closely (II-3) (68,69).

Evaluation of Recipient With Suspected Donor-Derived Infection

Although donor-derived disease transmissions are rare (estimated to involve ~0.2% of all transplants), it is critical to consider the donor as the source of any posttransplant infection or malignancy and report that concern to the local OPO and/or national competent authority (i.e. UNOS in the United States) immediately (II-2) (2). Unfortunately, recipients may be cared for by different teams within the same hospital or in a number of different hospitals; this may hamper recognition of a transmission. Additionally, as has been the case in several recent transmissions, the patients present with clinical symptoms at different times posttransplant; mechanisms to flag all recipients of a single donor with concern about a potential transmission should be in place but typically are not available. The OPO should have a mechanism in place to rapidly assess the status of all other recipients of organs, tissues or vessels from the same donor and report the concern to the OPTN (2,22). The recent allograft recipient with unexplained fever, leukocytosis, altered mental status, or other signs of occult infection is a candidate for donor-derived infection. Likewise, proven infections early posttransplant should prompt a careful review of donor cultures and donor origin of the infection should be considered (II-3). Common processes such as wound or surgical sites infections, graft rejection, anastamotic leaks, vascular compromise, drug toxicity, pneumonia, or *C. difficile* colitis must be evaluated for and treated if present. If donor origin is considered, the case should be immediately reported to the national transplant authority (UNOS in the United States), the local organ procurement organization and, if it is a reportable disease, the local public health authorities. This reporting should be done as early as possible to potentially alert providers of other recipients of the same donor to facilitate evaluation and initiate disease transmission mitigation strategies (III). It should be emphasized that reporting should not await confirmation of transmission. As part of the evaluation, it is prudent to contact the involved laboratory to save any residual blood, serum, CSF and donor tissues (such as vessels) to facilitate the investigation and insure that they will be held and not be inadvertently disposed of.

Lastly, it is critical that the transplant team work collaboratively to develop an evaluation and treatment plan for all recipients of donors with identified risk of infectious disease transmission. This should include a clear plan for who is responsible for follow-up testing (i.e. follow-up cultures or serology/PCR testing of the recipient) and treatment (III). In general, when an infection is identified in the donor, the recipient is treated with appropriate antimicrobial therapy directed against the pathogen for a duration that one would use if the recipient themselves had the infection (2,22). Further, it is currently recommended that all recipients of organs from donors with identified risk factors for HIV, HBV and HCV be tested posttransplant (III). While there is controversy as to the optimal timing of this testing, it is important to utilize assays that directly detect the presence of the virus (i.e. HIV and HCV NAT and HBsAg) since patients frequently fail to seroconvert (2,14,22). Reliance on serology alone may miss acquisition of a donor-derived viral infection.

Future Research

Since the topic of donor-derived infections is still relatively new, there is significant need for additional research. It is critical that more nations establish organ vigilance and surveillance systems to further define the epidemiology of donor-derived infections. This includes evaluation for geographically limited infections that may not have been transmitted in areas where surveillance is currently ongoing. Additionally, the relative importance of specific pathogens and risk mitigation strategies can only be assessed with collection of global data. Prospective studies of organ donors and recipients, similar to what was conducted as part of the Retrovirus Epidemiology Donor Study (REDS) in transfusion medicine, are needed to more completely define the true epidemiology and risk of donor disease transmission. Studies are also needed to assess the wide range of available diagnostic and screening assays that could be utilized to risk stratify potential organ donors. Lastly, specific registries of donors with potentially transmissible infections (i.e. Chagas, encephalitis, or bacteremia) are needed to inform which donors can safely be utilized and what risk mitigation strategies are most effective in prevent disease transmission.

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Special Article

Multidrug-Resistant Gram-Negative Bacteria Infections in Solid Organ Transplantation

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Key words: Antibiotic resistance, bacterial infection, multidrug resistance, posttransplant infection

Abbreviations: BCSA, *Burkholderia cepacia* selective agar; BOS, bronchiolitis obliterans; CF, cystic fibrosis; CLSI, Clinical and Laboratory Standards Institute; CR, carbapenem resistant; CRAB, carbapenem-resistant *Acinetobacter*; CRE, carbapenem resistant *Enterobacteriaceae*; ESBL, extended-spectrum beta-lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing; FDA, Food and Drug Administration; ICU, intensive care unit; IV, intravenous; MCBT, multiple combination bactericidal antibiotic testing; MDR, multidrug resistant; MIC, minimum inhibitory concentration; OFPBL, oxidation-fermentation, polymyxin B, lactose; OR, odds ratio; PC, *Pseudomonas cepacia*; PR, pan-resistant; SBP, spontaneous bacterial peritonitis; SOT, solid organ transplant; TMP/SMX-R, trimethoprim sulfamethoxazole resistant; UNOS, United Network of Organ Sharing; UTI, urinary tract infection; VIA, vancomycin, imipenem, amphotericin B.

Epidemiology

The prevalence of multidrug resistance (MDR) in Gram-negative bacteria isolated from clinical samples continues to increase globally (1,2). Several reports indicate a similar continued trend toward increased resistance in Gram-negative bacteria isolated from transplant patients (3–6). Clinically important MDR bacteria that have been reported in transplant recipients include nonlactose fermenters such as *Pseudomonas* species, *Burkholderia* species and *Stenotrophomas* species, as well as carbapenem-resistant (CR) *Acinetobacter* species, and MDR *Enterobacteriaceae*, with CR *Enterobacteriaceae* (CRE) being of particular concern. For the purposes of this paper, MDR is defined as nonsusceptibility to at least one agent in three or more antibiotic classes (7). Pan-resistance (PR) is defined as nonsusceptibility to all licensed, routinely available antibacterials. The impact of infection with MDR or PR bacteria on transplant recipient survival has become an important concern as several reports indicate significantly decreased survival of patients infected with such bacteria (8–12).

MDR Enterobacteriaceae and CR Acinetobacter (CRAB)

In several cohorts of transplant recipients, dramatic increases in percentages of *Enterobacteriaceae,* which are ciprofloxacin-resistant or produce extended-spectrum beta-lactamase (ESBL) or AmpC have been reported. Rates of ESBL producing *Enterobacteriaceae* ranged from 8% to 77% in these studies (3,4,13–15). In kidney transplant recipients, ESBL-producing *Enterobacteriaceae* were found to be associated with recurrent urinary tract infection (UTI); the incidence of ESBL producing *Enterobacteriaceae* increased from 13%, 38% to 45% for first, second, and third UTI episodes, respectively (15).

Prevalence data for CRE and CRAB in transplant populations are limited and highly variable by region. Most case series are from higher endemic areas for these MDR bacteria, resulting in relatively higher percentages of resistant bacteria reported, ranging from 18% to 50% (16–18). One year after transplantation, infection with CR *Klebsiella pneumoniae* was a predictor of time-to-death in 175 liver transplant recipients, (HR 4.9, 95%CI 1.5–15.6) (16). Mortality at 30 days was 42% in 12 transplant recipients infected with CR *K. pneumoniae*, with most deaths directly attributable to infection (17).

MDR Pseudomonas, Stenotrophomonas, Achromobacter and Burkholderia

Lung transplant recipients: MDR or PR *Pseudomonas aeruginosa* colonize the respiratory tract of especially cystic fibrosis (CF)-lung transplant recipients in up to 52% prior to transplantation, with posttransplantation colonization rates reaching 75% (19–21). *P. aeruginosa* also remains the most frequent microorganism identified during pneumonia after lung transplantation, being responsible in 25% (22). Despite early reports suggesting reduced survival, more recent studies suggest similar survival of CF-lung transplant recipients independently of pretransplant colonization by MDR or PR *P. aeruginosa*, with an overall survival similar to general results in the United Network of Organ Sharing (UNOS) registry (20,21,23). Pretransplant colonization with MDR or PR *P. aeruginosa* is therefore not

considered an absolute contraindication for lung transplantation in the "International Guidelines for the Selection of Lung Transplant Candidates". It is suggested to include colonization by such bacteria in a comprehensive evaluation including all other comorbidities to determine whether their combination increases the risk of transplantation above a safe threshold (24) (II-2). *P. aeruginosa* has also been suggested to participate in the pathogenesis of bronchiolitis obliterans (BOS), a major limiting factor for long-term survival after lung transplantation (19,23,25).

Colonization by *Burkolderia* species is less frequent, affecting 6–9% of lung transplant recipients, and colonization by PR *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* remains rare (26,27). Of the 17 genotypically distinct species forming the *Burkholderia cepacia* complex, *Burkholderia cenocepacia* (genomovar III) and *Burkholderia multivorans* (genomovar II) account for 85% of isolates in both the United States and France (27,28). Resistance is common with 86% of *B. cenocepacia* being MDR, including 43% PR isolates and 78% of non-*B. cenocepacia* isolates being MDR including 56% PR isolates (29).

Posttransplant survival among patients colonized by *Burkholderia* depends on the species. Colonization by *B. multivorans* is associated neither with a higher mortality risk nor with reduced survival (27,29–31), and patients colonized with these bacteria should therefore not be denied access to lung transplantation (II-2). In contrast several studies have shown reduced 1-year survival from 90% to less than 30% for patients colonized by PR *B. cenocepacia* (26,27,29,31). The International Guidelines updated in 2006 did not consider colonization by PR *B. cenocepacia* to be an absolute contraindication for transplantation, but suggested particular care to be taken in the identification of species and repeated antibiotic susceptibility testing (24). However, because of a deemed unacceptably high risk of fatal outcome, some more recent reports recommend to discontinue listing such patients for lung transplantation (29,31) (III). Whether an aggressive multidisciplinary management including reduced immunosuppression, improved nutrition and long-term antibiotic treatment might improve survival of these patients remains questionable (27,32). In the light of the present data we recommend that patients colonized by *B. cepacia* complex are referred to reference centers and that the different species and antibiotic susceptibilities are precisely determined using appropriate reference laboratories (II-2). Those patients colonized by PR *B. cenocepacia* should be evaluated for lung transplant with extreme caution due to the documented increased risk of morbidity and mortality (II-2). Adequate information should be provided to patients and relatives concerning the high risk of poor outcome (II-2).

Other solid organ transplant recipients: In nonlung transplant recipients *P. aeruginosa* is also a major pathogen. *P. aeruginosa* is responsible for up to 14% of all bloodstream infections in kidney, 6.5% in liver and 5% in pancreas transplant recipients in the Spanish RESITRA cohort (8). In these patients *P. aeruginosa* remains essentially an early nosocomial pathogen, being responsible for up to 23% of Gram-negative bacteremia within 1-month posttransplantation, but only for 3% of episodes after 12 months (3,33). Strikingly, as compared to nontransplant patients, MDR isolates among *P. aeruginosa* bloodstream infections are more frequent in transplant recipients reaching 43% in Pittsburgh and even 52% in China (11,34). *P. aeruginosa* is also a frequent cause of nosocomial pneumonia in both kidney and liver transplant recipients, with an incidence of MDR isolates in this setting between 50% and 65% (10,35). In renal transplant recipients, *P. aeruginosa* is also a frequent cause of UTI, being responsible for up to 10% of cases and frequently MDR (36,37).

Risk Factors

Specific risk factors for antibiotic resistance in transplant patients have not been systematically studied in large-scale multicenter analyses. General risk factors for acquisition of MDR bacteria are increasingly recognized to be shared among pathogens, and include prior antimicrobials, devices, longer length of hospital stay, and increased severity of underlying illness (38). As transplant recipients often have several of these risk factors, it is not surprising that organ transplantation has been reported as a risk factor for MDR Gram-negative bacteria with odds ratios ranging from 3.2 to 3.7 (34,39,40). An alarming trend toward increased prevalence of MDR bacteria in long-term care facilities has been noted in several studies (41–43). Therefore, the decision to discharge a transplant recipient to an extended care facility may have a substantial impact on their risk of acquiring MDR bacteria.

MDR Enterobacteriaceae and MDR Acinetobacter

Similar to the nontransplant population, risk factors for solid organ transplant (SOT) recipients to acquire MDR *Enterobacteriaceae* and *Acinetobacter* including previous use of antibiotics, prolonged intensive care unit (ICU) stay, and renal failure with or without dialysis, have been derived from single transplant center studies (6,13,44–46). Additional transplant-specific risk factors, which have been reported include combined kidney–pancreas transplantation as compared to isolated kidney transplant recipients, posttransplant dialysis or urinary obstruction and renal transplant versus other organs (13,44). In the pediatric transplant population, younger age and the placement of central venous catheters are additional risk factors (47). No studies specifically link antimicrobial prophylaxis for spontaneous bacterial peritonitis (SBP) to posttransplant MDR infections. However, prior antibiotic use is a consistent risk factor, and studies in patients with liver cirrhosis show that SBP prophylaxis is associated with increased rates of both

Table 1: Diagnosis

Organism	Recommendation	Level
All	Obtain cultures from appropriate sites	I
	Suspect MDR bacteria in the following:	
	Lack of clinical response	
	Presence of risk factors for MDR bacteria	
	Prior isolation of MDR bacteria	
Enterobacteriaceae		
ESBL-producing	Use current CLSI or EUCAST breakpoints for cephalosporins	II-1
	Alternative: ESBL screening by double disk diffusion assay or by broth dilution testing with and without a β-lactamase inhibitor	
Carbapenem-resistant	Use current CLSI or EUCAST breakpoints for carbapenems	II-1
	Alternative: carbapenemase screening by modified Hodge testing	
MDR *Acinetobacter*	Use varying assays based on specific antibiotic tested	II-1
	Test each carbapenem individually	
MDR *P. aeruginosa*	MacConkey agar	I
	Cetrimide agar	
	Etest or standardized disk diffusion tests	
MDR *B. cepacia* complex	BCSA, OFPBL or PC agar	I
	Use MCBT only in selected cases	II-3
MDR *A. xylosoxidans*	Etest or standardized disk diffusion tests	I
MDR *S. maltophilia*	MacConkey agar or VIA agar	I
	DNase confirmatory media or biochemical or molecular identification.	
	Etest or standardized disk diffusion tests	

BCSA = *Burkholderia cepacia* selective agar; CLSI = Clinical and Laboratory Standards Institute; ESBL = extended spectrum beta-lactamase; EUCAST = European Committee on Antimicrobial Susceptibility Testing; MCBT = Multiple combination bactericidal antibiotic testing; OFPBL = oxidation-fermentation, polymyxin B = bacitracin, lactose; PC = *Pseudomonas cepacia*; VIA = vancomycin, imipenem, amphotericin B.

ESBL-producing bacteria, as well as increased quinolone resistance (48,49).

MDR Pseudomonas, Stenotrophomonas, Achromobacter and Burkholderia

As for other MDR isolates, the main risk factor for acquisition of MDR *P. aeruginosa* is exposure to repeated and/or prolonged courses of antibiotic treatments. Selection of *P. aeruginosa* isolates with increased resistance toward the antimicrobial that have been previously used has been documented in the nontransplant population, with persisting resistance toward imipenem and ciprofloxacin despite their discontinuation (50,51). For both *P. aeruginosa* and *B. cepacia* complex, patient-to-patient transmission occurs mainly via the direct or indirect contact or droplet routes (52). Importantly transmission of the epidemic *P. aeruginosa* Liverpool strain has been linked to social networks among patients (52). Posttransplant acquisition in non-CF lung transplant recipients of both *P. aeruginosa* and *B. cepacia* complex has not been well documented. For both *S. maltophilia* and *A. xylosidans* there is also evidence of patient-to-patient transmission. For MDR *P. aeruginosa* blood-stream infections in nonlung transplant recipients, independent risk factors include admission to ICU in the previous year (Odds Ratio [OR]: 5.14), antibiotic treatments in the last 30 days (OR: 5.62) and hospital acquisition (OR 3.81) (34).

Diagnosis

When resistant bacteria are isolated from a patient, the clinical significance of the organism must be evaluated by assessing the source of the culture and the method of collection (II-2). Early involvement of an infectious disease specialist may aid in distinguishing colonization from infection and to help guide therapy. Identification of MDR Gram-negative bacteria may be complicated and it is important that isolates be evaluated in microbiology laboratories experienced in the recognition of these bacteria. If unusual susceptibility patterns are noted on routine screening of Gram-negative bacteria, further testing may be warranted. If the laboratory is not experienced in this testing, referral to a reference laboratory may be indicated (III) (Table 1).

MDR Enterobacteriaceae

Following the initiative of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the Clinical and Laboratory Standards Institute (CLSI) revised their interpretive criteria for cephalosporins in 2010 (53,54). If these new criteria are employed, further ESBL screening is no longer recommended for all isolates. However, as per CLSI, confirmatory testing may still be useful for epidemiological or infection control purposes (54). Differences between nonsusceptibility breakpoints between EUCAST and CLSI can

lead to differences in detection of ESBL-producing *Enterobacteriaceae* for instance for ceftazidime (55). In case new cephalosporin interpretive criteria have not been adopted by the clinical microbiology laboratory, ESBL screening will still need to be performed by either a double disk diffusion assay or by broth dilution testing with and without a β-lactamase inhibitor (54) (II-1). Some laboratories use the ESBL E-test strip, although there are no CLSI guidelines for interpretation. New CLSI breakpoints for carbapenem susceptibility in *Enterobacteriaceae* were also established. These are substantially lower than the previous breakpoints, for instance for ertapenem the breakpoint for susceptibility has been lowered from ≤2 to ≤0.5 μg/mL (54). However, the Food and Drug Administration (FDA) breakpoints have not yet been changed. This has resulted in a complicated situation for clinical microbiologists, who may be reluctant to use the new CLSI breakpoints. If the current CLSI carbapenem breakpoints are not yet adopted by the clinical microbiology laboratory, CLSI recommends screening for carbapenemase production by modified Hodge testing (54).

MDR Acinetobacter Baumannii

Identifying resistance in *Acinetobacter baumanii* is complicated and there may be poor concordance between disc susceptibility testing and microbroth dilution methods (56,57). The accuracy of breakpoints for susceptibility testing with regards to clinical outcomes may be variable. Consequently different assays may be required for different antibiotic classes (II-1). Because susceptibility to specific carbapenems may vary, each carbapenem should be tested individually.

MDR Pseudomonas, Stenotrophomanas, Achromobacter and Burholderia

Identification of MDR bacteria from CF respiratory tract secretions may be complicated by their mucoid and polymicrobial nature and the slow growth of some bacteria. Selective media and specific identification procedures are recommended for the isolation of *P. aeruginosa* (MacConkey agar, cetrimide), *B. cepacia* complex (OFPBL agar, PC agar, BCSA), *S. maltophilia* (MacConkey agar, VIA agar, DNase agar confirmatory media or biochemical or molecular identification) and *A. xylosoxidans* (MacConkey agar, biochemical identification assay) (58–61) (I). Identification of species of the *B. cepacia* complex, indicated because of differing clinical outcomes with infections caused by certain members of this class, may require molecular testing. Antibiotic resistance is common and susceptibility testing should be repeated at regular time intervals while patients are on the waiting list to allow adequate antimicrobial therapy at the time of transplant surgery. Automated susceptibility testing may be unreliable and either Etest or standardized disk diffusion tests should be used (62) (I). Multiple combination bactericidal antibiotic testing (MCBT) initially appeared a promising tool to design treatment combinations for CF patients infected by *B. cepacia* complex. However, the only controlled clinical trial testing MCBT to treat exacerbations in CF patients failed to show any improvement as compared to standard culture and sensitivity techniques (63). In the absence of clinical data supporting an advantage of *in vitro* synergy testing, MCBT cannot be routinely recommended, but might be useful in specific cases (64) (II-3).

Prevention

Various MDR Gram-negative bacteria are associated with different settings—for example MDR or PR *P. aeruginosa* and *B. cepacia* typically emerge in CF patients due to repeated antibiotic exposure over many years—and consequently preventive strategies for different bacteria vary (25–27,52). However, important areas of overlap in preventive efforts can be identified. Most importantly, prevention should include a reduction in antibiotic exposure before and after transplantation wherever it is safe to do so (6,13,38,65). All unnecessary exposure to antibiotics should be avoided, the length of antibiotic treatments should be kept as short as possible, and the spectrum of coverage as narrow as possible (III). Except for lungs, per transplant prophylactic antibiotics should not be used beyond 48 hours posttransplantation (III). Exposure to interventions and indwelling devices should similarly be restricted. Length of endotracheal intubation should be reduced, invasive devises and central venous and urinary catheters should be removed as soon as possible (10,38,65) (III).

MDR Enterobacteriaceae and Acinetobacter

While ESBL producing bacteria are also seen in increasing frequency in community-acquired infections, CRE and MDR *Acinetobacter* remain mostly associated with nosocomial infections. Traditionally, infection control efforts have focused on the hospital setting. However, increasing evidence supports that long-term acute and chronic care facilities serve as a reservoir for MDR bacteria (42). Therefore, increased efforts to limit long-term care exposure for transplant recipients and efforts to improve infection control in these settings are indicated.

A number of hospital outbreaks have been reported of infections with MDR *Enterobacteriaceae*, including CRE (45,66–69). Consequently, appropriate laboratory techniques coupled to responses from healthcare providers should lead to environmental control measures and antimicrobial strategies to limit spread (I). This should include contact isolation, defined as the use of gowns and gloves and patient placement in private rooms with dedicated bathroom facilities or cohorting of patients with others who are colonized or infected with the same organism (II-2). As with all patients, strict hand hygiene measures before and after contact with the patient or patient contaminated surfaces are critical to limiting the spread of MDR bacteria (II-2). Since there is the potential for prolonged

carriage of these bacteria in the intestinal tract, even following treatment, these patients should be identified and either isolated or cohorted upon readmission to the hospital or transfer to other facilities. Currently there is no recommendation for screening of asymptomatic patients as there are no data regarding the sensitivity or benefits of this screening. Because hospital-wide as well as community antimicrobial prescribing practices will impact the resistance patterns observed in transplant recipients, it is important to restrict antibacterial use to those patients in whom bacterial infection has been documented or strongly suspected (II-3). Donor-derived infections with MDR *Enterobacteriaceae* present a unique opportunity for prevention. Twelve recipients have been reported, of whom five experienced clinical donor-derived infection resulting in death in two patients, renal graft loss in two other patients and in one patient resolution of infection after prolonged combination treatment (70–73). If donor colonization or infection with CRE is known prior to transplantation, a risk-benefit evaluation should be made, taking into account the organ to be transplanted and the source of the positive donor cultures. Selective decontamination of the digestive tract has not been proven to be of benefit in transplant recipients or candidates, and cannot be recommended at this time for prevention of infections with MDR *Enterobacteriaceae* or MDR *Acinetobacter* (III).

MDR Pseudomonas, Stenotrophomonas, Achromobacter and Burkholderia

Efforts should be made to minimize the risk of pretransplant acquisition of MDR or PR bacteria in CF-lung transplant recipients. These should include parsimonious use of antibiotics and as much as possible nonantimicrobial management strategies to control CF exacerbations (III). The widespread transmission of epidemic clones of *P. aeruginosa* also underlines the importance of avoiding socialization among CF patients (52). The "3 foot rule" advocated as the minimal distance between CF patients has recently been suggested not to be sufficient, as infectious particles in small size droplets might remain in the air for several minutes to hours (52). Whether aerosolized colistin can promote emergence of antibiotic susceptible *P. aeruginosa* in pretransplant CF patients colonized by MDR *P. aeruginosa* needs confirmation (74). On the other hand aerosolized colistin might favor colonization by intrinsic colistin resistant *B. cepacia* complex. Home-use nebulizers have been identified as potential primary source of *B. cepacia* and *S. maltophilia* in CF patients. Clearly, strict nebulizer hygienic practices should be endorsed to avoid such acquisition routes (III). Some centers recommend sinus surgery (endoscopic frontosphenoethmoidectomy) to reduce bacterial seeding from the paranasal sinuses, acting as reservoirs for *P. aeruginosa* and *B. cepacia* complex, to the transplanted lungs. Whether this approach reduces the incidence of tracheobronchitis and the risk of bronchiolitis obliterans (BOS) remains controversial (75,76). Consequently, this approach cannot be routinely recommended at this time (II-3). Combined continuous sinonasal and bronchial colistin inhalation has been recently suggested to prevent pulmonary postlung transplant recolonization by *P. aeruginosa* (77).

Colonized lung transplant recipients are also a potential reservoir for transmission to other transplant patients. Contact isolation measures should therefore be considered for transplant recipients harboring MDR and/or PR bacteria (III). Cohorting of patients with MDR *P. aeruginosa* is so far not recommended. In contrast, because of the dramatic rise in serious posttransplant complications, separation of patients colonized with *B. cenocepacia* from those patients free of this pathogen seems justified (III). As previously noted, hand hygiene measures are critical to control the spread of these resistant bacteria (II-2). Additionally the previously noted caveats regarding maintaining the appropriate level of patient care despite isolation should also be considered. Recently, donor-derived infections with MDR *P. aeruginosa* have been reported (78,79). Obviously, all efforts should be made to identify organ donors with MDR *P. aeruginosa* infections in order to give preemptive antibiotics to the recipients (II-3).

Treatment

Source control–removal of infected devices, drainage of collections—is the most important predictor of a good outcome for many infectious syndromes (40,80). Therefore, adequate source control as allowed by clinical circumstances should be the first priority in all patients infected with MDR Gram-negative bacteria (II-2). Antimicrobial treatment should be selected on the basis of *in vitro* susceptibility, predicted levels at the site of infection, cost, method of administration and side effect profile. Empiric therapy for suspected Gram-negative bacterial infections in transplant recipients should be guided by the type of infection (nosocomial vs. community acquired), the local resistance patterns, known MDR Gram-negative colonizers for the specific patient, and the severity of the infection (III). Data to support recommendations regarding duration of antibiotic courses are lacking. In general, guidelines for specific infectious syndromes such as pneumonia or bloodstream infection may be followed. However, duration of treatment in transplant recipients infected with MDR Gram-negative bacteria should be individualized and guided by response to treatment and degree of source control, as well as by side effects of therapy (III) (Table 2).

MDR Enterobacteriaceae

For MDR *Enterobacteriaceae* that retain susceptibility to carbapenems, these are generally the drug class of choice. In selected infections with ESBL producing bacteria, cefepime and piperacillin/tazobactam may still be used upon documentation of *in vitro* susceptibility. However, the use of cefepime in such conditions should be restricted to

Table 2: Treatment recommendations

Organism	Recommendation	Level
All	Source control should be aggressively pursued Early transplant infectious disease consultation	I
ESBL-producing *Enterobacteriaceae*	Carbapenems Alternative: cefepime or piperacillin/tazobactam (if susceptible and low inoculum infection)	I III
Carbapenem-resistant *Enterobacteriaceae*	Systemic infections: Individualized combination regimen with two or more of the following: Colistin Tigecycline Aminoglycosides (if susceptible) High-dose, prolonged infusion carbapenems Uncomplicated UTI: Oral fosfomycin (if susceptible) IV aminoglycosides (if susceptible)	II-3
MDR *Acinetobacter*	Carbapenems (except ertapenem) if susceptible If carbapenem resistant consider combination therapy with: Colistin Ampicillin/sulbactam if sulbactam susceptible Tigecycline (if susceptible and no bloodstream or urinary infection) Rifampicin	II-3
MDR *P. aeruginosa*	Individualized combination regimen with two or more of the following: Antipseudomonal beta-lactam (consider high doses of prolonged or continous infusion) Aminoglycoside Ciprofloxacin Adjunctive aerosolized colistin or tobramycin	II-2
PR *P. aeruginosa*	Individualized combination regimen with three or more of the following: IV colistin Doripenem or another anti-pseudomonal beta-lactam (consider high doses of prolonged or continuous infusion) Aminoglycosides Fosfomycin Rifampicin Adjunctive aerosolized colistin or tobramycin	II-2
MDR *B. cepacia* complex	High dose TMP/SMX Alternatives if susceptible: Meropenem Ciprofloxacin	II-2
TMP/SMX-R or PR *B. cepacia* complex	Combination therapy with: Meropenem Aminoglycoside Ceftazidime (or trimethoprim sulfamethoxazole)	II-2
MDR *A. xylosoxidans*	Combination therapy: Piperacillin/tazobactam Carbapenems (except ertapenem) TMP/SMX	III
MDR *S. maltophilia*	High dose TMP/SMX Alternatives: Ticarcilline/clavulanate Moxifloxacine Doxycycline Tigecyline Consider combination therapy	II-2

IV = intravenous; MDR = multidrug resistant; PR = pan-resistant; TMP/SMX-R = trimethoprim/sulfamethoxazole resistant.

infections with a low bacterial inoculum (i.e. for a UTI but not for a pneumonia) (III). CRE present a greater therapeutic challenge, as CRE generally retain *in vitro* susceptibility only to colistin, tigecycline and fosfomycin, and display variable *in vitro* susceptibility to selected aminoglycosides. Side effects of colistin include nephrotoxicity and neurotoxicity. Tigecycline is an alternative choice, with a more attractive side effect profile. Its most common side effect is nausea, which may be quite severe. Tigecycline should not be used for UTI (81,82) (II-3). Also, low serum levels raise concern for its use as monotherapy for bloodstream infections (III). The FDA issued a warning regarding increased mortality risk associated with tigecycline in 2010. The outcomes of four meta-analyses trying to assess this risk have been conflicting (83–86). However, a small but significant increased mortality risk is likely to be associated with the use of tigecycline, most likely secondary to decreased efficacy. However, it should be noted that these studies did not specifically address the treatment of CR bacteria.

In the United States, fosfomycin is currently available only in oral form, and can be quite useful in the treatment of UTI in patients without renal failure caused by MDR *Enterobacteriaceae* (fosfomycin is not active against *Acinetobacter*). However, emergence of resistance has been reported (87). For UTI with CR bacteria susceptible to aminoglycosides, these are the agents with the highest response rate (82,88). However, their use is limited by nephrotoxicity as well as ototoxicity.

Limited data suggest that if the carbapenem MIC is $\leq$4 mg/L, high-dose carbapenems given by prolonged infusion may be beneficial in a combination regimen for the treatment of CRE (89). In addition, results from a murine model and *in vitro* data hint at potential efficacy of double-carbapenem therapy (90). There is a general lack of prospective data comparing treatment modalities not only in transplant recipients but also in the nontransplant population. Whether combination therapy improves outcomes has been insufficiently studied as well. In nontransplant populations, retrospective studies in CRE bloodstream infections have shown a survival benefit associated with combination therapy (91–94). The combination of meropenem, tigecycline and colistin was associated with lower mortality in one study (OR for 30-day mortality 0.27, $p = 0.009$) (92).

MDR Acinetobacter Baumannii

Carbapenem susceptible isolates should be treated with a carbapenem (except ertapenem) (II-3). CR *Acinetobacter* may remain susceptible *in vitro* to the sulbactam component of ampicillin/sulbactam. If this is documented, ampicillin–sulbactam may be used for treatment. Many isolates however are susceptible to colistin only (95). If susceptibility is documented, aminoglycosides may also be of use in the treatment. The use of tigecyline is limited by widespread resistance and reports of treatment failure (96–98). Although rifampin has been used in combination therapy where multiresistance may be anticipated, the risk of drug interactions with calcineurin inhibitors and mTOR inhibitors should limit its use (III).

MDR Pseudomonas, Stenotrophomonas, Achromobacter and Burkholderia

Transplant recipient specific studies concerning the treatment of MDR *P. aeruginosa*, *B. cepacia* complex, *Stenotrophomonas* and *Achromobacter* infections are lacking.

Optimal treatment for non-MDR *P. aeruginosa* infections remains controversial. In the nontransplant population it appears that initiation of therapy with a combination therapy (usually a beta-lactam combined with an aminoglycoside) for a limited time (3–5 days), followed by a beta-lactam monotherapy, might improve survival and limit the nephrotoxicity of aminoglycosides (99) (II-2). This is even more important after transplantation when renal failure and /or coadministration of other nephrotoxic drugs are common. In contrast, for MDR/PR *P. aeruginosa* infections in lung transplant recipients most experts recommend combination therapies including two or three different classes (beta-lactam + aminoglycoside $\pm$ fluoroquinolone) of antibiotics for 10–14 days (23,27,29,100) (II-2). In nonlung SOT patients, shorter treatment durations (7–10 days) might be possible depending on the infection site (III). In all cases the duration of therapy, as well as the timing of downgrading towards monotherapy, should always been guided by the clinical evolution and a careful reevaluation of the balance between reduced risk of recurrence versus selection of further resistance and drug dependent side effects associated with prolonged antibiotic therapy (III). Novel combination regimens may include colistin, doripenem, aminoglycosides, fosfomycine and rifampicin, however, most of the evidence is provided so far by *in vitro* studies and clinical experience is limited to small case series (64,100–102). In order to optimize pharmacokinetics, prolonged as well as continuous high-dose beta-lactam infusion therapy might be advantageous, as suggested for piperacillin–tazobactam, ceftazidime, meropenem and doripenem (102,103) (II-2). Evidence that adjunctive aerosolized colistin might be beneficial in combination with systemic antibiotics (colistin or beta-lactam) for the treatment of MDR *P. aeruginosa* infections has emerged in several studies, with success rates up to 88% (104,105) (II-3).

For *B. cepacia* complex infections, the drug of choice remains high dose trimethoprim sulfamethoxazole, and if susceptible meropenem or ciprofloxacin (II-2). Triple combination therapies including meropenem, aminoglycoside, and ceftazidime or trimethoprim sulfamethoxazole are recommended for MDR/PR *B. cepacia* infections (II-2). The clinical significance of *A. xylosoxidans* in transplant recipient remains uncertain. Treatment should be restricted to chronically colonized/infected patients with clinical decline (III). *A. xylosoxidans* is often resistant

to beta-lactams including cephalosporins and carbapenems, aminoglycosides, quinolones and trimethoprim-sulfamethoxazole (106). Treatment should be based on susceptibility testing and combination therapies including piperacillin–tazobactam, carbapenems and/or trimethoprim sulfamethoxazole should be favored. *S. maltophilia* infections should be treated with high dose trimethoprim sulfamethoxazole (II-2). Alternative antibiotics include ticarcillin–clavulanate, moxifloxacin and doxycline, as well as combination therapies including trimethoprim sulfamethoxazole and tigecycline (107,108).

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Special Article

Clostridium difficile Infections in Solid Organ Transplantation

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Abbreviations: ABHR, alcohol based hand rubs; CDI, *Clostridium difficile* infection; ELISA, Enzyme-linked immunosorbent assay; GDH, glutamate dehydrogenase; IVIG, intravenous immunoglobulin; NAAT, nucleic acid amplification test; NAP1, North American pulsed field gel electrophoresis type 1; PPI, proton-pump inhibitors; SOT, solid-organ transplant.

Clostridium difficile infection (CDI) is a commonproblem encounteredin solid-organ transplant (SOT) recipients and the incidence is increasing. SOT recipients have an incidence of CDI that is higher than other postoperative patients, and this group has several unique risk factors that may contribute to more severe disease. Recent publications in nontransplant patients have indicated that treatment choices should be based on the severity of the illness (1). Although there continues to be a lack of well-designed, randomized, controlled trials to support the management decisions that must be made for SOT recipients with CDI, the available evidence is reviewed and summarized for these treatment guidelines.

Epidemiology and Risk Factors

Clostridium difficile is a spore-forming, anaerobic, Gram-positive bacillus. It causes 6–25% of cases of antibiotic-associated diarrhea, up to 75% of antibiotic-associated colitis, and over 90% of cases of antibiotic-associated pseudomembranous colitis (1). *C. difficile* causes inflammatory diarrhea and colonic mucosal injury through production of two exotoxins, toxin A and toxin B, which trigger a cytotoxic response, neutrophilic infiltrate and cytokine release (1). The resulting inflammatory response results in the visible yellow plaques that form the characteristic pseudomembrane. This finding is less commonly seen in patients on immunosuppressive medications (2). Although most strains of *C. difficile* produce both toxins A and B (toxigenic *C. difficile*), some produce only toxin B, and some do not produce any toxin. Strains that produce only toxin B can produce the same spectrum of illness as those that produce both toxins and are considered toxigenic. Strains that do not produce toxins A or B (nontoxigenic) are not capable of causing *C. difficile* infection (CDI). Some *C. difficile* strains also produce a binary toxin; however, what role this toxin plays in humans disease is not known (1). It is also important to note that 50% or more of patients in healthcare settings colonized with toxigenic *C. difficile* never develop CDI (1,3,4). Whether this proportion differs in SOT recipients is not known.

The incidence and severity of CDI have increased dramatically since the year 2000 (5). These changes in CDI epidemiology have been associated with the emergence of the North American pulsed field gel electrophoresis type 1 (NAP1)/restriction enzyme analysis type BI/PCR-ribotype 027 (NAP1/BI/027) strain of *C. difficile* (5). CDI is a more frequently encountered problem in SOT recipients than other hospitalized populations. The incidence of CDI is estimated to be 3–19% in liver recipients, 3.5–16% in kidney recipients, 1.5–7.8% in pancreas–kidney recipients, 9% in intestinal recipients, 8–15% in heart recipients and 7–31% in lung recipients (6,7). This is higher than that seen in other hospitalized patient populations, where the incidence is typically <1% (8,9). Fulminant colitis develops in up to 8% of immunocompetent patients and 13% of SOT recipients with CDI (10). The incidence of CDI in SOT recipients is highest within the first 3 months after the procedure, probably because of more frequent antimicrobial exposure, intense immunosuppression and increased exposure to the healthcare setting (6,10). Late-onset CDI occurs months to years after the transplant and is usually associated with either antimicrobial exposure or intensified immunosuppression to treat graft rejection (10). It is not known how the NAP1/BI/027 strain has impacted the

incidence and severity of CDI in SOT recipients relative to the general hospital population.

Antimicrobial exposure is the most important risk factor for development of CDI (7). Any antimicrobial agent may predispose to CDI, but clindamycin, ampicillin, cephalosporins and fluoroquinolones are most frequently implicated (1). The use of multiple antimicrobial agents and extended treatment courses have also been identified as risk factors (1). Antimicrobial agent administration has been associated with CDI in nearly all immunocompetent inpatients with CDI. However, some studies have found only 80% of transplant recipients who develop CDI have recent antimicrobial exposures (11). The reduced relationship with antimicrobial exposure in SOT recipients may be secondary to alterations in the normal flora and impaired immunity due to immunosuppressive medications, severe pretransplant illness and surgical intervention.

Immune system dysfunction may also be an important factor in the development of CDI in SOT recipients. The importance of the humoral immune response is demonstrated by a fourfold greater incidence of symptomatic disease in patients who are newly infected and lack preexisting immunity (12). A brisk humoral response to *C. difficile* toxins after infection reduces the likelihood of symptomatic disease (13). The hypogammaglobulinemia commonly associated with lung, heart and liver transplants may result in a poor immune response and increase the incidence of CDI by fivefold in some patient subsets (14).

The use of medications that suppress gastric acid, such as proton pump inhibitors and H2 receptor antagonists, is common in SOT recipients and may also serve as a significant risk factor for the development of CDI. The acidic environment of the stomach is usually fatal to vegetative forms of *C. difficile* and may prevent germination of the spore form of the organism. Proton-pump inhibitors (PPIs) may also cause disturbances in the gastrointestinal flora that can allow *C. difficile* to more easily colonize the bowel. However, whether gastric acid suppression plays a causative role in CDI pathogenesis or is a marker for patients at risk for CDI remains unresolved (1). Other risk factors commonly cited in the literature include age greater than 65 years old, severe underlying disease, uremia, gastrointestinal surgery, presence of a nasogastric or endotracheal tube and prolonged hospitalization (15). SOT recipients frequently have a combination of these risk factors.

Of note, infants under the age of 1 are generally not thought to be at risk for CDI; however, asymptomatic carriage of *C. difficile* in this population is common (12). In this population, detection of *C. difficile* or its toxins should not be assumed to be the cause of diarrhea until alternate causes of diarrhea are ruled out.

- Antimicrobial exposure, advanced age, immune system dysfunction or immunosuppression and gastric acid suppression are important risk factors for CDI (II-2).

Diagnosis

CDI is diagnosed by confirming the presence of toxigenic *C. difficile* in the stool of a symptomatic patient. Recent evidence suggests that clinical information is critical when it comes to interpreting *C. difficile* test results, especially if more sensitive assays such as nucleic acid amplification tests (NAAT) are used (16). While SOT patients may have an atypical presentation, their transplant status should not affect diagnostic assays. The laboratory gold standard for *C. difficile* toxin detection in stool is the cytotoxicity cell assay, and the gold standard for detecting toxin producing *C. difficile* is toxigenic culture. Cytotoxicity cell assays detect biologically active toxin in stool. However cytotoxicity cell assays have fallen out of favor because it is relatively labor intensive and the delay of at least 24 h before interpretation (1). Toxigenic culture involves anaerobic culture of *C. difficile* followed by testing isolates for toxin production. It is rarely used for clinical diagnosis due to slow turnaround time and costs. However it is an important tool for epidemiological studies.

According to a 2008 College of American Pathologists survey, 45% of institutions in the United States currently use commercially available ELISAs for *C. difficile* toxin detection (17). These assays provide a rapid turnaround of results and are relatively inexpensive. ELISAs are generally only 60–90% sensitive compared with cytotoxicity assays, though newer assays continue to improve detection rates (18) and may provide better specificity (16). Even with the relatively low sensitivity, the negative predictive value of a negative toxin ELISA is greater than 95%, and repeat testing increases the likelihood of a false positive result. Therefore additional diagnostic and treatment decisions after an initial negative toxin assay should be based on the clinical suspicion of CDI rather than automatically repeating the test (1). It is important to note some ELISAs only detect toxin A. These assays will miss strains that produce only toxin B.

While ELISA may still be a common diagnostic modality for CDI, more hospitals are converting to a two-step algorithm that utilizes new molecular methods (17). Screening stool for the presence of glutamate dehydrogenase (GDH), a common cell wall protein produced by both toxigenic and nontoxigenic *C. difficile*, is the foundation for many of the new protocols. Testing for the presence of GDH allows for rapid and cost-effective screening; however, as GDH does not differentiate toxigenic strains from nontoxigenic strains, subsequent toxin testing is required for those stool specimens that are GDH positive (1). The presence of toxigenic *C. difficile* in GDH positive specimens has been evaluated by several different assays. In addition to the previously mentioned ELISA and cytotoxicity cell assays, NAAT have been evaluated both as a stand-alone test as

well as to confirm the presence of toxigenic *C. difficile* in GDH positive specimens (19). While the sensitivity of using NAAT testing alone for detecting *C. difficile* in stool approaches 93–100% (20,21), the positive predictive value can be as low as 63% for the diagnosis of CDI, and it is the most costly method of diagnosis (16). The low positive predictive value is due to detection of *C. difficile* in asymptomatic carriers. Regardless of what assay or algorithm an individual hospital uses, caution should be employed for only testing patients for whom there is a clinical concern for CDI.

In cases where the presentation of CDI is atypical or the presence of ileus results in a lack of diarrhea, clinicians will need to rely on physical examination and laboratory findings. Fever, abdominal pain and abdominal distension are typically present in severe colitis, even in the absence of diarrhea (1). Striking bandemia and a leukemoid reaction can be seen in SOT recipients with CDI. CT scan findings suggestive of severe colitis include significant bowel wall edema and ascites. These exam and laboratory findings usually precede organ dysfunction. A high index of suspicion for CDI is necessary in SOT patients with these otherwise unexplained exam and laboratory findings.

- Providers should be familiar with the *C. difficile* diagnostic modalities available at their institution and customize their clinical evaluations accordingly (III).
- Testing of stool for *C. difficile* and/or its toxins should only be performed in symptomatic patients who have stool that is not formed (II-2). If the initial ELISA test is negative, testing should be repeated only if there is a high index of suspicion for CDI and if test results will alter clinical management (II-2). Immediate repeat toxin testing is not indicated for cytotoxic tissue assays, GDH based algorithms and NAAT (II-2).
- Test of cure assays (i.e. testing stool for the presence of C diff toxin at the completion of therapy) should be avoided (III).
- Otherwise unexplained fever, abdominal pain and leukocytosis in a patient with ileus should prompt the clinician to consider CDI despite a lack of diarrhea (II-2). The presence of formed bowel movements indicates CDI is unlikely the cause of these symptoms (II-2).

Treatment

Severity of CDI can be divided into three categories: mild-to-moderate, severe and severe with complications (1). Of note, there are no validated methods to objectively categorize patients as such. Mild-to-moderate CDI is typically patients with diarrhea and possibly also with mild abdominal pain and minimal systemic symptoms. Severe CDI includes abdominal pain, leukocytosis and fever or other systemic symptoms along with profuse diarrhea. Advanced age and patients with hypoalbuminemia are at increased risk for severe disease (1). Severe disease with complications includes the symptoms of severe disease accompanied by life-threatening conditions such as paralytic ileus, toxic megacolon, refractory hypotension and/or multi-organ failure secondary to CDI. The disease severity may rapidly progress so clinicians should frequently reassess and adjust therapy accordingly.

The first intervention that should occur in any patient with CDI is cessation of the inciting antimicrobial agent whenever possible. Removing antimicrobial pressure on the normal flora was curative in roughly 15–25% of immunocompetent patients prior to the NAP1/BI/027 epidemic (1). If antimicrobial agents must be continued in order to treat another ongoing bacterial infection, clinicians may consider changing to a more narrow-spectrum regimen or an alternate antimicrobial agent with less association with CDI.

Previously published guidelines support basing the initial antibiotic choice on the severity of CDI (1) (Figure 1). Oral metronidazole is recommended for mild-to-moderate disease in both the general population and SOT recipients. Metronidazole undergoes biliary excretion and crosses the inflamed colonic mucosa so it also reaches adequate levels in the feces when given intravenously. This route of administration has not been rigorously studied, but is supported by several case series (22). There has also been a long-held concern that the use of oral vancomycin will increase the incidence of vancomycin-resistant enterococci, but recent studies have not substantiated this effect (23). A major disadvantage of metronidazole use in SOT recipients is an interaction with medications such as tacrolimus or sirolimus, so that levels of tacrolimus should be monitored during treatment. Readers are referred to the corresponding guidelines on interactions between antiinfective agents and immunosuppressants published in this supplement for further comment.

Oral vancomycin is the preferred therapy for severe CDI. Several studies demonstrated improved response rates with vancomycin compared to metronidazole in severe disease. Two randomized studies found that 85–97% of patients with severe CDI were cured with vancomycin therapy, but only 65–76% of patients were cured with oral metronidazole (24,25). These same studies continue to show no significant difference between the two antimicrobial agents in mild-to-moderate disease (24,25). Vancomycin typically is administered at 125 mg four times daily in adults because higher doses have increased cost and side effects without improved efficacy (26). This regimen achieves stool vancomycin concentrations that are hundreds of times greater than the minimum inhibitory concentration (MIC) of *C. difficile* (27). The usual dose of oral vancomycin for children is 40 mg/kg daily given in three or four divided doses. Many pharmacies now constitute oral vancomycin solution from IV vancomycin with marked cost savings yet no obvious impact in clinical outcomes.

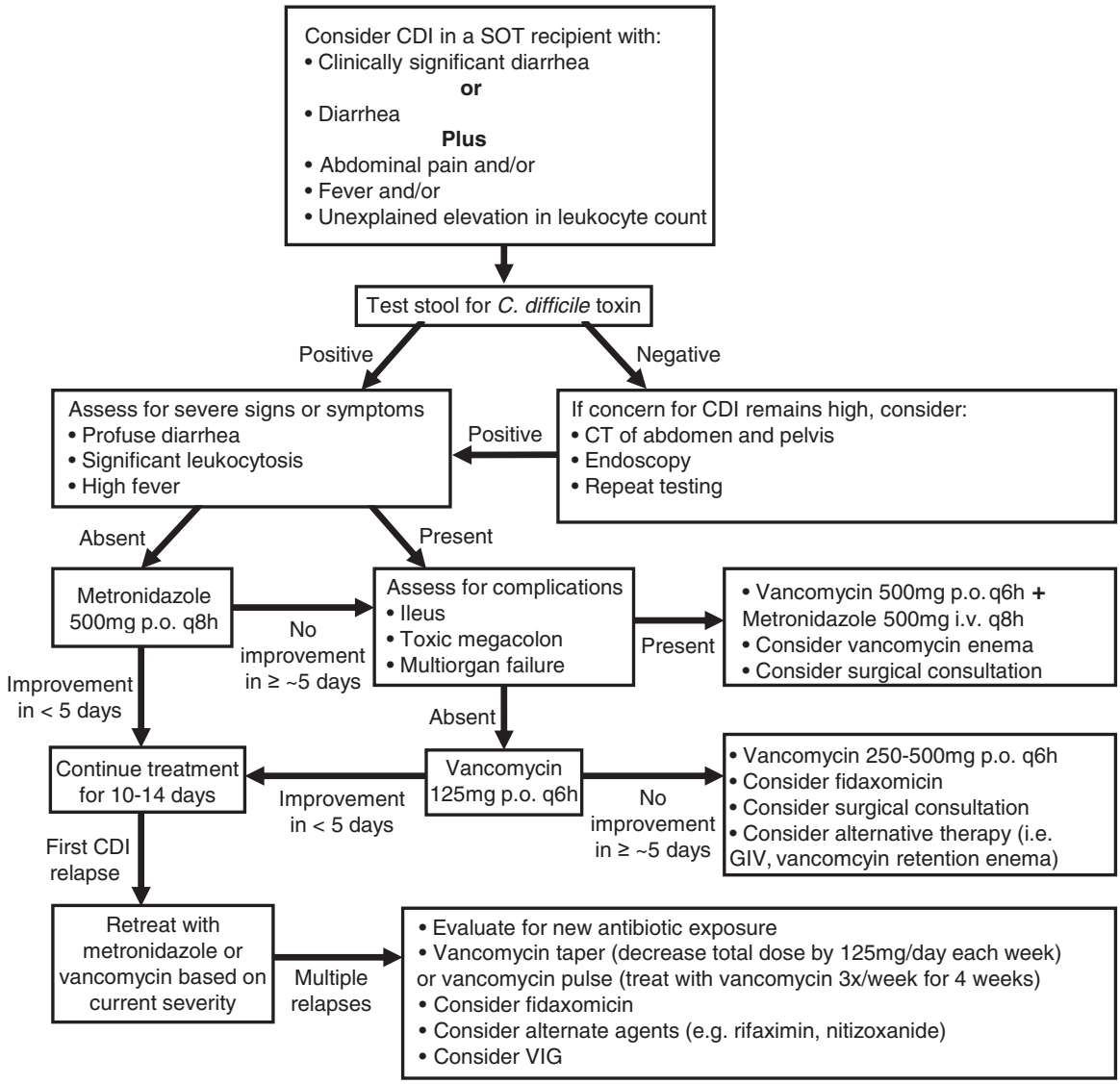

Figure 1: Recommended approach to the diagnosis and treatment of CDI presenting with diarrhea in adult SOT recipients.

In contrast to metronidazole, vancomycin does not reach adequate levels in the feces when given intravenously and should never be administered intravenously to treat CDI.

In 2011, fidaxomicin was FDA approved for the treatment of CDI (28,29). Fidaxomicin is a macrocycline (in the United States it is designated as a macrolid; in Europe as a macrocycle) antibiotic with minimal systemic absorption, high colonic concentrations and limited impact on normal gut flora. It has been evaluated in patients with no or 1 prior episode of CDI. Data reveal similar clinical response, but decreased rates of recurrent infection, as compared with vancomycin 125 mg orally every 6 h (28,29). Limitations to fidaxomicin include drug acquisition costs and lack of data in SOT recipients. One publication did suggest fidaxomicin has improved success rates in patients who are on concomitant antibiotics for other infections compared to vancomycin (30).

In cases of severe CDI with complications, decreased gastrointestinal motility may limit the efficacy of oral vancomycin by preventing the drug from reaching the site of infection. In these patients, 500 mg every 6 h of oral vancomycin may be warranted in an attempt to increase the probability that adequate levels of vancomycin will be achieved in the colon as quickly as possible. Several case reports also support the use of vancomycin administered by retention enema in cases of ileus (31). Novel surgical approaches such as diverting loop ileostomy are being studied though their exact role in the management of complicated CDI is still being determined (32). Bloodstream infections from colonic flora have been reported following

administration of vancomycin enemas so clinicians should exercise caution when considering this approach (31).

Intravenous metronidazole should also be administered with oral vancomycin in an attempt to ensure drug delivery to the site of infection in more severe cases. Antimicrobial therapy alone may be insufficient treatment in patients with severe CDI and surgical intervention may be a necessary addition. Less than 3% of immunocompetent patients with CDI develop fulminant pseudomembranous colitis that requires colectomy; however, colectomy is performed in up to 13% of SOT recipients with CDI (10). Surgical intervention within the first 48 h of a failure to respond to medical therapy, bowel perforation, or multiorgan failure may reduce mortality in patients with severe disease (10). Serum lactate levels and peripheral WBC count may be helpful in determining timing of surgical intervention. Lactate levels rising to 5 mmol/L and WBC count rising to 50 000 cells/μL are associated with perioperative mortality; thus intervention prior to reaching these cut offs should be considered. Patients at higher risk for postoperative mortality include those admitted for a diagnosis other than CDI, mental status changes, and vasopressor support prior to colectomy (33).

Intravenous immunoglobulin (IVIG) has been attempted with variable success in the treatment of CDI. IVIG is known to contain *C. difficile* antitoxin antibodies; but its use is supported only by case studies and series. A retrospective analysis of 18 pair-matched patients with severe CDI did not show any benefit to combining IVIG with standard antimicrobial therapy; however, this study did not control for the time from onset of symptoms to IVIG administration (34). In a retrospective review of heart transplant recipients with hypogammaglobulinemia, a lower incidence of CDI was noted in the patients treated with IVIG (14); however, these results were not statistically significant. At this time, IVIG remains a treatment option that is worth further study, but cannot be broadly recommended.

Twenty- to 30 percent of patients with CDI will suffer at least one recurrence (1). Patients treated with fidaxomicin have demonstrated less episodes of recurrent CDI, though studies to date have not included transplant recipients (29). Treatment of the first recurrence should again be guided by the disease severity as recurrence is not related to the development of antimicrobial resistance to the first course of treatment (1). Management of patients with multiple recurrences has not been thoroughly studied, but there are reports of success with either a prolonged tapering or pulse-dosing schedule of oral vancomycin. Metronidazole should not be tapered or pulsed (1). One suggested regimen for vancomycin tapering is included in Figure 1 and would include the following: after the usual dosage of 125 mg 4 times per day for 10–14 days, vancomycin is administered at 125 mg 2 times per day for a week, 125 mg once per day for a week, and then 125 mg every 2 or 3 days for 2–8 weeks (1). Pulse dosing recommendations include 125 mg every 2 or 3 days for 4 weeks. Studies have demonstrated similar outcomes between tapered dosing and pulse therapy. The hope of both the taper and the pulse therapy is that *C. difficile* vegetative forms will be kept in check while allowing restoration of the normal flora (1).

There has been great interest in the use of adjunctive therapies with conventional antibiotics in order to reduce the frequency of CDI recurrences. Several retrospective studies and case series in patients suffering from recurrent disease have revealed a modest benefit after treatment with IVIG or probiotics (1). Clear benefits have not been reported in placebo-controlled trials probiotics, and IVIG has not been studied with placebo-controlled trials. Probiotic use also carries the risk of superinfection (including bloodstream infections) from the organisms in probiotic formulas, but this complication appears rare (1,35). Fecal flora restoration therapy (e.g. fecal enemas) appears beneficial at preventing relapses in immunocompetent hosts (1). However, similar to recommendations supporting avoidance of probiotics in immunocompromised hosts because of risk of infection, it also appears prudent to avoid fecal flora restoration therapy in SOT recipients given the absence of supportive data in SOT recipients and theoretical potential for infection. Cholestyramine and colestipol have also been investigated as adjunctive therapy in case studies and series since they bind the *C. difficile* toxins *in vitro*, but have demonstrated inconsistent clinical results. Caution should be used when the binding resins are administered in conjunction with vancomycin since cholestyramine has been shown to complex with it *in vitro* and may result in subtherapeutic fecal concentrations in addition to having numerous other drug interactions. A small case series indicates rifaximin may be of benefit to prevent relapses; however there are concerns for the rapid development and dissemination of resistance (36,37).

Patients with confirmed CDI and continued diarrhea despite appropriate therapy should be evaluated for other causes of diarrhea, including coinfection with other pathogens. Parasites such as giardia or cryptosporidium, viral infection with CMV or HSV, bacterial coinfection with Salmonella, Shigella or Campylobacter and noninfectious causes such as laxative use, other concomitant antibiotics, or ischemic colitis may occur concomitantly. Appropriate diagnostic testing should be pursued.

- The first intervention that should occur in any patient with CDI is cessation of the inciting antimicrobial agent whenever possible (II-2).
- For mild-to-moderate CDI, oral metronidazole remains the drug of choice (I). The accepted dose of metronidazole is 500 mg TID for adults and 30–50 mg/kg/day divided TID for pediatric patients (not to exceed adult dosing).
- For severe CDI, oral vancomycin is the treatment of choice (I). The accepted dose of vancomycin is 125 mg

QID for adults and 40–50 mg/kg/day divided QID for pediatric patients (not to exceed adult dosing).
- In cases of severe CDI with complications, the dose of oral vancomycin may be increased up to 500 mg orally QID (III), vancomycin may be administered by retention enema (II-2), and intravenous metronidazole may be added (II-3).
- Surgical intervention should be considered in cases of complicated CDI (II-3).
- Patients suffering from multiple recurrences of CDI may respond to prolonged courses of oral vancomycin, either in a tapering or pulse dose schedule (II-2).
- Role of fidaxomicin in solid-organ transplant recipients is not yet clear.
- There is insufficient evidence to recommend routine use of IVIG (II-2), probiotics (I), or toxin-binding resins (I) in the treatment of initial or recurrent CDI. Probiotics and toxin-binding resins may be potentially harmful due to the risk of bacteremia or reducing the effectiveness of antimicrobial therapy, respectively.

Prevention and Prophylaxis

Prevention of CDI is a multidisciplinary effort, involving infection prevention and control, physicians, hospital administration, nursing, housekeeping, pharmacy and the microbiology laboratory (38). Transplant physicians should play an active role on the hospital CDI prevention team if CDI is problematic in their patients. In addition to infection control measures (discussed below), prevention of CDI must focus on reducing the risk factors for developing the disease in patients that acquire *C. difficile*. The most significant modifiable risk factor for CDI remains antimicrobial exposure, especially to broad-spectrum antimicrobial agents. Many institutions have succeeded in limiting the use of broad-spectrum antimicrobial agents through use of formulary restrictions and antimicrobial stewardship programs. This strategy was effective in reducing the incidence of CDI by 60% when a stewardship program was implemented during the nosocomial outbreak in Quebec (39). Programs that reduced broad spectrum antimicrobial agent use without altering overall antimicrobial use also resulted in significant reductions in the incidence of CDI (39). Other interventions that specifically limit only high-risk antimicrobial agents such as cephalosprins and clindamycin also meet with statistically significant reductions in CDI at many other centers (40).

There is no known effective prophylaxis against *C. difficile*. CDI can be caused by any antimicrobial therapy, including metronidazole and vancomycin, so it is recommended that no antimicrobial agent be given with the intention of preventing the disease. Preexisting colonization with *C. difficile* also appears to be protective against development of CDI after a patient is hospitalized, so the presence of the organism or its toxin in an asymptomatic patient would not be cause for preemptive therapy (41). The use of probiotics as a preventative measure has also had inconsistent success in several small studies, and there are currently no adequate studies that specifically support the use of probiotics as effective prophylaxis against CDI. Vaccines may be beneficial in the future; however vaccine development has not progressed beyond animal and phase II studies at this time.

- Limiting antimicrobial use through formulary restrictions and/or antimicrobial stewardship programs reduces the incidence of CDI (II-3).
- Other modifiable risk factors for the development of CDI, such as gastric acid suppression or prolonged hospitalization, should be reduced if possible (III).

Infection Control Issues

Both strict hand hygiene and appropriate contact precautions are essential in order to limit the spread of *C. difficile* within institutions. Patients with CDI should be placed into contact precautions as soon as possible to limit the spread of *C. difficile*. Contact precautions should be at least until diarrhea resolves, or a few days after diarrhea cessation, and possibly until discharge during outbreaks (38). An area of confusion and controversy when preventing CDI is the preferred method of hand hygiene after caring for a patient with CDI. Alcohol-based hand rubs (ABHR) do not kill *C. difficile* spores and are less effective than soap and water at removing *C. difficile* spores (42). However several studies have failed to demonstrate either an increase in CDI with ABHR or a decrease in CDI with soap and water (38). Conversely, several of these studies did demonstrate a reduction in infections due to other antimicrobial resistant organisms. Currently it is felt ABHR are an adequate form of hand hygiene when gloves are worn when caring for a patient with CDI. However, soap and water should be considered during outbreaks where other measures are not successful at reducing CDI incidence (38). *C. difficile* spores are known to contaminate the environment, are resistant to standard disinfectants, and are capable of surviving for months on dry surfaces within a hospital room. It is not yet clear if routine environmental decontamination with sporicidal agents is necessary, although it is reasonable to consider during disease outbreaks. Whether to use diluted bleach, or a new technology such as UVA or hydrogen peroxide vapor, to kill *C. difficile* spores should be individualized to the institution (38).

- The combination of strict hand hygiene and contact precautions significantly reduces the incidence of CDI through limiting patient acquisition of *C. difficile* (II-3).
- 1:10 dilution of household bleach solutions are sporicidal with $\geq$ 6 log reduction in viable *C. difficile* spores after 10 min contact time and may be used for environmental decontamination during outbreaks (II-3).

Future Research

There are many unknowns with regard to CDI, including the optimal method to diagnose CDI, optimal treatment strategies especially for recurrent and severe CDI with complications, and optimal methods to prevent CDI. This is true for both immunocompetent and immunocompromized patient populations. Studies on CDI diagnosis should include clinical information on the patient, as the detection of *C. difficile* from stool alone does not equate to CDI. Ideally, data on treatments the patient received and outcomes should be included as well. Studies are needed to better stratify severe from nonsevere CDI, with validation that treatment based on this stratification results in improved outcomes. Methods to predict patients at highest risk for CDI recurrence and methods to manage multiply recurrent CDI are needed. Higher quality data are needed to validate our current methods to prevent CDI and to determine if novel prevention approaches are needed.

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Disclosure

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Special Article

Methicillin-Resistant, Vancomycin-Intermediate and Vancomycin-Resistant *Staphylococcus aureus* Infections in Solid Organ Transplantation

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Key words: Antibiotic resistance, bacterial infection, multidrug resistance, posttransplant infection

Abbreviations: CA-MRSA, community-associated MRSA; CLSI, Clinical and Laboratory Standards Institute; EARSS, European Antimicrobial Resistance Surveillance System; HA-MRSA, healthcare-associated MRSA; HIV, human immunodeficiency virus; hVISA, heteroresistant VISA; ICU, intensive care unit; IDSA, Infectious Diseases Society of America; MALDI-TOF, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; MSSA, Methicillin-susceptible *S. aureus*; PCR, polymerase chain reaction; PNA-FISH, fluorescent *in situ* hybridization employing peptide nucleic acid probes; PVL, Panton-Valentine leukocidin; SCC*mec*, staphylococcal chromosome cassette *mec*; SSTIs, skin and soft-tissue infections; TMP-SMX, trimethoprim-sulfamethoxazole; VISA, vancomycin-intermediate *S. aureus*; VRE, vancomycin-resistant enterococci; VRSA, vancomycin-resistant *S. aureus*.

Epidemiology and Risk Factors

Staphylococcus aureus is a major cause of infection among solid-organ transplant recipients. After years of rising incidence, methicillin-resistant *S. aureus* (MRSA) infections have been decreasing. In the United States, the incidence of MRSA catheter-associated bloodstream infections has declined (1) as have rates of invasive healthcare-associated MRSA infections (2). Data from Europe are even more encouraging (3). According to the European Antimicrobial Resistance Surveillance System (EARSS), invasive MRSA infections are decreasing in nine countries (4). According to the HELICS surveillance network, the incidence of MRSA infections has decreased in the intensive care setting (5). Those data support the use of aggressive policies in infection prevention and control. Despite those positive data, MRSA still accounts for more than 25% of bacteremias caused by *S. aureus* in many European countries (4). However, among central venous catheter-associated bloodstream infections caused by *S. aureus* in United States intensive care units (ICUs), more than 50% are caused by MRSA (6). Thus, further efforts to decrease infection are needed.

S. aureus is a Gram-positive organism frequently causing infection following transplantation. It is commonly encountered within the first 3 posttransplant months. A significant number of those infections are caused by MRSA. *S. aureus* is one of the leading causes of Gram-positive bacteremia among transplant recipients reported in up to 25% of all isolated bacterial pathogens (7–10). *S. aureus* is a common cause of pneumonia after lung transplantation with rates of MRSA infection ranging from 40% to 80% in staphylococcal pneumonia (11–13). Surgical site infections following transplantation are also commonly caused by *S. aureus*. The true extent of MRSA colonization and incidence of infection after transplantation in adults and children varies among transplant centers reflecting the type of transplanted organs and the prevalence of carriage and infection in the nontransplant patient population.

Risk factors associated with MRSA infection include prolonged hospital stay, exposure to broad-spectrum antibiotics, admission to an ICU or burn unit, recent surgery, close contact to other patients with MRSA, presence of foreign bodies such as central venous catheters, and MRSA colonization (14). Factors specifically noted in liver transplant recipients include surgery within 2 weeks prior to infection, cytomegalovirus seronegativity or primary infection, extended posttransplant ICU stay, presence of other major posttransplant infections, peritonitis and increased prothrombin time (15–17). Patients on the waiting list and transplant recipients have an increased risk of becoming colonized with MRSA because of their illness and contact with the healthcare system. High rates of colonization have been reported for those undergoing hemodialysis (18) and patients with cystic fibrosis (19). Patients can become colonized following transplantation, as shown among liver

transplant recipients (20). MRSA acquisition is dependent on the local MRSA prevalence, infection control policies and the recipient's general state of illness (21).

Methicillin-susceptible and -resistant *S. aureus* colonization has been shown to increase the risk of subsequent infection (22), which is usually caused by the same strain. Among transplant patients specific data exist only for liver recipients. Liver transplant recipients colonized with MRSA on admission are at risk for subsequent MRSA infection. The reported incidence of infection in MRSA carriers ranges from 24 to 87% (15,23–25). MRSA carriage among liver transplant recipients does not seem to significantly affect mortality (24,25). In contrast, MRSA infection is associated with increased mortality (15,25). The incidence of MRSA infection seems to be higher in newly colonized patients than in chronic carriers (26), although data on transplant recipients are lacking. Donor-derived MRSA infection transmitted from a healthy living donor has been reported (27).

The increasing incidence of community-associated MRSA (CA-MRSA) is becoming a public health problem of great concern (28,29). CA-MRSA strains were originally isolated in patients who did not have contact with the healthcare system and were distinguished from healthcare-associated MRSA (HA-MRSA) through epidemiologic and antimicrobial resistance patterns. Most CA-MRSA strains carry staphylococcal chromosome cassette (SCC*mec*) type IV and genes for the exotoxin Panton-Valentine leukocidin (PVL) (30). CA-MRSA has a worldwide distribution, but its prevalence varies geographically. In a study conducted in 12 US emergency departments, the prevalence of MRSA was 59% among all skin and soft-tissue infections (SSTIs) and clone USA300 accounted for almost all isolates (31). Clone USA300 also causes an increasing proportion of hospital-onset invasive MRSA infections (28,29,32,33). CA-MRSA prevalence is lower in Europe and currently the most important risk factor is traveling to or origin from high-prevalence countries (34,35). Isolated cases and small outbreaks caused by different clones have been documented in many European countries (3,36). Furthermore, CA-MRSA is spreading from the community into hospitals, and the incidence of CA-MRSA infections and outbreaks in hospitalized patients is increasing (28,36,37). An increasing prevalence of CA-MRSA colonization in livestock with the potential of human spread has also been reported (3,28,29,36).

CA-MRSA can be transmitted from person to person. In US studies, the following groups were found to be at risk for colonization or infection: neonates and children; athletes who participate in contact sports; injection drug users; men who have sex with men; military personnel; persons living in correctional facilities, nursing homes, or shelters; adults 65 years or older; veterinarians; pet owners; pig and horse farmers. HIV infection, cystic fibrosis and household contact with a person known to be colonized or infected with MRSA are additional risk factors. The presence of SSTI or a history of recent severe pneumonia should raise the suspicion of CA-MRSA colonization (28,38). In the general population, CA-MRSA is typically associated with uncomplicated SSTIs but can also cause severe disease, such as necrotizing fasciitis or necrotizing pneumonia (28,29,38). Certain strains, notably USA300, often produce PVL, whose role in the virulence of MRSA remains controversial. Infection with CA-MRSA has been reported among transplant patients; very few epidemiologic data exist (27,39) but possibly follow the trends of the general population. In a single-center study from Canada, among 17 cases of MRSA colonization and/or infection, all strains were found to be hospital-associated (13). Considering the increasing incidence, infection with CA-MRSA should be suspected even in low-prevalence areas.

The prevalence of vancomycin-intermediate *S. aureus* (VISA) and heteroresistant VISA (hVISA) is increasing worldwide with major regional differences (40,41). Lacking a standardized detection method, findings on prevalence depend on study methodology. Data on transplant recipients are sparse. In a French study, heterogeneous glyocopeptide intermediate *S. aureus* strains were found in 13 (27%) of 48 patients (42). Vancomycin-resistant *S. aureus* (VRSA) has been shown to occur through transfer of the *vanA* gene from vancomycin-resistant enterococci (VRE) to MRSA. Few cases of VRSA have been reported to date in the United States (43) and Asia (44,45); none among transplant recipients. Factors that have been associated with VRSA infection are colonization or infection with MRSA or VRE, prior use of vancomycin, presence of chronic cutaneous ulcers and diabetes mellitus (43). Transplant recipients have multiple comorbidities and are potentially at risk for VRSA infection.

Diagnosis

S. aureus infections occurring in the first 3 posttransplant months are typically related to the surgical procedure and use of medical devices such as intravenous catheters and endotracheal tubes (7,9,10,46). MRSA most commonly causes bloodstream, lower respiratory tract, wound and intraabdominal infections. Diagnosis is established by isolation of the organism from affected sites. In general, isolation of *S. aureus* from a normally sterile body site or blood culture is diagnostic of infection. Depending on the clinical context, MRSA isolated in sputum, wound culture or fluid obtained from a drainage catheter may represent infection or mere colonization. In the absence of consistent clinical symptoms, signs and/or radiographic findings, isolation of the pathogen is more likely to represent colonization than infection and antibiotic treatment is not required.

Detection of Gram-positive cocci in clusters on Gram stain of the direct specimen provides an early clue to diagnosis. Rapid diagnostic assays, such as real-time PCR (47), fluorescent *in situ* hybridization employing peptide

nucleic acid probes (PNA-FISH) (48) and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF) (49) can expedite the characterization of Gram-positive cocci in blood cultures. For infection control purposes, surveillance cultures may be obtained from the anterior nares, throat, axillae, rectum or open wound areas. Traditional culture techniques provide results within 24–72 h. Chromogenic agar can be used to detect MRSA with a very high negative predictive value after only 24 h of incubation. A longer incubation period of 48 h slightly increases the sensitivity of the assay (50). Molecular techniques targeting DNA sequences within *SCCmec*, a mobile element carrying the methicillin-resistance gene *mecA*, allow for MRSA detection within 2–6 h (51).

Isolates with oxacillin MIC ≥4 μg/mL or methicillin MIC ≥16 μg/mL are considered methicillin-resistant. A 30 μg cefoxitin disk is more sensitive in detecting methicillin resistance than a 1 μg oxacillin disk (52). Molecular methods can be used to detect the *mecA* gene which codes for penicillin binding protein 2a and has been associated with resistance to beta lactams. In 2006, the Clinical and Laboratory Standards Institute (CLSI) lowered the vancomycin breakpoints for MRSA. Current breakpoints are ≤2 μg/mL for susceptible, 4–8 μg/mL for intermediate and ≥16 μg/mL for resistant isolates (53). Vancomycin has been considered the drug of choice for MRSA infections (I). However, strains with reduced susceptibility have emerged. VISA strains are homogeneous bacterial populations with MIC of 4–8 μg/mL. hVISA strains are susceptible using standard broth microdilution, but contain a small subpopulation of bacteria ($1/10^5$–10^6) that show intermediate susceptibility to vancomycin. VISA and hVISA strains are difficult to detect with automated standard MIC methodology and disk diffusion testing. E-test can improve the detection of VISA. Routine use of alternative methods for hVISA detection is not routinely recommended. Clinicians and microbiology laboratory personnel should be aware of this pitfall, as those strains have been associated with treatment failures (54). For an insufficient or failed response to vancomycin, particularly with strains at the upper end of the susceptible range (2 μg/mL), hVISA and VISA should be suspected. This should be communicated to the microbiology laboratory. If necessary, the strain can be further tested at a reference laboratory. A more detailed review is beyond the scope of this text; please refer to IDSA Guidelines (55), Centers for Disease Control VISA/VRSA guide (56) and recent reviews (40,54,57). Finally, pulse field gel electrophoresis and/or genotyping of the *SCCmec* gene can be performed to differentiate CA-MRSA from HA-MRSA and is mainly used for epidemiologic and research purposes.

Treatment

Clinical practice guidelines for the treatment of MRSA infections have been published by the Infectious Diseases Society of America (55). A summary of antimicrobial agents used in the management of staphylococcal infections, with an emphasis on transplantation issues, is provided in Table 1. Vancomycin is the drug of choice for serious infections caused by MRSA (I). Vancomycin is a bactericidal agent that inhibits bacterial cell wall synthesis. For methicillin-susceptible *S. aureus* (MSSA) the rate of bacterial killing is slower compared to β-lactams.

Guidelines have been published on the therapeutic use of vancomycin (58). Dosages should be calculated based on actual body weight. Target trough concentrations were selected with the aim of optimizing pharmacodynamics and efficacy and to minimize selection of resistant strains. For complicated MRSA infections, such as endocarditis, bacteremia, meningitis and pneumonia, serum trough concentrations of 15–20 μg/mL are advised (III). In most patients with normal renal function, these concentrations are achieved with a dose of 15–20 mg/kg every 8–12 h. In seriously ill patients, a loading dose of 25–30 mg/kg should be considered (58) (III).

In the case of isolates with an MIC value of 2 μg/mL, therapeutic serum levels cannot be achieved even with trough concentrations of 15–20 μg/mL. As demonstrated in a meta-analysis of 22 studies, vancomycin MIC values of ≥1.5 μg/mL were associated with higher mortality rates, particularly among patients with bloodstream infections (59). Higher MIC values were also predictive of treatment failure. The optimal treatment in case of high MIC and vancomycin failure is controversial, as there are currently no data to support better survival rates with the use of alternative antimicrobial agents, even though this practice has been recommended by several experts (60–66) (III). Infectious disease consultation is strongly advised (II-2).

Daptomycin, a bactericidal agent, is approved for use in complicated SSTIs, bacteremia and right-sided endocarditis (67). Further data are needed to extend the experience in the treatment of left-sided endocarditis (68). Daptomycin should not be used to treat pulmonary infections as it is inactivated by the lung surfactant. For prolonged bacteremia or documented microbiological failure while on daptomycin therapy, susceptibility testing should be repeated because of the risk of emergence of resistance. Of note, nonsusceptibility to daptomycin has been seen in isolates with increased MIC to vancomycin (69). The standard dose for treatment of bacteremia in patients with normal renal function is 6 mg/kg/day. Dosages of 8–10 mg/kg/day may be safe and effective in patients with severe complicated infections and have been suggested by some experts (61).

Linezolid, a bacteriostatic agent, is approved for use in uncomplicated and complicated SSTIs and nosocomial MRSA pneumonia. The drug is not approved for use in *S. aureus* bacteremia or endocarditis. Adverse events include thrombocytopenia, lactic acidosis, peripheral and optic neuropathy, particularly after prolonged use (more than 28 days).

Table 1: Therapeutic options for methicillin-resistant *Staphylococcus aureus* (MRSA) infections (please see the text for details)

Antimicrobial	Dosing	Comments
Vancomycin	15–20 mg/kg (actual body weight) q12h. For younger patients consider dosing q8h. Do not exceed 2 g/dose Consider 25–30 mg/kg load for serious infections and in critically ill patients Cr_{cl} 20–49: 15–20 mg/kg q24h Cr_{cl} ≤20: redose based on serum concentrations Initial load for critically ill with renal impairment should not be reduced IHD: loading 15–25 mg/kg, then 5–10 mg/kg or 500–1000 mg after each dialysis session (3 times per week)	• Treatment of choice for susceptible MRSA (I) • Dosing should be adjusted based on serum trough concentrations; obtain trough at steady-state conditions (just before the fourth dose) • Target trough concentrations for bacteremia, endocarditis, osteomyelitis, meningitis and hospital-acquired pneumonia: 15–20 μg/mL (III) • If no adequate clinical/microbiological response despite adequate debridement, or MIC >2 μg/mL, an alternative drug is recommended. ID consultation is advised (II-2) • Nephrotoxicity mostly if concomitant use of other nephrotoxic medications, preexisting renal impairment, dehydration, advanced age • Red person syndrome may be reduced by prolonging infusion rate and premedication with antihistamine
Daptomycin	Cr_{cl} ≥30: 4 mg/kg q24h for complicated SSTI; 6 mg/kg q24h for bacteremia, endocarditis, bone/joint infection Some experts advocate 8–10 mg/kg for endocarditis and complicated bacteremia Cr_{cl} <30, IHD: 4 mg/kg q48h for complicated SSTI; 6 mg/kg q48h for bacteremia, endocarditis, bone/joint infection Not evaluated in severe hepatic impairment (Child–Pugh class C)	• Do not use for pneumonia. Inactivated by surfactant • Reduced susceptibility can emerge during therapy; recheck MIC if inadequate response. Risk factors: previous vancomycin therapy and high vancomycin MIC. Observed especially in left-side endocarditis and deep-seated infections • Can cause myopathy. Monitor creatine phosphokinase at least weekly during therapy. Avoid concomitant use of statins
Linezolid	600 mg PO/IV q12h No renal adjustment required Metabolites may accumulate in patients with renal impairment but clinical significance unknown Not adequately evaluated in severe hepatic impairment (Child–Pugh class C)	• Indicated in SSTI and nosocomial pneumonia • Myelosuppression (mainly if used for >2 weeks). Monitor complete blood count weekly • Lactic acidosis • Peripheral and optic neuropathy (in long-term therapy) • Serotonin syndrome (avoid use with SSRIs, triptans)
Trimethoprim-sulfamethoxazole	One double strength (DS) tablet contains 160 mg of trimethoprim 8–10 mg/kg daily based on trimethoprim component in 2 divided doses (usually 1–2 DS tab twice daily) Cr_{cl} 10–30: 50% of usual dose Cr_{cl} <10, IHD: avoid or use 1 DS tab q48h	• Indicated for SSTI. Unlabeled use: osteomyelitis, septic arthritis • Avoid use in bacteremia, endocarditis • May reduce serum concentration of cyclosporine • Rare but life-threatening adverse events: hepatotoxicity, severe dermatologic reactions, hematologic dyscrasias • Do not use in third trimester of pregnancy
Clindamycin	300–600 mg po/iv q8h No renal adjustment required Use caution with severe hepatic impairment	• Indicated for SSTI. Unlabeled use: pneumonia, osteomyelitis, septic arthritis • Avoid use in bacteremia, endocarditis • May decrease serum concentration of mycophenolate • Diarrhea, including *Clostridium difficile* infection • Myelosuppression • Hepatotoxicity
Tigecycline	100 mg load, then 50 mg q12h No renal adjustment required Child–Pugh class C: 100 mg load, then 25 mg q12h	• Indicated for SSTI, intraabdominal infections, community acquired pneumonia caused by MSSA. Not approved for MRSA pneumonia • Avoid use in bacteremia and endocarditis • May increase serum concentration of cyclosporine • Nausea and vomiting are common adverse events • Do not use in pregnancy and children <8 years
Doxycycline	200 mg load, then 100 mg twice daily No renal adjustment required	• Unlabeled use: cellulitis due to community-associated MRSA • Do not use in pregnancy and children <8 years
Ceftaroline	600 mg q12h Cr_{cl} 31–50: 400 mg q12h Cr_{cl} 15–30: 300 mg q12h Cr_{cl} <15, IHD: 200 mg q12h	• Indicated for complicated SSTI. Not approved for healthcare-associated pneumonia • No data for bacteremia • Use with caution in patients with penicillin allergy
Quinupristin-dalfopristin	7.5 mg/kg q12h for complicated SSTI 7.5 mg/kg q8h for bacteremia No renal adjustment required	• Unlabeled use: persistent bacteremia associated with vancomycin failure • Quinupristin may increase the serum concentration of cyclosporine • Severe myalgias and arthralgias limit drug use • Phlebitis when infused via peripheral line • Hyperbilirubinemia
Telavancin	Cr_{cl} ≥50: 10 mg/kg q24h Cr_{cl} 30–50: 7.5 mg/kg q24h Cr_{cl} 10–29: 10 mg/kg q48h Cr_{cl} <10 or IHD: no data available. Use caution or avoid Not evaluated in severe hepatic impairment	• Indicated for complicated SSTI • Combination with tacrolimus may cause QT_c prolongation • Women of childbearing age should have serum pregnancy test prior to use
Rifampin	Prosthetic-valve endocarditis: 300 mg three times daily Device-associated osteoarticular infection: 600 mg once daily or 300–450 mg twice daily Cr_{cl} <10 or IHD: give 50–100% of usual dose	• Use only in combination with other antistaphylococcal agent if hardware retention (I) • Rifampin may significantly increase the metabolism of tacrolimus, sirolimus, cyclosporine and corticosteroids (use caution, monitor concentrations). Avoid combination with mycophenolate mofetil

Cr_{cl} = creatinine clearance in mL/min, IHD = intermittent hemodialysis; MIC = minumum inhibitory concentration; MSSA = methicillin-susceptible *S. aureus*; SSRI = selective serotonin re-uptake inhibitor; SSTI = skin and soft tissue infection; VISA = vancomycin-intermediate *S. aureus*.

Renal insufficiency can increase drug toxicity. Concomitant use of selective serotonin reuptake inhibitors should be avoided to prevent serotonin toxicity. In a retrospective study, linezolid appeared to be safe and effective for the treatment of gram-positive infections in liver transplant recipients despite those patients' increased risk of thrombocytopenia (70). In a single randomized controlled trial, linezolid demonstrated greater clinical efficacy compared to vancomycin for the treatment of nosocomial MRSA pneumonia, even though 60-day mortality was similar between the two drugs (71).

Trimethoprim-sulfamethoxazole (TMP-SMX), a bactericidal agent, is used in the treatment of SSTIs and osteomyelitis. Due to its use for prophylaxis in transplant recipients, susceptibility may not be universal. TMP-SMX can increase the myelotoxicity of methotrexate and nephrotoxicity of cyclosporine. TMP-SMX may decrease the renal excretion of creatinine and thus increase serum creatinine levels without causing actual renal impairment. Clindamycin, a bacteriostatic agent, has a role in complicated SSTIs, pneumonia and osteomyelitis. Susceptibility to MRSA may vary by geographic region. TMP-SMX and clindamycin are not recommended for the treatment of bacteremia or endocarditis. Tigecycline, a bacteriostatic agent, is approved for use in complicated SSTIs (72). Because of a rapid decline of the drug serum concentration between dose intervals (73), tigecycline is not recommended in the treatment of serious infections, such as bacteremia or endocarditis In a meta-analysis of randomized controlled trials, tigecycline was associated with increased mortality compared to active comparator antibiotics (74). Doxycycline and minocycline are alternative oral agents. Quinupristin-dalfopristin is bactericidal if the organism is susceptible to both drug components. The drug is approved for use in complicated SSTIs. Its use has been limited by severe arthralgias and myalgias.

Ceftaroline and telavancin are two recently approved bactericidal agents with activity against MRSA. Their role in invasive MRSA infections remains to be determined. Ceftaroline, a fifth-generation cephalosporin, is approved for complicated SSTIs (75) and community-acquired pneumonia (76). For pneumonia, it has been approved for MSSA but not MRSA. Telavancin, a semisynthetic lipoglycopeptide, is approved only for complicated SSTIs (77). Teicoplanin and fusidic acid are antimicrobials with activity against MRSA which are marketed in several countries but are not currently available in the United States.

Combination treatment is considered in certain infections. For prosthetic valve endocarditis (II-3) and device-associated osteoarticular infection with hardware retention (I), rifampin is typically combined with other antistaphylococcal agents (55,78). In transplant recipients receiving rifampin, immunosuppressive drug serum concentrations should be monitored closely due to the potential drug–drug interactions, especially with calcineurin inhibitors (III). Addition of gentamicin to vancomycin is not recommended for bacteremia or native valve endocarditis (II-1). Aminoglycosides may be used in combination with vancomycin as synergistic agents for prosthetic valve endocarditis (III); however, the potential for nephrotoxicity, especially with calcineurin inhibitors, should be considered. For severe necrotizing pneumonia, combination therapy that includes toxin-suppressing agents (clindamycin or linezolid) has been suggested in nontransplant patients based on *in vitro* studies (79) (III).

Duration of treatment depends on the type of infection. For uncomplicated SSTIs treatment for 5–10 days is generally recommended. Abscesses should be drained and complicated deep-seated infections should be debrided. Pneumonia should be treated for 7–14 days depending on the extent of the infection and patient's clinical response. Longer courses are generally advised for necrotizing pulmonary infection. Patients meeting the criteria for uncomplicated bacteremia (exclusion of endocarditis; no implanted prostheses; clearance of bacteremia within 2–4 days; defervescence within 72 h of initiating effective therapy; and no evidence of metastatic sites of infection) should be treated for a minimum of 2 weeks (55). Patients who do not meet the above criteria have complicated bacteremia and should be treated for 4–6 weeks. Infective endocarditis is also treated for 4–6 weeks (80). There are no data to support longer antibiotic treatment courses for MRSA in transplant recipients compared to immunocompetent patients. Reducing immunosuppressive therapy is advised in the case of severe infection (III).

In patients with persistent bacteremia, endovascular infection must be excluded. Patients should undergo evaluation for endocarditis with transesophageal echocardiography. Septic thrombophlebitis should be considered in the presence of intravenous catheters. If possible, indwelling devices should be removed. Appropriate imaging studies can identify a potential metastatic focus of infection. Serial blood cultures should be obtained to document clearance of bacteremia and determine duration of treatment.

Prevention/Infection Control

Several studies have demonstrated the positive impact of infection control measures for the prevention of MRSA infection. Transplant recipients are at high risk for MRSA infection due to surgical procedure, ICU stay, multiple comorbidities and immunocompromised status. Few studies have specifically addressed the issue of prevention in the transplant population and data on efficacy of infection control strategies are often extrapolated from studies conducted in other high-risk groups. Published guidelines provide the framework for the prevention of nosocomial transmission of MRSA (81–84), VISA and VRSA (56). Infection control strategies, aimed to reduce transmission of MRSA and other multidrug-resistant bacteria, include active surveillance, contact isolation, hand hygiene,

environmental cleaning, decolonization of carriers and antimicrobial stewardship. Each transplant program should adopt infection control practices based on the local epidemiology and available resources.

Universal active surveillance screening for MRSA colonization has been a matter of debate and not generally recommended (II-1). The approach can be considered in facilities with unacceptably high MRSA transmission rates despite optimized prevention practices (81) Using a reporting system, healthcare workers should be notified of patients with known MRSA colonization or recent infection. These patients should be isolated until their status can be confirmed or disproved (II-2).

In the hospital setting, healthcare workers are the main source of patient-to-patient MRSA spread. Hand hygiene is the most important measure for limiting the spread of resistant organisms, and programs that increase adherence and compliance with hand washing or use of alcohol-based sanitizers should be implemented (85) (II-1). To reduce MRSA spread to noncolonized patients, contact precautions are recommended for patients who are known to be colonized or infected, especially those with draining wounds or infected airways (II-1). Contact precautions include placement of patients in private rooms or in rooms with other similarly colonized individuals (cohorting), gloving and use of impermeable gowns for every patient contact, and additional barrier protection (e.g. masks, face shields and eye protection) if exposure to contaminated body fluids is anticipated (II-1). Medical equipment and patient care surfaces should be cleaned and disinfected (II-1). Whenever possible, the dedicated use of noncritical equipment for the affected patient is preferable, as well as cleaning and disinfecting of shared equipment before use in patients not known to be colonized with MRSA (III).

The efficiency of universal decolonization of hospitalized patients in preventing transmission has been a matter of debate. MRSA colonization has been associated with subsequent development of infection in patients undergoing surgical procedures. Decolonization has been associated with a decrease in postoperative *S. aureus* infections (86,87) (I). Pretransplant identification of colonized patients and subsequent eradication of MRSA may be a valuable strategy for limiting infection. However, decolonization may not be permanent; hence it is difficult to determine when to decolonize a patient awaiting transplantation. The benefit of decolonization may vary depending on the type of transplanted organ. For instance, Gram-positive organisms may play a greater role in surgical site infections among cardiothoracic transplant patients. Colonized patients can be identified by using nasal/cutaneous swab cultures or a rapid identification method such as PCR or chromogenic agar. A typical decolonization protocol includes the intranasal application of 2% topical mupirocin twice daily for 5 days combined with chlorhexidine baths for 7 days (88) (II-1). Long-term use of antistaphylococcal agents is not recommended for decolonization (II-2). Patients with known MRSA colonization or previous infection without documented eradication should receive perioperative prophylaxis against MRSA (89) (II-2).

Liver transplant candidates and recipients colonized with MRSA are at increased risk of infection (24,25). Transmission of MRSA to patients not previously colonized may occur after transplantation (20). In a single institution study, isolated nasal decolonization of liver transplant candidates was not shown to reduce posttransplant infections due to MRSA (90). In a more recent study, active surveillance, cohorting, contact isolation precautions and nasal decolonization reduced MRSA infection rates among liver transplant recipients (21) . Eradication measures are most successful when implemented in patients with a limited extent of colonization (i.e. the absence of open wounds colonized with MRSA) shortly before surgery (82) (II-2).

Antimicrobial stewardship programs that promote judicious antibiotic use are critical in reducing selective pressure and limiting the spread of resistant pathogens (II-2). Consequently, it is preferable to limit empirical antimicrobial therapy, avoid unnecessary prolonged regimens for perioperative prophylaxis, favor narrow spectrum antibiotics, adopt narrow spectrum antibiotics once a specific pathogenic organism is identified, and avoid excessive duration of treatment (84).

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Special Article

Vancomycin-Resistant *Enterococcus* Infections in Solid Organ Transplantation

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Abbreviations: BEAV, bile esculin azide agar; CLSI, Clinical and Laboratory Standards Institute; CNI, calcineurin inhibitor; LVAD, left ventricular assist device; MIC, minimum inhibitory concentration; mTOR, mammalian target of rapamycin; VRE, vancomycin-resistant *Enterococcus*.

Despite advances in surgical technique and immunosuppression, bacterial infections remain a significant source of morbidity in organ transplantation. Organ transplant recipients are at increased risk for acquisition of multidrug-resistant organisms due to critical illness, prolonged hospitalizations, extensive antimicrobial exposure and frequent device utilization. After staphylococci, *Enterococcus* species are the most common etiology of healthcare-associated infections in the United States (1). Although not traditionally considered virulent, enterococci are commonly implicated in catheter-associated bloodstream infections, catheter-associated urinary tract infections and surgical site infections. Of great concern is the incidence of vancomycin resistance among enterococci, particularly *E. faecium*. Infections with vancomycin-resistant *Enterococcus* (VRE) are associated with increased healthcare expenditures and significant mortality. Although antimicrobials exist with *in vitro* activity against these organisms, clinical outcomes are less than ideal and resistance to available agents is increasing (2,3).

Epidemiology and Risk Factors

Enterococcus is a commensal of the gastrointestinal tract and asymptomatic colonization often precedes infection (4). The first descriptions of vancomycin resistance among enterococci were in the mid to late 1980s subsequent to the introduction of third generation cephalosporins (5,6). Between 1989 and 1993, the Centers for Disease Control and Prevention reported a 20-fold increase in VRE in US hospitals (7). Prior exposure to antimicrobials, including vancomycin, cephalosporins, and agents with anti-anaerobic activity, is associated with both asymptomatic gastrointestinal carriage as well as invasive infections with VRE (8–12). Other cited risk factors include prolonged length of stay, indwelling devices, close proximity to another patient with VRE, especially in the setting of diarrhea, and placement in a contaminated room (13–15).

In the late 1980s, an increase in the isolation of *Enterococcus* species in abdominal organ transplant recipients was noted (16). In these early accounts, vancomycin susceptibility appeared to be universal. However, in the 1990s transplant centers observed increasing recovery of *E. faecium* and an associated increase in vancomycin resistance (17,18). Many studies evaluating the epidemiology of VRE in organ transplantation are limited to abdominal organ transplantation (e.g. liver and kidney transplantation) and are prior to the clinical introduction of quinupristin–dalfopristin and linezolid. In these initial reports, mortality rates associated with VRE infections were unacceptably high, ranging between 33–82% (3,18–23).

Between 1985 and 1993, 13% of liver transplant recipients at Mayo Clinic developed vancomycin-susceptible enterococcal bloodstream infections (16). In the setting of a selective bowel decontamination protocol at the same institution between 1995 and 1997, targeted surveillance identified VRE in 52 (11.7%) abdominal organ transplant recipients (23). The prevalence of gastrointestinal VRE colonization among liver and kidney transplant patients (pre- and posttransplantation) is reported to be between 3.4% and 55% with the highest rates among hospitalized liver transplant recipients in outbreak settings (23–28). Early outbreak investigations in transplant units confirm that colonized patients serve as reservoirs for horizontal transmission of VRE (22,23). Reported rates of VRE infections among colonized liver transplant patients range between 11.5–32% (23,26,27).

Most VRE infections present early posttransplantation in the setting of surgical complications and critical care. These include bloodstream infections, intra-abdominal

infections, urinary tract infections and surgical site infections (3,21). Mediastinitis and endocarditis are also reported (18,29–31).

Antimicrobial use and biliary complications (e.g. leaks and strictures), specifically those requiring re-exploration or percutaneous intervention, are common risk factors for development of VRE infections postliver transplantation (3,18–22,26). Hepatitis C infection, simultaneous kidney–pancreas transplantation, need for posttransplant renal replacement therapy, re-exploration and nephrostomy placement are associated with multidrug-resistant bacterial infections, including VRE, in kidney transplantation (32). Prior infections associated with left ventricular assist devices (LVAD) may be associated with posttransplantation invasive VRE infections including mediastinal infections and primary bloodstream infections (30). It is unclear, however, if this association is related to other factors including length of stay and antimicrobial exposures.

Diagnosis

Infection with VRE should be considered in a symptomatic patient growing Gram-positive cocci in pairs and chains with the aforementioned risk factors including prior infection or documented colonization with VRE. Isolation of VRE from an aseptically collected specimen from a normally sterile site is consistent with an infection. Specimens taken from longstanding drainage catheters may represent colonization rather than infection and their significance must be interpreted in conjunction with the patient's clinical status. Asymptomatic bacteriuria should not be routinely treated unless clinically indicated after kidney or pancreatic transplantation (III) (33). Endocarditis should be considered in patients with prolonged bacteremias or bloodstream infections without an obvious primary source in the setting of valvular abnormalities or cardiac devices (II-2).

Although great progress has been made in molecular diagnostics, most clinical laboratories rely on traditional culturing techniques in combination with automated systems to identify *Enterococcus* species and perform susceptibility testing. *E. faecalis* often demonstrates no hemolysis or rare β-hemolysis whereas *E. faecium* typically demonstrates α-hemolysis on sheep's blood agar. Enterococci produce a positive PYR test (a cherry red color produced after exposure to L-pyrrolidonyl-beta-naphthylamide [PYR] substrate with the addition of N, N methyl aminocynnamaldehyde). PYR testing may assist with early antimicrobial management. It should be noted that *Streptococcus pyogenes* is also PYR positive but is β-hemolytic.

Currently the Clinical and Laboratory Standards Institute (CLSI) recommends that enterococcal isolates with a minimum inhibitory concentration (MIC) to vancomycin of ≥32 μg/mL be reported as resistant. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) considers an MIC >4 μg/mL as vancomycin-resistant. In general, current automated susceptibility platforms are accurate at identifying high-level vancomycin resistance. Earlier generations of these systems, however, were not considered as sensitive at detecting low to intermediate levels of vancomycin resistance (34).

Glycopeptide (i.e. vancomycin and teicoplanin) resistance is primarily due to alterations in peptidoglycan precursors and is mediated by the presence of *van* gene clusters. To date, eight different genotypes have been described. VanA and VanB types are the most clinically relevant. The *van*C gene cluster is responsible for the intrinsic low level of vancomycin resistance found in *E. gallinarum* and *E. casseliflavus–E. flavescens* (35). The characteristics distinguishing these gene clusters were recently reviewed, but it should be noted that levels of phenotypic vancomycin resistance are variable (36).

Vancomycin resistance in *E. faecium* is commonly mediated by *van*A and is associated with high levels of resistance to both vancomycin and teicoplanin (37,38). *Van*B has been associated with outbreaks of VRE and demonstrates variable levels of vancomycin resistance (typically in the range of 16–64 μg/mL) and usually tests susceptible to teicoplanin (23,37). Both of these resistance determinants have been localized to transmissible elements and transfer of *van*A from *E. faecalis* is responsible for high-level vancomycin resistance in *Staphylococcus aureus* (VRSA; (39).

Despite little change in the handling of clinical specimens by microbiology laboratories, there have been several advances in rapid screening techniques for gastrointestinal carriage of VRE. Culture remains the gold standard for detection of VRE and is required for further susceptibility testing (40). Screening media for gastrointestinal colonization of VRE include Campylobacter medium containing supplemental vancomycin and bile esculin azide agar with supplemental vancomycin (BEAV). These media require additional testing to differentiate between *Enterococcus* species. Over the past several years, chromogenic agars have been studied and compared to BEAV. Most demonstrate high sensitivity and specificity and can differentiate between *E. faecalis* and *E. faecium* based on colony pigmentation (41–45). Real-time polymerase chain reaction (PCR) for *van*A and/or *van*B is both rapid and sensitive thus ideal for outbreak settings. Of note, due to acquisition of *van*B by anaerobic bacteria, the specificity of some of these PCR assays is not ideal and may require confirmatory testing (46). In institutions where a large percentage of *E. faecalis* is vancomycin susceptible, rapid differentiation between enterococcal species by peptide nucleic acid fluorescent *in situ* hybridization (PNA-FISH) may aid in early antimicrobial management (47).

Treatment

Enterococci are intrinsically resistant to traditional cephalosporins, anti-staphylococcal penicillins, and clindamycin and readily acquire mutations conferring resistance to other antimicrobial classes. A large percentage of *E.*

faecalis remain susceptible to ampicillin. In the setting of vancomycin resistance and retained susceptibility to ampicillin, ampicillin should be used (I). In the United States, the majority of *E. faecium*, however, are both ampicillin and vancomycin resistant with high-levels of aminoglycoside resistance. Although a handful of commercially available drugs demonstrate *in vitro* activity against VRE (Table 1), growing resistance threatens to compromise this limited armamentarium. With the exception of infective endocarditis, recommendations regarding antimicrobial duration remain undefined (48). Antibiotic choice and duration should be individualized based on source of infection, clinical severity and the potential for drug interactions and adverse events (III). Prolonged treatment courses of antimicrobials are seldom required.

Source control is paramount in the treatment of VRE. This includes removal of unnecessary catheters and devices as well as either percutaneous or open drainage of abscesses and debridement of wounds (II-2; Ref. 49). Prior to the advent of quinupristin-dalfopristin and linezolid, a variety of agents were used alone or in combination to treat serious VRE infections. Chloramphenicol is among these and many isolates continue to remain susceptible (37,50–52). With the availability of more specific therapy, clinical use of chloramphenicol is less common.

In 1999, the US Food and Drug Administration (FDA) approved the use of quinupristin-dalfopristin (Q/D) for the treatment of vancomycin-resistant *E. faecium*. Q/D is a combination of streptogramins which inhibits protein synthesis and demonstrates bacteriostatic activity against *E. faecium* with no appreciable activity against non-*E. faecium* enterococci. In prospective, noncomparative studies the overall treatment success rate was around 65–83% (53–55). Success varied by indication and lower response rates were reported in patients with intra-abdominal infections. Q/D is only available for parenteral administration and due to the risk of phlebitis, administration through a central venous catheter is recommended. Nausea and hyperbilirubinemia are common. However, debilitating arthralgias and myalgias can lead to premature discontinuation of therapy (54,55). Two reports describe an association between arthralgias and liver disease (56,57). A series of pediatric liver transplant recipients treated with Q/D did not support this finding (58). It should be noted that Q/D inhibits CYP450-3A4 and can potentially lead to calcineurin inhibitor toxicity. Levels of tacrolimus and cyclosporine should be monitored. Q/D resistance has been described (59). Clinical use of Q/D has substantially decreased with the introduction of better-tolerated agents.

Linezolid, an oxazolidinone, is bacteriostatic against both *E. faecium* and *E. faecalis* and is FDA-approved for the treatment of VRE infections. A moderate sized open-label noncomparative emergency use study reported clinical cure rates in 78% of patients with VRE; however, lower rates of clinical success were observed in patients with endocarditis (60). An evaluation of organ transplant recipients receiving linezolid described a modest improvement in overall survival of 62.4% with the highest attributable mortality rates in those patients requiring multiple surgeries and with polymicrobial infection (61).

Linezolid is available in both a parenteral and an oral formulation. The latter achieves appreciable levels in tissue and is an attractive option for patients with limited intravenous access and tolerating enteral nutrition. Adverse effects include myelosuppression (i.e. leukopenia and thrombocytopenia) that usually appears after two weeks of treatment. Peripheral neuropathy and optic neuropathy have been reported with extended use and may not be reversible with discontinuation of therapy (62–65). Caution should be used when administering linezolid to patients on serotonergic agents, including selective serotonin reuptake inhibitors, due to linezolid's potential to inhibit monoamine oxidase (66). Lactic acidosis is uncommon but has been reported with prolonged linezolid administration and serial serum chemistries monitoring for evidence of acidosis should accompany periodic complete blood counts while on therapy (III; Ref. 67). Linezolid resistance has been reported in organ transplant recipients both in the setting of protracted courses of linezolid as well as in the setting of cross-transmission (61,68–70).

Daptomycin, a lipopeptide, demonstrates rapid concentration-dependent bactericidal activity against most clinically relevant Gram-positive cocci including enterococci. Currently daptomycin is FDA-approved for the treatment of skin and skin structure infections, including those with vancomycin-susceptible *E. faecalis*, and for bloodstream infections. Despite not being a licensed indication, it has been used frequently in the treatment of VRE infections with some anecdotal success (71,72). Per CLSI, *E. faecalis* with a daptomycin MIC >4 μg/mL is resistant and *E. faecium* tends to have higher MICs than *E. faecalis*. Daptomycin is only available in a parenteral formulation. Although the dose of 6 mg/kg is recommended for bloodstream infections, higher doses have been used in severe infections (73,74). Myalgias and rhabdomyolysis are potential side effects with prolonged daptomycin use and serial monitoring of creatinine phosphokinase is recommended especially with higher doses and in the setting of renal failure or concomitant therapy with agents with similar side effect profiles (e.g. HMG CoA-reductase inhibitors; III). Although VRE pneumonia is unusual, due to inactivation by surfactant, daptomycin should not be used whenever a pulmonary source of infection is suspected. Like both Q/D and linezolid, resistance is described both in the setting of active treatment and possible antimicrobial pressure (75–77). Institutional daptomycin resistance rates of up to 15% have been reported in VRE isolates (76).

Tigecycline, a glycylcycline, is FDA-approved for the treatment of complicated skin and skin structure infections and abdominal infections with vancomycin-susceptible *E.*

Table 1: Agents with potential use in the treatment of infections with vancomycin-resistant *Enterococcus* (VRE) in the absence of susceptibility to ampicillin

Drug	Recommended adult dosing (with normal creatinine clearance)	Notable adverse events	Drug interactions in the transplant setting	Additional comments
Quinupristin-dalfopristin[1]	• 7.5 mg/kg IV every 8 hours through central venous catheter	• Infusion site reactions • Hyperbilirubinemia • Treatment limiting arthralgias and myalgias	Inhibits CYP3A4 and can increase levels of CNIs[2] and mTOR[3] inhibitors	• Bacteriostatic • No appreciable activity against *E. faecalis*
Linezolid[1]	• PO or IV dose 600 mg every 12 hours	• Myelosuppression usually after 2 weeks of therapy • Lactic acidosis • Peripheral and optic neuropathy • Sertonin syndrome	Risk of serotonin syndrome in the setting of concomitant use of serotonergic agents	• Bacteriostatic • Monitor weekly complete blood count (myelosuppression) • Monitor weekly anion gap (lactic acidosis) • Avoid use of serotonergic agents • Resistance has been reported and has been associated with prior exposure to linezolid
Daptomycin[4]	• Skin and soft tissue infections-4 mg/kg IV every 24 hours • Bloodstream infections 6 mg/kg IV every 24 hours	• Myopathy • Acute eosinophilic pneumonia (101)	Use with caution with HMG-CoA reductase inhibitors due to similar adverse event profile	• Bacteriocidal • Cannot be used for primary pulmonary infections • Monitor weekly creatine phosphokinase • Higher doses have been used in the setting of severe infections
Tigecycline	• 100 mg IV once then 50 mg IV every 12 hours • Dose adjustment required in the setting of severe liver disease	• Nausea and gastrointestinal complaints		• Bacteriostatic • Not recommended for bloodstream infections or urinary tract infections • Avoid in pregnancy (category D) and children • Use of tigecycline has been associated with increased mortality with comparator agents (102)
Telavancin[4]	• 10 mg/kg IV every 24 hours	• Red Man Syndrome • QT prolongation • Nephrotoxicity • May interfere with coagulation tests (e.g. PT/INR, aPTT)	Use with caution with other potentially nephrotoxic agents	• Bacteriostatic against VanB expressing isolates • Avoid in pregnancy (Category C) • Safety and efficacy not established in children • Clinical data for treatment of infections with VRE are limited
Ceftaroline[4]	• 600 mg IV every 12 hours	• Similar to other cephalosporin agents		• Bacteriocidal • No appreciable activity against *E. faecium* • Safety and efficacy not established in children • Paucity of clinical data for the treatment of VRE

[1]FDA-approved for the treatment of infections with VRE.
[2]Calcineurin inhibitor (e.g. cyclosporine and tacrolimus).
[3]Mammalian target of rapamycin.
[4]Dose adjustment recommended for alterations in creatinine clearance.

faecalis and is bacteriostatic against susceptible enterococci (78,79). Due to rapid concentration of drug into tissue, serum concentrations may not be adequate to treat primary bloodstream infections and use in urinary tract infections is also controversial.

Telavancin is a long-acting lipoglycopeptide recently FDA-approved for complicated skin and skin structure infections including those with vancomycin-susceptible *E. faecalis* (80). Telavancin lacks appreciable activity against *van*A harboring strains of VRE although there is some evidence of bacteriostatic activity against *van*B expressing strains (81,82). Clinical data for the treatment of VRE infections are limited. Oritavancin is an investigational lipoglycopeptide with promising concentration-dependent *in vitro* bactericidal activity against a wide spectrum of Gram-positive bacteria, including enterococci expressing either *van*A or *van*B (83).

The novel cephalosporins, ceftobiprole and ceftaroline, demonstrate *in vitro* activity against other clinically relevant but traditionally cephalosporin-resistant Gram-positive organisms, notably MRSA (84). Both agents demonstrate activity against vancomycin-susceptible and -resistant *E. faecalis* but no appreciable *in vitro* activity against *E. faecium*. Ceftaroline recently received FDA approval for the treatment of complicated skin and skin structure infections and pneumonia.

Fluoroquinolones, nitrofurantoin and fosfomycin may be used to treat symptomatic VRE cystitis (III; Refs. 85-88).

Prevention and Infection Control Issues

In the United States, clinical isolation of VRE is uniformly associated with healthcare exposure. Epidemiologic surveys inclusive of organ transplant candidates and recipients cite antimicrobial exposure as a common risk factor for VRE. Unfortunately, antimicrobial use in organ transplantation is unavoidable. Broad-spectrum antimicrobials increase susceptibility for VRE acquisition by inadvertent suppression of normal gastrointestinal flora. Increases in stool concentration of VRE may increase the probability of environmental contamination and thus horizontal transmission.

Formal antimicrobial stewardship programs charged with promoting judicious and appropriate use of all antimicrobials are crucial in combating increased resistance (III). Long courses of antibiotics are rarely necessary and reevaluating continued administration of broad-spectrum agents or antimicrobials in general, is recommended (II-2; Refs. 9,11,89-91). Due to the prevalence of MRSA, empirical use of vancomycin may be inevitable in certain patient populations and in the appropriate clinical scenario. However, prolonged use in the absence of supportive culture data is discouraged (III).

Organ transplant patients are subject to general recommendations for the prevention of horizontal transmission of epidemiologically significant multidrug-resistant organisms (II-2; Ref. 92). The colonized patient remains the primary reservoir for VRE, but transmission is facilitated by healthcare workers and the soiled environment (93–95). When there is a high prevalence of VRE (i.e. colonization pressure), other risk factors for colonization may be less important (96).

Cleansing of patients with chlorhexidine may decrease the bioburden of VRE thus decreasing healthcare-associated VRE infections and horizontal transmission. However, chlorhexidine cleansing has been studied primarily in the ICU setting and its role in organ transplantation remains unclear but deserves further investigation (97). Removal of unnecessary catheters is encouraged (II-2).

Mandating routine active surveillance for VRE among organ transplant patients cannot be recommended (III; Ref. 98). A possible outbreak of VRE or a high prevalence of VRE, however, warrants implementation of active surveillance to identify asymptomatic colonization (II-2; Refs. 40,99) Isolation and contact precautions are recommended for all patients with a history of VRE colonization or infection during the index hospitalization as well as subsequent readmissions (II-3). This includes use of single rooms or cohorting as well as hand hygiene using either alcohol-based sanitizer or antiseptic soap before and after all patient contact (II-2). Gloves and gowns should be worn when entering the room and for all patient contact and discarded promptly when exiting the room (II-2). Dedicated equipment (e.g. stethoscopes, thermometers, sphygmomanometers) should be used for isolated patients and shared equipment requires disinfection prior to subsequent use (II-2). Monitoring for compliance with contact isolation precautions and hand hygiene with immediate feedback and continuing education is recommended (II-1).

Since asymptomatic colonization can persist for months to years, the optimal duration for maintaining contact precautions remains unclear. CDC recommendations for discontinuation of contact precautions suggest that in the absence of active antimicrobial agents, demonstration of at least three negative peri-rectal or stool specimens collected over several weeks may be sufficient (III; Ref. 99). In the setting of limited resources, including private rooms, and in the presence of other epidemiologically significant multidrug-resistant organisms, requiring such a labor-intensive process for historical colonization or infection with VRE may not be feasible. Policies for discontinuation of contact precautions are often institution-specific. It should be noted that rates of spontaneous decolonization in organ transplant recipients appear to be lower than that in the general population (23). Attempts at decolonization of high-risk patient are not recommended (III) and selective bowel decontamination may be a risk factor for VRE (100).

A history of VRE colonization or past infection is not a contraindication to organ transplantation (III). Despite the absence of specific recommendations for adjusting perioperative prophylaxis based on history of VRE colonization or infection, it may be something to consider (III).

Although not historically considered as virulent as other multidrug-resistant pathogens, VRE remains challenging not only because of its environmental resilience but its increasing resistance to available agents. A multidisciplinary approach that includes transplant program leadership is required to continue to educate and reinforce healthcare workers' understanding of the importance of complying with infection control practices as well as recommendations of antimicrobial stewardship programs. Administrative support for education, research, infection control and antimicrobial stewardship is crucial to continue to combat the rise and persistence of multidrug-resistant pathogens.

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Special Article

Mycobacterium tuberculosis Infections in Solid Organ Transplantation

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Abbreviations: BCG, Bacillus calmette-guerin; IGRA, interferon-gamma release assay; LTBI, latent tuberculosis infection; MTB, *Mycobacterium tuberculosis*; SOT, solid organ transplant; TB, tuberculosis; TST, tuberculin skin test.

Introduction

The diagnosis and treatment of tuberculosis (TB) in organ transplant recipients presents several challenges. Impediments to rapid and accurate diagnosis may lead to treatment delay and include negative or indeterminate tuberculin skin tests (TST) or interferon-gamma release assays (IGRA), negative sputum smear results despite active disease and atypical clinical presentations (1–3). Therapeutic challenges arise from drug related toxicities, metabolic interactions between immunosuppressive and antituberculous drugs and side effects from antituberculous medications (4). Increasing drug resistance and inadequate immune responses to *Mycobacterium tuberculosis* (MTB) due to exogenous immunosuppression increase the complexity of treating TB in this population (5).

Recommendations for the diagnosis and treatment of latent TB infection and active TB disease in organ transplant recipients are made based on consensus guidelines formulated by experts in the field (6–11). Only a few controlled studies of treatment of latent or active TB in organ transplant candidates or recipients are available (3,12–14). Case series and epidemiologic surveys of organ transplant patients with TB are often used for guidance in this area (15–26).

Epidemiology

It should be noted that the rates of TB reported in the transplant literature often reflect cumulative rates in populations of patients followed over a number of years and cannot always be compared to or converted to annual incidence rates.

The frequency of active TB disease among solid organ transplant (SOT) patients is estimated to be 20–74 times that of the general population, but differs according to the organ transplanted (1). For active TB disease, the prevalence among SOT recipients in most developed countries is 1.2–6.4%, while the prevalence in SOT recipients in highly endemic areas has been reported to be up to 12% (1,27). Over two-thirds of reported cases of active TB disease in transplant recipients occur in the first posttransplant year, with the median time for presentation of disease reported as 6–11 months (2,28). Posttransplant TB has a crude mortality of 20–30% (2,29). One study from Spain reported an attributable mortality of 10% (11), but this may be higher in other countries due to the challenges associated with diagnosis in a highly immunosuppressed population.

In most cases, active TB disease is thought to arise by reactivation of old foci of infection, because primary infection has only been documented in a small number of cases posttransplant. TB may also be transmitted from the donor through transplantation. The US Organ Procurement and Transplant Network's Disease Transmission Advisory Committee (OPTN/DTAC) reviewed 22 recent donor reports of potential TB transmission. Acquisition of MTB from the donated organ was substantiated in at least 16 of 55 recipients of organs from these 22 donors. Donor-derived TB transmission has been reported in renal, hepatic and lung transplantation (2,30–33). Although donor-derived TB accounts for less than 5% of all active TB cases in transplant recipients, it may result in significant morbidity and mortality. TB can be acquired after transplant, with the rate of primary infection likely greater in developing countries, although this has not been carefully evaluated. Nosocomial acquisition of MTB has been documented during an outbreak on a renal transplant unit, though such events appear to be uncommon (34,35). Surprisingly, only 20–25% of all cases of active TB disease occurring after transplantation are in patients who had positive TST reactions before transplantation (1). This may in part be due to anergy in patients with end-stage organ failure and likely does not reflect posttransplant acquisition of infection. The precise frequency at which TST positive patients later develop active TB after transplantation has not been determined.

Few risk factors have been defined for the occurrence of active TB disease after transplantation (1,2,10,11). In

general, TB risk increases with TB incidence in one's country of origin, and social and medical risk factors such as homelessness, incarceration, cigarette smoking, diabetes mellitus, chronic kidney disease, malnutrition and known contact with TB. Reported risk factors for active TB after transplantation include prior residence outside the United States, history of untreated TB, the presence of findings on chest radiographs suggestive of healed TB and intensified immunosuppression for treatment of allograft rejection. It is clear that certain immunosuppressive drugs (e.g. T cell depleting antibodies) are associated with a greater risk of TB than others (1). Risks after kidney transplant appear to be increased in those with longer pretransplant hemodialysis treatment and in those with hepatitis C (36). Lung transplant recipients have a greater risk of active TB compared to other transplanted organs, with a 5.6-fold increased risk seen in a large Spanish cohort (11). The same study found recipient age to be an independent risk factor for post transplant TB, at least in Spain, where TB in the general population has decreased significantly in recent years. It may be that older persons are more likely to have latent TB; this may be true in other regions where TB control programs have been successful.

Clinical Manifestations and Diagnosis

The clinical manifestations of TB in transplant recipients can differ from those in normal hosts (1,2). Among SOT recipients, lung transplant patients are most likely to develop pulmonary manifestations of TB. However, about one-third to one-half of all cases of active TB disease after transplantation are disseminated or occur at extra-pulmonary sites, compared to only about 15% of cases in normal hosts (2). Classic symptoms of TB such as fever, night sweats and weight loss are usually seen, but may not always be present. One large series reported fever in 91% of transplant recipients with disseminated disease and in 64% of those with pulmonary disease (2). Atypical presentations may also be noted, such as pyomyositis, cutaneous ulcers or tenosynovitis.

A minority of transplant patients have classic cavitary changes on chest radiograph. Radiographic findings of pulmonary TB in SOT recipients may demonstrate a focal opacity, a miliary pattern, nodules, pleural effusions, diffuse interstitial opacities and cavities. The mortality of TB after transplantation is increased compared to immunocompetent hosts, especially in patients who have disseminated disease, those with prior rejection or after receipt of anti-T cell antibodies (1,2).

The diagnosis of active TB disease after transplantation requires a high index of suspicion and in practice is frequently delayed. A diagnostic invasive procedure, such as bronchoscopy with bronchoalveolar lavage or lung biopsy in pulmonary TB, or biopsy of skin lesions or abscess fluid in patients with skin and soft tissue involvement is often required (37). Specimens should be sent for smear and culture for acid-fast bacilli, along with histopathological evaluation. The use of rapid nucleic acid amplification techniques, such as Xpert MTB/RIF (Cepheid Inc, Sunnyvale, CA, USA), an automated molecular test for MTB and resistance to rifampin (RIF), can increase the sensitivity and decrease the time to diagnosis. However, such tests may be falsely negative when low levels of mycobacteria are present.

A diagnosis of latent TB infection may be made by documenting a positive TST or IGRA in a person without signs, symptoms, or chest radiographic evidence of active TB. IGRAs, including QuantiFERON-Gold (QFT, Cellestis) and T-SPOT TB (Oxford Immunotec Ltd, Abingdon, UK) have emerged as alternatives to the TST in the general population (38,39). The use of these tests in transplant candidates and donors is discussed later. It should be noted that neither the TST nor IGRA assays can distinguish latent TB infection from active disease. Both IGRA and TST should be interpreted with caution in patients receiving high levels of immunosuppressive drugs as they may yield falsely negative or indeterminate results (40,41). Therefore screening for LTBI should be done prior to administration of immunosuppressives. That said, the QFT and T-SPOT TB tests are highly specific, and a positive test should be interpreted as evidence of MTB infection. Compared to QFT, T-SPOT TB appears to have a slightly higher sensitivity for detecting MTB infection (42,43).

Prevention of Active TB Disease

Evaluation of transplantation candidates and donors

A careful history of previous exposure to MTB should be taken from all transplant candidates, including details about previous TST results and exposure to individuals with active TB in the household or workplace (III) (8,44). Further inquiry about possible institutional exposure and travel to areas highly endemic for TB is also helpful. Any history of active TB should be documented, as well as details regarding the length and type of treatment. It is also important to document previous treatment for latent TB and obtain relevant records. A chest radiograph should be examined for evidence of old healed TB. All transplant candidates, including those with a history of BCG vaccination, should undergo evaluation for latent TB infection (III). Conventional TST can be used in all situations, with a test being considered positive if there is $\geq$5 mm of induration at 48–72 h (III). If feasible, patients with negative reactions should have a second skin test performed 2 weeks later, as the TST can convert from being falsely negative to positive due to "boosting" in some individuals with remote MTB exposure. For individuals not highly immunosuppressed, the QFT and T-SPOT TB are alternatives to TST, and should be interpreted according to manufacturers' guidelines. IGRA testing may be preferred to TST in transplant candidates with a prior history of BCG vaccination, as IGRA results will not be impacted by prior receipt of BCG. Studies of the performance of the QFT in liver transplant candidates indicate their utility in patients with advanced liver disease, with indeterminate results more common in candidates with higher MELD scores (43,45,46). The T-SPOT TB test

may be more sensitive than TST in detecting LTBI in kidney transplant candidates (47). A Korean study of kidney transplant recipients revealed T-SPOT TB to be helpful in predicting risk for post transplant active TB in patients who were TST negative prior to transplant (10,48). In transplant candidates with epidemiologic evidence of high risk for latent or asymptomatic active TB, careful radiographic assessment with CXR and thoracic CT may be helpful if results of TST and IGRA are negative or indeterminate (3,49). Unfortunately, none of the available screening tests are infallible in diagnosing latent or active infection with MTB; therefore treatment decisions must be individualized based on the clinical likelihood of infection and a careful review of the available data. The management of discordant TST and IGRA test results also requires a thorough assessment of the candidate's individual TB risk (50). Since the sensitivities of TST and IGRA do not overlap fully, both modalities can be employed in screening, with appropriate timing to avoid the potential induction of false positive IGRA results (51). This should only be considered in transplant candidates with high pretest probability of LTBI in whom a single positive test result might change clinical management. Patients with a prior history of positive TST or IGRA testing may be screened for active TB and then treated as appropriate without retesting. A current negative screening test, especially in patients with organ failure awaiting transplantation, does not negate a prior positive test result. Individuals having a reliable prior history of treated latent TB infection or treated TB disease need not undergo TST, QFT or T-SPOT TB. However, these individuals should have a symptom review and chest X-ray, as well as additional testing if indicated, to screen for active TB.

Living donors should undergo an evaluation similar to that described for transplant recipient candidates (III). For living donors, the TST should be interpreted as positive or negative according to CDC guidelines for the general population (52). QFT and T-SPOT TB are alternatives and should be interpreted according to manufacturers' specifications. If a test reveals evidence of MTB infection, then active disease should be ruled out, starting with a symptom review and chest x-ray (III). For living donors with latent TB infection, treatment for latent TB infection should be considered prior to organ donation, especially for recent TST or IGRA converters. Organs from potential donors, whether living or deceased, with active TB disease should not be used. Also, a well-founded suspicion of active TB should contraindicate donation, and residual pulmonary lesions should contraindicate lung donation (10). It is not possible to accurately perform TST or IGRA on deceased donors, but a history should be obtained from the donor's family or relatives of previous active TB and any associated treatment. Ideally, it would also be desirable to know if the donor had exposure to active TB within the last 2 years.

Treatment of Latent TB

Public health authorities recommend treatment of latent TB in persons who are actively immunosuppressed (7). In highly endemic areas where TB transmission is common, some transplant experts recommend universal isoniazid prophylaxis for the first year posttransplant during the period of maximum immunosuppression (14). Treatment options for latent TB are listed in Table 1. The data supporting various treatment options for latent TB are extensive, with a paucity of information devoted to the management of transplant candidates (53–55).

The mainstay of latent TB treatment is isoniazid, but its use in transplant recipients was controversial in the past due to a high rate of hepatotoxicity reported in older studies (56–58). More recent data, however, show a low risk of hepatotoxicity due to isoniazid in renal transplant recipients without serious underlying liver disease (59), and in patients with compensated liver disease awaiting liver transplantation (60,61). A 4-month course of rifampin monotherapy can be used for the treatment of latent TB (62), but is limited by drug–drug interactions that preclude continuation of treatment posttransplant, thus it is preferable to complete the course of rifampin prior to transplantation. A previously recommended regimen of pyrazinamide and rifampin daily for 2 months has been associated with a high rate of hepatotoxicity and is no longer recommended. A promising new regimen for treatment of LTBI is a 12-week course of isoniazid and rifapentine (63). It is recommended weekly as directly observed therapy in otherwise healthy individuals $\geq$12 years of age who have a risk factor for developing active TB (64). However, it has not been studied in patients with organ failure, such as those awaiting transplantation. Use of this regimen after transplantation is limited by severe drug interactions between rifamycins and immunosuppressive agents.

The rationale for latent TB treatment in this setting is supported by the fact that active TB disease is difficult to diagnose in transplant recipients, the cause of appreciable morbidity and mortality and a potential public health risk. LTBI treatment significantly reduces the incidence of TB reactivation in transplant recipients (65). It must be stressed that a thorough clinical evaluation to rule out active TB must be performed prior to initiating treatment for LTBI. Neither TST nor IGRA testing can distinguish active from latent infection. With this in mind, the following recommendations are made regarding candidates for treatment and timing the following recommendations are made:

(1) Isoniazid preventive treatment for 9 months—given daily, or twice weekly by directly observed therapy (DOT)—should be considered for all transplant patients who have a positive TST or IGRA (II-1), unless they have received a prior adequate course of treatment for LTBI or active TB. Pyridoxine (vitamin B6) 25–50 mg daily should be administered concomitantly with isoniazid to all transplant candidates and recipients, since they are at increased risk of neurotoxicity (III). Because 9 months of treatment confers additional protection over 6 months, a 6-month course of isoniazid is not routinely

Table 1: Treatment of latent TB

Medication	Adult dose	Pediatric dose	Duration	Notes
Isoniazid (INH) (daily)	5 mg/kg (max 300 mg/day)	10–15 mg (max 300 mg/day)	9 months preferred over 6 months due to additional protection	Pyridoxine 25–50 mg/day with INH to decrease risk of neurotoxicity. Some recommend INH dose adjustment with renal insufficiency, but generally do not change dose with hemodialysis.
Isoniazid (twice weekly by directly observed therapy)	15 mg/kg (max 900 mg/dose)	20–25 mg/kg (max 900 mg/dose)	Same	Same
Rifampin	10 mg/kg (maximum of 600 mg	10–20 mg/kg (maximum of 600 mg) for children.	4 months	Best to complete prior to transplant due to immunosuppressive drug interaction.
Isoniazid (INH) with Rifapentine (RFP) (63,64)	INH: 15 mg/kg q week (max 900 mg/dose) RFP: <50 kg 750 mg/week; >50 kg 900 mg/week	Recommended for ≥12 years of age. INH: same as adult RFP: 25–32 kg: 600 mg/week, 32–50 kg: 750 mg/week	Once weekly for 12 weeks, only studied as directly observed therapy, with at least monthly clinical assessment	Pyridoxine 25–50 mg/day should be given with INH. Best to complete prior to transplant due to drug interactions. Not studied in patients with organ failure or transplant recipients.

recommended in transplant patients (II-1). Regimens that employ rifampin for 4 months are not preferred due to limited data on efficacy (II-3), but may be used prior to transplantation; after transplantation they are to be avoided due to drug interactions with immunosuppressive agents (III) (52). If standard treatment is not tolerated, alternative regimens such as ethambutol plus either levofloxacin or moxifloxacin have been used and could be considered for high-risk individuals (III) (10). If no alternative treatment is possible, then careful clinical follow-up with prompt diagnostic attention to protracted fever or pulmonary symptoms is likely the best course (III).

(2) Most of the patients who develop active TB disease after transplantation have a negative TST before transplantation. For this reason, most authorities in low TB prevalence areas recommend the use of isoniazid preventive therapy in TST negative (or IGRA negative/indeterminate) patients who: (i) have radiographic evidence of previous TB and no history of adequate treatment, (ii) have received an organ from a donor who is TST positive, had recent exposure to active TB or had radiographic evidence of untreated TB or (iii) have had close and prolonged contact with a case of active TB, a circumstance in which the risk of de novo infection may be 50% or higher (III).

(3) If either the recipient or donor has recently converted their TST or IGRA from negative to positive, then prompt recipient evaluation and treatment for LTBI is indicated if there is no evidence of active TB disease (III).

(4) Underlying liver disease limits use of isoniazid preventive therapy in transplant recipients. Latent TB therapy should still be strongly considered in patients with liver disease if they are known to be recent TST converters (III), since the risk of progression to active TB disease is high in this setting. The interaction between isoniazid and calcineurin inhibitors is not clinically significant enough to preclude the use of isoniazid. If candidates cannot tolerate treatment prior to transplantation, then treatment should be initiated as soon as possible following transplantation.

(5) The timing of isoniazid administration requires balancing risks and benefits for individual patients. Factors that require consideration include the current medical condition, transplant urgency, risk of progression to active TB and anticipated timing of transplantation (if not yet performed). Individuals with recent TB exposure and/or recent TST conversion should receive evaluation and LTBI treatment as soon as medically practicable, due to heightened risk for progression to active TB. Renal transplant candidates awaiting deceased donor transplantation should be treated before transplantation, as they may face long waiting times and renal failure is itself a risk factor for active TB disease. Treatment should be considered before lung transplantation in TST or IGRA positive individuals, because active TB may be difficult to diagnose in the presence of chronic lung disease (III). In some transplant candidates it may be preferable to delay the administration of isoniazid until after transplantation, at which time the risk for active TB is higher and the patient may be more stable medically. The administration of isoniazid to liver transplant recipients is somewhat controversial. In this population, it may be prudent to delay the initiation of isoniazid until liver function is relatively stable

(III). In liver transplant recipients who are taking isoniazid, rise in serum transaminase levels should not be automatically ascribed to isoniazid. A specific diagnosis should be sought, with liver biopsy, if necessary.

(6) Transplant recipients receiving isoniazid should routinely be monitored for hepatotoxicity. A suggested approach is to monitor at 2-week intervals for 6 weeks and then monthly. A single blood test (ALT) should suffice. Low-grade elevations of hepatic transaminases to 1.5–3 times normal are relatively common during the first months of isoniazid use and may not require immediate discontinuation, but should prompt more frequent laboratory monitoring (III). LTBI treatment should be discontinued with a threefold increase in hepatic transaminases and signs and symptoms of hepatotoxicity, or fivefold elevation without symptoms (52).

(7) Organ transplantation may be performed in patients who are receiving treatment for LTBI, especially if the potential benefit of early transplantation outweighs the risk of reactivation TB (III). After transplantation, latent TB treatment should be resumed as soon as medically possible and continued until completion of originally planned course.

(8) If treatment of LTBI has been delayed until after transplantation, then the selected regimen should be initiated as soon as medically possible after the recipient is stabilized (III).

Treatment of Active TB

Because of the challenges of treating active TB disease after transplant, every effort must be made to diagnose and treat active TB pretransplant. A major challenge when screening transplant candidates is distinguishing latent TB from clinically asymptomatic active TB. Should asymptomatic candidates not receive a diagnosis of active TB until after transplant, successful treatment is still possible with early aggressive management (66). Drugs commonly used to treat active TB disease are listed in Table 2. Also noted are their standard adult and pediatric doses, the degree of dose adjustment required for renal dysfunction, and common side effects (6,7). Drug interactions are addressed in Chapter 32.

The standard treatment recommendation for active TB disease in the general population is to administer a four-drug regimen of isoniazid, rifampin, pyrazinamide and ethambutol for the first 2 months ("intensive phase") followed by isoniazid and rifampin alone for an additional 4 months ("continuation phase") (I). Ethambutol can be discontinued if the MTB isolate is susceptible to isoniazid, rifampin and pyrazinamide. Fluoroquinolones including moxifloxacin and levofloxacin have potent activity against MTB, and while not recommended for use as "first-line" therapy, they can be useful components of multidrug regimens in individuals who have hepatotoxicity on standard TB therapy or who have poor liver function.

With respect to dosing interval, daily TB therapy is recommended. Twice- or thrice-weekly administration of TB therapy is not recommended due to the increased risk of relapse associated with intermittent dosing (II-2) (67) and the potential for wide fluctuations in immunosuppressive drug levels due to drug–drug interactions with rifamycins. With respect to treatment duration, published data in renal transplant recipients indicate that 6 months of treatment should be adequate; however, some experts disagree (10,17). A longer duration of therapy is recommended for the treatment of bone and joint disease (6–9 months) (I), central nervous system disease (9–12 months) (II-2), and should be considered in individuals with severe disseminated disease (6–9 months) (II-1). In addition, 9 months of treatment is recommended for individuals with cavitary pulmonary TB in whom sputum at completion of 2 months of treatment is still culture-positive for MTB (I). Longer treatment duration should always be considered if the response to treatment is slow. Longer treatment courses are mandated if second line drugs are used to replace first line drugs, or if there is resistance to rifampin ± other drugs (III). For drug susceptible TB, when treatment is extended beyond 6 months, the intensive phase remains two months in duration and the duration of the continuation phase is extended.

DOT programs have been shown to improve adherence and outcome in TB patients and are recommended for transplant recipients (II-2). If a transplant recipient receives antituberculous medication in a public health clinic, close communication with the health clinic is necessary to ensure that clinic personnel are aware of transplant specific issues. Consultation with a TB expert is recommended for any patient with active TB, and is imperative for patients whose TB is complicated by drug resistance or drug intolerance, as well as those who require nonstandard treatment for whatever reason.

The major difficulty in administering antituberculous therapy to transplant patients is drug–drug interactions involving rifampin. Nevertheless, a rifamycin-containing regimen is strongly preferred due to the potent MTB sterilizing activity of this drug class. Rifampin is a strong inducer of the microsomal enzymes that metabolize cyclosporine, tacrolimus, sirolimus, and everolimus. To some extent rifampin may also interfere with corticosteroid metabolism. It may be difficult to maintain adequate levels of immunosuppressive drugs while using rifampin, and rejection episodes occurring in conjunction with rifampin use have been widely reported. Successful use of rifampin has been reported in transplant recipients, but doses of cyclosporine, tacrolimus and sirolimus will have to be increased at least two- to fivefold (II-3). An option is to replace rifampin with rifabutin (another rifamycin) (I). Rifabutin has

Table 2: Medications for treatment of active tuberculosis

Drug	Daily dose (Adults)	Daily dose (Pediatrics)[1]	Dose alteration for renal dysfunction[2]	Common adverse events
First line drugs				
Isoniazid	5 mg/kg PO or IV (maximum 300 mg)	10–15 mg/kg (maximum 300 mg)	Minimal	Hepatotoxicity Neurotoxicity (peripheral neuropathy, optic neuritis, seizures) Cytopenias Drug interactions
Rifampin	10 mg/kg PO or IV (maximum 600 mg)	10–20 mg/kg (maximum 600 mg)	None	Hepatotoxicity Cytopenias Red-orange body fluids Interstitial nephritis Severe rash Major drug interactions
Pyrazinamide	40–55 kg: 1000 mg 56–75 kg 1500 76–90 kg 2000 mg (Use lean body weight)	Over 2 years old, <40 kg: 15–30 mg/kg/day	Mild	Hepatotoxicity Cytopenias Hyperuricemia Interstitial nephritis
Ethambutol	15–25 mg/kg PO (maximum 1.6 g)	15–20 mg/kg PO (maximum 1.0 g)	Mild	Hepatotoxicity Neurotoxicity (optic neuritis, visual loss) Cytopenias
Streptomycin	15 mg/kg (max 1 g) IM or IV[3] given 2–5 times/week	20–30 mg/kg IM or IV (max 1 g)	Major	Nephrotoxicity Ototoxicity (auditory and vestibular) Neuromuscular blockade Cytopenias
Second line drugs				
Kanamycin	15 mg/kg (maximum 1.0 g) IM or IV[3]	15–30 mg/kg (maximum 1.0 g) IM or IV[3]	Major	Nephrotoxicity Ototoxicity (auditory and vestibular) Neuromuscular blockade
Amikacin	15 mg/kg (maximum 1.0 g) IM or IV[3]	15–30 mg/kg (maximum 1.0 gm) IM or IV[3]	Major	Nephrotoxicity Ototoxicity (auditory and vestibular) Neuromuscular blockade
Rifabutin	5 mg/kg PO (maximum 300 mg)	Appropriate dosing for children is unknown	None	Cytopenias Red-orange colored body fluids
Levofloxacin	750 mg/day PO or IV	N/A	Moderate	*C difficile*-associated diarrhea QT prolongation Tendonitis
Ethionamide	15–20 mg/kg (maximum 1.0 g; usual daily dose 500–750 mg)	15–20 mg/kg (maximum 1.0 g)	Mild	Hepatitis Neurotoxicity (peripheral neuropathy and optic neuritis) Hypothyroidism
Cycloserine	10–15 mg/kg (maximum 1.0 g/d in two doses; usual dose 500–750 mg/d in two doses)	15–20 mg/kg (maximum 1.0 g/d in two doses)	Moderate	Neurotoxicity (seizures, psychosis) Congestive heart failure Transaminitis
Capreomycin	15 mg/kg (maximum 1.0 g) IM or IV[3]	15–30 mg/kg (maximum 1.0 g) IM or IV[3]	Major	Nephrotoxicity Ototoxicity (auditory and vestibular) Neuromuscular blockade

Dosing was adapted from Ref. (6).

[1]Children weighing more than 40 kg should be dosed as adults.

[2]The degree of drug dose alteration for renal dysfunction reflects the creatinine clearance at which dose reduction is first necessary: Thus it is minimal when dose reduction is first necessary for CrCl $\leq$ 10 cc/min, mild for CrCl $\leq$ 30 cc/min, moderate for CrCl $\leq$50 cc/min and major for CrCl $\leq$ 70 cc/min.

[3]Smaller doses (10 mg/kg) are generally used in adults over the age of 50. Streptomycin is usually not given more than five times a week and frequency may be reduced to 2–3 times a week as patients clear their infection.

activity against MTB that is similar to rifampin, but rifabutin is a much less potent inducer of cytochrome P3A4, and therefore immunosuppressant levels may be easier to maintain (68). There is relatively little published clinical experience using rifabutin after transplantation, since active TB is relatively uncommon in transplant recipients in the United States and rifabutin is generally not available in parts of the world in which TB is more common. However, in HIV-infected individuals, the effectiveness of rifabutin-containing regimens appears no different than that of rifampin-containing regimens. Rifabutin dose is 5 mg/kg (maximum 300 mg) given once daily. With either rifampin or rifabutin, immunosuppressant levels should be monitored closely when the rifamycin is started (as higher doses of the immunosuppressant will be required) and when it is stopped (as the dose may then need to be reduced). Management of posttransplant TB with nonrifamycin regimens has been successful in countries where rifabutin is not available (69,70). When prescribing medications for treatment of latent or active TB a careful review of all drug-drug interactions is recommended. Refer to Chapter 32 in the guidelines for further information.

The hepatotoxicity of isoniazid, rifampin and pyrazinamide used in combination is greater than isoniazid alone and noted to be particularly severe in liver recipients (57). Liver function tests should be closely monitored. Isoniazid use may be associated with peripheral neuropathy and other neurotoxicity. Ethambutol use can impair visual acuity; early detection with periodic ophthalmologic monitoring for toxicity is recommended.

Future Directions and Research

Transplant physicians can derive valuable information about the management of TB after transplantation from ongoing research in nontransplant populations. Since immunosuppression may eliminate TST and IGRA responses, development of diagnostic tests for LTBI that do not rely on an intact T cell response would greatly improve diagnosis and clinical management, especially in the case of donor derived infections. Another important advance would be the development and/or clinical validation of antituberculous drugs that are free of significant organ toxicities and drug–drug interactions. New treatment regimens are on the horizon, including potent drugs that may have the potential to shorten and simplify anti-TB therapy (4). Evaluation of these in transplant candidates and recipients may provide useful treatment alternatives for this population in the future.

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Disclosure

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Special Article

Nontuberculous Mycobacterial Infections in Solid Organ Transplantation

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Key words: Mycobacteria, mycobacterium avium complex, nontuberculous mycobacteria, pulmonary infection, transplant

Introduction

With the availability of advanced microbiologic techniques for the detection and identification of nontuberculosis mycobacteria (NTM) over the last 10 years, the number of NTM species has swelled to over 125 (1). Over half of these have the potential to cause human disease, but fewer than two dozen account for most reported cases. Due to impaired cell-mediated immunity, transplant recipients are susceptible to infection with these organisms. There are no prospective studies or registries of these infections, so our understanding of these infections in solid organ transplant (SOT) recipients comes from case reports and a few case series (2–7). While relatively rare compared to other posttransplant infections, these infections are important due to the difficulty in establishing the diagnosis, the need for multidrug, long-term treatment and the interaction between treatment regimens and the drugs used to prevent rejection. This guideline will focus on the common NTM causing infection following transplantation including *Mycobacterium avium-intracellulare* complex (MAC), *M. kansasii*, *M. marinum, M. haemophilum* and the rapid growing mycobacteria (RGM): *M. fortuitum, M. chelonae* and *M. abscessus*. The most frequently encountered species causing pulmonary disease include *M. avium* complex, *M. kansasii, M. xenopi* and *M. abscessus* (6).

Epidemiology

Most NTM are ubiquitous free living saprophytic organisms which have been recovered from a wide variety of environmental sources including soil, water, dust, aerosols, plant material, animals and birds (8). They are often resistant to disinfection and thus can be recovered from drinking water distribution systems including those in hospitals. Most infections are felt to arise following exposure in the environment although nosocomial infections of water contaminated medical devices have been described (8). Until recently there was no compelling evidence of either person to person or animal to person transmission; however, a recent report describes an outbreak of *M. abscessus* ssp *massiliense* infection in a lung transplant and cystic fibrosis center where person to person transmission may have occurred (9). These organisms can be recovered worldwide but most reports of infection are from the developed world (10).

Since NTM infections are not reportable, incidence data in the transplant population can only be estimated. Limited data suggest an incidence rate for NTM infections to be between 0.16% and 0.38% among kidney transplant recipients, 0.24% and 2.8% among heart transplant recipients and 0.46–8.0% in lung transplant recipients (4–6). In a series of 253 patients with a median of 25 months follow-up after lung transplant, 22% had NTM isolated from at least one culture, but only 2.5% required treatment (4). Among liver transplant recipients the rate appears to be at least 0.04% but this is based on even more limited data (2). It is unclear why the incidence of NTM in liver transplant recipients appears to be lower than other SOT groups.

The timing of infection after transplantation can vary from early to very late. In a series of 82 transplant patients with NTM infection, onset of infection was a mean of 48 months after transplant but with a range of 10 days to 269 months (5).

In the nontransplant patient population, four categories of increased risk for NTM infection have been identified. First, among HIV infected persons, a CD4+ T cell count of less than 50/μL is associated with increased risk of disseminated NTM infection. Among non-HIV infected patients, genetic syndromes affecting the interlukin-12/interferon-γ pathways, treatment with antitumor necrosis factor-α agents and structural lung disease from chronic obstructive pulmonary disease (COPD), cystic fibrosis and bronchiectasis all confer increased risk (10,11). In the current era of induction with antilymphocyte agents and 2–3 agents for immunosuppression, a formal risk analysis for infection has not been performed, but disruption of mucocutaneous barriers, structural abnormalities and the net state of immunosuppression are likely contributing factors. In a recent

study of 36 lung transplant recipients diagnosed with NTM infection, both NTM colonization and disease were associated with a significantly increased risk of death (12). A risk factor analysis for NTM infection after lung transplantation found cystic fibrosis, NTM infection before transplantation and the use of rabbit antithymocyte globulin as significant risk factors (13).

Clinical manifestations

Most clinical manifestations of NTM infection fall into one of six categories: pulmonary disease, skin and soft tissue infection, musculoskeletal infection, disseminated disease, catheter associated disease and lymphadenitis, with pulmonary and cutaneous involvement being the most common. (2,5). The spectrum of pulmonary disease includes a solitary nodule, pulmonary infiltrates, abscesses and cavitary nodules with symptoms varying according to the syndrome and may include chronic cough, sputum production, dyspnea and, less commonly, hemoptysis (6,10). Fever may or may not be present (3).

Apart from pulmonary infection in lung recipients, skin and soft tissue infection is the most common (5). Typical findings include painful to minimally painful erythematous to violaceous subcutaneous nodules most commonly on the extremities or in the region of the surgical wound occurring singly or in clusters (4). Lesions will commonly ulcerate and can also have a lymphangitic distribution resembling sporotrichosis (10). Tenosynovitis, osteoarticular disease and osteomyelitis have all been reported (2). The most common species causing skin and soft tissue and musculoskeletal infections are the RGM, *M. fortuitum, M. abscessus* and *M. chelonae* (6). *M. marinum* can produce a lymphangitic eruption resembling sporotrichosis identical to that seen in nontransplant patients after water exposure particularly fish tank water (7).

Disseminated disease with NTM infection has been reported in all SOT types but is most common among kidney recipients (2,5). Nearly half of patients with pulmonary disease will have evidence of dissemination (2,6). Sites of dissemination can include skin, lymph nodes, bone marrow, visceral organs including the allograft and musculoskeletal sites (6). *M. abscessus, M. chelonae* and *M. kansasii* have been the species most frequently associated with dissemination (6). Gastrointestinal tract infection, catheter associated infection and lymphadenitis have been reported infrequently in SOT recipients (2,5,6).

Diagnosis

Establishing the diagnosis of NTM infection can be quite difficult and giving it careful consideration in the differential diagnosis is the critical first step. Although recovery of an NTM from a sterile source such as blood or skin biopsy provides straightforward evidence of invasive disease, in contrast, differentiation of colonization from disease in the respiratory tract can be a formidable challenge.

Table 1: American Thoracic Society/Infectious Diseases Society of America Criteria for diagnosing NTM lung disease

Clinical (both required)
Pulmonary symptoms, nodular or cavitary opacities on chest radiograph or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules (A, 1), AND
Appropriate exclusion of other diagnoses (A, 1)
Microbiologic
Positive culture results from at least two separate expectorated sputum samples (A, II). If the results from 1 are nondiagnostic, consider repeat sputum AFB smears and cultures (C, III), OR
Positive culture result from at least one bronchial wash or lavage (C, III), OR
Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum sample or bronchial washing that is culture positive for NTM (A, II).

Adapted from Ref. (10).

Data presented by Knoll and colleagues suggest that in the respiratory tract, colonization is more frequently encountered than invasive disease by a factor of ten (4). The American Thoracic Society/Infection Diseases Society of America have published have published clinical and microbiological criteria for diagnosing NTM lung disease (10) (Table 1). Although developed for patients with generally normal immune function, these criteria provide a useful reference point for diagnosing pulmonary infection in SOT recipients. Nevertheless, applying these criteria too rigorously may lead to under diagnosis of invasive disease in SOT patients. For example, *M. gordonae* is a common laboratory isolate and generally regarded as a nonpathogen; however, among immunocompromised patients including SOT recipients, there are reports of both pulmonary and extrapulmonary invasive disease with this organism (14). There are no specific criteria for diagnosis of extrapulmonary disease and an assessment of the clinical, histopathological and microbiologicalfindings must be performed to establish a diagnosis.

When NTM infection is suspected, clinical specimens from involved sites such as abscess fluid, synovial fluid, cerebrospinal fluid and bronchoalveolar lavage fluid should be submitted to the mycobacteriology laboratory for mycobacterial culture and staining and biopsy specimens submitted for culture, staining and histopathology. The RGM, NTM that typically grow within about 7 days, can be isolated from routine bacterial cultures, but most cultures are usually not incubated long enough for other NTM to grow. Most NTM will grow on standard mycobacteria media at standard temperatures but several species require special processing in the mycobacteriology laboratory to reliably recover them from clinical specimens (10,15). For example, *M. marinum* and several other NTM grow at temperatures lower than standard incubation temperatures, thus if these

organisms are suspected on clinical grounds, the specimens should be incubated at both 28–30°C and standard temperature (9,14). Other NTM such as *M. hemophilum* and *M. genavense* require special supplementation of the media for growth to occur. Finally, the incubation period for NTM can vary from as short as a few days for the RGM, while others such as *M. genavense* should be incubated for at least 8–12 weeks (10,15). Unless these specialized laboratory techniques for detection of growth are routinely performed by the mycobacteriology laboratory, laboratory personnel should be notified to insure optimal efforts for recovery are being utilized. It is critically important that NTM be identified to the species level and recently the importance of identifying the related species of the *M. abscessus* complex has been recognized (16). Commercially available DNA probes, PCR-based methods and high-performance liquid chromatography are used to rapidly identify some NTM species once growth on media has occurred (10). Unlike the turnaround time for a microbiology report with identification and susceptibilities of a few days for aerobic bacteria, a final report with susceptibilities may take 4 months or longer for some NTM. Although the commercially available interferon gamma release assays (IGRA) have no role in the diagnosis of NTM infection, it is worth noting the antigens used in these assays are present in *M. marinum, M. kansasii* and *M. szulgai,* hence, the potential exists for cross-reaction with these three NTM and possibly other unrecognized unsequenced NTM (17).

Treatment

The treatment of NTM depends on multiple factors including the organism isolated, the extent of the patient's disease, the type of SOT received, overall immunosuppression and the patient's tolerance to medications prescribed. Antimicrobial treatment usually requires a multidrug regimen and therapy must be continued for months to years based on national guidelines and case series, as given the rarity of these infections no controlled trials are available to guide length of therapy or the agents recommended (10). Two drug therapy is generally standard, but three agents may be indicated when the illness is life threatening, the burden of organisms is high or the patient has a RGM and susceptibility or identification to species level is not yet available. Treatment recommendations for NTM encountered infrequently are anecdotal. Cultures should be performed during therapy to judge response, predict the duration of therapy and monitor for resistance to antimycobacterial agents. Consideration should be given to tapering of the immunosuppression regimen, but immune reconstitution syndrome may occur, as it has been reported in SOT patients after therapy for other granulomatous diseases including tuberculosis (18).

Antimicrobial treatment options vary according to species, so the first step is to accurately identify the species or the species group (MAC, includes *M. avium* and *M. intracellularae*) (10). The value of using *in vitro* susceptibility testing to guide treatment decisions is variable depending on the species of NTM. Multiple drug susceptibility testing is generally useful only for RGM. In other cases susceptibility testing can be misleading, and is recommended only for specific "drug-bug" combinations (for example, clarithromycin for MAC and rifampin for *M. kansasii* both of which have established criteria for reporting as susceptible or resistant) (19). For most of the slow growing mycobacteria the susceptibility of the organism can be predicted based on the identification (19). For RGM, empiric initial treatment should be guided by species, but once susceptibility testing to specific agents is available, therapy may need to be modified. For other species, even if criteria are established for a few specific agents, clinical correlation is not available (19) (Tables 2 and 3). For therapy of the slow growing NTM in patients not treated previously, no clear correlation exists between treatment efficacy outcome and susceptibility in patients treated with more than a single agent (10,20). One possible reason for this is that *in vitro* testing is done with single antimicrobials and when certain agents are used in combination they are efficacious despite the results of the testing. For example, ethambutol increases the mycobacterial cell wall permeability (19,21) and there is *in vitro* synergy with rifampin. Combination therapy with at least two or more a antimicrobials is standard in most NTM infections in transplant patients. However, in patients with prior treatment, susceptibility testing may be used as a guide despite the lack of available evidence (III).

A major problem with the treatment of NTM is interactions between immunosuppressive agents and the rifamycins and macrolides (see chapter 32). Rifampin will markedly decrease the levels of the calcineurin inhibitors and sirolimus and its use may result in rejection due to the difficulty in obtaining adequate immunosuppression. For this reason rifabutin is preferred over rifampin in SOT patients and azithromycin over clarithromycin even though the ATS/IDSA guidelines statement suggests rifampin and clarithromycin as preferred agents for MAC treatment. In addition, interactions between the antimycobacterial agents occur. Rifampin is a potent inducer of CYP3A4 enzymes and clarithromycin is an inhibitor. Rifabutin is a less potent inducer of CYP3A4 and therefore has less effect on the metabolism of cyclosporine, tacrolimus, and sirolimus. Clarithromycin, partially, but not completely, offsets the effect of the rifamycins on the calcineurin inhibitors. Another problem is the intolerance of the patient to the medications. Many of these agents cause gastrointestinal toxicity and patients with disseminated disease to the GI tract or intrabdominal lymph nodes are often the most difficult to treat with oral agents. All of these agents may have toxicities, Examples include aminoglycoside related nephrotoxicity and ototoxicity, isoniazid related hepatotoxicity, ethambutol related visual toxicity and quinolone related tendon rupture. Clinicians should consult the ATS.IDSA guidelines for guidance. Many agents are available in an IV form (Table 4).

Table 2: Recommended treatment agents and use of susceptibility testing for slow growing and fastidious NTM in SOT patients on cyclosporine, tacrolimus or sirolimus based on guidelines for all patients from ATS/IDSA and expert opinion

Pathogen (level of evidence non-SOT patients)	Recommended regimen (see reference for details and Table 4 for dosing regimens)	Second line or additional agents[1]	Routine susceptibility testing for initial treatment	Special considerations	Length of treatment
M. avium complex (A or B, II depending on severity)	Azithromycin Rifabutin Ethambutol	Clarithromycin Rifampin Amikacin or Streptomycin	Only for clarithromycin as class drug for macrolides	Never use macrolides alone. Start ethambutol at 25 mg/kg	At least 12 months after negative cultures
M. kansasii (A, II)	Rifabutin Ethambutol Isoniazid plus pyridoxine	Rifampin Clarithromycin or azithromycin Sulfamethoxazole Moxifloxacin Amikacin or streptomycin	Rifampin If rifampin resistant or the patient is failing treatment	May be reported as resistant to isoniazid but inhibited by achievable concentrations	18 months with at least 12 months of negative cultures
M. marinum (B, III)	Azithromycin Ethambutol Consider adding Rifabutin for extensive disease	Rifampin Clarithromycin or azithromycin Sulfonamides Doxycycline or minocycline	Not unless patient is failing treatment	Some strains are resistant to ciprofloxacin, moxifloxacin may have better *in vitro* activity	3–4 months with at least 2 months after symptoms resolve
M. hemophilum (C,III)	Azithromycin Rifabutin Ciprofloxacin	Rifampin Clarithromycin or azithromycin Sulfonamides Doxycycline	Use with caution as methods not standardized	All resistant to ethambutol. For doxycycline and sulfonamides susceptibility is variable	Unknown

[1]For patients in whom drug interactions with calcineurin inhibitors or mTOR inhibitors is not a consideration, there is more data to support the use of clarithromycin to treat MAC (10). Although there is no demonstrated superiority of one rifamycin over the other, rifampin is recommended by most experts due to fewer adverse events than with rifabutin (10).

Table 3: Generally useful treatment agents for empiric therapy and treatment after *in vitro* susceptibility testing for rapid growing NTM in SOT patients on *cyclosporine, tacrolimus or sirolimus*

Pathogen (level of evidence SOT patients)	Regimens should be based on *in vitro* susceptibility data for the patient's isolate (see reference for details and table 4 for dosing regimens)	Second line or additional agents	Special considerations
M. abscessus (C, III)	Azithromycin Plus amikacin, imipenem, or cefoxitin Or two parenteral agents	Clarithromycin Linezolid Tigecycline	Lung infection is difficult to cure May want to start 3 drug therapy until susceptibility available
M. chelonae (C, III)	Two drugs: Azithromycin Plus Amikacin or tobramycin, linezolid, tigecycline or imipenem	According to susceptibility results	Surgery should be considered for drainage of abscesses or resection of infected tissue. Infected foreign material should be removed
M. fortuitum (C, III)	Two drugs: Amikacin Ciprofloxacin or other quinolones Sulfonamides	Sulfonamides Doxycycline or minocycline Imipenem Tigecycline	All isolates contain an inducible erythromycin methylase gene; use macrolides with caution (10)

Table 4: Dosing regimens and drug interactions

Drug	Adult dose	Drug interactions		Dose adjust for renal insufficiency
		Rifamycins	Cyclosporine Tacrolimus Sirolimus	
Azithromycin	250–300 mg daily PO or IV 500 mg daily PO or IV, three times a week (MAC) 1200 mg po/week prophylaxis	Yes	Yes	No
Clarithromycin	500 mg BID PO 1000 mg PO three times a week (MAC)[1]	Yes	Yes	Yes, mild
Ethambutol	15 mg/kg/daily 25 mg/kg three times a week (MAC)	No	No	Yes, mild
Rifabutin	150–300 mg/daily or three times a week (MAC)[1]	n/a	Yes	No
Rifampin	600 mg daily or three times a week (MAC)[1] PO or IV	n/a	Yes	No
Ciprofloxacin	500 mg PO (400 mg IV) BID	Yes	Yes, mild	Yes, moderate
Levofloxacin	500–750 mg daily PO or IV	Yes	Yes, mild (CsA)	Yes, moderate
Moxifloxacin	400 mg daily PO or IV	No	No	
Amikacin	10–12 mg/kg daily or three times a week IV or IM[1]	No	Potentiate renal toxicity	Yes, major
Streptomycin	500–1000 mg daily or three times a week IV or IM[1]	No	Potentiate renal toxicity	Yes, major
Tobramycin	5 mg/kg daily or three times a week IV or IM[1]	No	Potentiate renal toxicity	Yes, major
Linezolid	600 mg BID PO or IV	No	No	None
Isoniazid	5 mg/kg/daily up to 300 mg daily PO give with pyridoxine 50 mg daily PO	No	No	Minimal
Doxycycline	100 mg BID PO or IV	No	No	None
Minocycline	100 mg daily PO	No	No	None
Tigecycline	100 mg IV × one then 50 mg IV q 12 h	No	Yes, mild	None
Cefoxitin	8–12 g daily in divided doses IV	No	No	Yes, moderate
Imipenem	500 mg q 6 h IV	No	No	Yes, moderate
Sulfamethoxazole Trimethoprim/ sulfamethoxazole	1000 mg BID to TID 800–1600 mg (sulfa component) BID PO or IV	No	Possible potentiation of renal toxicity	Yes, moderate

[1]Intermittent therapy (thrice weekly) with aminoglycosides may decrease toxicity. For other agents less frequent dosing may have inconsistent effects on immunosuppressive agents and is not usually recommended as initially therapy or for patients with cavitary lung disease. In many patients therapy will need to be individualized due to renal function, GI toxicity, site of infection and species of NTM.

Because infections may persist despite antimycobacterial therapy, surgery may be required to treat localized skin infections due to NTM. Resection of cutaneous NTM infections in SOT patients has been successful, usually in combination with drug treatment. Surgery has not been as useful in lung transplant patients as in cases of refractory lung disease due to NTM in nonimmunocompromised patients since transplant patients are more likely to have more extensive disease (4). Lung transplant patients with surgical site or pleural infection have required chronic suppressive therapy (22). Because transplant recipients often have more disseminated disease, surgical resection of affected lung is considered only when disease is predominantly localized to one lung. Treatment needs to be continued for many months to years in most patients with NTM infections. The length of treatment is shortest for cutaneous infections with *M. marinum* and longest for lung infections with almost any species of NTM. In treating MAC and *M. kansasii* the goal of therapy is 12 months of negative sputum cultures so sputum must be collected periodically during therapy (A, II) (10). This goal is similar for *M. abscessus* (C, III) but may be less attainable. Many experts regard pulmonary infection with *M. abscessus* to be nearly incurable and the goal of therapy should be control of infection rather than cure. Repeat cultures and susceptibilities are warranted in patients failing therapy or who relapse and require repeat treatment.

Prevention/prophylaxis

Rifabutin, clarithromycin and azithromycin are effective prophylactic agents for MAC in individuals with AIDS (A, I) (23,24). Prophylaxis has not been systematically studied for other NTM species. In lung transplant recipients, there is emerging evidence that NTM colonization especially with *M. abscessus* or MAC pretransplant may be associated with overt NTM disease posttransplant (12). Some centers exclude patients with NTM infection from transplantation until the patient completes at least 3 months of

therapy for NTM (12). Patients with cystic fibrosis undergoing lung transplantation and known to be colonized with RGM should be considered for posttransplant chemoprophylaxis with azithromycin to prevent surgical site infections (III). Similarly, patients infected or colonized with MAC prior to lung transplant should be considered for multidrug MAC therapy prior to lung transplantation (12) (III). For patients who have completed therapy for a documented NTM infection, some experts extrapolate from the HIV data and recommend secondary prophylaxis, but for this and for other situations, there is insufficient evidence to recommend routine prophylaxis (III).

Future directions

More information is needed to improve understanding of NTM related infections in all patients but especially after SOT. Better understanding of epidemiology and diagnosis is needed. Laboratory susceptibility testing needs to be further developed and standardized for all species and antimycobacterial agents. Prospective multicenter trials of prophylaxis in prelung transplant patients who are colonized with MAC or RGM are warranted. New agents and regimens are need for therapy of the most difficult to treat species. A registry of SOT patients with NTM disease and their treatment and disease outcomes, including the function of the transplanted organ after therapy, would help us better understand these infections over time.

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Special Article

Nocardia Infections in Solid Organ Transplantation

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Key words: Abscess, brain abscess, nocardiosis, opportunistic infection, pneumonia, trimethoprim-sulfamethoxazole

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; CNS, central nervous system; HIV, human immunodeficiency virus; MIC, minimum inhibitory concentration; MRI, magnetic resonance imaging; TMP-SMX, trimethoprim-sulfamethoxazole; TNF, tumor necrosis factor; US, United States.

Epidemiology and Risk Factors

Nocardia species are ubiquitous saprophytic gram-positive bacteria in the 'aerobic actinomycetes' group (1), which also includes *Corynebacterium, Rhodococcus, Gordonia, Tsukamurella*, *Actinomadura* and *Mycobacterium* (2–4). More than 30 of the 80 *Nocardia* species characterized have been associated with disease (2,5,6). The most important causes of infection in transplant recipients are *Nocardia asteroides sensu stricto* (1), *N. farcinica*, *N. nova*, *N. brasiliensis*, *N. otitidiscaviarum* and the *N. transvalensis* complex (4,6–9). Molecular methods have now identified many new species which cause infection (2,6,10), including *N. veterana* (11), *N. abscessus* (12), *N. paucivorans* (13), and *N. wallacei and N. blacklockiae* of the *N. transvalensis* complex (9). *Nocardia cyriacigeorgica* is becoming a commonly identified human pathogen (2,5,14–19). From 1995 to 2004, the most common *Nocardia* species of 765 United States (US) isolates were *N. nova* (28%), *N. brasiliensis* (14%), *N. farcinica* (14%) and *N. cyriacigeorgica* (13%) (19).

Nocardia infections appear to have increased in the last two decades, likely due to better detection and identification procedures as well as to an expanding immunocompromised population (2,20), since the majority of patients with nocardiosis are immunosuppressed (2,14). The frequency of nocardial infections in solid organ transplant recipients varies between 0.7% and 3.5%, and historically, infections were mostly reported in heart, kidney and liver transplant recipients and less frequently in lung transplant recipients (21–23). However, several recent reviews have shown greater rates of nocardial infection among lung transplant recipients. A review of 5126 organ transplant recipients demonstrated a 3.5% rate of *Nocardia* infection among lung transplant recipients, with rates in heart, intestine, kidney or liver recipients of 2.5%, 1.3%, 0.2% and 0.1%, respectively (23). Two other large retrospective studies showed rates of nocardial infection in lung transplant recipients of 1.9% (24) and 1.78% (25), and in the latter study this accounted for 37% of all the transplant-associated *Nocardia* infections. Regarding species in solid organ transplant recipients, Peleg et al. identified *N. nova* in 49% of 35 patients, followed by *N. farcinica* in 28%, *N. asteroides* in 23%, and *N. brasiliensis* in 3% (23). Among the nocardial infections reported by Santos, nine (47%) were due to *N. asteroides*, two each to *N. brevicatena* and *N. brasiliensis* and one to *N. otitidiscaviarum*, but five cases (26%) were not speciated (25). However, the prevalence of nocardial infections in general, and specific *Nocardia* species appears to vary by geography (19,26,27). In the US it has been observed that nocardial infections are more frequent in the dry, windy climates of the southwest, possibly because such conditions facilitate aerosolization and dispersal of the organisms (27). Furthermore, there may be species and strain differences in virulence due to cell wall composition, inhibition of immune responses and a variety of other virulence factors (1,2,28). *N. farcinica*, for example, has been demonstrated to be more virulent than other *Nocardia* species in a mouse model (29).

The protective immune response to *Nocardia* is primarily a T-cell-mediated one (30). Therefore, the organism is most commonly seen in solid organ transplant recipients, persons with human immunodeficiency virus (HIV) infection (CD4 counts < 100 cells/mm^3), those with lymphoreticular malignancy and individuals treated with chronic corticosteroid therapy, with more than 60% of reported cases associated with one of these conditions (31). Although nocardial infection in transplant recipients often develops within the first year posttransplant, infection rarely occurs within the first month (14,18,23,32). Nocardiosis has also occurred late posttransplant in some series, with a median time to infection from transplant of 34–38 months (25,33). The diagnosis should be considered at any time posttransplant if intensified immunosuppression (including antilymphocyte globulin or high-dose steroids) has recently been used (23,34). Studies have demonstrated that up to 63% of solid organ transplant recipients with nocardiosis develop infection in the first year after transplant, and receipt of

high-dose steroids, cytomegalovirus disease in the preceding 6 months and intensified immunosuppression, including a high median calcineurin inhibitor level in the preceding 30 days, were independently associated with subsequent *Nocardia* infection (14,18,20,23,33).

Newer immunosuppressive therapies may also be risk factors for nocardiosis. Despite the fact that animal studies have demonstrated little contribution from B lymphocytes in preventing *Nocardia* infection (35), rituximab, a monoclonal antibody used to prevent and treat antibody-mediated rejection, was recently described as a potential risk factor for the development of cerebral nocardiosis (36,37). In addition, profound hypogammaglobulinemia in combination with transplant immunosuppression has been implicated as a factor in the development of nocardial infections (38). It is therefore possible that deficits in B-cell function may affect cell-mediated immune responses against *Nocardia*. Tumor necrosis factor (TNF) has also been shown to be important in clearing *Nocardia* in animal models (39), and there have been recent reports of *Nocardia* infections complicating immunomodulatory treatment of rheumatologic diseases with TNF blockers (40,41). Finally, alemtuzumab, a humanized monoclonal antibody against the CD52 antigen found on mononuclear cells, is increasingly being used for the prevention or treatment of organ rejection and it has been associated with a number of opportunistic infections including nocardiosis (42,43).

Diagnosis

The main route of *Nocardia* infection is via the respiratory tract, and subsequent spread to other tissues, particularly the brain, may occur. Nocardial infection predominantly causes pneumonia in transplant recipients (14,23,25,44) and disease is typically subacute with symptoms often present for weeks (6). Nocardiosis should be considered a diagnostic possibility in any transplant recipient patient with an indolent pulmonary process. The host response to infection may range from a granulomatous to purulent reaction (1,45). Radiological examination usually demonstrates irregular nodular lesions (18,23,33) that may cavitate, and may be accompanied by a 'halo sign' (46). However, the infection may also appear as diffuse pneumonic infiltrates or consolidative with pleural effusions (14,23,25,44,46,47). Local spread to contiguous structures including the chest wall has been observed (46), and hematogenous dissemination is not uncommon. In fact, because extrapulmonary disease complicates up to 50% of cases of pulmonary nocardiosis, the diagnosis of *Nocardia* pulmonary infection should prompt a search for disseminated disease, and further investigation should include magnetic resonance imaging (MRI) of the brain to exclude cerebral abscess, as up to one-third of cases have central nervous system (CNS) involvement (1,6,33,44,48,49). Nocardiosis should always be considered a diagnostic possibility in patients with nodular lesions of the lungs and brain.

Nocardia can also infect the skin and subcutaneous tissues, either via direct inoculation or hematogenous spread. Primary cutaneous infection has occurred in immunocompromised and immunocompetent persons after penetrating injuries, especially with outdoor activities (14,25,50). Subcutaneous nodules and a sporotrichoid type of infection can develop, as well as mycetoma, pyomyositis and bone abscess (1,33). While much less common, other typical locations for *Nocardia* dissemination include the eyes, kidneys and bone or joints (1,33,51,52). Unusual manifestations of systemic disease in transplant recipients include epididymo-orchitis (53) and pericarditis (54). Despite its propensity to disseminate hematogenously, *Nocardia* is isolated from blood cultures only rarely (25), and this is often associated with central venous catheters, particularly in cancer patients (55,56).

The definitive diagnosis of nocardial disease requires demonstration of the organism on culture from a suspected site, particularly given the broad differential diagnosis of pulmonary, brain and soft tissue infections in transplant recipients. It is important to obtain lung or skin biopsies, where possible, to confirm the diagnosis. Biopsy of brain abscesses, while preferred, may not always be feasible, but the finding of brain lesions in the setting of confirmed pulmonary or soft tissue *Nocardia* infection is a strong indication of CNS nocardiosis, as is radiographic improvement of brain abscesses during treatment for nocardiosis.

Contamination of clinical specimens may occur, as can colonization of the respiratory tract, but the latter is typically in patients with underlying lung disease who are not on immunosuppressive therapy (1,2,6,14). Isolation of *Nocardia* from a transplant recipient should always be carefully investigated (1,31). *Nocardia* is a branching bacterium (57) and will usually stain with a modified acid-fast (Kinyoun) stain (1). Organisms appear in tissue sections as gram-positive branching and beaded rods surrounded by a pyogenic inflammatory reaction (2,57). *Nocardia* grow in non-selective media and can form aerial hyphae, but the laboratory should be informed of the possibility of *Nocardia*, because growth can be obscured in a mixed specimen such as sputum, and cultures may need to be incubated for a longer time period (1). Growth is generally inhibited at 50°C (9) and the yield of a culture for *Nocardia* can be increased by use of selective media such as Thayer–Martin agar with antibiotics (58). *Nocardia* may take 2 days to several weeks to grow in culture but growth is often seen in 3–5 days, and colonies appear chalky white if producing aerial hyphae (1,3). Determination of the species of *Nocardia* can help guide therapy (see below), and accurate identification generally requires molecular methodology (10). Only a few species, such as *N. brasiliensis*, *N. farcinica*

and *N. pseudobrasiliensis*, can be reliably identified by biochemical methods (6,59).

Treatment

The mainstay of treatment of nocardial infections in transplant recipients is antibiotic therapy. Because there are no controlled clinical trials comparing treatment regimens for nocardiosis, initial selection of antibiotic therapy should take into account the site and severity of disease, the potential for drug interactions and the species of *Nocardia* (2). Antimicrobial susceptibility testing is strongly recommended [III] (Table 1) (60,61). In 2003, the Clinical and Laboratory Standards Institute (CLSI) published the first approved methods for susceptibility testing of aerobic actinomycetes (6,62), and this standard was updated in 2011 (60). The primary antimicrobials recommended for susceptibility testing include amikacin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, linezolid, minocycline, sulfamethoxazole or trimethoprim-sulfamethoxazole (TMP-SMX) and tobramycin. Secondary agents include cefepime, cefotaxime, doxycycline, gentamicin, gatifloxacin and moxifloxacin (6,60,62). However, few laboratories routinely do this testing and data correlating susceptibility results with clinical outcomes are sparse (2,6,14). Still, testing is recommended particularly when there is disseminated infection, treatment failure or relapse, or when newly identified or relatively resistant species of *Nocardia* (e.g. *N. farcinica, N. abscessus*) are isolated [III] (2). The possibility of antimicrobial resistance among certain species of *Nocardia*, the demonstration of synergy with certain combinations of antibiotics in animal models (63), and the high mortality that may be associated with nocardiosis (14) have all led to the recommendation that a combination of agents be used as initial therapy in persons who are seriously ill, those who have disseminated or CNS disease and those who are immunocompromised [III] (Table 2) (1,6,18,44,54,64,65).

High-dose sulfonamides such as sulfadiazine (1.5 g qid) and sulfisoxazole (2 g qid) were used successfully for many years in the treatment of nocardial infections [II-3] (2,6,80), although these agents can be associated with renal toxicity (1). Obtaining sulfonamide serum drug levels can ensure adequate drug absorption and provide dosing guidance when there is concern for drug toxicity (1). Target serum levels should be 10–15 mg/dL (1). Although antimicrobial regimens for nocardiosis have not been compared by controlled clinical trials, TMP-SMX is now generally the preferred agent in treating most nocardial infections (Table 2) [II-2]. The synergy observed *in vitro* between TMP and SMX against various *Nocardia* isolates may confer an advantage over older sulfonamide agents, and TMP-SMX has demonstrated clinical efficacy and achieves high tissue concentrations in lung, brain, skin and bone (15,80,81). Recommended initial treatment dosing with TMP-SMX is 15 mg/kg/day orally or intravenously in two to four divided doses, particularly if there is disseminated or CNS disease, although there appears to be a range of effective doses, depending on the site and severity of infection (2,80). The main side effects are nausea/vomiting, rash, including erythema multiforme, myelosuppression, hyperkalemia and crystalluria (1). Organ transplant recipients may be at higher risk for myelosuppression and nephrotoxicity as their immunosuppressive medications (e.g. mycophenolate mofetil, azathioprine and calcineurin inhibitors) often have overlapping side effects.

Some species of *Nocardia*, such as *N. farcinica, N. nova* and *N. otitidiscaviarum*, may have high-grade resistance to sulfonamides, so species identification and susceptibility testing are especially recommended (2,6,14,16,19,61,82,83). A 10-year retrospective evaluation of US *Nocardia* isolates performed by the Centers for Disease Control and Prevention noted a very high rate of TMP-SMX resistance (42% overall) (19). However, clinical correlation of TMP-SMX susceptibility test results with outcomes were not provided, and other recent studies have not corroborated extensive resistance to TMP-SMX among *Nocardia* isolates (5,15,61). There may be regional and strain differences to account for the discrepant results (5), but another possibility is interlaboratory variability in susceptibility testing methodology and interpretation of *in vitro* MICs (15,61). Proficiency testing for labs performing *Nocardia* susceptibility testing is advocated, as is the use of control strains of *Nocardia* with known susceptibility patterns (15,61).

Alternative treatment regimens have been less well studied but there is enough evidence to suggest there are options for patients with allergy or intolerance to sulfonamides or for *Nocardia* species that are less susceptible to sulfonamides. Amikacin is very active against all species of *Nocardia*, although it may have variable activity against *N. transvalensis* (2,19), and imipenem displays good activity *in vitro* except against *N. brasiliensis*, *N. abscessus* and *N. otitidiscaviarum* (6) (Table 1). Imipenem coadministered with amikacin alone or in a three-drug regimen with TMP-SMX has been increasingly accepted as initial therapy for cerebral disease and for very ill patients with nocardiosis [III] (1,6,44,45), particularly while susceptibility testing is pending. The combination of imipenem and amikacin has demonstrated additive or synergistic effects *in vitro* (95,96) and has been effective in human cases (18,24,97). Both imipenem and amikacin appear to display synergy against *Nocardia* when combined with sulfa medications as well (84). Tripodi et al. also showed that amikacin combined with imipenem, moxifloxacin or TMP-SMX displayed rapid *in vitro* bactericidal activity against multiple *Nocardia* isolates (18). Imipenem and amikacin dosing must be adjusted for creatinine clearance. Also, caution is required in using amikacin in transplant recipients taking cyclosporine or tacrolimus as aminoglycoside nephrotoxicity may be enhanced. In critically ill patients with significant renal

Table 1: Antimicrobial susceptibility of *Nocardia* spp. (% isolates susceptible)

Antibiotic	*N. asteroides complex*	*N. farcinica*	*N. nova*	*N. brasiliensis*	*N. transvalensis*	*N. otitidiscaviarum*	*N. cyriacigeorgica*
TMP-SMX	79–100	20–100	47–100	80–100	44–88	68–100	78–100
Imipenem	70–100	65–100	95–100	0–52	48–90	0–32	77–100
Amikacin	85–100	100	83–100	99–100	20–82	94–100	99–100
Minocycline	43–100	9–66	16–100	31–90	16–54	38–100	14–40
Ceftriaxone	64–100	0–73	47–100	19–100	50–68	0–26	82–96
Ciprofloxacin	0–50	19–90	0–17	0–30	24–60	0–32	0–7
Amoxicillin/clavulanate	0–70	40–100	5–50	65–100	30–56	0–24	0–38
Linezolid	100	100	100	98–100	98–100	100	96–100
Moxifloxacin	50	26–88	2	X	X	X	4
Tigecycline*	X	23	58	X	X	X	X

*There are no established interpretive categories for susceptibility testing for tigecycline; these data come from reference (16) where a minimum inhibitory concentration (MIC) ≤ 1 mcg/mL was arbitrarily designated as susceptible.
X—not enough data for accurate results.
Composite data from the following references: (2,5,8,14,16,19,26,61,66–79).
Interpretation of data based on CLSI breakpoints for bacteria that grow aerobically (60,62,66).

Table 2: Suggested therapy for *Nocardia* infections in transplant patients

Disease	Primary/empiric therapy§	Alternative‡§	Duration of therapy
Pulmonary—stable	TMP-SMX** [II-2]	Imipenem†** + amikacin** [III] or minocycline [III] or linezolid [III]	6–12 months [III]
Pulmonary—critical	Imipenem†** + amikacin** [III] or TMP-SMX** [II-2]	Linezolid [III]	6–12 months [III]
Cerebral*	Imipenem†** + amikacin** [III] or TMP-SMX** [II-2]	Linezolid [III] or ceftriaxone [III] or cefotaxime** [III] or minocycline [III]	Parenteral therapy 3–6 weeks then change to oral therapy for at least 9–12 months of treatment [III]
Disseminated*(>1 organ +/− cerebral disease)	Imipenem†** + amikacin** [III] or TMP-SMX** [II-2]	Ceftriaxone, cefotaxime**, linezolid or minocycline [III] after initial therapy	9–12 months [III]

*Based on animal studies and numerous case reports (1,2,21,49,52,64,66–75,78,80–82,84–94).
**Adjust therapeutic agents based on patient's renal function.
§Antibiotic dosing: TMP-SMX 15mg/kg in 3–4 divided doses, either IV or PO, imipenem 500 mg IV q6 h, amikacin 10–15 mg/kg/day, minocycline 200mg PO or IV q12 h, linezolid 600mg PO or IV q12 h, ceftriaxone 2 g iv q12 h, cefotaxime 2 g iv q8 h.
†Meropenem (1 g q8h) may be an alternative agent depending on species [III].
‡This table is only a guide and the choice of treatment depends on antimicrobial susceptibility, severity of condition, immunosuppression of the patient and allergy history. Alternate agents such as amoxicillin-clavulanate, ceftriaxone, fluoroquinolones and macrolides may be effective [III] but there is insufficient information to support their use as initial therapy. These agents should be considered only if standard therapy is ineffective.
Note: Sulfonamides may be substituted for TMP-SMX; however there are reports of more resistance to sulfonamides than to TMP-SMX (19).

dysfunction where it may be desirable to avoid TMP-SMX and aminoglycosides, treatment with linezolid is an option until susceptibility test results are available (Tables 1, 2 and discussion below) [III].

There are increasing reports of successful outcomes with the use of meropenem in the treatment of *Nocardia*, generally in combination with other agents and especially for treatment of brain abscesses (14,64,85,98–100). Susceptibility studies suggest meropenem is less active against the more common *N. asteroides* complex organisms than imipenem; however it is more active against *N. brasiliensis* and *N. otitidiscaviarum* (93). With its less frequent dosing, good penetration of the blood brain barrier and reported lower incidence of seizures (101), meropenem is an additional option for therapy. However, until there are further data, including studies associating outcomes with *Nocardia* susceptibility testing, its use should be on an individual patient basis [III]. Of note, ertapenem has significantly less activity *in vitro* against *Nocardia* species than imipenem and meropenem (102).

Third-generation cephalosporins such as ceftriaxone and cefotaxime are additional options for intracranial nocardial infections [III]; these agents obtain excellent CNS penetration and there are case reports of successful therapy with their use, typically in combination with other active agents (65,103,104). However, some *Nocardia* species such as *N. farcinica, N. transvalensis* and *N. otitidiscaviarum* are relatively resistant to cephalosporins (5,6,19,54,74,105).

Minocycline has been a popular alternative to TMP-SMX in the treatment of *Nocardia* [III] (54,86,106). Given 200 mg twice daily either orally or intravenously, it achieves adequate intracerebral levels (106) but CNS dissemination has occurred in patients on lower dose minocycline (107). Minocycline has activity against the majority of *Nocardia* species, but many strains are resistant or not fully susceptible (11,77,102), and therefore the use of minocycline should be guided by susceptibility testing. Toxicities include photosensitivity, headache, nausea, disequilibrium, esophageal ulceration and skin discoloration with prolonged use. It cannot be given to pregnant patients or children, due to bone and dental toxicity (86).

At present, the data supporting the use of ampicillin, macrolides, or the fluoroquinolones for treatment of nocardial infection are not as robust as for the antimicrobials discussed above, and their *in vitro* activities appear more variable (Table 1). Case reports of both treatment success and failure with amoxicillin alone or in combination with clavulanate regimens have been published (44,50,90,108,109). The use of amoxicillin-clavulanate should be guided by *in vitro* sensitivity testing given its variable activity against *Nocardia* spp. (Table 1 and Ref.18). Tigecycline and fluoroquinolones have demonstrated *in vitro* activity against many species of *Nocardia* (16,18,102,110). Gatifloxacin and moxifloxacin appear to have better activity against *Nocardia* spp. compared to ciprofloxacin (76), although ciprofloxacin has been used successfully as combination therapy (18,85). Moxifloxacin treatment has demonstrated mixed results. It was successful in two cases of *N. farcinica* CNS infection (111,112) and as continuation therapy after parenteral antibiotics in two heart transplant recipients (18). However, there is a report of recurrent *N. farcinica* CNS infection in a patient receiving moxifloxacin despite *in vitro* susceptibility and high levels of the drug in the abscess material (113). Moxifloxacin appears to be more active *in vitro* against *N. farcinica* than against other common *Nocardia* species (6,16,76) and has shown bactericidal effect against *Nocardia* isolates when combined with either imipenem or TMP-SMX (18). Nemonoxacin, a novel oral nonfluorinated antibiotic, appears to have the lowest MICs for *Nocardia* compared to moxifloxacin, ciprofloxacin and levofloxacin (5).

Linezolid is an oxazolidinone antibiotic which is gaining more attention as a primary therapy for nocardial infections [III] (87). Antimicrobial sensitivity testing has shown that it has excellent activity against all species of *Nocardia*, including *N. farcinica* (16,18,19,26,61,78). A recent review of the literature summarizing 16 patients with nocardiosis treated with linezolid as monotherapy or in combination with other agents reported a high success rate with 12/16 cures and 3 improvements (including cerebral and disseminated disease), although anemia and myelosuppression were common (114). *In vitro* studies have shown an antagonistic effect against *Nocardia* isolates for combinations of linezolid with amikacin and imipenem but linezolid is bactericidal when used in combination with moxifloxacin (18). Linezolid is given 600 mg twice a day either intravenously or orally, with few significant drug interactions (115). Serious toxicities include thrombocytopenia, aplastic anemia, peripheral neuropathy, lactic acidosis and serotonin syndrome in the setting of concomitant serotonin-reuptake inhibitor use (78,87,115,116). Myelosuppression in particular, as well as the drug's expense may limit its widespread use for nocardiosis (23,33,116,117).

Surgical drainage may be required in the treatment of nocardial infection, especially for cerebral nocardiosis not responding to antibiotic therapy, and for other large soft tissue abscesses. Surgical therapy should be performed in conjunction with antibiotic treatment (1,64,85,90). A reduction of immunosuppression may be a helpful adjunctive measure (18,32), particularly in progressive or severe disease, such as cerebral or disseminated infection (2) [III]. However, several authors have deemed that this maneuver is not mandatory, finding good treatment outcomes despite continuing immunosuppressive agents without dosage adjustments (44,118,119). Reduction of immunosuppression should be considered on a case-by-case basis.

The optimal duration of therapy for nocardiosis is unknown, but recommendations are guided by the tendency of *Nocardia* to recur and reports of relapse after varying durations of therapy (2). In addition, the type and level of the patient's immunosuppression is an important consideration. Most patients will show a clinical improvement within 1 week of starting therapy (18). If the patient is very ill at presentation, parenteral therapy should be continued for 3–6 weeks and clinical improvement should be seen before changing to an oral regimen [III] (2,4). While extrapulmonary localized abscesses have been cured with short courses (~8 weeks) of parenteral antibiotics (2), and there is a recent report showing successful treatment of pulmonary nocardiosis with approximately 2–4 months of therapy in a small series of heart transplant recipients using an initial 3–4 weeks of combination parenteral therapy (18), the standard recommended treatment for nocardiosis is generally much longer. Cerebral nocardiosis should be treated at least for 9–12 months [III] (1,2,4,80) and exhibit improvement of lesions radiographically prior to stopping therapy. Pulmonary and soft tissue infections should be treated for 6–12 months depending on the response to therapy and resolution of disease [III] (1,2,48,80). Catheter-associated bloodstream infection with *Nocardia* should be treated with catheter removal and several months of antibiotics (55). Intensification of immunosuppression for allograft rejection during nocardial therapy may warrant an extension of the duration of antimicrobials. Following discontinuation of therapy, patients should be monitored for relapse of disease. Patients with cerebral nocardiosis should undergo repeat computed tomography or magnetic resonance imaging of the brain. Some centers continue prophylaxis for *Nocardia* once therapy is complete, as described below [II-3] (49).

The response of nocardial infections to therapy is dependent on the patient's underlying disease and the extent and site of infection. Skin and soft tissue infections are often treated successfully, whereas cerebral infection has been described to carry mortality rates of 30 to 55% (81,120). Among 23 published reports, each of which had at least 5 cases of nocardiosis, the mean mortality rate was 26% (14). Among organ transplant recipients there is a range of survival rates reported; Santos et al. noted a 53% mortality rate (25), but several other series have reported cure rates of almost 90% or more among organ transplant recipients (14,18,23,44). Delay in diagnosis and early discontinuation of therapy have been associated with poor outcomes (121).

There have been few reported cases of nocardial infection in pediatric transplant recipients, making it difficult to draw conclusions regarding optimal therapy (122–124). In fact, nocardiosis appears to be a rare infection in children (122,123,125) and in one series of 43 patients with *Nocardia* isolated from 1995 to 2006 at a large tertiary center, none were children (14). There are only 51 children with *Nocardia asteroides* reported in the literature from 1895 to 1981, with only two of these noted to be transplant recipients (123). Antibiotics reported useful in these pediatric cases are similar to those used in adults, including linezolid, meropenem, amikacin, amoxicillin-clavulanate and TMP-SMX, with therapy generally given for months to more than 1 year. Due to the possibility of tooth discoloration and potential for adverse effects on skeletal development, tetracyclines should not be used in children younger than 8 years, and fluoroquinolones are also generally avoided in children due to potential musculoskeletal toxicity.

Prevention/Prophylaxis

When used daily for the prevention of *Pneumocystis jiroveci* pneumonia in the first 6 months posttransplantation (see *Pneumocystis* prevention guidelines), TMP-SMX reportedly reduces the rate of nocardial infection among solid organ transplant recipients [III] (44,89), similar to what has been observed among persons with HIV infection (126). TMP-SMX prophylaxis is a very cost-effective prophylactic agent, as its benefits extend to the prevention of *Toxoplasmosis gondii*, *Listeria monocytogenes* and many common respiratory, urinary and gastrointestinal pathogens (32,126). However, there are increasing reports of breakthrough infections by TMP-SMX-susceptible *Nocardia* isolates in patients taking TMP-SMX prophylaxis (14,21,23–25,31,54,88,126–128), creating some doubt about the utility of this agent for prevention of nocardiosis (14). Minero and colleagues found that 21.6% in their series were on TMP-SMX prophylaxis at the diagnosis of nocardiosis, of which 62.5% were still susceptible to the drug (14). Similarly, in their series of 19 *Nocardia* cases, Santos and colleagues noted that most had received TMP-SMX prophylaxis for *Pneumocystis jiroveci* (25). In many of these reports, dosing of TMP-SMX prophylaxis is not provided. Because the dosing of TMP-SMX employed at some centers for prophylaxis of *Pneumocystis jiroveci* in organ transplant recipients is two to three times per week rather than daily, insufficient blood levels due to intermittent dosing could explain breakthrough infections, although nocardiosis has also been reported despite daily TMP-SMX prophylaxis (54). Additional factors may be sulfa resistance, posttransplant immunosuppression and possibly other comorbidities that affect a patient's immune response (23,24,127).

Given the observations regarding the imperfect efficacy of lower dose TMP-SMX for primary prevention of nocardiosis, it is equally difficult to make definitive recommendations on the dose and duration for secondary prophylaxis. Relapse after initial *Nocardia* infection has been reported in the setting of solid organ transplantation, but typically after abbreviated therapy (2–2.5 months) (24,128). As noted in the Treatment section above, some centers choose to continue TMP-SMX for long-term prophylaxis against *Nocardia* to prevent relapse [II-3], although few published reports provide dosing details. One double-strength TMP-SMX tablet daily (24) and a double-strength table three times weekly (129) have been used indefinitely (24,81,129) [III].

Transmission and Infection Control Issues

Inhalation of *Nocardia* from environmental sources is likely to be the main route of transmission, although penetrating cutaneous injury is another potential route of inoculation (1,31,45,50). Animal-to-human transmission has not been reported (2). While most patients develop sporadic infections, clusters of nosocomial infection have occurred, including among transplant recipients, with possible sources being construction, contaminated air or healthcare worker hands (130–132). However, these studies have often lacked strain testing with molecular methods to confirm the relationship between infections (6). In some cases however, investigators have demonstrated common source environmental and person-to-person transmission in health care facilities by using ribotyping and pulsed-field gel electrophoresis methods (131,132). Although there are no measures that effectively prevent inhalation, TMP-SMX prophylaxis in high-risk populations, as noted above, may reduce the incidence of the disease (31). However, the efficacy of prophylaxis may depend on the dose of TMP-SMX administered, antimicrobial resistance to TMP-SMX and the immunologic status of the host.

Future Studies

In the future, rapid diagnostic testing may become widely available to assist in identifying *Nocardia* to the species

level using methods such as polymerase chain reaction directly on clinical specimens (133). Gene probes, ribotyping and restriction endonuclease analysis could also be used to provide rapid diagnosis and assist in early institution of therapy (75). Further studies will also allow for a broader selection of antibiotics to be used in the treatment of the condition, especially if the newer fluoroquinolones and macrolides prove effective, as they will provide potent oral alternatives to the regimens we presently use, with less toxicity.

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Special Article

Cytomegalovirus in Solid Organ Transplantation

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Key words: Cytomegalovirus (CMV), donor-to-host transmission, ganciclovir, posttransplant infection, valganciclovir, viral infection

Abbreviations: CMV, cytomegalovirus; GCV, ganciclovir; NAT, nucleic acid testing; SOT, solid organ transplantation; VGCV, valganciclovir.

Introduction and Epidemiology

Cytomegalovirus (CMV) is a ubiquitousherpes virus that infects the majority of humans (1). The seroprevalence rates of CMV ranges from 30–97% (2,3). Primary infection manifests as an asymptomatic or self-limited febrile illness in immunocompetent individuals, after which CMV establishes life-long latency in various cells (2,3), which serve as reservoirs for reactivation and as carriers of infection to susceptible individuals (4,5).

CMV is a major cause of morbidity and a preventable cause of mortality in solid organ transplant (SOT) recipients (4). Without a prevention strategy, CMV disease typically occurs during the first 3 months after SOT; this onset has been delayed in SOT patients receiving CMV prophylaxis (6–10). Various terminologies have been used to describe CMV infection and disease in SOT recipients (11,12). To ensure uniformity of reporting in research publications, the following definitions are recommended:

- CMV infection: Presence of CMV replication regardless of symptoms (this should be distinguished from latent CMV). CMV replication is detected (1) nucleic acid testing (NAT; Ref.2), antigen testing and (3) culture. Depending on the method used, CMV infection can be termed as CMV DNAemia or RNAemia (NAT), CMV antigenemia (viral antigen testing) and CMV viremia (culture).
- CMV disease: CMV infection accompanied by clinical signs and symptoms. CMV disease is categorized into (1) CMV syndrome, which manifests as fever and/or malaise, leukopenia or thrombocytopenia, and (2) tissue-invasive CMV disease (e.g. gastrointestinal disease; pneumonitis; hepatitis; nephritis; myocarditis; pancreatitis; retinitis, others). CMV infection without any clinical manifestations should be labeled "asymptomatic CMV infection."

CMV has a predilection to invade the allograft, likely in part due to aberrant immune response within the allograft (13). It also has numerous indirect effects due to its ability to modulate the immune system. CMV has been associated with other infections such as bacteremia (14), invasive fungal disease (15) and Epstein–Barr virus-associated posttransplant lymphoproliferative disease (16). CMV infection is an important contributor to acute and chronic allograft injury (13), including chronic allograft nephropathy (or tubulointerstitial fibrosis in kidney recipients; Ref.17), bronchiolitis obliterans (lung recipients; Ref.18) and coronary vasculopathy (heart recipients; Refs.19,20).

Risk Factors

CMV disease risk is highest when primary CMV infection occurs in an SOT recipient with no preexisting CMV-specific immunity (21), such as the CMV donor-seropositive, recipient-seronegative (D+R–) patient (5). Other risk factors are the overall state of immunosuppression as determined by the immunosuppressive protocol (e.g. type of drug, dose, timing, duration), host factors (e.g. age, comorbidity, leukopenia and lymphopenia, genetic factors) and others (e.g. cold ischemia time, critical illness, stress; Ref.21). Use of lymphocyte-depleting agents such as antilymphocyte antibodies is associated with CMV disease, particularly when these are used for rejection therapy (22). The risk of CMV disease varies by the transplant type, likely in part due to the amount of lymphoid tissue in transplanted organs and the intensity of immunosuppression. Lung and small intestinal recipients are considered at highest risk among SOT recipients. Coinfections with human herpes virus (HHV)-6 and HHV-7 have been suggested as risk factors (23).

CMV D–/R– SOT recipients have the lowest risk of CMV disease, and they should receive CMV-negative blood or leuko-depleted blood products. The use of mTOR inhibitors (everolimus, sirolimus) is associated with a lower risk of CMV disease (24).

Recommendations for CMV risk assessment

- All donors and transplant candidates should be tested for CMV serology prior to transplantation in order to allow for risk stratification and guide prevention strategies (II-1).
- Serologic test that measures CMV-IgG is recommended (II-1).
  - Unless clinically indicated (i.e. if primary infection is suspected), CMV-IgM measurement is not recommended due to potential for false-positivity (III).
- In patients with borderline or indeterminate CMV serology results, the assignment of serostatus should assume the most conservative approach (III).
  - If a donor CMV serology is borderline or indeterminate, it should be considered as positive (III).
  - If the recipient CMV is borderline or indeterminate, the result should be considered in the context of donor serology to assign the most conservative designation (III). If the donor CMV serology is positive, the recipient will be considered seronegative (i.e. CMV D+/R– mismatch) (III). If the donor CMV serology is negative, the recipient will be considered seropositive.
- Transplant recipients who receive treatment with lymphocyte-depleting drugs, especially if given for the treatment of rejection, should be considered at high risk for CMV disease (II-1).

Laboratory Diagnosis

The laboratory methods to confirm CMV infection are (1) histopathology, (2) culture, (3) serology, (4) antigenemia and (5) molecular assays that detect and quantify CMV nucleic acid (NAT).

Histopathology confirms the presence of tissue-invasive CMV disease. However, this entails an invasive procedure to obtain tissue for diagnosis. Its use has declined due to the availability of non- or less-invasive tests to document CMV infection in the blood (25). However, histopathology is recommended in cases where another concomitant pathology (e.g. graft rejection) or copathogens are suspected, especially when patients do not respond to anti-CMV treatment. Histopathology may be needed when CMV disease is suspected but CMV testing in the blood is negative, such as in some cases of gastrointestinal CMV disease (25). However, repeated histopathology to document clearance of CMV infection in the affected organ, such as the gastrointestinal tract, is generally not clinically necessary (25).

CMV serology to detect CMV-IgM and IgG antibodies has a limited utility for diagnosis of CMV disease after transplantation. Because of immunosuppression, SOT recipients may have delayed or impaired ability to mount an antibody response to CMV infection (26).

Viral culture is highly specific for the diagnosis of CMV infection. However, its use is limited by its modest sensitivity and slow turn-around time (27). Tissue culture may take weeks before the virus can be detected. Shell-vial centrifugation assay has a relatively more rapid turn-around time, but it remains less sensitive compared to molecular assays (27). Nonetheless, culture is still used in isolating CMV in nonblood clinical specimens, partly because molecular methods are not yet optimized for these clinical samples. Viral culture of urine is of low clinical utility in the adult SOT population (see below for its use in pediatric population; Ref.27). Viral culture is needed when phenotypic antiviral drug resistance testing is requested, although genotypic assays are the method of choice for detecting drug resistance (see below; Ref.28–32).

The antigenemia assay is a semiquantitative assay that detects pp65 antigen in CMV-infected peripheral blood leukocytes (27). Antigenemia has higher sensitivity than culture, and is comparable to NAT by polymerase chain reaction (PCR; Ref.27,33). Depending on the number of CMV-infected cells, one can estimate the magnitude of viral replication. The CMV antigenemia assay is useful to guide preemptive therapy, for rapid and sensitive diagnosis of CMV disease, and to guide treatment responses (27). The main disadvantage is the need to process the clinical sample within few hours, and since the test relies on leukocytes, it has limited utility in leukopenic patients (27).

Molecular tests that detect CMV DNA or RNA are the preferred methods for the diagnosis of CMV after SOT (27). Generally, detection of CMV RNA is indicative of active CMV replication. In contrast, detection of CMV DNA may or may not reflect CMV replication since a highly sensitive NAT may amplify latent viral DNA. Hence, quantitative NAT (QNAT) assays have been developed to potentially differentiate active viral replication (typically associated with high viral load) from latent virus (low-level CMV DNAemia if using highly sensitive tests; Ref.27).

Higher CMV load values are generally associated with tissue-invasive disease, while lower values are seen with asymptomatic CMV infection, and intermediate-range viral loads are seen with CMV syndrome; however, there is wide overlap between these categories (34). Higher viral loads are generally observed in CMV D+/R– compared to CMV R+ SOT recipients. The rate of rise in viral load is an equally important marker of CMV disease risk (34–36); the faster the rise in CMV load, the higher is the risk of CMV disease (35,36). There are occasional patients (most often CMV R+ SOT recipients) with tissue-invasive disease (especially late-onset gastrointestinal CMV disease and retinitis) with very low to undetectable viral load in the blood (37); these cases may be due to CMV disease compartmentalization, or the use of less sensitive QNAT assays.

Table 1: Characteristics of antiviral prophylaxis and preemptive therapy

	Prophylaxis	Preemptive therapy
Efficacy	Yes: large randomized trials	Yes: smaller trials; fewer D+/R–
Ease	Relatively easy to coordinate	More difficult to coordinate Viral load thresholds not standardized
Late-onset CMV disease	Occurs commonly in CMV D+/R– transplant recipients	Occurs much less commonly
Cost	Higher drug costs	Higher laboratory costs
Toxicity	Greater drug toxicity (myelosuppression)	Potential for less drug toxicity with shorter courses of antivirals
Indirect effects (graft loss, mortality and opportunistic infections)	Positive impact based on meta-analyses and limited comparative trials	Very limited data that preemptive therapy affects indirect effects
Drug resistance	Yes	Yes

QNAT is useful for guiding preemptive therapy, for rapid and sensitive diagnosis of CMV infection, and to guide treatment responses (27). The major drawback to QNAT is the lack of (until recently) an international reference standard (38,39). Accordingly, the viral load results of one assay cannot be directly extrapolated as equal that of another assay (38,40). An up to a 3-$\log_{10}$ variation among different CMV QNAT has been demonstrated (39), due to differences in assay platform, samples, calibrator standards, gene target, extraction techniques, among others (38).

The lack of standardization in CMV QNAT testing limited the generation and implementation of widely applicable viral thresholds for preemptive therapy, disease prognostication and therapeutic monitoring. Hence, it is recommended that each transplant center should work with their clinical laboratories to define the relevant viral load thresholds for their clinical applications. In 2011, the WHO released the first International Reference Standard for the quantification of CMV nucleic acid, and laboratory and commercially developed CMV QNAT assays should now be calibrated to this standard. This may ensure uniformity in viral load reporting, thereby facilitating to define viral thresholds for various clinical applications (i.e. preemptive therapy, disease prognostication, therapeutic monitoring).

Recommendations for CMV diagnosis in SOT recipients

- Viral culture of blood and urine has limited clinical utility for prediction, diagnosis and management of CMV disease in adult patients (II-2).
- Serologic assays to detect CMV-IgM and IgG antibodies should not be used for the diagnosis of CMV disease (III).
- CMV QNAT or pp65 antigenemia should be used for rapid diagnosis of CMV disease (II-2).
- CMV QNAT or pp65 antigenemia should be performed once weekly for monitoring the response of CMV disease to antiviral treatment (II-2).
- CMV QNAT or pp65 antigenemia should be performed once weekly to predict risk of CMV disease, if preemptive therapy is used for CMV prevention (II-2).
- CMV QNAT assays should be calibrated based on the WHO International Reference Standard (III).
  - Studies should report CMV load in IU/mL using QNAT assays that have been calibrated to the WHO International Reference Standard (III).
- Patients suspected to have tissue-invasive CMV disease but with negative QNAT or pp65 antigenemia should have tissue biopsy and histopathology to confirm the clinical suspicion of CMV disease (III).

Prevention of CMV Disease

The approaches to CMV prevention inSOT recipients vary among different transplant populations and risk profile. The two major strategies for CMV prevention are: (1) antiviral prophylaxis and (2) preemptive therapy. A comprehensive review of these strategies has recently been published (1).

Antiviral prophylaxis is the administration of antiviral drug to all "at-risk" patients for a defined period after SOT. Preemptive therapy is the administration of antiviral drug only to asymptomatic patients with evidence of early CMV replication in order to prevent CMV disease. For preemptive therapy to be effective, SOT recipients are monitored at regular intervals (usually once weekly) for evidence of early CMV replication using a laboratory assay such as CMV QNAT or pp65 antigenemia. Although most centers employ either of these two major strategies for CMV prevention, others use a hybrid approach wherein short-term antiviral prophylaxis is followed by preemptive therapy during the period of CMV disease risk (41).

Antiviral prophylaxis and preemptive therapy have advantages and disadvantages (Table 1; Ref.21). Preemptive therapy may be associated with lower drug costs and adverse toxicities, but this is offset by the cost of laboratory testing and increased logistic coordination in order to obtain, receive and act upon results in a timely fashion. Preemptive strategy may therefore be a difficult approach for patients who reside at considerable distance from the transplant center. Due to a lack of QNAT standardization (38,39), there is currently no widely acceptable viral load threshold that can guide preemptive therapy. Antiviral

prophylaxis has the advantage of preventing reactivation of other herpes viruses, and has been associated with a lower incidence of indirect CMV effects (42,43). Meta-analyses have demonstrated that antiviral prophylaxis is associated with lower rates of allograft loss and opportunistic infections, and improvement in allograft and patient survival (8,42,43). However, antiviral prophylaxis is associated with late-onset CMV disease, particularly among CMV D+/R– patients (6–9,44). CMV drug resistance has been observed with both strategies (28–31,45,46).

There are only few randomized trials directly comparing preemptive therapy versus antiviral prophylaxis (47–50). These few studies, which were performed mainly in kidney recipients, demonstrate that both are similarly effective for CMV disease prevention. However, long-term graft survival was significantly higher with antiviral prophylaxis (48,49). The conduct of larger multicenter trials to assess the impact of CMV prevention strategies on indirect outcomes is warranted.

Antiviral prophylaxis

Antiviral drugs for CMV prophylaxis are valganciclovir and oral or intravenous ganciclovir. For kidney recipients, valacyclovir is an alternative. In selected patient populations (heart and lung recipients), immunoglobulin preparations are occasionally used as an adjunct in combination with antiviral drugs. Acyclovir should NOT be used for anti-CMV prophylaxis.

The efficacy of ganciclovir, valganciclovir and valacyclovir prophylaxis has been demonstrated in randomized clinical trials (6–9). Among them, valganciclovir is most commonly used for prophylaxis (6,9,51). In a randomized controlled trial of 372 CMV D+/R– kidney, liver, pancreas and heart recipients, CMV disease rate was comparable between patients who received 3 months of oral ganciclovir versus valganciclovir prophylaxis (17.2% valganciclovir vs. 18.4% ganciclovir at 12 months; Ref.6). The improved bioavailability of valganciclovir and its lower pill burden makes it the preferred drug for prophylaxis, even in liver recipients (52). Because of the concern for late-onset CMV disease with 3 months of antiviral prophylaxis in CMV D+/R– patients (6), a trial was performed to compare 200 versus 100 days of valganciclovir prophylaxis (9). In this study of 318 CMV D+/R– kidney recipients, the incidence of CMV disease was 16.1% versus 36.8% in the 200 days versus 100 days groups, respectively (9). Similar studies to assess the optimal duration in liver, heart and pancreas transplant recipients have not been performed, although many centers have already extrapolated these results in the prevention of CMV disease in liver, heart and pancreas recipients.

There are less data on CMV prevention in lung transplant recipients. Previous studies demonstrated that the rates of CMV viremia and disease are high with 3 months or short courses of antiviral prophylaxis (less than 6 months; Ref.53). Another study reported that the rate of CMV disease was significantly lower with at least 6 months of antiviral prophylaxis (54). In a recent multicenter trial, CMV D+/R– and CMV D+/R+ lung recipients that received 12 months of valganciclovir prophylaxis had significantly lower rates of CMV disease and CMV viremia (4% and 10%) compared to patients who received 3 months of valganciclovir prophylaxis (34% and 64%; Refs.55,56). Others have observed higher rates of CMV disease in CMV D+/R– lung recipients despite 12 months of antiviral prophylaxis, and have adapted an even longer course of antiviral prophylaxis (e.g. anticipated lifelong) in high-risk CMV D+/R– lung recipients (57). However, this was associated with significant myelotoxicity that required temporary or permanent discontinuation of valganciclovir prophylaxis (57). There is currently no good evidence to guide the duration of antiviral prophylaxis in intestinal and composite tissue allograft transplantation.

The efficacy of prophylaxis with either CMV immunoglobulin (CMV-Ig) or intravenous immune globulin (IVIg) in SOT recipients was suggested in a few trials (58,59). A pooled analysis of previous studies suggest that the addition of Ig preparations to antiviral prophylaxis may reduce severe CMV disease and mortality (60), but this finding has been debated (61). Hence, further research is needed to delineate the benefits of Ig preparation as an adjunct to antiviral prophylaxis.

Late onset CMV disease: The potential options for the prevention and management of late-onset CMV disease are:

(1) *Careful clinical follow-up with early treatment of CMV disease when symptoms occur.* SOT recipients (especially CMV D+/R–) should be advised of the risk of CMV disease upon discontinuation of antiviral prophylaxis and that they should immediately seek medical assistance when signs and symptoms of CMV disease occur. Clinicians should have a low threshold for considering CMV disease as a diagnosis in SOT patients presenting with compatible signs and symptoms.
(2) *Virologic monitoring after completion of antiviral prophylaxis.* Patients who completed antiviral prophylaxis should be monitored using pp65 antigenemia or QNAT periodically for a period of time. However, the optimal duration and frequency of CMV monitoring are not defined. In a few studies, this approach has poor sensitivity and specificity for predicting CMV disease in CMV D+/R– SOT recipients (62,63).
(3) *Prolong antiviral prophylaxis.* As discussed earlier, extending the duration of antiviral prophylaxis from 3 months to 6 months in CMV D+/R– kidney recipients (9) or 12 months (56) in lung recipients has resulted in further reduction in the incidence of CMV

Table 2: Antiviral drugs for CMV prevention and treatment in solid organ transplant recipients

Drug	Treatment[1]	Prophylaxis	Comments on use and toxicity
Valganciclovir	900-mg[2] p.o. twice daily	900 mg[2] p.o. once daily	Ease of administration Leukopenia is major toxicity
Oral Ganciclovir	NOT recommended	1 g p.o. three times daily	Low oral bioavailability High pill burden Leukopenia and risk of resistance development NOT recommended for preemptive therapy
IV Ganciclovir	5-mg/kg IV every 12 h	5 mg/kg IV once daily	Intravenous access and complications Leukopenia is major toxicity
Valacyclovir	NOT recommended	2 g p.o. four times daily	Use in kidney transplant recipients only NOT recommended for heart, liver, pancreas, lung, intestinal and composite tissue transplant recipients High pill burden High risk for neurologic adverse effects NOT recommended for preemptive therapy
Foscarnet	60 mg/kg IV every 8 h (or 90 mg/kg every 12 h)	NOT recommended	Second-line agent for treatment Highly nephrotoxic Used for UL97-mutant ganciclovir-resistant CMV disease NOT recommended for preemptive therapy
Cidofovir	5 mg/kg once weekly × 2 then every 2 weeks thereafter	NOT recommended	Third-line agent Highly nephrotoxic Used for UL97-mutant ganciclovir-resistant CMV disease NOT recommended for preemptive therapy

CMV-immune globulin has been used by some centers as an adjunct to antiviral prophylaxis, especially in heart and lung transplant recipients. The efficacy of this approach is debatable.
The doses of the antiviral drugs are for adults and should be adjusted based on renal function.
[1]These treatment doses are also recommended for preemptive therapy of asymptomatic CMV replication. Foscarnet, valacyclovir, oral ganciclovir and cidofovir are not recommended for preemptive therapy.
[2]Pediatric valganciclovir dose is mg = 7 × BSA × Creatinine clearance.

infection and disease (9,56). Data to support extending the antiviral prophylaxis beyond 3 months in CMV D+/R– liver, heart, and pancreas recipients do not yet exist, but many centers have extrapolated and adapted the clinical practice of prolonging antiviral prophylaxis in these patient groups.

Specific recommendations for antiviral prophylaxis:

- Antiviral prophylaxis can be administered to any at-risk SOT recipient to prevent CMV disease after transplantation. The antiviral drugs that can be used for prophylaxis are listed in Table 2. Specific recommendations for various organ recipients are listed in Table 3.
- Valganciclovir is the preferred drug for prophylaxis in adults (level of evidence varies from I-III depending on transplant type). The US FDA has cautioned against valganciclovir prophylaxis in liver recipients due to high rate of tissue-invasive disease compared to oral ganciclovir. However, many experts still recommend its use as prophylaxis in liver recipients (52). Alternative options are intravenous ganciclovir, oral ganciclovir, and for kidney recipients only, valacyclovir. Unselected IVIg and CMV Ig may also be used, but only as an adjunct to antiviral therapy in lung (II-2), heart (II-2) and intestinal (III) transplant recipients.
- In general, antiviral prophylaxis should be started as early as possible, and within the first 10 days after transplantation (I).
- The duration of prophylaxis vary depending on the CMV donor and recipient serologies and the transplant types.
- CMV-specific antiviral prophylaxis is not recommended for CMV D–/R– SOT recipients as long as they receive CMV-negative blood or leuko-depleted blood products (III).

Preemptive therapy

With preemptive therapy, SOT patients are monitored weekly for evidence of early CMV replication, which is then treated with valganciclovir or intravenous ganciclovir to prevent its progression to symptomatic disease (64). Preemptive therapy has the potential advantage of targeting antiviral therapy only to the highest risk patients and thereby decreasing drug costs and toxicity. An algorithm for preemptive therapy is depicted in Figure 1.

There is concern regarding the use of preemptive therapy in highest risk CMV D+/R– and lung recipients, due to the potential failure of once weekly surveillance in the face of rapid viral replication (35). Nonetheless, preemptive therapy has been shown to be effective for preventing CMV disease (50).

Table 3: Recommendations for CMV prevention in SOT recipients

Organ	Risk category	Recommendation/options (see Table 2 for dose and text for special pediatric issues)	Evidence
Kidney	D+/R−	*Antiviral prophylaxis* is preferred	I
		Drugs: valganciclovir, oral ganciclovir, intravenous ganciclovir or valacyclovir	
		Duration: 6 months	
		Preemptive therapy is an option (see Figure 1).	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg [1]p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
	R+	*Antiviral prophylaxis*	I
		Drugs: Valganciclovir, oral ganciclovir, intravenous ganciclovir or valacyclovir	
		Duration: 3 months	
		Preemptive therapy (see Figure 1).	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg[1] p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
Pancreas and kidney/pancreas	D+/R−	*Antiviral prophylaxis* is preferred	I (3-month prophylaxis)
		Drugs: valganciclovir, oral ganciclovir or intravenous ganciclovir	III (6-month prophylaxis)
		Duration: 3–6 months	
		Preemptive therapy is an option (see Figure 1).	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg [1]p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
	R+	*Antiviral prophylaxis*	II-2
		Drugs: Valganciclovir, oral ganciclovir or intravenous ganciclovir	
		Duration: 3 months	
		Preemptive therapy (see Figure 1).	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg[1]p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
Liver	D+/R−	*Antiviral prophylaxis* is preferred:	I (3-month prophylaxis)
		Drugs: valganciclovir (note FDA caution[2]), oral ganciclovir or intravenous ganciclovir	III (6-month prophylaxis)
		Duration: 3–6 months	
		Preemptive therapy is an option (see Figure 1).	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg [1]p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
	R+	*Antiviral prophylaxis*	I
		Drugs: Valganciclovir (note FDA caution[2]), oral ganciclovir or intravenous ganciclovir	
		Duration: 3 months	
		Preemptive therapy (see Figure 1).	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg[1] p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	

Continued

Table 3: Continued

Organ	Risk category	Recommendation/options (see Table 2 for dose and text for special pediatric issues)	Evidence
Heart	D+/R–	*Antiviral prophylaxis* is preferred. Drugs: valganciclovir, oral ganciclovir or intravenous ganciclovir. Some centers add adjunctive CMV immune globulin. Duration: 3–6 months *Preemptive therapy* is an option (see Figure 1). Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg[1] p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	I (3-month prophylaxis) III (6-month prophylaxis) II-2 (immune globulin)
	R+	*Antiviral prophylaxis* Drugs: Valganciclovir, oral ganciclovir or intravenous ganciclovir. Some centers add adjunctive CMV immune globulin. Duration: 3 months *Preemptive therapy* (see Figure 1). Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg[1] p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	II-2
Lung, heart–lung	D+/R–	*Antiviral prophylaxis* Drugs: valganciclovir or intravenous ganciclovir Duration: 12 months. Some centers prolong prophylaxis beyond 12 months. Some centers add CMV immune globulin.	I (12-month prophylaxis) II-2 (>12 months) II-2 (immune globulin)
	R+	*Antiviral prophylaxis* Drugs: valganciclovir or intravenous ganciclovir Duration: 6–12 months	II-2
Intestinal	D+/R–, R+	*Antiviral prophylaxis* Drugs: Valganciclovir or intravenous ganciclovir Duration: 3–6 months.	III
Composite tissue allograft	D+/R–, R+	*Antiviral prophylaxis* Drugs: valganciclovir or intravenous ganciclovir Duration: 3–6 months.	III

The above recommendations do not represent an exclusive course of action. Several factors may influence the precise nature and duration of prophylaxis or preemptive therapy.
Antiviral prophylaxis should be started as soon as possible, and within 10 days after transplantation. Preemptive therapy is NOT recommended for lung, heart–lung, intestinal and composite tissue allograft transplantation.
[1]Pediatric valganciclovir Dose is mg = 7 × BSA × Creatinine clearance.
[2]The US FDA has cautioned against valganciclovir prophylaxis in liver recipients due to high rate of tissue-invasive disease compared to oral ganciclovir. However, many experts still recommend its use as prophylaxis in liver recipients. CMV D–/R– SOT recipients do not require anti-CMV prophylaxis. Instead, CMV D–/R– should receive anti-HSV prophylaxis during the early period after transplantation (see chapter on HSV). If blood transfusion is required, CMV D–/R– SOT patients should receive CMV-seronegative or leuko-reduced blood products.

There is debate as to the optimal method for monitoring (pp65 antigenemia or QNAT), the viral load threshold to guide antiviral therapy, the duration of antiviral therapy, and the duration of laboratory monitoring (27). Either pp65 antigenemia or QNAT may be used for monitoring CMV replication (27). However, due to the current lack of standardized assays and reporting (as discussed earlier), site-specific and assay-specific viral load threshold values for initiation of preemptive therapy should be locally validated prior to institution of a preemptive protocol (34). The availability of a WHO CMV International Reference Standard, to which CMV QNAT assays should be calibrated to, should facilitate defining such clinically relevant thresholds. It is likely that such viral load thresholds may be specific for various risk groups, patient populations and immunosuppression-dependent. Clinical research to define these viral thresholds for initiation of preemptive therapy is encouraged.

Once pp65 and QNAT is positive above a defined threshold, treatment with oral valganciclovir (900-mg twice daily) or intravenous ganciclovir (5-mg/kg twice daily) should be initiated. In a clinical trial, viral decay kinetics was similar between valganciclovir and intravenous ganciclovir for preemptive treatment of asymptomatic CMV reactivation (65,66). Since preemptive therapy should treat low-level

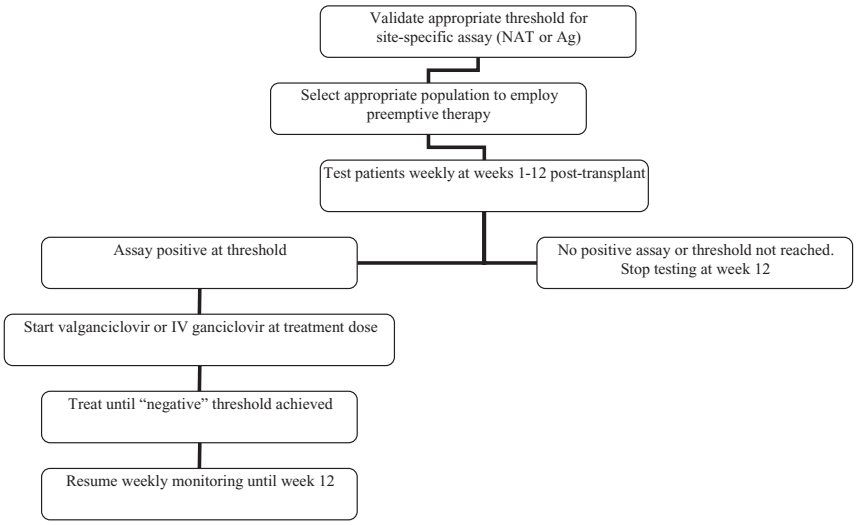

Figure 1: Suggested algorithm for preemptive therapy. CMV monitoring may be extended beyond 12 weeks in patients who remain severely immunocompromised, as assessed by the clinician.

asymptomatic viremia, experts recommend oral valganciclovir as preferable compared to intravenous ganciclovir for logistic issues.

Preemptive therapy recommendations:

- Preemptive therapy is effective for CMV prevention in patients at risk for CMV disease (I).
  - There is ongoing debate on whether preemptive therapy can be highly effective in high-risk populations. Many authorities prefer antiviral prophylaxis for D+/R– and lung transplant recipients while recognizing the clinical utility of preemptive therapy in CMV R+ kidney, liver, pancreas and heart recipients (Table3).
- The laboratory test for CMV monitoring is CMV QNAT or a pp65 antigenemia assay (II-2).
  - The recommended monitoring frequency is once weekly for 12 weeks after transplantation (II-2).
  - The viral load threshold for initiation of preemptive therapy remains center specific in the absence of standardized QNAT reporting system (II-2).
  - Future studies should define the clinically-relevant viral load threshold in IU/mL for the initiation of preemptive therapy (III).
- The recommended antiviral drugs for preemptive therapy are valganciclovir (900 mg twice daily) or intravenous ganciclovir (5 mg/kg every 12 h) (I).
  - Antiviral therapy should be continued until CMV DNAemia or antigenemia is no longer detectable (II-2). Many authorities recommend treating until two consecutive negative weekly pp65 antigenemia or QNAT testing has been attained (III).
  - Laboratory monitoring for CMV by QNAT or pp65 antigenemia is recommended once weekly during antiviral therapy (II-2).
- Further studies are required to determine the efficacy of preemptive therapy versus prophylaxis in reducing the indirect sequelae of CMV (III).

CMV prevention during ALA therapy and/or treatment of rejection

The use of lymphocyte-depleting therapy is a major risk factor for CMV disease especially when used for rejection treatment (22,67,68). The administration of intravenous ganciclovir was associated with lower incidence of CMV disease in kidney recipients receiving anti-lymphocyte antibodies (67,68).

Recommendations for CMV prevention with use of lymphocyte-depleting agents:

- Antiviral prophylaxis should be given to patients receiving antilymphocyte antibody therapy either as induction or for the treatment of rejection (I).
  - The optimal duration of antiviral prophylaxis is not known, but has been given for 1–3 months (II-2).
  - Options include valganciclovir (900-mg once daily) (III), oral ganciclovir (1-g p.o. thrice daily) (III) or intravenous ganciclovir (5-mg/kg every 24 h) (I).
- Alternative approach to CMV prevention in patients receiving antilymphocyte antibody therapy is a preemptive therapy protocol (III). See Figure 1.
- For patients treated for acute rejection with high-dose steroids, resumption of antiviral prophylaxis or a preemptive strategy may be considered (III).

Treatment of CMV Disease

The antiviral drugs for treating CMV disease are intravenous ganciclovir and valganciclovir (Table 2; Ref.66). Oral ganciclovir should NOT be used for treatment of CMV disease because its poor oral bioavailability will lead to insufficient systemic levels. Cautious reduction in the degree of immunosuppression should be considered in SOT patients presenting with CMV disease, especially if the disease is moderate to severe.

The efficacy of intravenous ganciclovir for the treatment of CMV disease has been demonstrated in numerous trials. The duration of therapy varied from 2 to 4 weeks, although recent data suggest that this should be based on clinical and virologic response (66,69,70). Valganciclovir achieves blood levels that are comparable to intravenous ganciclovir treatment, and has been used for the treatment of mild to moderate CMV disease. In a randomized controlled trial that compared 3 weeks of oral valganciclovir to intravenous ganciclovir for the treatment of CMV disease in 321 SOT recipients with mild to moderate CMV disease, both drugs had similar efficacy for the eradication of viremia at 21 days (66). In this study, there were many patients who remained viremic at day 21, suggesting that longer courses of antiviral therapy are needed in many patients (66).

The duration of antiviral therapy should be individualized based on resolution of clinical symptoms and virologic clearance (66,69–71). Generally, SOT recipients with CMV disease should be monitored once weekly using pp65 antigenemia or QNAT to assess virologic response. The risk of CMV relapse is lower among patients with undetectable CMV load at the end of antiviral therapy (69–71). Therefore, patients with CMV disease should remain on full therapeutic dose of antiviral therapy until CMV DNAemia or antigenemia has declined to undetectable levels or negative threshold value for a given test. The duration of treatment is therefore dependent on the sensitivity of the assay being used. An ultrasensitive assay may lead to a more prolonged treatment compared to less-sensitive assays (38,40,72,73). Standardization of CMV QNAT assays should facilitate the derivation of a clinically relevant viral threshold that is safe for discontinuation of antiviral therapy. Further research in this area is encouraged.

Summary recommendations for treatment of CMV disease

- CMV disease should be treated with intravenous ganciclovir (5 mg/kg every 12 h) (I) or oral valganciclovir (900 mg twice daily) (I).
  - Intravenous ganciclovir is the recommended initial treatment for severe or life-threatening CMV disease, those with high viral load, and those with questionable gastrointestinal absorption (I).
  - Oral valganciclovir is an effective initial therapy for mild to moderate CMV disease (I).
- Treatment of CMV disease should be continued until the following criteria are met (I):
  - Resolution of clinical symptoms, and
  - Virologic clearance below a threshold negative value (test specific; see text) based on laboratory monitoring with CMV QNAT or pp65 antigenemia once a week and
  - Minimum 2 weeks of antiviral treatment.
- Transplant recipients with CMV disease treated initially with intravenous ganciclovir may be switched to oral valganciclovir once there is adequate clinical and virologic control (III).
- Acyclovir and oral ganciclovir should NOT be used for treating CMV disease (II-2). Oral ganciclovir treatment of active CMV replication may lead to emergence of ganciclovir resistance (II-2).
- It is unclear whether addition of IVIg or CMV Ig to existing antiviral treatment regimens has a benefit but may be considered for patients with life-threatening disease, CMV pneumonitis and possibly other severe forms of disease (II-2).
- After completion of full-dose antiviral treatment, a 1–3 month course of secondary prophylaxis may be considered depending on the clinical situation (II-3). Alternatively, patients should have close clinical and/or virologic follow-up after discontinuation of treatment to assess the risk of relapse (II-2).
- Cautious reduction in immunosuppression should be considered in SOT patients presenting with CMV disease, especially if the disease is moderate to severe (II-2).

Ganciclovir Resistant CMV

Ganciclovir is not active *per se* against CMV unless it has been activated through a process of phosphorylation. The initial phosphorylation of ganciclovir is carried out by a kinase encoded by CMV gene *UL97*. Subsequent phosphorylation by cellular enzymes leads to the active ganciclovir-triphosphate, which competitively inhibits CMV DNA polymerase encoded by the viral gene *UL54*. Therefore, mutations in *UL97* and less commonly in *UL54* can confer ganciclovir resistance (32). The degree of resistance to ganciclovir by CMV *UL97* mutants depends on the site of mutation, which could confer either a low-level or high-level resistance (32). Combined mutations (*UL97* and *UL54*) often have high-level resistance to ganciclovir. Isolated *UL54* mutation (in the absence of *UL97* mutation) is rare (32).

Therapeutic options for ganciclovir-resistant CMV are limited. Because of limited antiviral drugs for treatment, it is highly recommended that the degree of immunosuppression be cautiously reduced. Foscarnet is often the first line for the treatment of *UL97*-mutant ganciclovir-resistant CMV (32). There are only a few studies of foscarnet use in SOT recipients; however, the majority of transplant recipients treated with foscarnet, either alone or in combination with ganciclovir, did improve (29,74–76). The major problem with foscarnet in transplant patients is significant nephrotoxicity (29,74–76). Cidofovir is another alternative for the treatment, although controlled studies in SOT recipients are not available. Cidofovir is highly nephrotoxic (29,74–76). Generally, ganciclovir-resistant CMV isolates with *UL97* mutations remains susceptible to foscarnet and cidofovir. Since ganciclovir, foscarnet and

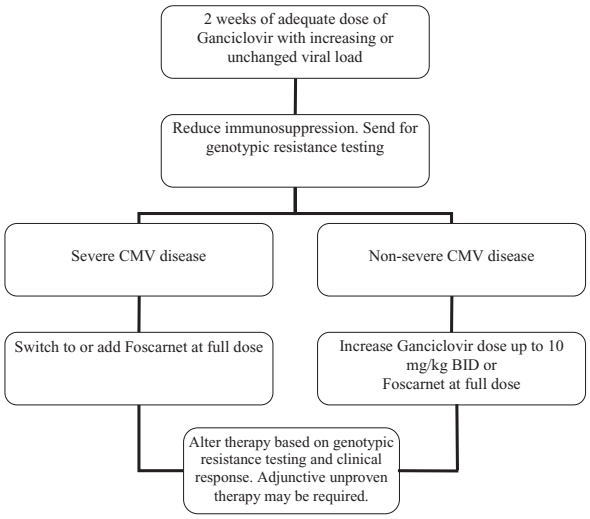

Figure 2: Algorithm for treatment of ganciclovir resistance.

cidofovir act by competitively inhibiting *UL54*-encoded CMV DNA polymerase, mutations in the *UL54* may result in resistance to any or all of these drugs depending on the site of the mutation. Treatment should therefore be guided by genotypic assays (32). Because of the complexity in the management of drug-resistant CMV disease, referral to clinical experts in the field for guidance may be warranted.

The incidence of ganciclovir-resistant CMV remains low (32). It was 1.9% in SOT patients who received 3 months of oral ganciclovir prophylaxis and 0% in patients who received 3 months of valganciclovir prophylaxis (77). The incidence of ganciclovir resistance may theoretically increase with prolonged antiviral administration, however, this was not significantly different between CMV D+/R– kidney recipients who received 3 months compared to 6 months of valganciclovir prophylaxis (28). Certain SOT subpopulations, such as lung transplant recipients, have higher rates of resistance (31,78). Risk factors for resistance include prolonged low-dose oral prophylaxis, D+/R– serostatus, increased intensity of immunosuppression and lung transplantation (79). Resistance has also been demonstrated in patients receiving preemptive therapy, where it was reported in 2.2% of patients (46). Resistance should be suspected if (1) the patient has received prolonged antiviral therapy, either as antiviral prophylaxis or preemptive therapy, (2) the viral load fails to decline or it increases despite 2 weeks of adequate dose antiviral therapy and (3) patients have other risk factors for resistance. Genetic resistance testing should be very helpful in managing resistant CMV. An algorithm for treatment of ganciclovir resistant CMV disease is presented in Figure 2.

Several investigational and off-label drugs have been used for the treating resistant CMV disease. Letermovir (AIC246), which inhibits CMV replication through a specific mechanism that targets viral terminase (80–82), has been used in a lung transplant recipient with CMV disease that was resistant to treatment with ganciclovir, foscarnet and cidofovir (82). An oral formulation of cidofovir, CMX001, is being investigated for the treatment of ganciclovir-resistant CMV disease (83). Another drug in clinical development is cyclopropravir, which is a DNA polymerase inhibitor with anti-CMV activity (84). Leflunomide and artesunate have been used off-label for treatment of a few cases of drug-resistant CMV disease (85,86). The clinical development of maribavir is uncertain due to disappointing results of clinical trials conducted in bone marrow transplant and liver transplant populations, although it has been used for the treatment of few cases of drug-resistant CMV disease (87). Finally, sirolimus and other mTOR inhibitors have been associated with a lower risk of CMV disease and may be a useful adjunct in the immunosuppressive management of SOT recipients with drug resistant CMV disease.

Recommendations for ganciclovir resistant CMV

- Patients who develop CMV disease after prolonged courses of ganciclovir or valganciclovir administration, either as prophylaxis or preemptive therapy, and those failing to respond to standard ganciclovir treatment should be suspected of having ganciclovir resistant virus. Genotypic testing for resistance should be performed, and this is preferred over phenotypic resistance testing (II-2).
- Immunosuppression should be cautiously reduced in patients with drug-resistant CMV disease (III).
  - Switch to sirolimus-containing regimen may be an option due to the reportedly lower risk of CMV disease in patients receiving mTOR inhibitors (III).
- Options for the empiric treatment of drug-resistant CMV disease include increasing the dose of intravenous ganciclovir (up to 10-mg/kg two times a day) or full-dose foscarnet (see Figure 2) (II-2). Definitive treatment should be guided by the results of genotypic testing (II-2).
  - Other therapeutic options are cidofovir or its new oral formulation that may be available for compassionate release (CMX001), compassionate release letermovir (AIC246), compassionate release maribavir, off-label leflunomide and off-label artesunate (III).
- CMV Ig may be used as adjunct to antiviral drugs (III).

Pediatric Issues

There are only limited data to support definitive recommendations for pediatric transplant populations with regards to CMV prevention and treatment. In addition, other issues such as prevention of EBV-related PTLD may be of primary importance, and may affect the choice of CMV strategies. Overall, proportionately more pediatric patients are at risk of primary and potentially severe CMV disease by virtue of being CMV-seronegative prior to transplantation. Although many donors for pediatric patients

will also be seronegative, the use of living-related or split deceased-donor organs (as in liver transplantation) may result in a marked higher frequency of D+/R– recipients. The following are recommendations specific to pediatric patients:

Pretransplant screening (pediatrics)

- Pediatric SOT recipients <18 months of age may have passively acquired maternal antibody, and hence CMV serology may not be reliable. In these patients, CMV culture of urine specimen should be performed (III). If urine CMV culture positive, the recipient is considered infected. If negative, assign the recipient serostatus based on the highest risk level for the purposes of CMV prevention (III). The role of urine CMV QNAT, instead of urine culture, in CMV risk assessment has not been fully investigated. For donors <18 months age, if the CMV serology is positive, the donor should be assumed as truly seropositive (II-2).

Prevention and treatment (pediatrics)

The principles and recommendations for the use of antiviral prophylaxis and preemptive therapy in adult recipients are generally applicable to pediatric recipients, with the following qualifying statements.

- Data are limited data regarding the efficacy of preemptive therapy in pediatric patients.
- Data are still limited on the appropriate dose and efficacy of oral ganciclovir and valganciclovir in children. Hence, treatment and prevention strategies continue to be primarily intravenous ganciclovir especially in younger children (II-1). However, oral valganciclovir may be used for prophylaxis and treatment in stable pediatric patients following an initial course of intravenous ganciclovir (III).
- The duration of intravenous ganciclovir treatment is influenced by the risk of catheter-associated bloodstream infections in some settings. The duration of antiviral prophylaxis is also influenced by other factors that vary across centers. These factors include the types of organ transplanted, the institution's experience with CMV disease in their patient population, immunosuppressive practices and the institution's consensus-driven EBV prophylaxis regimen (88) (III).
- There is no single standard of care as this relates to the optimal duration of prophylaxis. The duration of intravenous ganciclovir prophylaxis in major centers varies from a minimum of 14 days to 3 months (II-2).
- Treatment of CMV disease is with intravenous ganciclovir due to a lack of efficacy data of oral therapy in the pediatric population.
- CMV Ig is considered by some experts in combination with intravenous ganciclovir for the treatment of CMV disease in young infants and for treatment of more severe forms of CMV disease (III).
- Intravenous ganciclovir treatment of CMV disease in pediatric SOT recipients may be transitioned to oral valganciclovir in clinically stable patients with well-controlled viremia and clinical symptoms (III).

Future Research Directions

There are a number of areas that are being actively explored in basic, translational and clinical research fields related to CMV disease diagnosis, prevention and treatment. An urgent need that can now be realized is the derivation of clinically relevant viral load threshold that should guide risk stratification, preemptive therapy and therapeutic assessments. Clinical and commercial laboratories are encouraged to calibrate CMV QNAT assays based on the recently available WHO International Reference Standard. Studies using calibrated QNAT assays are encouraged to facilitate the derivation of much-needed viral load thresholds.

A number of in-house and some commercially available assays for the assessment of T cell immunity to CMV are being evaluated for their ability to predict the development of CMV disease (89–91). Recent studies have been promising, although more confirmatory tests are needed. It is hoped that these assays will allow better risk-stratification of patients and allow more targeted prevention strategies.

Large clinical trials that will compare antiviral prophylaxis and preemptive therapy remain lacking and should be encouraged in all SOT groups. To attain this, a multicenter collaboration would certainly be needed. An NIH-funded clinical trial comparing antiviral prophylaxis and preemptive therapy has started in five centers in the United States. Recent comparative trials conducted in a modest-sized cohort of kidney recipients demonstrate the potential for antiviral prophylaxis to offer benefits of better long-term allograft survival (47–50).

There are novel preventive and therapeutic options in the horizon. Several CMV vaccine candidates are being tested in early to midphase clinical trials (92). A recent CMV vaccine trial, based on the CMV glycoprotein B with MF59 adjuvant, was found to be highly immunogenic in phase II clinical trials, and was associated with lower rates of antiviral drug use and lesser degree of viremia among vaccines (92). Several novel antiviral drugs are in various stages of clinical development, including letermovir (AIC246), cyclopropravir, maribavir, CMX-001 and others (82,84,87). The successful clinical development of these drugs, some with unique mechanisms of action, will expand the therapeutic armamentarium for the prevention and treatment of CMV in SOT recipients. Finally, studies of CMV prevention and treatment are required for pediatric SOT recipients.

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Special Article

Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorder in Solid Organ Transplantation

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Abbreviations: ACVBP chemotherapy, (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone); ANZDATA, Australia and New Zealand Dialysis and Transplant Registry; ATP, adenosine triphosphate; BAL, bronchoalveolar lavage; CHOP, Cyclophosphamide, Hydroxydaunorubicin (also called doxorubicin or Adriamycin), Oncovin (vincristine), Prednisone or prednisolone; CMV, cytomegalovirus; CNS, central nervous system; CT, computerized tomography; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group; HHV6, human herpesvirus type 6; HIV, human immunodeficiency virus; IL6, interleukin 6; IVIG, intravenous immune globulin; LDH, lactate dehydrogenase; PET, positron emission tomography; PNCL, primary central nervous system lymphoma; PTLD, posttransplant lymphoproliferative disorder; RSST, risk stratified sequential treatment; SRTR, Scientific Registry of Transplant Recipients.

Introduction

Posttransplant lymphoproliferative disorder (PTLD) is recognized as potentially one of the most devastating complications of organ transplantation. The Epstein–Barr virus (EBV) genome is found in the majority (>90%) of B cell PTLD occurring early (within the first year) after solid organ transplantation The entity referred to as EBV-associated PTLD encompasses a wide spectrum of clinical conditions characterized by lymphoproliferation after transplantation, which may or may not be symptomatic. These syndromes range from uncomplicated infectious mononucleosis to true malignancies (1–3). Disease may be nodal or extranodal, localized, often in the allograft, or widely disseminated. PTLD may resemble a self-limited infection or be indistinguishable from non-Hodgkin's lymphoma. Lesions may be localized and progress slowly or the patient may present with a fulminant multisystem sepsis-like syndrome.

EBV is known to play a major role in the development of PTLD (4). The pathogenesis of these disorders is complex, and related to EBV's ability to transform and immortalize B lymphocytes, sometimes combined with secondary genetic or epigenetic events that occur during uncontrolled proliferation. Host and viral genomics affecting the response to EBV infection, local environmental factors including chronic antigenic stimulation, and the presence of other infections may impact outcome. Immunomodulation caused directly by EBV viral proteins, the coordinated effects of viral and cellular miRNAs (5) and exogenous immunosuppressive drugs alter the proliferative response and survival of infected cells (6,7) and the innate and adaptive immune responses, particularly the EBV-specific cytotoxic T lymphocyte (CTL) responses critical for controlling EBV infection.

Although B cell transformation and PTLD are a result of latent EBV infection, lytic EBV infection appears to be extremely important during primary EBV infection prior to the development of the CTL response (8). For a patient experiencing EBV infection for the first time in the early posttransplant period, delay in development of the immune response theoretically would prolong the one-way self-amplifying circuit of naïve B cell infection, latency in memory cells and reactivation with infectious virus production. The resulting high virion peak results in massive infection of the B cell pool and perhaps other cells not normally infected (T cells, NK cells, memory B cells), thereby setting the stage for secondary events that lead to malignancy. Although the role of EBV in EBV-negative PTLD is uncertain, recent data support the hypothesis that over time, immune escape occurs in initially EBV-driven lymphoproliferation, with cellular mutations replacing the functions of EBV oncogenes (9).

This document summarizes current recommendations and supporting data that guide the prevention, diagnosis and treatment of PTLD in the solid organ transplant recipient. The recent literature was reviewed, including recommendations for the diagnosis and management of PTLD that were published by notable groups (e.g. the British Transplantation Society [10,11]). Although the focus is largely on PTLD, relevant aspects of non-PTLD EBV syndromes are addressed, as appropriate.

Epidemiology

Humans are the only known hosts of EBV. In immunocompetent individuals, this virus is transmitted in the community by exposure to infected body fluids such as saliva. Although infection may also be acquired in the community by the traditional routes of transmission seen in immunocompetent patients, for solid organ transplant recipients, EBV that is transmitted from the seropositive donor organ is an important source of infection. Transmission is also possible when nonleukoreduced blood products are used. In the least affluent nations, greater than 90% of individuals are EBV-seropositive before the age of 5 years (12). However, in more affluent developed nations, this level of seropositivity is not attained until the fourth decade of life.

The diagnosis of PTLD requires tissue examination. In many settings tissue is not available or accessible. When laboratory evidence of EBV infection is present and other causes have been ruled out, investigators have used the term EBV "disease" to describe a number of clinical syndromes where EBV is believed to play a causative role.

Although the highest rate of PTLD in the solid organ transplant setting is seen in the first year after transplant, recent analyses suggest that the incidence of early PTLD is decreasing (13,14). However, cases occurring in the first year after transplant represent only one-fifth of the total cumulative 10-year post transplant PTLD burden (15). Analyses of both French and ANZDATA renal PTLD registries suggest a biphasic pattern of disease with a second peak occurring in years 7–10 after transplant after a period of reduced incidence in years 2–7. A significant proportion of late B cell PTLD is monomorphic and may be EBV-negative (~20%), with the relative proportion of EBV-negative lesions increasing over time after transplant; NK or T cell PLTD (approximately 37% are EBV positive) may also occur late after transplant (16). As transplant patient survival improves, late and EBV-negative PTLD will represent an increasing proportion of cases seen in adult populations. Although historically the median time of onset of primary EBV infection after solid organ transplantation is 6 weeks and reactivation/infection events were most often observed in the 2–3-month period after transplantation, recent studies in patients monitored serially using EBV viral load, note later initial detection of EBV DNAemia at a median of 110 days (17) and a mean of 276 days (18). PTLD incidence is also dependent on the type of organ transplanted, which may reflect immunosuppressive regimens, lymphoid load in the allograft and chronic antigenic exposure when organs directly communicate with the environment (8). Small intestine transplant recipients are at the highest risk for development of PTLD (up to 32%), while recipients of pancreas, heart, lung and liver transplants are at moderate risk (3–12%). Renal transplant recipients are at relatively low risk (1–2%). Recently, Caillard also described a temporal sequence of sites of PTLD involvement in adult renal allograft recipients, with disease localized to the graft occurring within the first two years, CNS disease occurring between years 2 and 7 and gastrointestinal disease occurring between years 6 and 10 and becoming the predominant site of late disease (13). Although PTLD in solid organ transplant recipients is most often of recipient origin (19), PTLD limited to the graft occurring early after transplant is predominantly donor in origin (20).

Table 1: Risk Factors for PTLD in solid organ transplant recipients

Early PTLD
Primary EBV infection
Type of organ transplanted
OKT3 and polyclonal antilymphocyte antibodies
Young recipient age (i.e. infants and young children)
CMV mismatch or CMV disease
Late PTLD
Duration of immunosuppression
Type of organ transplanted
Older recipient age (i.e. adults)

Contradictory/controversial evidence exists for the role of the following as risk factors for primary disease: Tacrolimus in pediatric recipients; HLA matching; certain cytokine gene polymorphisms; preexisting chronic immune stimulation; Hepatitis C infection; viral strain virulence (EBV1 vs. EBV-2 and LMP1 deletion mutants).

Risk Factors

The risk factors for the development of early (<12 months after transplant) and late PTLD (>12 months after transplant) in solid organ transplant recipients are shown in Table 1 (21–24). Analyses of risk factors for PTLD have used both smaller single center and larger registry datasets. Both approaches have limitations and often involve specific subsets of patients, adults versus children or specific allograft types. Many of the risk factors are interrelated and multivariate analysis is required to identify independent risk factors. Even using this approach, results are not always consistent (25). An overwhelming risk factor in most analyses is primary EBV infection, placing pediatric populations at higher risk of developing PTLD than their adult counterparts (14,26). Surprisingly, in a recent Collaborative Transplant Study database analysis, pretransplant EBV seronegativity in liver transplant recipients, unlike other allograft types, was not associated with an increased risk of developing non-Hodgkin's lymphoma. However, a subsequent analysis of the SRTR data in the United States confirmed that being EBV seronegative was a risk

factor for PTLD development even in liver transplant recipients (but less so than in kidney and heart transplant recipients) because of a higher baseline risk in seropositive liver transplant recipients (27). Individuals who are R+ are not devoid of PTLD risk, and account for up to 25% of PTLD cases in children (28). Intestinal transplant recipients who are EBV-seropositive remain at a high risk of PTLD. Although, PTLD rates increased after calcineurin inhibitors became the backbone of most immunosuppressive regimens in the 1990s, it is likely that the net state of immunosuppression, an entity difficult to measure, is a major risk factor. Attempts to quantify the risk associated with specific immunosuppressive agents used for induction or maintenance therapy have often led to inconsistent results (25,29). Antilymphocyte globulins that result in selective T cell depletion, particularly when used in high dose or repetitive courses, have historically been associated with increased PTLD risk. Among the newer biologic agents, alemtuzumab does not seem to be associated with an increased PTLD risk. Very high rates of PTLD presenting predominantly as primary CNS lymphoma were observed in renal transplant patients who received belatacept and were EBV seronegative prior to transplant, leading to prohibition of the use of this agent in this subset of patients (30–32). The duration of immunosuppression and older recipient age are risk factors for late PTLD development. This highlights the need for studies to optimize minimization of long term immunosuppression in individual patients including the accommodation of immunosenescence associated with aging in patients surviving for long periods after transplant. Cytomegalovirus infection may contribute to the net state of immunosuppression and is known to be a risk factor for PTLD.

Manifestations of Non-PTLD EBV Syndromes

Although the most feared EBV-associated disease after transplantation is PTLD, patients may experience non-PTLD-related disease. The features of this might include the manifestations of infectious mononucleosis (fever, malaise, exudative pharyngitis, lymphadenopathy, hepatosplenomegaly and atypical lymphocytosis), specific organ diseases such as hepatitis, pneumonitis, gastrointestinal symptoms and hematological manifestations such as leucopenia, thrombocytopenia, hemolytic anemia and hemophagocytosis. Some of these manifestations may be identical to the features of PTLD (Table 2). EBV-associated posttransplant smooth muscle tumors can occur *de novo* or after PTLD at a median interval of 48 months after transplant and develop earlier in children than adults. They can be of donor or recipient origin, and appear in atypical sites such as solid organs. When involving multiple sites, disease is multifocal rather than metastatic in origin (33). HHV6 reactivation may theoretically be an indirect cofactor for PTLD due to the potential for interaction with CMV (34).

Table 2: Presenting symptoms and signs in patients with lymphoproliferative disorder

Symptoms/complaints	Signs
Swollen lymph glands	Lymphadenopathy
Weight loss	Hepatosplenomegaly
Fever or night sweats	Subcutaneous nodules
Sore throat	Tonsillar enlargement
Malaise and lethargy	Tonsillar inflammation
Chronic sinus congestion and discomfort	Signs of bowel perforation
Anorexia, nausea and vomiting	Focal neurologic signs
Abdominal pain	Mass lesions
Gastrointestinal bleeding	
Symptoms of bowel perforation	

Manifestations and Diagnosis of PTLD

Clinical assessment

Relevant clinical information includes, but is not limited to the following:

- EBV serostatus of transplant recipient and donor.
- CMV donor/recipient serostatus.
- Time from transplantation to PTLD diagnosis.
- Type of allograft.

An adequate physical examination is required to detect the manifestations of PTLD, which may be quite nonspecific (Table 2). Given the predilection for the reticuloendothelial system to be involved, this clinical examination should include a meticulous assessment for lymphadenopathy and adenotonsillar hypertrophy. The general physical examination might elicit signs referable to the site(s) of organs affected by PTLD.

Laboratory tests

Blood tests (Non-EBV): Initial tests include a complete blood count with white blood cell differential. In the case of the latter, lymphopenia might suggest less overall CTL activity, which is essential in containing EBV-driven lymphoproliferation. In some patients with PTLD, there may be evidence of anemia, which is usually normochromic, normocytic, but may be hemolytic. In patients with gastrointestinal tract PTLD and occult bleeding over a prolonged period of time, there may be evidence of iron-deficiency anemia with hypochromia and microcytosis. The source of bleeding can be determined by performing additional testing, such as examination of the stools for occult blood. Thrombocytopenia has also been observed in non-PTLD EBV disease.

Depending on the location of PTLD lesions, there may be evidence of disturbances in serum electrolytes, liver and renal function tests. Elevations in serum uric acid and lactate dehydrogenase may occur. Serum immunoglobulin levels may be elevated as part of an acute phase reaction.

CMV infection status should be determined using CMV pp65 antigenemia assays, plasma or whole blood quantitative nucleic acid testing for CMV DNA as well as the examination of biopsy tissue for viral inclusions, CMV DNA or CMV antigens by immunohistochemistry.

Other adjunctive tests that might predict PTLD risk have been investigated. Promising initial results have been obtained for biomarkers that include serum 1L-6 (35), serum/plasma free light chains (36), serum sCD30 (37), serum CXCL13 (38) and host genetic polymorphisms particularly in cytokine genes (25) but require further validation. How these markers relate to each other and to EBV viral load in predicting PTLD risk should be the subject of future research.

Blood tests (EBV-related)

EBV serology: In immunocompetent patients, primary EBV infection can be determined by measuring EBV antiviral capsid antigen IgM and IgG antibodies, antibodies to early antigen (EA) and Epstein–Barr nuclear antigen. Persistence of anti-EA antibodies has been shown to be more likely in PTLD patients (39) and patients who are known to be seropositive before transplantation may have falling anti-EBNA-1 titers in the setting of elevated EBV loads and the presence of PTLD (40). Serology is unreliable as a diagnostic tool for either PTLD or primary EBV infection in immunocompromised patients, due to delayed or absent humoral responses. Another important drawback is that if these patients are receiving blood products, the passive transfer of antibodies may render EBV IgG antibody assays difficult to interpret. The most important role of EBV serology in the setting of transplantation is the determination of pretransplant donor and recipient EBV serostatus for PTLD risk assessment.

Detection of EBV nucleic acids or protein in tissue: Documenting the presence of EBV-specific nucleic acids in tissues is of value in the diagnosis of EBV-associated PTLD. RNA *in situ* hybridization targeting EBV-encoded small nuclear RNA (EBER; Refs.41,42) is the preferred approach and is more sensitive for detecting EBV-infected cells than *in situ* hybridization directly targeting viral DNA because EBERs are expressed at levels several orders of magnitude higher in infected cells. EBV latent or lytic antigens can also be detected in fixed tissues by immunohistochemistry using commercial antibodies directed against EBNA-1, EBNA-2 and LMP-1 or BZLF1, respectively (41,43) and used to document the presence of EBV although these techniques are less sensitive than *in situ* hybridization. Direct EBV DNA amplification from tissue is less useful as it does not allow cellular localization or differentiation of EBV in lesions from that present in passenger lymphocytes.

Viral load determination: The optimal way to perform, interpret and utilize quantitative EBV viral load assays for surveillance, diagnostic and disease monitoring purposes remains uncertain (44). In October 2011, the World Health Organization approved the 1st International Standard for EBV created by the National Institute for Biological Standards and Controls for calibration of the wide array of commercial and in house developed assays currently being used for EBV nucleic acid testing. This international reference standard should reduce the significant and extreme interlaboratory variability in both qualitative and quantitative viral load results previously documented (45,46). Until the impact of the standard on result harmonization among assays is validated, interinstitutional result comparison requires formal crossreferencing of assays between institutions. Data suggest that in most laboratories intralaboratory result reproducibility and result linearity over the dynamic range of the assay is reasonable. Therefore trends in patients over time within individual institutions using a single assay are valid and more useful than single values (45,46). Optimal extraction methods, gene targets and instrument platforms for EBV viral load assessments have not been determined. Although EBV viral load in whole blood and lymphocytes appears comparable and normalization of reporting units to cellular DNA does not change dynamic trending in individual patients (reporting IU/mL of whole blood is adequate), controversy with respect to preferred sample type (whole blood vs. plasma) remains and should be the focus of future research studies (47–49). Whole blood or lymphocyte EBV viral load monitoring is more sensitive than plasma for detection of early EBV reactivation. Although, generally, EBV DNA becomes detectable in plasma as EBV viral load rises in matched whole blood samples, the quantitative correlation between EBV viral load measured in whole blood or lymphocytes versus plasma is suboptimal.

Studies of the sensitivity and specificity of quantitative EBV viral load for the diagnosis of early PTLD and symptomatic EBV infection are limited (50–53). Pediatric populations have been the focus of many of these studies. Data from prospective studies targeting adult patients are limited (54,55). In high-risk asymptomatic solid organ transplant recipients being serially monitored, the use of EBV viral load as a diagnostic test (i.e. levels above a specific quantitative threshold being diagnostic of PTLD) has good sensitivity for detecting EBV-positive PTLD but misses EBV-negative, some cases of localized and donor-derived PTLD. However, it has poor specificity, resulting in good negative (greater than 90%) but poor positive predictive value (as low as 28% and not greater than 65%) in these populations. When used in the diagnostic context, this would result in significant unnecessary investigation of patients for PTLD.

Formal evaluation of EBV viral load assessments as a diagnostic tool using a single evaluation in patients presenting with symptoms and/or signs (usually mass lesions) with no history of recent or previous monitoring have not been carried out in populations at high risk for PTLD. In low-risk seropositive adult transplant recipients presenting for

investigation with signs and symptoms compatible with PTLD, high EBV viral load lacked sensitivity, understandably missing all cases of EBV-negative PTLD and some cases of localized EBV-positive PTLD, but was highly specific for EBV-positive PTLD (52). EBV viral load measured in plasma appears to improve the specificity of the test as a diagnostic tool for EBV-positive PTLD while not significantly lowering its sensitivity relative to assessments in cellular blood compartments (50–53,56). Preliminary data suggest that EBV viral load testing in samples other than peripheral blood, that is, broncoalveolar lavage (BAL) fluid or CSF may be useful. Among pediatric lung and heart lung transplant patients in whom the lung is often the primary site of PTLD, high quantitative levels of EBV load in BAL fluid may be a more sensitive predictor of PTLD than peripheral viral load assays (57). However, EBV DNA, often at high levels were detected in BAL fluid of adult lung transplant recipients in the absence of PTLD (58). Similarly, extrapolating from experience in HIV-infected patients, qualitative and quantitative EBV testing in CSF is performed to assist in the diagnosis of CNS lymphoma (59). However, further data regarding the sensitivity and specificity of testing in BAL and CSF are required in order to meaningfully interpret testing at these sites.

Adjunctive laboratory testing may improve the specificity of high viral load as a predictor of PTLD. The best studied and most promising are assays measuring T cell restoration or EBV-specific T cell responses (60). Although data suggest that the specificity and positive predictive value of EBV viral load can be significantly improved by using concomitant EBV-specific T cell ELISPOT and tetramer assays, these assays are complex, costly and difficult to implement in a routine diagnostic laboratory (10). Simpler rapid assays to measure global and EBV-specific T cell immunity using commercial ATP release assays (Cylex Immuknow and T Cell Memory) have undergone preliminary evaluation as adjunct markers of PTLD risk when combined with viral load testing in pediatric thoracic transplant recipients but require further validation (61). Viral gene expression profiling in peripheral blood as an adjunctive test of PTLD risk has been studied (62) and is still the subject of research. To date no distinctive pattern that is indicative of PTLD or PTLD risk has been demonstrated.

Radiographic imaging: Most centers employ a total body CT scan (head to pelvis) as part of the initial assessment of PTLD. Beyond this, the choice of tests depends largely on the location of suspected lesions and the historical sequence of prior radiographic testing. Many experts recommend that a head CT or MRI be included as part of the initial work-up, as the presence of central nervous system lesions will significantly influence treatment and outcome. CT scanning of the neck may help to define the extent of involvement or detect subtle early changes that necessitate biopsy to rule out PTLD. Depending on the location (e.g. CNS lesions), MRI may be a more suitable modality than CT scanning due to radiation concerns with CT scans and more precise lesion delineation with MRI.

Pulmonary lesions that are visible on chest radiographs may require high-resolution CT scanning for better delineation prior to biopsy. Furthermore, CT of the chest may reveal mediastinal adenopathy and small pulmonary nodules that are not visible on the plain chest radiograph. Suspected intra-abdominal lesions may be evaluated with ultrasonography and CT scanning. This is in addition to other modalities of assessment, including GI endoscopy in the case of intestinal hemorrhage, persistent diarrhea and unexplained weight loss, where necessary.

Positron emission tomography–computerized tomography (PET–CT) is emerging to be a useful test in the evaluation of PTLD (63,64), although additional data are needed on its utility across the known heterogenous spectrum of PTLD lesions. It may be more useful for monitoring response to therapy than for initial diagnosis. A major disadvantage is that the amount of radiation exposure is significantly greater than that associated with regular CT scans.

Histopathology: Pathology remains the gold standard for PTLD diagnosis (2,65). Although excisional biopsy is preferred, needle biopsy is acceptable when larger biopsies are impractical as in the case of allograft organ biopsy. The tissue specimen should be interpreted by a hematopathologist or pathologist familiar with histopathologic features of PTLD. Institutional protocols should be put in place to ensure that tissue is handled appropriately for ancillary diagnostic tests.

It is essential that reactive conditions such as plasma cell hyperplasia and infectious mononucleosis be clearly segregated in the classification process from potentially neoplastic lesions, which contain monoclonal elements. The Society for Hematopathology has published a working categorization of PTLD under the auspices of the World Health Organization (65) and is recommended for use (III). Table 3 summarizes the key features of this classification system. Intrinsic weaknesses are present in the purely histologic classification of PTLD. Additional pathologic tools have provided a better understanding of the pathogenesis of PTLD with the goal of developing more effective and more targeted therapy. Use of ancillary diagnostic tests identified as essential is strongly recommended if available (AIII). In addition to EBER and the detection of latent antigens as outlined previously, these tests are as follows:

- Immunophenotyping to determine lineage and therapy dependent markers (i.e. CD20) (essential).
- EBV clonality studies (rarely required/research).

Table 3: Categories of posttransplant lymphoproliferative disorder (PTLD)

Early lesions[1]
Plasmacytic hyperplasia
Infectious mononucleosis-like lesion
Polymorphic PTLD
Monomorphic PTLD
(classify according to the lymphoma they resemble)
B cell neoplasms
Diffuse large B cell lymphoma
Burkitt lymphoma
Plasma cell myeloma
Plasmacytoma-like lesion
Other[2]
T cell neoplasms
Peripheral T cell lymphoma, NOS
Hepatosplenic T cell lymphoma
Other[2]
Classical Hodgkin lymphoma-type PTLD

[1]Some mass-like lesions in the posttransplant setting may have the morphologic appearance of florid follicular hyperplasia or other marked but non-IM-like lymphoid hyperplasias.
[2]Indolent small B cell lymphomas arising in transplant recipients are not included among the PTLD.

- Molecular genetic markers of antigen receptor genes to assess clonality (useful).
- Donor versus recipient origin (useful).
- Fluorescent *in situ* hybridization or gene profiling by microarray to detect alterations in oncogenes, tumor suppressor genes or chromosomes (rarely required/research).

Recurrent PTLD may represent true recurrences (morphologically and clonally identical to the original tumor), PTLD in a more aggressive form or the emergence of a second primary tumor such as an EBV-associated posttransplant smooth muscle tumor. For this reason, biopsy of such recurrences is encouraged (III) (2).

Clinical staging of PTLD

No staging system currently exists for PTLD and no single system totally captures the full spectrum of what is classified as PTLD. Although the Ann Arbor staging has been used with the Cotswold's modifications, other staging approaches such as the Murphy system have been used in children (66). At the very minimum, staging should document the presence or absence of symptoms, the precise location of lesions, the involvement of the allograft and the presence of CNS involvement. Additional investigations such as a bone scans, a bone marrow biopsy and a lumbar puncture may assist in ruling out bone, bone marrow and CNS disease, respectively. In cases of EBV-positive PTLD documented by immunohistochemistry or *in situ* hybridization, an EBV viral load assay should be performed in order to better document the incidence and natural history of EBV viral load negative but EBV positive PTLD cases.

Prevention of PTLD

Although some centers employ chemoprophylaxis and/or preemptive strategies using EBV viral load as a surveillance tool, for the prevention of this complication, published data in the form of prospective controlled trials in support of these protocols are currently limited and the role of antiviral agents is controversial. Potential strategies for prevention are listed below.

General

Identification of patients who are also at risk of primary CMV infection or severe CMV disease or receiving antithymocyte globulin for induction or rejection would select a particularly vulnerable subgroup of recipients since these factors have been identified as risk factors for PTLD. Such patients should be monitored carefully for clinical symptoms/signs (fever, diarrhea, lymphadenopathy, allograft dysfunction, etc.) and investigated aggressively for PTLD. Allograft biopsies from these patients should be reviewed carefully for evidence of early PTLD. Wherever appropriate, immunosuppression should be minimized and aggressive immunosuppression should only be employed in the presence of biopsy proven acute rejection (65) (II-2). Because PTLD frequently presents with allograft dysfunction, it is important to make a pathologic diagnosis of rejection using standardized criteria and clearly distinguish early PTLD from rejection prior to the use of more potent antirejection therapy. The use of techniques to identify EBV-infected cells in tissues would be useful in this setting.

Antiviral prophylaxis

Chemoprophylaxis: Some centers have adopted antiviral prophylaxis as standard of care for high-risk patients (EBV D+R–). Although the antiviral agents, acyclovir and ganciclovir, have been employed as prophylaxis for the prevention of PTLD, data to support this are limited and a definitive recommendation regarding their use cannot be made at this time (I). Because CMV disease is a cofactor in PTLD development, if employed, the use of ganciclovir is preferable to acyclovir use (67). However, PTLD has been documented in patients receiving antiviral prophylaxis. Although a case-control study in renal transplant recipients suggest antiviral therapy may reduce PTLD risk (II-2) (67), analysis of the Collaborative Transplant Study database suggested that the use of antiviral drugs does not reduce the risk of posttransplant lymphoma (70). EBV load has been shown to progressively rise in some patients while patients were on ganciclovir prophylaxis (68). The impact of antiviral drugs on lytic virus could potentially decrease the recruitment of newly infected cells and the subsequent generation of latently infected memory cells, leading to a long term decrease in viral load measured in cellular blood compartments; these responses might not be readily apparent in the short term as assessed by EBV viral load monitoring. Antiviral therapy may have an indirect benefit on PTLD development by eliminating other viral infections which act

as cofactors in the lymphoproliferative process (III). However, these theoretical considerations remain unproven and there is currently no definitive evidence that such antiviral effects would be beneficial in preventing PTLD.

Immunoprophylaxis: Prospective randomized trials of CMV–IVIG, and ganciclovir plus CMV–IGIV, respectively have been inconclusive (68,69). An epidemiologic study by the Collaborative Transplant Group found that the use of anti-CMV IVIG reduced the incidence of non-Hodgkin lymphoma in kidney transplant recipients but only in the first posttransplant year (70). Thus, although prophylaxis with immune globulin may have some effect in reducing the short-term risk of PTLD, data are limited. At this time an all-encompassing recommendation of the utility of this approach cannot be made (I). Preventing EBV infection by vaccination is currently the subject of research (71). A phase I/II study indicated transient humoral immune response to an EBV recombinant gp350/alhydrogel vaccine among children with chronic kidney disease (potential transplant candidates; Ref.72).

Preemptive management

Since high viral load states often antedate the clinical presentation of PTLD, there are data to support quantitative EBV viral load monitoring for PTLD prevention in high-risk populations (50,53). Data to support this approach in populations at low risk of PTLD such as adult transplant recipients seropositive for EBV before transplant are lacking. Optimal monitoring frequency is uncertain. Since EBV viral load doubling times as short as 49–56 h have been documented, frequent (weekly) monitoring over the high-risk period has been recommended by some investigators. However, there are no data to suggest that less frequent monitoring (i.e. biweekly or at even longer intervals later in the first year after transplant) negatively impacts preemptive management. Weekly to biweekly monitoring over the first year after transplant is recommended, although this may be logistically difficult to implement over the entire period (II-3). There are insufficient data to support routine monitoring beyond the first transplant year. Data regarding the natural history of EBV viral load in transplant recipients in the absence of intervention are limited. This, along with lack of assay harmonization, prevents clear definition of "trigger points" that can be applied across all organ types that are predictive of PTLD development and at which preemptive intervention should take place.

Preemptive strategies in the solid organ transplant setting most commonly involve the use of reduction of immunosuppression and antiviral agents ± immune globulin (73) or the reduction of immunosuppression as the sole strategy (74). Some centers have reported a reduction in incidence of PTLD when routine viral load monitoring and these preemptive strategies were applied compared to historical cohorts (II-2). A retrospective study of EBV adult mismatched renal transplant recipients suggested that preemptive rituximab may have had an impact on PTLD development (75). The absence of a control group and the inability to differentiate between rituximab and the influence of viral load monitoring itself on immunosuppression management in this study precludes any firm conclusions regarding the efficacy of preemptive rituximab. More aggressive interventions involving the use of low dose rituximab (76) and adoptive immunotherapy (77) have been studied primarily in hematopoietic stem cell transplant recipients; some measure of success has been observed. Data regarding adoptive immunotherapy use in the solid organ transplant setting are more limited; proven efficacy remains uncertain (78,79) (II-3). Reduction in immunosuppression remains the best-validated preemptive strategy. Currently, there is insufficient evidence to recommend the use of either preemptive rituximab or adoptive immunotherapy for preemptive management (III).

Treatment of PTLD

The treatment of PTLD remains a challenge. Currently, there is no unifying consensus that dictates the specific treatment approaches that should be undertaken for all categories of patients. Controlled interventional studies are lacking. The general approach to therapy involves a stepwise strategy that starts with reduced immunosuppression, with plans for further escalation of treatment based largely on the clinical response and the histopathologic characteristics of the PTLD. Due to the highly specialized nature of the diagnosis, staging and treatment of PTLD, the initial evaluation and management of such patients should be done by or under the supervision of a tertiary transplant center and involve a multidisciplinary team that includes transplant physicians, oncologists and infectious disease specialists.

Reduction of immunosuppression

Over the past 25 years, reduction in immunosuppression has been a common initial approach to PTLD management, but reported response rates have been highly variable (0–73%), likely reflecting the heterogeneity and size of the populations studied and the nonstandardization of immunosuppression reduction. Among the largest studies examining this issue is a recent single center report that retrospectively analyzed outcomes in 67 adult solid organ transplant PTLD patients managed with a standardized approach to immunosuppression reduction alone as initial therapy (80). An overall response rate of 45% (37% complete response) was observed; patients who achieved complete remission had relapse rates of 17%. Although neither EBV-seronegativity nor B cell histologic subtype influenced outcome, bulky disease, advanced stage and older age predicted lack of response. Of concern were the high rates of acute rejection (32%) observed. It is unclear whether these data are applicable to pediatric populations who are more likely to experience PTLD in the context of primary infection. In patients who do not have rapidly

progressive disease and who lack predictors of poor response to immunosuppression reduction, reduction of immunosuppression to the lowest tolerated level is recommended as initial therapy for early and late B cell PTLD (II-3). The optimal strategy for immunosuppression reduction is uncertain and may be allograft specific, depending on the comfort of the physicians in risking acute rejection events. Suggestions for reducing immunosuppression based on expert opinion are outlined in the British Transplantation Society PTLD management guidelines (10). The period one should wait before proceeding to alternative therapeutic interventions is also uncertain. Most patients would be expected to show evidence of a clinical response to reduced immunosuppression within 2–4 weeks (81) but since the median time to failure in nonresponders was 45 days in the study by Reshef et al. (80), waiting up to 6 weeks in stable patients without evidence of progressive disease could be considered (II-3).

Surgical resection/local irradiation

Complete or partial surgical resection, as well as local radiotherapy, have been used as adjunctive therapy along with reduced immunosuppression (82). When surgical excision or radiotherapy has been used for localized disease, long-term remission in the absence of additional therapy has been observed (81,83). Surgery is an essential component of the management of local complications such as gastrointestinal hemorrhage or perforation (III).

Antiviral agents (acyclovir, ganciclovir)/passive antibody (IVIG)

Acyclovir and ganciclovir have been used in the management of early PTLD, alone or in combination with immune globulin (1,3,28). Currently, when antiviral agents are employed, the agent of choice is ganciclovir, as *in vitro* it is 10 times more active against EBV compared with acyclovir. The efficacy of this approach is uncertain and there is no evidence to support the use of antiviral agents in the absence of other interventions such as decreasing immunosuppression or anti-CD20 therapy (III). Arginine butyrate, a histone deacetylase inhibitor induces the lytic cycle of EBV, making EBV-infected cells sensitive to ganciclovir. A phase I/II trial of arginine butyrate combined with ganciclovir demonstrated overall response rates in 10 of 15 patients with EBV+ lymphoid malignancies; one third had PTLD (84). Unfortunately this agent is no longer available for use in clinical settings. Another chemotherapeutic agent, the proteosome inhibitor bortezomib, also induces lytic virus replication in EBV infected cells and is currently being evaluated in clinic trials of gamma-herpesvirus associated malignancies including PTLD (85).

Monoclonal B cell antibody therapy (Anti-CD20)

Although single agent rituximab, an anti-CD20 humanized chimeric monoclonal antibody, is rarely effective in the treatment of high grade B cell lymphomas in the immunocompetent patient, complete and sustained responses have been observed using this treatment approach in PTLD. Three prospective phase II rituximab monotherapy trials demonstrated a combined overall response rate of 55% (86) and in a large retrospective review early rituximab therapy improved progression free and overall survival (87). Gonzalez-Barca (88) reported complete response rates improving from 34.2% to 60.5% with a further four doses of rituximab in patients who achieved partial remission with the initial four doses. Although treatment is well tolerated, relapse is not infrequent after four courses of rituximab, with 25% of patients who had partial or complete responses showing evidence of disease progression by one year after treatment in one study (89). There is limited evidence to suggest that relapsed patients can be successfully retreated with single agent rituximab (90). Choquet proposed a prognostic score composed of age >60, Eastern Cooperative Oncology Group prognostic index of 2–4 and raised LDH that predicted survival after rituximab monotherapy and suggested that patients with one or more of these risk factors would benefit from rituixmab in combination with chemotherapy as initial therapy. In a prospective PTLD treatment trial of 4 weeks of rituximab therapy followed by four sequential cycles of rituximab/CHOP every 3 weeks (cyclophosphamide, doxorubicin, oncovin and prednisone) called sequential therapy, interim analysis suggested that response to the first 4 weeks of rituximab correlated with survival (86). An approach known as risk-stratified sequential therapy (RSST) is an alternate more tailored approach, whereby patients who achieved complete remission with an initial four doses of rituximab received a second course of rituximab without chemotherapy. Optimal number and timing of doses is unclear when this rituximab monotherapy approach is used. British guidelines suggest 8 weeks of rituximab (10); the future RSST trial proposed will use four additional courses or rituximab at three weekly intervals in patients who achieve complete remission after four initial weekly courses of rituximab (86). There is a growing body of evidence in support of the use of rituximab as the next step in the treatment of CD20+ B cell PTLD after reduction in immunosuppression in low risk patients who lack risk factors outlined by Choquet above (II-1). Potential adverse events include a tumor-lysis like syndrome, prolonged depletion of B cells with protracted hyprogammaglobulinemia, intestinal perforation, CMV reactivation, and progressive multifocal leukoencephalopathy. Although experience with the use of this agent is increasing, there is an ongoing need for data from prospective clinical trials.

Cytotoxic chemotherapy

In studies usually retrospective and involving a relatively small number of patients, cytotoxic combination chemotherapy, usually CHOP but also ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone) and ProMACE CytoBOM (mechlorethamine, doxorubicin, cyclophosphamide, etoposide, vincristine, prednisone, procarbazine, methotrexate, cytarabine, bleomycin) has been used to treat PTLD. Complete remission rates varying from 42–92% (87). Although this approach offers

better long-term disease control than rituximab monotherapy, treatment related mortality is high at 13–50%, usually from infectious complications. Outcomes in the largest prospective PTLD treatment trial, in which sequential treatment with rituximab and CHOP as described above was used in 74 adult patients with ECOG >2, have recently been reported (86). The overall response rate 90%, complete response rates 68%, and median response duration was >79.1 months in the 53 patients who responded. This was better than the response of rituximab monotherapy followed by chemotherapy at relapse, and the authors argue that this approach should be applied to all patients not responding to immunosupression reduction. However, CHOP associated treatment-related mortality at 11% predominantly related to infection was observed, suggesting that a more tailored approach that identifies patients who may sustained responses to rituximab monotherapy alone and avoids the toxicity of chemotherapy might be preferred. In pediatric populations, multicenter prospective studies using six cycles of low dose cyclosphosphamide and prednisone with and without rituximab after failure of initial therapy, most often reduction of immunosuppression have been reported (91,92). Response rates (67%, 69%) and relapse rates (19%, 8%) without and with rituximab, respectively were observed. Addition of rituximab therapy appeared to add efficacy to the management of fulminant disease which was not responsive to low dose chemotherapy alone. The use of chemotherapy should be considered after failure of reduction in immunosuppression in adults who have risk factors predicting poor response to rituximab monotherapy, patients who fail to achieve complete remission after initial rituximab therapy (II-1), and in the setting of T cell, Burkitt or Hodgkin lymphoma (III).

Other treatment modalities

Adoptive immunotherapy: Adoptive immunotherapy using donor derived cloned EBV-specific cytotoxic T cells has been used successfully for both the prevention and treatment of PTLD in allogeneic stem cell transplant recipients (76), but in the solid organ transplant setting experience is limited. Obstacles include the fact that PTLD lesions are usually of recipient origin in contrast to donor origin in the stem cell transplant recipient. Cost and time required to clone cell lines may also limit the utility of this approach. Although dramatic and sustained responses (52%) of PTLD, including CNS PTLD, that had failed conventional therapy including chemotherapy and rituximab, have been observed using HLA-matched unrelated donor EBV–CTL in a prospective multicenter trial, these biologic products are currently not readily available (93). Thus, additional research is needed to define the role of adoptive immunotherapy in the solid organ transplant setting and create the infrastructure, which might produce and distribute such products.

Immunomodulatory/Anticytokine therapy: Alpha interferon has both antiviral and antiproliferative activity, and additionally affects the host immune response via its activity as a T helper type 1-associated cytokine. Limited data in solid organ transplant recipients indicate that some patients may respond to alpha interferon in conjunction with a reduction in immunosuppression (94) (III). However, there are concerns that interferon therapy could precipitate rejection. Thus, this agent is no longer commonly employed in the treatment of PTLD and its place in the stepwise management of PTLD has been largely replaced by anti-CD20 monoclonal antibody. Anti-IL6 therapy has been explored in the treatment of early PTLD (95). Data are limited and additional research is needed.

CNS disease: Because CNS PTLD is a rare disease, clinical trial data and standardized management approaches that might inform optimal treatment approaches are lacking. Current recommendations that include the use of whole brain irradiation or high dose methotrexate as first line therapy rely heavily on the experience in immunocompetent patients with primary CNS lymphoma (PCNSL) (10,87,96). However, the former approach is associated with significant neurotoxicity particularly in older patients and when the latter approach is used, renal and hepatotoxicity can be difficult to manage in a transplant setting. The inability of rituximab to cross the blood–brain barrier has raised concerns that levels achieved with systemic use alone are unlikely to have clinical efficacy in CNS PTLD. However, Cavaliere (97) observed surprisingly good outcomes in seven of eight SOT recipients with PCNSL treated with primary rituximab monotherapy, often with reduction in immunosuppression in the absence or either chemotherapy or radiotherapy. Over the past decade there has been an increasing number of additional case reports in transplant recipients with PCNSL achieving complete remission using either standard or escalating doses of rituximab alone (98). Although high dose methotrexate or local radiotherapy should be considered as treatment options in patients with CNS disease who are able to tolerate therapy (II-3), in stable patients systemic rituximab therapy and initial reduction in immunosuppression might be considered as an initial therapeutic strategy (III).

Use of viral load to monitor response to PTLD therapy and predict relapse: Although data are limited, in the short term, PTLD patients with high viral load as well as those receiving preemptive therapy, often demonstrate a fall and clearance of viral load coincident with clinical and histologic regression in response to interventions that include reduction of immunosuppression and adoptive immunotherapy (93,99). In contrast, some clinicians have observed that when rituximab is used, viral load measured in cellular blood components fell dramatically and remained low even in the face of progressive disease and disease relapse (100,101).

In pediatric patients, particularly those experiencing primary infection after transplant, asymptomatic intermittent or persistent viral load rebound occurs frequently with no

short-term consequences. Adult PTLD patients have been observed to relapse in the presence of persistently low viral load (101). However, recent data suggest that the sample type may influence the usefulness of viral load testing to monitor treatment response and predict relapse as plasma monitoring appears to correlate better with treatment response and relapse than monitoring in the cellular compartment (54,102). Further studies to confirm this observation are required (54,102).

A significant number of transplant recipients who experience primary EBV infection or EBV-positive PTLD have sustained elevation of EBV viral load after asymptomatic infection or resolution of EBV disease or PTLD (chronic high load carriers). The pathogenesis of this state is unknown. The detectable viral load appears to be predominantly in memory B cells with type 0 gene expression (103–105). Recent studies in thoracic pediatric chronic high load carriers suggest that these patients have high frequencies of activated but functionally exhausted EBV-specific cytotoxic T cells exhibiting unexpected immunopolarization. Whether this exhausted immune phenotype is also present in nonthoracic transplant recipients with chronic elevations in viral load and how this immune phenotype relates to PTLD risk is uncertain. Although a study in pediatric thoracic transplants suggest that patients who are chronic high viral load carriers (105) may be at significantly increased risk of late onset EBV-positive PTLD (106), this risk appears in part to be organ-specific with intermediate risks observed in intestinal transplants (107) and low risk in pediatric liver transplant patients from the same center (108). However, even among specific allograft types such as pediatric liver transplant recipients, reported long-term risks differ among centers (109,110). Additional data from prospective studies are required to determine allograft-specific long-term risks, the pathogenesis and evolution of this phenotype in relationship to PTLD risk in order to guide patient management and the usefulness of ongoing viral load monitoring in this setting.

Prognostic Indicators of PTLD

Several variables have been identified as indicators of prognosis in the management of PTLD. The extent to which findings can be generalized across centers is limited by the absence of a standardized approach to the pathologic diagnosis and treatment of PTLD. Table 4 summarizes some factors that have been associated with poorer outcomes.

Table 4: Factors associated with poorer outcomes from PTLD

Poor performance status
Multisite disease
Central nervous system disease
T or NK cell PTLD
Spindle cell PTLD
EBV-negative PTLD
The abnormal cells leading to PTLD of recipient origin as opposed to donor-origin
Coinfection with hepatitis B or C
Monoclonal disease
Presence of mutation of proto-oncogenes or tumor suppressor genes

Prognostic factors not always consistent among studies.

Summary of Key Recommendations/Statements

(1) Primary EBV infection and high or repetitive doses of antilymphocyte globulin represent the best-documented risk factors for the development of early PTLD (II-2).

(2) EBV serostatus should be determined on all transplant recipients and donors in order to identify the patients at high risk for PTLD development. Seropositive candidates <18 months of age should be considered seronegative for purposes of risk stratification (II-2). Patients seronegative prior to transplantation should be rescreened while on the waitlist and yearly after transplant to determine ongoing susceptibility to primary infection (III).

(3) The establishment of an international standard for EBV viral load assessment should reduce interlaboratory variability in reported results; this requires validation. In the interim, formal cross-referencing is required for interinstitutional result comparison (II-2). Serial monitoring of high risk (usually seronegative recipients) with EBV viral load as part of preemptive strategies for PTLD prevention is the best validated use of these assays (II-2); monitoring of low risk seropositive populations is not routinely recommended (II-3). The clinical benefit of EBV viral load assays for monitoring response to therapy, predicting relapse and for disease diagnosis is uncertain. Results obtained in these settings should be interpreted with caution; interpretation may be sample type dependent (II-3).

(4) Histopathology remains the gold standard for the diagnosis of PTLD (III).

(5) Antivirals ± immune globulin are sometimes employed as EBV prophylaxis after transplantation among EBV D+R– patients. There is insufficient evidence to support or refute this strategy (I). Where employed, a prophylaxis strategy similar to that for CMV may be considered (III).

(6) The use of preemptive strategies in high-risk populations may lower PTLD incidence rates; reduction in immunosuppression is the best documented intervention strategy (II-2). There are insufficient data to determine the efficacy of other intervention strategies such as antivirals, anti-CD20 antibody or adoptive immunotherapy (III).

(7) Additional data from prospective studies are needed to determine the significance of chronic, sustained elevations of EBV loads after transplantation (III).

(8) In patients who do not have rapidly progressive disease and who lack predictors of poor response to immunosuppression reduction, reduction of immunosuppression to the lowest tolerated level is recommended as initial therapy for early and late B cell PTLD (II-2). Other modalities of therapy depend in part of on the histopathologic characteristics of PTLD and location of lesions.
(9) In adult patients with PTLD, rituximab therapy should be considered as the next step in the treatment of CD20+ B cell PTLD after reduction in immunosuppression in patients who lack risk factors that predict rituximab failure (II-1).
(10) The use of chemotherapy should be considered for PTLD treatment after failure of reduction in immunosuppression in patients who have risk factors predicting poor response to rituximab monotherapy, patients who fail to achieve complete remission after initial rituximab therapy (II-1), and in the setting of T cell, Burkitt or Hodgkin lymphoma (III). Treatment of CNS disease requires special consideration (III).

Future Research Priorities

It is clear that several areas relating to EBV infection in the setting of transplantation are in need of further research. Additional research or consensus is needed to address and to enhance the levels of evidence for or against different aspects of the diagnosis, prevention and treatment of PTLD. A list of potential research targets include, but are not limited to the following:

(1) Understanding the pathogenesis of the full spectrum of PTLD.
(2) Standardization of the format used to report PTLD incidence trends.
(3) EBV vaccine evaluation for transplant candidates.
(4) Evaluation and standardization of EBV viral load measurement.
(5) Optimal use of antiviral ± immune globulin in patients at risk of EBV diseases posttransplantation.
(6) Enhancement of screening/diagnostic strategies to enhance the early detection of PTLD, beyond the use of viral load testing.
(7) Controlled trials of preemptive management modalities, including role of reduced immunosuppression with/without rituximab.
(8) Prospective studies of the significance of chronic viral load carriage.
(9) Continued research on optimal treatment for specific categories of PTLD, include the specific chemotherapy regimens with/without rituximab.
(10) Factors influencing susceptibility to EBV and EBV-related outcomes, including host and viral genetic variation.

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Special Article

Herpes Simplex Virus in Solid Organ Transplantation

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Key words: Prevention, transplantation, treatment

Abbreviations: BAL, broncheoalveolar lavage; CMV, cytomegalovirus; CSF, cerebrospinal fluid; DFA, direct florescent antibody; EBV, Epstein-Barr virus; GFR, glomerular filtration rate; HSV, herpes simplex virus; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction; SOT, solid organ transplant.

Herpes Simplex Virus (HSV) 1 and 2

Epidemiology

Herpes simplex virus type-1 and 2 (HSV-1, HSV-2) are α-herpesviruses which establish latency in nerve root ganglia. Infection with HSV-1, the classic oro-labial herpes virus, is acquired from early childhood through adulthood, with prevalence in the United States of 44% in 12–19 year olds and approximately 80% by the age of 60 (1). Associated primarily with genital herpes, HSV-2 has a seroprevalence that increases rapidly at the age of sexual debut, infecting 1.6% of persons aged 14–19 years and 26.3% of persons aged 40–49 years in the United States (2). In recent years, HSV-1 is an increasing cause of genital lesions, though typically with less frequent recurrences (3,4). Most adult transplant patients are infected with HSV-1 or HSV-2, or both, with prevalence similar to the distribution by age in the general population.

A minority of immunocompetent persons infected with HSV develop symptomatic lesions; however, most will shed virus on mucosal surfaces (5). Compared with immunocompetent persons, solid organ transplant (SOT) recipients shed virus more frequently, have more frequent and severe clinical manifestations of HSV (6,7), and may be slower to respond to therapy. Most symptomatic HSV disease in adult transplant recipients results from reactivation of previously acquired virus, particularly early after transplantation and in the setting of antirejection therapy (8–10). Primary infection from the allograft is rare but described in liver, kidney and other organ transplant types (10–13). Patients may present early after transplant with a fulminant course with hepatitis and poor outcome. HSV seronegative SOT recipients may also acquire HSV from intimate contacts.

The most common clinical presentation of HSV is orolabial, genital or perianal disease (8,9). Lesions can be classic vesicular and/or ulcerative and may extend locally. Visceral or disseminated disease can occur, including disseminated mucocutaneous disease, esophagitis, hepatitis and pneumonitis (14,15). Fever, leucopenia and hepatitis are the most common presenting signs of disseminated disease. Pneumonitis is described in recipients of all organ types, but is most common in heart–lung transplant recipients (15). Rarely, visceral disease may occur in the absence of cutaneous or mucosal findings.

Keratitis (infection of the cornea) is the most common manifestation of HSV in the eye (16). Keratitis presents in a variety of pathophysiologic entities. Superficial infection has historically been thought to result from HSV infection in the trigeminal nerve. However, other, pathology may be the result of deeper infection of corneal tissues (e.g. stromal keratitis) with resultant inflammatory reaction and/or immune mediated responses to remaining antigen (17).

Risk factors

Recipient HSV IgG serostatus should be determined prior to transplant (II-1). It should be noted that there is limited utility in testing infants and children in the first 6–12 months of life when they may still harbor maternal antibodies. HSV seropositive recipients are at risk of clinical reactivation posttransplant in the absence of antiviral prophylaxis even if they had not had prior clinical HSV disease. The incidence of clinically apparent HSV disease in HSV-seropositive adult transplant patients who are not receiving antiviral prophylaxis ranges from 35% to 68% (9,10,18). Because severe HSV disease can occur in HSV-seropositive or in seronegative persons who newly acquire the infection, HSV infection should be considered in the differential diagnosis of clinically appropriate syndromes regardless of serostatus prior to transplantation. Knowledge of serostatus may be important to determine the possibility of primary HSV acquisition, either from the allograft or from natural sources after transplant, which may be more clinically severe and prolonged due to lack of immunologic memory (19,20).

Table 1: Laboratory methods for diagnosis of HSV

Test	Advantage	Disadvantage
Direct fluorescent antibody (DFA)	• Rapidly available • Virus-specific	• Lower sensitivity than PCR • Limited sample types (needs cells to stain; e.g. not CSF)
PCR	• Most sensitive • Done on most sample types	• Not available at all centers • Positive result, other than in CSF, requires interpretation
Culture	• Type-specific • Able to isolate virus for drug susceptibility testing	• Takes longer • Less sensitive, only ~25% of PCR positive depends on level of virus (Ref. 23)
Tzank smear	• Rapid • Direct visualization	• Requires experience • Nonspecific (HSV or VZV)
Histopathology with immunohistochemistry	• Can prove tissue-invasive disease	• Samples more difficult to acquire • Long turnaround
Serology	• Useful to guide pretransplant risk stratification and prevention	• Not useful posttransplant, insensitive marker for acute infection • False-positive IgM with HSV reactivation

The incidence of HSV reactivation with specific immunosuppressive regimens has not been formally assessed. Historically, use of anti-CD3 antibody muromonab (OKT3) and mycophenolate mofetil have been associated with an increased risk of HSV reactivation after transplantation (10,21,22). There have been no evaluations to date comparing different induction regimens (T cell depleting agents such as rabbit-antithymocyte globulin or alemtuzumab vs. nondepleting agents such as basiliximab or daclizumab) or maintenance immunosuppressive regimens with regards to HSV reactivation rates. However, there are some data to suggest use of the mTOR inhibitors (e.g. rapamycin) with reduced calcineurin inhibitor exposure leads to reduced herpes virus infections (23,24).

Diagnosis (Table 1)

Although most patients present with typical orolabial and genital lesions, HSV in immunocompromised hosts may be atypical, thus, laboratory confirmation may be helpful. HSV grows well in tissue culture so that most isolates are identified within 5 days. Timing of sampling is important with mucocutaneous lesions: for example, sampling of genital lesions >5 days old had a yield of less than 35% (25). Direct fluorescent antibody (DFA) testing of mucocutaneous lesions, bronchoalveolar lavage (BAL) and other clinical samples, can provide rapid results. Compared with virus isolation the sensitivity has been reported between 60% and 75% and specificity of 85–99% (25–27). Polymerase chain reaction (PCR) assays are up to fourfold more sensitive than tissue culture for diagnosing mucocutaneous HSV and have replaced viral culture as the preferred diagnostic test (28–32), culture and DFA remain options for mucocutaneous lesions. The use of PCR in cerebrospinal fluid to diagnose HSV encephalitis is the diagnostic test of choice, with a sensitivity of 98% and specificity approaching 100% (33). HSV DNA is also detected in the blood of immunocompetent patients with primary ulcerative infection (34) and in those with significant reactivation disease (34,35); however, the clinical significance of finding HSV DNA in the blood outside of patients with clinical syndromes consistent with disseminated disease has not been well established (36). Tissue histopathology with immunocytochemistry for HSV, can be helpful and is recommended to confirm a diagnosis where PCR or other tests (e.g. culture) may represent contamination from another site (e.g. BAL contaminated from oropharynx). Serologic testing is rarely useful for diagnosing acute infections as most patients will be HSV seropositive and IgM positivity in HSV infection may indicate reactivation and not new acquisition. Nevertheless, serology (by IgG) is useful to acquire pretransplant for appropriate posttransplant risk stratification.

Diagnosis of HSV keratitis remains primarily a clinical diagnosis based on characteristic features of the corneal lesion on slit lamp microscopy. Referral to an ophthalmologist is requisite for appropriate diagnosis and treatment of HSV ocular disease (Table 1).

Prevention

Currently, many transplant recipients receive antiviral medication to prevent CMV replication (see CMV guidelines). Ganciclovir (Grade I for HSV prevention), acyclovir (I), valacyclovir (I) and valganciclovir (III), prevent most HSV replication when given in standard doses for CMV prevention. HSV-specific prophylaxis should be considered for all HSV-1 and HSV-2 seropositive organ recipients not receiving antiviral medication for CMV prevention (Grade I). Some centers use EBV-specific prophylaxis in pediatric transplant recipients not receiving prophylaxis for CMV infection. The antivirals used for EBV prevention also likely prevent HSV reactivation, so additional prophylaxis may not be necessary (Grade III). In the unusual circumstance of a patient who is not receiving CMV antiviral prophylaxis and is also HSV seronegative, the risk of early posttransplant HSV infection is not well defined, though probable cases of HSV transmission from organs have been described (11). In this setting, some clinicians may choose to give antiviral prophylaxis while others may consider close clinical monitoring (Grade III).

Immunosuppression intensification for organ rejection has been associated with HSV recurrence, though usually not life threatening. Limited data suggest that prophylaxis during rejection episodes treated with OKT3 is effective (21);

and the utility of HSV prophylaxis is likely similar for other types of immunosuppressive regimens (Grade II-2). Patients may also be receiving antivirals for CMV prophylaxis during treatment of rejection so HSV-specific prophylaxis may not be required.

Unfortunately, a vaccine to prevent primary HSV infection has been elusive, therefore current prevention techniques are focused on behavioral and antiviral methods to prevent acquisition of HSV. Seronegative transplant recipients should be counseled regarding the risks of HSV-1 and HSV-2 acquisition. It is important to avoid contact with persons with active lesions as these patients are most infectious (Grade III). However, persons may acquire HSV from asymptomatic individuals so care should be taken in intimate contact, particularly during periods of most intense immune suppression (Grade III). Condoms may be effective, but do not completely protect against HSV transmission (37). A majority of persons infected with HSV have never had symptomatic lesions, so the virus may be acquired from persons who have never had lesions. Where appropriate, HSV-2 seronegative transplant recipients in new sexual relationships should consider having their partner tested for HSV-2 (Grade III). In serodiscordant couples, daily antiviral therapy taken by the seropositive partner has been shown to prevent HSV-2 transmission to the seronegative partner (38), so this may be considered as an option (Grade III), but has not been evaluated in the SOT population. There are no controlled studies looking at the efficacy of postexposure prophylaxis to prevent HSV acquisition so it is not routinely recommended.

Antiviral dosing for prophylaxis (Table 2): The only randomized trials of HSV prophylaxis in SOT recipients were published in the 1980s and showed effective HSV suppression with acyclovir administered at doses of 200 mg three (9) or four (8) times a day. In a meta-analysis comparing these regimens with higher doses of acyclovir and valacyclovir for CMV prevention, HSV was well suppressed at all evaluated doses of acyclovir, with no difference between these "low-dose" (<1 g/day) and the higher dose regimens (39); Table 2). In this meta-analysis, the use of acyclovir resulted in a significant reduction (OR, 0.17; 95% CI, 0.12–0.24; $p < 0.001$) in HSV disease (39).

Compared with these initial HSV prevention trials in SOT, higher doses of acyclovir administered less frequently (e.g. 400–800 mg 2×/day) have been shown to be safe and effective in other similarly immunocompromised populations (e.g. hematopoietic stem cell transplant, HIV), and are recommended for SOT recipients due to their safety and ease of administration (Grade II). Because SOT-specific studies have not been done, the level of evidence reported herein is extrapolated from studies performed in populations of other patients with similar levels of immune compromise (40–42). Patients with a history of frequent severe clinical HSV reactivations prior to transplant should be given doses in the higher range (Grade III). Valacyclovir given twice daily was found to be superior to once daily when used as prophylaxis against HSV in immunocompromised patients so once daily administration is generally not recommended (43). Dosage adjustment for renal insufficiency is necessary if GFR is <50.

Famciclovir, the oral prodrug of penciclovir, is also effective in preventing recurrent HSV in immunocompromised and immunocompetent hosts (44,45) and is an alternate option for prophylaxis.

HSV prophylaxis in pediatric patients is not universal. Dosing for seropositive patients or patients who have had prior occurrences is derived from studies of HIV positive and stem cell transplant recipients. For children ≥2 years of age requiring oral therapy a typical quantity is 600–1000 mg/day in 3–5 divided doses. For intravenous therapy, 5 mg/kg every 8 h is recommended (46).

Duration of prophylaxis: The majority of severe HSV disease occurs within the first month after transplant (9), so antiviral prophylaxis should continue for at least a month (Grade I). In addition, resumption of prophylaxis may be considered for patients being treated for rejection (with T cell depleting agents) (Grade III). For patients receiving CMV antiviral prophylaxis (typically continued for ≥100 days), additional HSV prevention is not necessary. In patients who experience bothersome clinical recurrences (≥2) after discontinuation of antiviral therapy, suppressive antiviral therapy should be continued until such time as the level of immunosuppression can be decreased (Grade I). Of note, suppressive therapy can be safely continued for many years and is associated with less frequent acyclovir resistant HSV than episodic therapy in immunocompromised patients (41), and thus is the preferred approach (Grade III). If cessation of prophylaxis is unsuccessful, then lifelong suppressive therapy may be necessary (Grade III).

Treatment (Table 2)

Disseminated, visceral, or extensive cutaneous or mucosal HSV disease should be treated with intravenous acyclovir (Grade II-1) at a dose of 5–10 mg/kg every 8 h (11,14,42,47,48). Mucocutaneous disease in the immunocompromised patient can be treated with the lower dose of 5 mg/kg. When there is a concern for disseminated, visceral or cerebral involvement doses of up to 10 mg/kg every 8 h should be initiated (with adjustment for reduced GFR) (Grade II). Rapid initiation of acyclovir therapy is associated with improved outcome for HSV disease in transplantation (11), and can be life-saving in cases of HSV hepatitis or dissemination. Reduction in immunosuppression should be considered for life-threatening HSV disease (Grade III). More limited mucocutaneous disease can be treated with oral acyclovir (I), valacyclovir (I) or famciclovir (I). Therapy should be continued for minimum of 5–7 days or until complete healing of the lesions depending on the clinical circumstances. Therapy in severe disease (e.g. encephalitis) should be continued for a minimum of 14 days (Grade III) although some clinicians favor longer courses up to 21 days (49–51).

Table 2: Recommendations for HSV prevention and treatment in HSV seropositive solid organ transplant recipients

Indication	Treatment	Evidence	Comments
Prevention			
Adult:	CMV prophylaxis[1] or	Grade I	• Administer for at least 1 month
	ACV 400–800 mg p.o. 2×/day	Grade I	• During treatment of rejection episodes (for at least 1 month)
	VACV 500 mg p.o. bid	Grade I	
	FCV 500 mg p.o. bid	Grade I	
Pediatric:	ACV 30–80 mg/kg p.o. in 3 divided doses	Grade III	• For recurrent infection: Lower doses for recurrent labialis, higher doses for recurrent genital or ocular disease.
	VACV 15–30 mg/kg/p.o. tid		
Treatment Mucocutaneous disease			
Adult:	ACV 400 mg p.o. 3×/day	Grade I	• Because prompt initiation of therapy is associated with improved outcome, therapy should be started based on clinical diagnosis, pending laboratory confirmation
	VACV 1 g p.o. 2×/day	Grade I	
	FCV 500 mg p.o. 2×/day	Grade I	
	ACV 5 mg/kg i.v. every 8 h (if unable to take p.o.)		• Therapy should be continued until complete healing of all lesions or at least 5–7 days
Pediatric:	ACV 10 mg/kg i.v. every 8 h		• Severe mucocutaneous.
	ACV 1000 mg/day p.o. in 3–5 divided doses for 7–14 days		• Limited disease. Treat for 7–14 days.
Severe, visceral/ disseminated/CNS disease			
Adult:	ACV 10 mg/kg i.v. every 8 h	Grade II-1	• i.v. Therapy should be continued until resolution of disease, or 14 days, then oral medication may be given. For CNS infection may consider 21 days of IV therapy.
Pediatric:	ACV IV 60 mg/kg/day in 3 divided doses	Grade II-2	• Continue for 21 days for disseminated or CNS infection.
HSV Keratitis	Topical:		• Topical steroids should also be considered for stromal keratitis.
	Ganciclovir 0.15%	Grade I	
	Trifluorothymidine 1%		• Ganciclovir given 5 × a day until healing then 3 × daily for 1 week
	Acyclovir 3% ointment (Grade III)		
	Acyclovir, 400 mg five times daily		• One drop every 2 h for 2 weeks. Limited by epithelial toxicity
	Valacyclovir and Famciclovir	Grade I	
	Oral:	Grade III	Avoids topical toxicity
			No comparative or dose finding studies.
Acyclovir-resistant HSV	Foscarnet 80–120 mg/kg/day IV in 2–3 divided doses until healing is complete	Grade I	• Resistance should be laboratory-confirmed, although empiric therapy can be started
	Intravenous cidofovir	Grade II-3	• Reduce immunosuppression, if possible
	Topical cidofovir	Grade III	
	Topical trifluridine	Grade II-3	

ACV = acyclovir; CMV = cytomegalovirus; FCV = famciclovir; HSV = herpes simplex virus; i.v. = intravenously; p.o. = per orally; SOT = solid organ transplant; VACV = valacyclovir.

[1]CMV prophylaxis with recommended doses of ganciclovir, valganciclovir, valacyclovir or acyclovir are adequate for HSV prevention. Due to lack of SOT-specific studies, the level of evidence is extrapolated from populations of other patients with similar levels of immune compromise. Dosages are for GFR ≥ 50, adjustment is necessary for renal insufficiency.

Children clear acyclovir more rapidly than adults, and thus need higher doses of acyclovir. There are no controlled clinical trial data for dosing of anti-HSV medications in the SOT pediatric population. In neonates, the recommended dose of acyclovir for encephalitis is 20 mg/kg/dose every 8 h for 21 days (52). Persistent HSV PCR in CSF has been associated with poor outcome in neonatal infection and it is suggested to confirm a negative CSF PCR prior to completing therapy (53) (Grade III). A similar dose is recommended for children from 3 months to 12 years, although some clinicians prefer 15 mg/kg/dose every 8 h (46). Localized, mucocutaneous, progressive disease is treated with IV acyclovir at a dose of 10 mg/kg/dose every 8 h for a minimum of 14 days (Grade III). For less severe localized disease oral acyclovir may be used at a dose of 1000 mg/day in 3–5 divided doses for 7–14 days; maximum dose: 80 mg/kg/day not to exceed 1 g/day (46). Acyclovir is associated with greater toxicity in the pediatric population; thus, close monitoring is recommended. Data for oral valacyclovir come from healthy immunocompetent patients: a dose of 20 mg/kg/dose twice daily is recommended for children 3 months to 11 years of age (54). Valacyclovir is

FDA approved for treatment of herpes labialis in children over 12 years of age and for children ≥2 years of age for the treatment of varicella infection though is not always easily available to the pediatric population as it needs to be reconstituted soon before use to be in liquid form.

HSV keratitis treatment includes both topical and/or systemic therapy. The various forms of topical therapy appear equally effective (55). Topical agents such as trifluridine solution and vidarabine ointment may result in epithelial toxicity with prolonged use. Topical ganciclovir gel has also been shown to be effective and has the advantage of less toxicity and less frequent applications. A study in immunocompetent individuals showed acyclovir at a dose of 400 mg five times a day was equivalent to topical therapy (56) and avoids the epithelial toxicity. Alternate HSV medications such as valacyclovir or famciclovir are possibly as effective as acyclovir, but have not been studied in comparative trials (57). Stromal keratitis and endotheliitis is treated with a combination of antivirals and topical steroids (58).

Resistance

The estimated prevalence of acyclovir resistance in immunocompromised hosts ranges from 3.6% to 6.3% (59,60) and needs to be considered in patients whose lesions are not responding clinically to appropriate doses of acyclovir, valacyclovir or famciclovir therapy. The most common mechanism of resistance in clinical practice is due to diminished or absent thymidine kinase (TK) activity that is conferred by resistance mutations. Thus, drugs that utilize TK (acyclovir, famciclovir and valacyclovir) are all affected. Initial evaluation should include laboratory confirmation of HSV disease including a viral culture as testing for acyclovir resistance generally relies on phenotypic assays—most commonly a plaque reduction assay. Given that testing relies on growth of the virus, results may be delayed for days to weeks and when strongly suspected, alternate therapy should be considered prior to confirmation of resistance (Grade III). Genotypic testing for known resistance mutations is available in some settings and may have a more rapid turnaround time.

Foscarnet is recommended for acyclovir resistant HSV infections (Grade I) (61). Intravenous cidofovir (Grade II-3) has also been associated with improvement (62), but both of these drugs are associated with significant renal toxicity and appropriate care should be taken to monitor for toxicities of these alternative regimens. Probenecid is usually given with cidofovir to potentiate the toxicity. Topical imiquimod has also been used for resistant anogenital HSV in immunocompromised hosts (63,64). Topical cidofovir (Grade III)) and trifluridine (Grade II-3) have also been used. Oral lipid-ester formulations of cidofovir (CMX-001) and helicase-primase inhibitors (e.g. ASP2151) are currently in later stages of development and may be available in the near future (65,66). To the extent possible, doses of immunosuppressive therapy should be reduced in patients with acyclovir resistant disease (Grade III). Recurrent acyclovir-resistant HSV disease may require repeated courses of foscarnet. However, after complete healing, subsequent recurrences may be again susceptible to acyclovir therapy (67).

Research Issues

The utility of molecular diagnostic testing in tissue and fluids other than CSF (i.e. blood, ascites, BAL) for diagnostic and monitoring purposes requires additional research to establish its role in routine care. Research into the epidemiology and natural history of HSV, in addition to controlled treatment trials are sorely needed in the pediatric population. It is important to further elucidate the effect of different immunosuppressive regimens on the natural history of herpes simplex reactivation and disease, and the potential benefit of suppressive therapy during long-term immunosuppression. As new therapeutic agents become available for HSV, they should be evaluated in the setting of transplant and other immunocompromised hosts. Should a therapeutic or prophylactic vaccine become available, the efficacy and, in the setting of a live virus vaccine, safety in the transplant population will need to be evaluated. The optimal method and duration for HSV prevention in seronegative recipients who are not taking CMV antiviral prophylaxis should be investigated.

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Special Article

Human Herpesvirus 6, 7 and 8 in Solid Organ Transplantation

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Key words: Exanthem subitum, ganciclovir, herpes virus, kaposis sarcoma, lymphoma, roseola infantum

Abbreviations: CI, chromosomally integrated; CMV, cytomegalovirus; CNI, calcineurin inhibitor; HHV, human herpesvirus; IL, interleukin; KS, Kaposi sarcoma; MCD, multicentric Castleman disease; mTOR, mammalian target of rapamycin; NK, natural killer; PEL, primary effusion lymphoma.

Human Herpesviruses 6 and 7

Epidemiology and risk factors

Human herpesvirus (HHV)-6A, HHV-6B and HHV-7 arelymphotropicβ-herpesviruses that are almost universally acquired during the first few years of life and establish latency in mononuclear cells, which eventually serve as reservoirs for endogenous viral reactivation during times of immune suppression or as potential vectors of transmission to susceptible individuals (1). In less than 1% of infected individuals, HHV-6 persistence occurs as a result of integration of the virus into the host chromosome, a condition known as chromosomally integrated CI-HHV-6 (2,3), with as yet undefined clinical significance (4,5). Previously felt to be two variants of the same virus, HHV-6A and HHV-6B were recognized as two distinct viruses by the International Committee on Taxonomy of Viruses in 2012, due to increasing virologic and epidemiologic evidence to support this distinction (6). HHV-6B has been implicated in most primary infections in children (7,8) and reactivation events after transplantation (9), whereas HHV-6A predominates in the lymph nodes of HIV-infected adults (10). Compared to HHV-6, less is known about the clinical implications of HHV-7 infection.

Primary HHV-6 and HHV-7 infections in immunocompetent children range from asymptomatic to self-limited febrile illnesses with rash, diarrhea, respiratory symptoms, or seizures (7,11–13). Transmission of HHV-6 and HHV-7 are thought to occur through saliva (14–16) or perinatally (17,18). Seroconversion occurs within the first 6–24 months of age for HHV-6 (7,17) and by 3 years for HHV-7 (19,20). However, an HHV-6 mononucleosis-like illness has been reported in adults (21,22) and primary infections of HHV-6 and HHV-7 can also occur in solid organ transplant recipients through allograft-transmission (23) or as a result of natural transmission in the community (24,25). Because seroprevalence studies typically show that over 90% of adults are infected with both viruses (1,11,17,19), most infections after transplantation are thought to result from the reactivation of endogenous latent virus (26,27). The estimated rates of HHV-6 reactivation after solid organ transplantation have varied widely between 20–82% (9,28–30), due to the variation of the diagnostic assays used and the inability of some tests to distinguish active from latent infection. In one review, HHV-6 infections were mostly reported in heart and lung (66–91%), liver (22–54%) and kidney (23–55%) transplant recipients, with few reports in kidney–pancreas and intestinal transplant recipients (31). There is less information on the rate of active HHV-7 infection after solid organ transplantation, though it has been estimated to occur in 0–46% of patients (9). Reactivation of both viruses occurs relatively early, generally within the first 2–4 weeks after solid organ transplantation (9,28,29,32,33).

Active infection by HHV-6 and HHV-7 in solid organ transplant recipients is usually asymptomatic. Overt clinical disease directly due to HHV-6 is estimated to occur in less than 1% of solid organ transplant recipients (27,32), reportedly causing fever and rash (34), hepatitis (35), gastroduodenitis (36,37), colitis (38,39), pneumonitis (34,40,41), and encephalitis (42). It may also present as a CMV-like syndrome, with fever and some degree of bone marrow suppression (43,44). Acute HHV-6 infection has also developed in patients who received liver transplantation for HHV-6 associated acute fulminant liver failure (45). Although HHV-6B causes more infections in transplant recipients, HHV-6A has been associated with giant cell hepatitis (35) and fatal disease in two renal transplant recipients (23,46). Of all the reported cases thus far, CI-HHV-6B is seen more commonly in solid organ transplant recipients whereas CI-HHV-6A has been described in hematopoietic stem cell transplant recipients, but the clinical significance is unclear (47). In contrast to HHV-6, symptomatic disease

due solely to HHV-7 to date has not been documented in heart (48), intestinal (49) or renal (50) transplant recipients, and the clinical associations in liver transplant recipients are controversial (51,52).

In addition to the direct effects described above, HHV-6 and HHV-7 appear to have immunomodulatory properties that result in important indirect effects on viral co-infections, fungal infections and allograft rejection. Both HHV-6 and HHV-7 have been associated with an increased risk of CMV disease (40,48,53–60). HHV-6 has also been associated with fungal and other opportunistic infections (61–63), early fibrosis due to hepatitis C virus recurrence after liver transplantation (64,65), and a higher mortality rate after liver (62,66) and heart–lung transplantation (63). HHV-6 and HHV-7 infections have been associated with allograft rejection and dysfunction (51,56,67,68), but the presence of CMV may confound the association. In addition, both HHV-6 and HHV-7 have been detected in bronchoalveolar lavage fluid (69), though the association between virus detection and bronchiolitis obliterans syndrome after lung transplantation is controversial (70,71). Although CI-HHV-6 has not been clearly associated with a clinical syndrome, there are data suggesting indirect effects. For example, a significantly higher rate of bacterial infections in liver transplant recipients (71.4% vs. 31.4%; $p = 0.04$) was noted among those with CI-HHV-6 than in the HHV-6 negative group, with a corresponding nonsignificantly higher rate of allograft rejection in the CI-HHV-6 group (72).

As with other herpesviruses transmitted through saliva, risk of infection with HHV-6 is associated with lower socioeconomic status and having more than one sibling, whereas seasonality and black race are associated with a higher prevalence of HHV-7 infection (73). Though data are limited, it is assumed that the intensity of pharmacologic immunosuppression is a risk factor for HHV-6 and HHV-7 reactivation and disease (74), potentially through prolonged suppression of memory responses (75). Certain agents, including muromunab-CD3 (49) and alemtuzumab have been associated with active HHV-6 infection after transplantation (76).

Diagnosis

Several factors complicate the diagnosis of clinically relevant HHV-6 infection. Diagnostic tests to detect HHV-6 and HHV-7 include serology, culture, antigenemia, immunohistochemistry and nucleic acid amplification assays. In general, these tests are not well standardized. In addition, many tests are unable to differentiate latent versus active infection or to distinguish between HHV-6A and HHV-6B, and there may also be cross-reactivity between HHV-6 and HHV-7. Specific testing for HHV-7 is mainly performed for research purposes, as there have not been any clear clinical syndromes associated with HHV-7 infection. Perhaps the most difficult aspect of interpreting HHV-6 and HHV-7 testing, however, is determining whether detection of the virus implies causality in a given clinical syndrome; the diagnosis of symptoms directly related to HHV-6 or HHV-7 infection typically requires the exclusion of other more likely etiologies.

Due to high HHV-6 and HHV-7 seroprevalence rates in adults, serology is of limited benefit for the diagnosis of active infection in solid organ transplant recipients (III; Ref. 9). Viral culture of HHV-6 is laborious and not routinely used. HHV-6 antigenemia assays can detect HHV-6 viral antigens in peripheral blood mononuclear cells using monoclonal antibodies (33) and can distinguish between HHV-6A and HHV-6B. Antigen-based assays are also rapid, relatively easy to perform, and may discriminate between active and latent infection. However in one study of adult liver transplant recipients, HHV-6 and HHV-7 active infection were detected in up to 39.2% and 14.2% of patients, respectively, at a median of 9 days posttransplantation, usually preceding CMV antigenemia; however, the cut-off level to determine clinically significant active infection is unknown (77). Further studies are needed to determine clinically significant levels of antigenemia posttransplantation. Polymerase chain reaction (PCR) may be preferred for the detection of HHV-6 and HHV-7 viremia after solid organ transplantation (II-2; Ref. 78). PCR assays can distinguish between HHV-6A, HHV-6B and HHV-7, but they may not differentiate active from latent infection (79,80). Quantitative real-time PCR assays on noncellular samples are often used for the diagnosis of active HHV-6 and HHV-7 infection (81–85); however, recent evidence suggests that HHV-6 DNA in plasma reflects the presence of infected blood cells (86). Therefore, quantification of viral DNA in whole blood, reverse transcriptase PCR on whole blood, or methods to detect messenger mRNA may be more specific for the diagnosis of active HHV-6 infection (III; Refs. 86–88). However, there are limited data linking reactivation of HHV-6 using whole blood clinical samples with clinical disease. It is also important to consider the potential detection of CI-HHV-6 in blood samples, characterized by persistent HHV-6 viral loads of over a million copies per mL of whole blood, which may be misinterpreted as substantial active infection leading to unnecessary treatment (4,72). Recent guidelines suggest that HHV-6 levels in whole blood exceeding 5.5 $\log_{10}$ copies/mL are strongly suggestive of CI-HHV-6, which is confirmed by the ratio of viral to human genomes of 1:1 (3). Qualitative or quantitative HHV-6 PCR of the cerebrospinal fluid is useful to diagnose HHV-6 encephalitis in patients with the appropriate clinical signs (42). Immunohistochemistry to detect viral antigens in biopsy specimens may be more informative than viremia in cases where tissue-invasive HHV-6 disease is suspected (34,89,90). However, HHV-6 antigen may be found commonly in tissue in the absence of symptoms (91,92). Because of the apparent low rate of clinical disease and the relatively high rate of subclinical viral reactivations, routine monitoring for HHV-6 or HHV-7 infection after solid organ transplantation

is not recommended based on the current evidence. Diagnostic testing should be limited to scenarios where symptomatic HHV-6 infection is plausible, and to assist in guiding treatment decisions, including response to therapy (III; Ref. 93).

Treatment

The majority of HHV-6 and HHV-7 infections are subclinical and transient, and therefore treatment of asymptomatic viral reactivation is not recommended (II-2). However, treatment directed against HHV-6 should be initiated in the setting of HHV-6 encephalitis and should be considered for other clinical syndromes attributable to HHV-6 (III). Especially in cases of moderate or severe disease, antiviral treatment may be complemented by a reduction in the degree of pharmacologic immunosuppression (III). Furthermore, HHV-6 and HHV-7 co-infections with CMV generally do not require therapy in addition to the treatment given for CMV infection and disease (III; Ref. 94). Currently, no antiviral compounds have been approved for the treatment of HHV-6 and HHV-7 infections in solid organ transplant recipients, though foscarnet, ganciclovir and cidofovir have been used clinically, based on *in vitro* data and anecdotal clinical reports in stem cell transplant recipients (30,95–97). However, there are no randomized controlled trials demonstrating antiviral efficacy in the treatment of HHV-6 or HHV-7 infections. *In vitro*, HHV-6 is sensitive to achievable concentrations of ganciclovir, foscarnet and cidofovir, though HHV-6A and HHV-6B demonstrate different susceptibilities (95). HHV6-B is usually susceptible to both ganciclovir and foscarnet, whereas HHV6-A is more resistant to ganciclovir though mutations in U69 and U28 genes (98,99). Of note, a cidofovir-resistant isolate of HHV-6 has been reported (100). HHV-7 appears resistant to ganciclovir *in vitro*, and may not be inhibited with achievable concentrations of ganciclovir (95). Both HHV-6 and HHV-7 are resistant to acyclovir and penciclovir (95).

Prevention

Specific antiviral prophylaxis or pre-emptive therapy for HHV-6 infection is not recommended due to insufficient evidence, and because the vast majority of HHV-6 and HHV-7 infections after solid organ transplantation are subclinical (III). Antiviral prophylaxis for CMV with ganciclovir or valganciclovir does appear to reduce the incidence of HHV-6 viremia in solid organ transplant recipients (101,102), though a similar effect has not been observed for HHV-7 (94).

Research issues

A full understanding of the clinical impact of HHV-6 and HHV-7, including the direct effects as well as interactions with CMV, impact on allograft dysfunction, and other immunomodulatory effects, requires large prospective clinical studies. A comprehensive assessment of the magnitude of their clinical impact would be required to estimate the potential benefits of interventions such as routine monitoring for these viruses after transplantation. Standardization of diagnostic methods to allow for more precise determination of the burden of active infection and association with clinical disease is warranted, along with further characterization of CIHHV-6 and its association with disease. Finally, determining the *in vivo* efficacy of currently available antiviral compounds against HHV-6 and HHV-7, preferably through randomized controlled trials, would be beneficial.

Human Herpesvirus 8

Epidemiology and risk factors

Human herpesvirus8 (HHV-8) is a γ-herpesvirus that causes Kaposi sarcoma (KS) and, much less commonly, primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD; Refs.103–106). HHV-8 has also been reported as a cause of fever and other constitutional symptoms, bone marrow suppression, hemophagocytic syndrome and clonal gammopathy after transplantation (106–109). HHV-8 infects B cells, oral epithelial cells, as well as cells of endothelial origin ("spindle cells") present in KS lesions (110). As with all herpesviruses, HHV-8 infection is lifelong, and the virus alternates between latency and active lytic replication, during which infectious virus is produced. Natural transmission of HHV-8 primarily occurs through saliva, but infection may also be acquired via sexual intercourse, blood transfusion and organ transplantation (111,112).

The prevalence of HHV-8 infection varies widely depending on the geographic region; seroprevalence is estimated to be between 0–5% in North America, northern Europe, and Asia, between 5–20% in the Mediterranean and Middle East, and >50% in parts of Africa (111,112). In high-prevalence areas, acquisition of HHV-8 frequently occurs during early childhood, in contrast to low-prevalence areas where seropositivity of children is extremely rare (113,114). The incidence of active HHV-8 infection and disease after solid organ transplantation reflect these geographic differences in seroprevalence. Between 23 and 68% of HHV-8 seropositive transplant recipients develop KS (115–117). As such, the cumulative risk of KS in transplant recipients has been reported to range from as low as 0.4% of patients in North America to 6% in the Mediterranean and Middle East (112,115–124). Furthermore, in Saudi Arabia, KS accounted for 87.5% of all tumors detected in kidney transplant recipients, compared to only approximately 3–6% in North America (118,124). The onset of KS is most often within the first 1–2 years after transplantation, though it may occur as early as a few weeks to as late as 18 years after transplantation (103,106,124–127).

The risk and manifestations of symptomatic HHV-8 infection in transplant recipients are likely dependent on the presence of pretransplant HHV-8 immunity, level of immunosuppression and type of organ. KS and other

HHV-8-related diseases may occur as a result of either primary infection in recipients of allografts from HHV-8-infected donors (106,107,128–132), or viral reactivation in recipients infected with HHV-8 before transplant (132–134). Primary HHV-8 infection in liver transplant recipients may result in particularly high rates of disease and death, based on a recent prospective Italian cohort study in which three of five patients who acquired HHV-8 posttransplant died from multiorgan failure or MCD, and a fourth developed KS (106).

The risk of HHV-8-related disease is greatly increased by pharmacologic immunosuppression or HIV infection, likely due to impaired control of HHV-8 replication (110,135). The intensity of immunosuppression, including use of antilymphocyte agents, has been associated with the risk of KS after transplantation (115). HHV-8 T cell responses were notably absent in a case series of transplant patients at the onset of KS, but became detectable following reduction in immunosuppression, which coincided with remission of KS (136). NK cells (137) and B cells may also be protective against KS; low levels of HHV-8 neutralizing antibodies have been associated with KS in HIV infection (138,139), and treatment of MCD with rituximab has been reported to increase HHV-8 reactivation and exacerbate KS (140). Use of calcineurin inhibitors (CNIs) for immunosuppression has been indirectly implicated as a risk factor for KS, since regression of KS lesions has occurred after reduction of CNIs or switching to the mTOR inhibitor sirolimus (also known as rapamycin; Refs.136,141). Older age and male gender have also been identified as risk factors for KS (117,120,124). Risk factors for less common clinical manifestations of HHV-8 infection in transplant recipients have not been well defined.

Diagnosis

A variety of serological assays are available to test for HHV-8 infection. However, use of these assays, which are based on a variety of viral antigens, is not standardized, and their sensitivity ranges from approximately 80% to greater than 90% (142,143). Although donor and recipient serological screening prior to solid organ transplantation may help stratify the risk of HHV-8-related disease after transplantation, how this information should be used is poorly understood at present (II-2). Similarly, the value of testing for seroconversion or an IgM response to HHV-8 post-transplantation is uncertain (III). For the detection of active HHV-8 infection, quantitative PCR testing of peripheral blood may be informative (II-2; Ref. 135). As HHV-8 viremia is associated with the development of KS, PCR could be used to monitor for risk of disease as a part of a preemptive strategy (see below; Refs.135,144,145). In addition, the use of HHV-8 viral load measurements to follow patients with KS and to assess response to therapy has also been suggested (115,142,146), though studies are needed to determine the clinical utility of these approaches (III).

KS presents in transplant patients as red or violaceous lesions of the skin or oral mucosa, but may also involve the lymph nodes or visceral organs, including the transplanted allograft (130,134,147,148). Presenting symptoms of PEL are dependent on the location (primarily the pleural, peritoneal or pericardial spaces) and size of the effusion. MCD is characterized by fever and other systemic symptoms of inflammation, lymphadenopathy, and anemia. Histopathology is required for definitive diagnosis of HHV-8-related tumors, and should be performed whenever possible. Testing for the presence of HHV-8 in biopsy or fluid samples (e.g. tumor tissue for KS, lymph node for MCD, pleural or ascitic fluid for PEL) using immunohistochemistry, in situ hybridization, or PCR is also valuable (II-2; Refs. 103,135).

Treatment

A multidisciplinary approach is recommended, including early consultation with oncology, infectious disease, and dermatology specialists, as appropriate. Cautious reduction or cessation of pharmacologic immunosuppression is the first line therapy for the treatment of KS if feasible (II-3; Refs. 136,149–151). The degree to which immunosuppression is reduced should be individualized based on the type of organ transplanted and the severity of KS in each case. For patients receiving a CNI as a part of their immunosuppression regimen, switching to sirolimus should also be considered (II-3). In addition to its ability to block T cell activation through inhibition of IL-2 response, sirolimus has antitumor properties, and conversion to sirolimus has led to regression of KS lesions in some patients (130,134,141,152,153). In addition, sirolimus blocks HHV-8 replication, which may provide additional clinical benefits (154).

Patients whose KS lesions do not regress with reduction in immunosuppression or change to sirolimus may require intralesional chemotherapy, surgical excision or radiation therapy or other local treatment for isolated lesions, or systemic chemotherapy for visceral or severe disease, using liposomal doxorubicin, paclitaxel, or other agents (II-2; Ref. 155). Chemotherapy may also ameliorate the risk of allograft rejection due to reduction of immunosuppression (148,156). It should be noted that no controlled KS treatment trials have been performed in transplant recipients. Data regarding treatment of MCD and PEL in transplantation is even more limited. As such, decisions regarding systemic chemotherapy may benefit from evaluation of evidence from the HIV literature (104,111,157,158). The benefits of antiviral therapy in transplant recipients with established KS or other manifestations of HHV-8 infection are not defined (135). However, numerous case reports suggest a benefit of antivirals for HHV-8 related diseases, including one in which foscarnet was used successfully for the treatment of bone marrow suppression and hemophagocytosis related to primary HHV-8 infection after kidney transplantation (108,135).

Table 1: Summary of recommendations for the diagnosis, prevention and treatment of human herpes viruses 6, 7 and 8 after solid organ transplantation

	Recommendations	Level of evidence
HHV-6 and HHV-7		
Diagnosis	Viral serologies are not helpful in the diagnosis of HHV-6 and HHV-7 infections after solid organ transplantation	III
	Direct methods, such as the detection of viral nucleic acids in blood or CSF by PCR, or viral antigen in tissue by immunohistochemistry, are preferred methods for diagnosis of HHV-6 and HHV-7	II-2
	Quantitative PCR on whole blood, reverse transcriptase PCR on whole blood, or methods to detect mRNA may be more specific for the diagnosis of active HHV-6 and HHV-7 infection	III
	Routine monitoring for HHV-6 and HHV-7 infections after solid organ transplantation is not recommended, except to assist in guiding decisions regarding treatment of symptomatic HHV-6 infection, including response to therapy	III
Treatment	The majority of HHV-6 and HHV-7 infections are asymptomatic, transient, and do not require antiviral treatment	II-2
	Antiviral treatment with ganciclovir, foscarnet or cidofovir should be initiated in the setting of HHV-6 encephalitis and should be considered for other syndromes attributable to HHV-6	III
	Treatment of symptomatic HHV-6 and HHV-7 infections should include reduction in the degree of immunosuppression, especially for moderate or severe disease	III
	HHV-6 and HHV-7 co-infections with CMV do not require additional therapy	III
Prevention	Antiviral prophylaxis or preemptive antiviral therapy for HHV-6 or HHV-7 infections is not recommended after transplantation	III
HHV-8		
Diagnosis	Serology is of limited utility in the diagnosis of HHV-8 after solid organ transplantation	III
	Pretransplant donor and recipient HHV-8 serology may stratify the risk of disease after transplantation in endemic areas	II-2
	Immunohistochemistry using monoclonal antibodies against HHV-8 antigens is useful for the pathological diagnosis of KS and other angiogenic proliferative diseases	II-2
	Nucleic acid amplification assays to quantitate HHV-8 load in clinical samples is preferred for the diagnosis of active HHV-8 replication	II-2
	Quantification of HHV-8 load could be used for monitoring transplant patients with KS	III
Treatment	Reduction or cessation of immunosuppression should be a first line therapy, especially for moderate or severe disease	II-3
	Conversion of immunosuppressive regimen from calcineurin inhibitors to sirolimus (rapamycin) should be considered	II-3
	Current evidence does not support the use of antivirals for the treatment of KS	II-2
	Patients whose lesions do no not regress despite reduction in immunosuppression or conversion to sirolimus may require local interventions or systemic chemotherapy	II-2
Prevention	HHV-8 serologic screening of donors and recipients may be considered to assess risk, especially in geographic regions with high rates of infection	II-2
	In HHV-8 seropositive recipients or those who receive an organ from HHV-8 seropositive donor, monitoring of HHV-8 load after transplantation may be useful to determine the risk of disease	III
	Avoidance of over-immunosuppression in high risk individuals and in those with detectable HHV-8 viremia may be beneficial	III
	The use of antivirals with activity against HHV-8 (e.g. valganciclovir) to prevent KS in selected high risk transplant recipients with detectable HHV-8 viremia may be beneficial based on studies in HIV-infected patients	III

PCR= polymerase chain reaction; HHV= human herpesvirus; CSF= cerebrospinal fluid; CMV= cytomegalovirus; KS= Kaposi sarcoma.

Prevention

Although serologic screening of donors and recipients is not routinely performed, it may be considered, especially in those from geographic regions with high rates of infection (II-2). However, seropositivity in either the donor or the recipient is not typically regarded as a contraindication to transplantation (132). In recipients who are seropositive for HHV-8 or receive an organ from a seropositive donor, monitoring of HHV-8 viral load after transplantation may be a useful strategy to determine the risk of clinical disease (III). Avoidance of over-immunosuppression in high-risk individuals and in those with detectable HHV-8 viremia is advisable (III). However, the frequency and duration of monitoring or the level of clinically relevant HHV-8

replication has yet to be determined. Moreover, once HHV-8 is detected, current data are insufficient to define a beneficial preemptive strategy (135). *In vitro* studies demonstrate that HHV-8 replication is inhibited by ganciclovir, foscarnet and cidofovir at concentrations achieved in plasma (135). Furthermore, clinical trials have reported that valganciclovir can suppress HHV-8 replication *in vivo*, and that ganciclovir reduces the incidence of KS by 75–93% in people infected with HIV (159,160). Although these antivirals are effective prophylaxis in organ transplant recipients at risk for HHV-8-related disease or as preemptive treatment of a patient with active HHV-8 replication has not been studied. Use of immunosuppression regimens containing sirolimus rather than a CNI might theoretically lower the risk of KS because of the anti-proliferative properties of mTOR inhibitors and their association with lower overall risk of malignancy in some studies (161,162). However, adequately powered studies have not been performed to determine whether sirolimus prevents KS, and incident KS cases have been reported in patients receiving sirolimus (153,163).

Research issues

Additional prospective studies are needed to evaluate the use of pretransplant donor and recipient serology to stratify risk among recipient of different organ types in regions of HHV-8 endemicity. The use of HHV-8 viral load monitoring after transplantation to predict individuals at high risk of disease should be evaluated, with the goal of assessing the optimal frequency of testing and viral load threshold that accurately predict disease. The potential clinical utility of antiviral drugs for targeted prophylaxis, or as preemptive treatment of asymptomatic HHV-8 reactivation or replication, should be subjected to prospective controlled clinical trials. The benefits of immunosuppression regimens containing sirolimus or other mTOR inhibitors for the prevention and treatment of KS after transplantation should be investigated in randomized clinical trials. Conducting these trials in regions where HHV-8 infection is prevalent has obvious advantages, and should therefore be encouraged and supported (Table 1).

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Disclosure

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Special Article

Varicella Zoster Virus in Solid Organ Transplantation

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Abbreviations: CMV, cytomegalovirus; DFA, direct fluorescent assays; HSV, herpes simplex virus; HZ, herpes zoster; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; PCR, polymerase chain reaction; PHN, postherpetic neuralgia; SOT, solid organ transplant; VZV, varicella zoster virus.

Epidemiology and Risk Factors

Epidemiology

Varicella zoster virus (VZV) is an exclusively human virus that exposure either through direct contact with a skin lesion or through airborne spread from respiratory droplets leads to acute varicella or "chickenpox" (1,2). More than 90% of adults in the United States acquired the infection in childhood; in recent years most children and many young adults have been vaccinated with the live virus vaccine (1,3). The incubation time after primary exposure is approximately 10–21 days. Primary varicella typically presents with fever, constitutional symptoms and a vesicular, pruritic, widely disseminated rash that primarily involves the trunk and face (4); symptoms usually resolve within 7–10 days. Rates of hospitalization and mortality due to varicella have dropped with the institution of routine childhood varicella vaccination (5,6).

After initial infection, VZV establishes lifelong latency in cranial nerve and dorsal root ganglia, and can reactivate years to decades later as herpes zoster (HZ) or "shingles" (7). Nearly all patients with HZ develop an exanthem of vesicular lesions in a dermatomal distribution. The annual incidence of HZ in the general population is 1.5–3.0 cases per 1000 persons (1), and is estimated to occur in up to 20% of individuals during their lifetime (8). Secondary complications such as bacterial superinfection and postherpetic neuralgia (PHN) lead to increased morbidity (9).

All patients being considered for transplant should undergo serologic testing before transplantation to document prior exposure to VZV. Over 90% of adult solid organ transplant (SOT) recipients will be seropositive for VZV. Rates of seropositivity are lower in pediatric transplants (10,11), but may improve with increased emphasis on varicella vaccination before transplantation (3). Primary infection is rare in adult SOT recipients, but can be devastating, with visceral involvement, severe skin disease, and disseminated intravascular coagulation (12–17). HZ is a frequent infectious complication in SOT recipients with an incidence of approximately 8–11% during the first 4 years posttransplant (18–20). Dissemination similar to that seen in primary VZV infection is uncommon but has been reported in SOT and other immunocompromised populations; the level of immunosuppression may alter the risk of developing this complication (15,21,22). Rates of PHN in SOT recipients may also be higher than in immunocompetent populations (19).

Risk factors

Primary varicella: Susceptible seronegative patients are at risk for primary varicella. Studies have showed that approximately 2–3% of adult SOT recipients are seronegative for VZV (11,23). Donor transmitted VZV infection is rare but has been reported in a case where the donor had recently been treated for primary varicella (24). Breakthrough varicella can occur in vaccinated patients but is usually a milder presentation when compared to wild-type primary infection (25,26). Data on risks of breakthrough varicella in immunocompromised patients who have previously been vaccinated for varicella are unknown.

Herpes zoster: Patients with previous VZV infection or VZV vaccination are at risk for the development of HZ. Because there are no large prospective trials that have evaluated HZ in SOT, risk factors are not well defined. Similar to the general population, longitudinal studies have showed that older transplant recipients are at greater risk for the development of HZ (18,20). Heart and lung transplant patients have increased rates of HZ compared to other transplant recipients, possibly related at least in part to more intensive immunosuppression (19,20,27,28). The use of mycophenolate mofetil (MMF) has also been suggested as a potential risk factor for the development of HZ (22,29,30). It is unknown whether the development of HZ before transplant lessens the risk for posttransplant recurrence. Similar to varicella, HZ can occur in patients who have previously received varicella immunization, but the episodes are thought to be milder than in patients who acquire natural infection with wild-type virus (31).

Diagnosis

In general, both primary varicella and HZ have typical clinical presentations that allow for a presumptive clinical diagnosis. Primary varicella presents as a disseminated pruritic rash that often starts on the face and spreads down the trunk, with relative sparing of the hands and soles of the feet; mucosal involvement can occur. One distinctive feature is that new lesions appear over several days so that most patients have papules, vesicles, and crusted lesions at the same time. HZ most often presents as a painful vesicular rash that involves ≤2 adjacent unilateral dermatomes (1). Presentations vary as patients may present with pain as a prodrome before the development of lesions, and pain may be less frequently seen in children and young adults. Herpes zoster ophthalmicus (trigeminal ganglion), herpes zoster oticus (Ramsay-Hunt syndrome – geniculate ganglion), and other unique HZ presentations have been described elsewhere (32,33).

Immunocompromised patients with HZ may develop disseminated skin lesions that can mimic primary varicella during periods of potent immunosuppression (34,35). SOT recipients are more likely to present atypically (34–36), may present with multi-organ involvement (34,37) and can rarely develop invasive complications with delayed or absent rash (36,38). In SOT recipients, who may develop a multitude of other infectious and noninfectious rashes, laboratory testing is even more important than in the normal host, as a diagnosis may be more difficult to establish on clinical grounds alone.

Definitive laboratory testing can be used for atypical cases of VZV or HZ and should routinely be used for suspected disseminated, visceral disease or central nervous system disease. Rapid diagnostic methods, including polymerase chain reaction (PCR) and direct fluorescent assays (DFA), are the methods of choice (39). PCR testing, the most sensitive test for VZV (40), can be used for detecting visceral involvement, and detects VZV in vesicle fluid, serum, spinal fluid, and other tissues. DFA is performed on scrapings taken from the base of a skin lesion, and is a rapid and reliable method for diagnosing VZV. Viral culture is specific and can help distinguish VZV from other viral pathogens such as Herpes simplex virus (HSV). Culture provides slower results and is less sensitive for VZV (41), but remains an important diagnostic entity, particularly because other viral infections (e.g. HSV) often do grow well in culture.

The majority of patients even without a history of clinical VZV infection will be seropositive (42,43). Regardless, all patients should undergo serologic testing to document prior exposure to VZV during their pretransplant evaluation process. Serology results can be used to determine posttransplant risk as patients who are seronegative before transplant are at risk for the development of primary VZV, and seropositive patients are at risk for developing posttransplant HZ. It is important to note that in acute infections serologic testing should be interpreted with caution. False-negative serologic results are more common in immunocompromised patients and may be seen during primary infection, and false-positive results can also occur after transfusions; serology should not be used for diagnosing acute infections in this population (39).

Treatment

Treatment recommendations are listed in Table 1. It is important to note that doses given in the table are given for patients with preserved renal function. In patients with renal dysfunction dosing should be reviewed before administration, because most agents will require appropriate dose modification.

Varicella

Posttransplant patients who develop primary varicella are at risk for developing severe infection and should be treated with intravenous acyclovir (I) (Table 1; Refs. 44–46). Therapy initiated early in the course of the illness, especially within 24-hours of rash onset, maximizes efficacy (39). Reduction in immunosuppressive therapy should be considered (III) (16), but to facilitate an appropriate stress response, steroid dosing should be maintained or may need to be temporarily boosted based on clinical findings. Nonspecific intravenous immunoglobulin (IVIG) or VZV immunoglobulin are unlikely to provide additional benefits to those with established infection and are therefore not recommended (39). However, IVIG and varicella zoster immune globulin (VZIG) have been used anecdotally in those with severe infection (III) (15,47–50).

Herpes zoster

Patients with disseminated or organ invasive disease should be treated with IV acyclovir (II-2) (44,46). Localized nonsevere dermatomal HZ can be treated with oral valacyclovir or famciclovir as an outpatient in most adults with close follow-up (II-1) (51,52). Two notable exceptions for those with localized infection are those within the trigeminal ganglion (herpes zoster ophthalmicus) which may be sight-threatening, and involvement of the geniculate ganglion (herpes zoster oticus/Ramsay-Hunt syndrome) which can lead to facial palsy (53). These patients should preferably receive IV acyclovir therapy, and in cases of trigeminal involvement, prompt ophthalmologic consultation to avoid major ocular complications (III). There are no data that support adding glucocorticoids to patients on steroid-sparring regimens to prevent late PHN complications so this is not recommended (III).

Prevention/Prophylaxis

Suggestions for prevention and prophylaxis are listed in Table 2. Doses given in the table are given for patients with

Table 1: Recommendations for VZV treatment in solid organ transplant recipients

Disease	Treatment	Evidence	Comments
Outpatient treatment			
Herpes zoster localized (dermatomal)	**Acyclovir** 800 mg PO five times daily (adults and children ≥12 years) OR **Valacyclovir** 1 gram PO three times daily (adults) 20 mg/kg PO four times daily (children ≥2 and ≤18) years)[1] OR **Famciclovir** 500 mg PO three times daily (adults only)	Evidence II-1	• Oral therapy is not recommended for young children <2 years of age, or patients with evidence of dissemination, tissue invasion, HZ ophthalmicus or oticus, or those with severe symptoms. These patients should be treated with IV therapy (see below) • Antivirals are typically given for at least 7 days or until lesions have crusted over, which may be delayed in immunocompromised hosts • Valacyclovir and Famciclovir are not FDA approved for treatment of herpes zoster, but are commonly used in clinical practice • Valacyclovir is only recommended for children ≥2–18 years of age • IV acyclovir is recommended in children <2 yrs of age or those who cannot tolerate oral therapy (see below for dosing) • Careful monitoring of renal function is needed while on high-dose acyclovir therapy, and dosing should be adjusted for renal insufficiency
Inpatient treatment			
Acute varicella	**Acyclovir** 30 mg/kg IV in 3 divided doses (adults and children <1 year) OR 1500 mg/m^2 IV per day in 3 divided doses (children ≥ 1 year of age)[2]	Evidence I	• IV therapy can be changed to oral therapy once the patient has significantly improved • Careful monitoring of renal function is needed while on IV therapy, and dosing should be adjusted for renal insufficiency
Herpes zoster Disseminated or Invasive disease or Herpes zoster ophthalmicus or Ramsay-Hunt syndrome/ Herpes zoster oticus	**Acyclovir** 30 mg/kg IV in 3 divided doses (adults and children)		• In disseminated disease IV therapy should be given for at for at least 7 days, but may need to be given for longer in patients with extensive involvement or CNS disease • Ophthalmology consultation is recommended for patients with ophthalmic involvement • Consideration for switch to oral therapy dependent on patient's clinical status • Careful monitoring of renal function is needed while on IV therapy, and dosing should be adjusted for renal insufficiency

Data supporting IV therapy for herpes zoster ophthalmicus and oticus are Evidence level III.
[1]FDA approved dosing for children only in varicella not herpes zoster, maximum 3200 mg/day.
[2]Some experts recommend 30 mg/kg in 3 divided doses for this age as well Ref. (39).

preserved renal function, so patients with renal dysfunction dosing may need appropriate dose modification.

VZV prevention

Antiviral therapy: Oral acyclovir and its pro-drugs have been shown to prevent VZV reactivation in other immunosuppressed populations, but they have not been systematically studied in SOT recipients (Table 2; Ref. 54). During the early posttransplant period, many current regimens used for cytomegalovirus (CMV) prevention will likely prevent VZV reactivation, and therefore additional antiviral prophylaxis for VZV is not needed during periods of CMV prophylaxis [valganciclovir, ganciclovir, or high dose acyclovir] (55–57). In patients who do not receive CMV prophylaxis, short term antivirals [acyclovir, valganciclovir] given for herpes simplex (HSV) prophylaxis may also be effective against VZV during the period immediately posttransplant (III). Prophylactic antiviral agents for patients who are both CMV/HSV seronegative but VZV seropositive have not been studied, but it seems prudent to consider similar strategies to patients receiving HSV prophylaxis to provide at least minimal protection during the high-risk posttransplant period (III). Because the length of immunosuppression is life-long in most SOT recipients, an increased risk for HZ is continuous after transplantation (18–20). Although effective for short-term use (58), insufficient data exist to recommend routine use of long-term VZV prophylaxis in SOT recipients (III).

Table 2: Recommendations for VZV prevention in solid organ transplant recipients

Strategy	Pretransplant	Posttransplant	Dosing	Comments
Varicella/HZ prevention				
Antiviral Prophylaxis Acyclovir (and pro-drugs)	N/A	Short- term prophylaxis is recommended for patients who are HSV positive in patients not receiving CMV prophylaxis (Evidence I). Prophylaxis in VZV seropositive CMV/HSV seronegative recipients has not been studied but can be considered (Evidence III)	**Acyclovir** 600–1000 mg/day PO in 3–5 divided doses (adults and children ≥ 2 years) Max dose in children is 80 mg/kg/day not to exceed 3200 mg/day See reference 39 for dosing in children <2 yrs OR **Valacyclovir** 500 mg PO twice daily (adults only)	• Evidence in other populations for effectiveness against VZV, minimal data in SOT recipients. • IV acyclovir is recommended in children <2 yrs of age [5 mg/kg IV every 8 hours] or those who cannot tolerate oral therapy • Alternate less frequent dosing (BID) for acyclovir has been described but has not been evaluated in SOT populations • Patients receiving CMV prophylaxis generally should be protected from VZV reactivation • Valacyclovir is only recommended for children ≥2 and ≤18 years of age and has not been studied as a prophylactic agent in children post-SOT • Lifelong risk of HZ limits use of these agents for long-term prevention.
Vaccination				
Varicella Vaccine (Varivax®)	YES, if seronegative (Evidence II-1)	Consider if susceptible in select populations (Evidence III)	**Varivax®** 0.5 mL administered SQ	• Vaccination has been shown to be safe in ESRD and ESLD patients • Seroconversion rate reduced in immunosuppressed individuals • Caution should be used in posttransplant patients because live virus vaccine • 2nd dose can be given 4–8 weeks after first in adults, but must be delayed till ≥ 3 months after 1st dose in children <13 years of age (see package insert for guidelines)
Zoster Vaccine (Zostavax®)	No for most transplant recipients (Evidence III), unless patient meets label indications (Evidence I)	No (Evidence III)	N/A	• Follow label indications, as no evidence that vaccine is safe in severe organ dysfunction or posttransplant since is live virus vaccine • If patient meets label indications can be considered, but should be given at least 3–4 weeks before transplant.

Continued

Table 2: Continued

Strategy	Pretransplant	Posttransplant	Dosing	Comments
Postexposure Prophylaxis (serongative patients only)				
Immunoprophylaxis VZV immunoglobulin (VZIG, VariZIG™)	YES, if seronegative (Evidence II-1)	YES, if seronegative (Evidence II-1)	**VariZIG** 125 units/10 kg body weight in single IM dose (Max dose is 625 units, min 125 units)	• VariZIG is only available through IND protocol[1] • Must be given as soon as possible – no efficacy if given more than 10 days postexposure • Not 100% effective in clinical studies of preventing VZV, so close observation is suggested • If varicella develops, patient should be treated with antiviral therapy
IV immunoglobulin (nonspecific IVIG)	Consider, if seronegative and VZIG or VariZIG not available (Evidence III)	Consider, if seronegative and VZIG or VariZIG not available (Evidence III)	**IVIG** 400 mg/kg IV single dose	• Amount of anti-VZV antibodies in IVIG is variable, and should only be considered if VZV specific immunoglobulin therapy is not available
Antiviral Prophylaxis Acyclovir[2] (and pro-drugs)	Consider, if seronegative and VZIG or VariZIG not available or in addition to immunoprophylaxis (Evidence III)	Consider, if seronegative and VZIG or VariZIG not available or in addition to immunoprophylaxis (Evidence III)	**Acyclovir** 800 mg PO four times daily (adults) 20 mg/kg PO four times daily (maximum 800 mg four times a day, ≥ 2 yrs of age) 30 mg/kg IV per day in 3 divided doses (adults and children) OR **Valacyclovir** 1 gram PO three times daily (adults)	• Given 7–10 days after exposure for 7 days • Alternatively, some experts recommend dosing being given days 3–22 after exposure (or till day 28 if given immunoprophylaxis) • Caution with patients with underlying renal dysfunction as dosing may need to be reduced • IV acyclovir is recommended in children <2 years of age or those who cannot tolerate oral therapy • Valacyclovir is only recommended for children 2 to <18 years of age and has not been studied as a prophylactic agent in children post-SOT

ESLD = end-stage liver disease, ESRD = end-stage renal disease, HSV = herpes simplex virus, HZ = herpes zoster, SOT = solid organ transplant, VZV = varicella zoster virus.
[1]Contact information for VariZIG is available online at: (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm55e224a1.htm).
[2]Valacyclovir is preferred as oral acyclovir may have poor bioavailability and unpredictable absorption.

Pretransplant vaccination: Potential transplant patients who are susceptible to VZV, should be given varicella vaccination with the live attenuated Oka vaccine (Varivax®, Merck & Co, Inc., Whitehouse Station, NJ, USA) provided no contraindications are present (II-1). Multiple nonrandomized studies in subjects with end-stage renal disease have showed that the Oka vaccine is safe and effective before transplant (23,59–61). Although fewer data are available in subjects with end-stage liver disease, the Oka vaccine also appears to be safe if given pretransplant to these patients (II-2) (62–64). Little data exist for other pretransplant patients but the vaccine is likely safe in these populations (III) (65). Patients with end-stage organ disease have reduced seroconversion rates to varicella vaccination [~60%] (59–61,64), so two doses should be given before transplantation if practical with a minimal interval of 4–6 weeks (57,66,67). Patients should be vaccinated at least 2–4 weeks before transplant (67), but if the vaccine is given in conjunction with measles, mumps, rubella vaccine (MMR and Varicella combined vaccine [ProQuad®, Merck & Co., Inc.]) it should be administered at least 4 weeks before transplant.

The current HZ vaccine (Zostavax®, Merck & Co., Inc.) contains approximately 10–12 times more plaque forming units of live-virus then current Oka varicella vaccines. This vaccine has not been studied in patients with end-organ disease awaiting transplant, but could be considered on a case-by-case basis for those who meet current criteria for HZ vaccination (III).

Posttransplant vaccination: The Oka varicella vaccines have been shown to be safe in select children undergoing chemotherapy and small studies have showed that they can be given safely to posttransplant recipients receiving immunosuppression (68–71). Although varicella vaccination has been given safely to small numbers of susceptible SOT recipients (70,71), caution should be used with the use of this live-virus vaccine as it is currently not approved for immunocompromised patients (III). In addition, rates of seroconversion in immunocompromised patients may not be as robust as in those with intact immune systems. The HZ vaccine poses a risk of disseminated infection in immunosuppressed patients and therefore is contraindicated for posttransplant recipients (III).

Postexposure prophylaxis

Seronegative transplant recipients are at risk for developing severe primary infection after exposure and should, after a significant exposure, receive postexposure prophylaxis (II-1). In the outpatient environment significant exposure to VZV has been defined as exposure to a household contact or nontransient face-to-face contact indoors with a playmate or other contact. In the hospital significant exposure to VZV is defined as exposure in the same two to four bedroom, face-to-face contact with an infectious staff member or patient, or a visit by a person deemed contagious (39). VZV can be spread through direct contact and airborne contacts from a person with active varicella. Patients with HZ may transmit VZV to a person who has never had varicella through direct contact with the rash. There is emerging evidence that VZV may be spread through an airborne route even from localized HZ (2,72–74).

Options for postexposure prophylaxis include passive immunoprophylaxis and/or antiviral therapy. VZIG is no longer available in most centers, and a non-FDA-licensed VZV immune globulin VariZIG™ (Cangene Corporation, Winnipeg, Canada) may be the only VZV specific immunoprophylaxis available (3). In the United States it is available only through an investigational new drug application, lack of rapid access may further limit the use of VariZIG at many centers (75). If available, VZIG or VariZIG is recommended in susceptible patients exposed to VZV and should be given as soon as possible but within at least 10 days of exposure (II-1) (39,76). Immunoprophylaxis alone does not prevent all immunosuppressed patients from developing clinical varicella but lessens the severity of infection (77–79). Although not studied in clinical trials, nonspecific IVIG has been suggested as an alternate postexposure prophylaxis when VariZIG is not available (39); combination use of IVIG with antiviral therapy in immunocompromised patients can also be considered (III).

The use of antiviral agents as postexposure prophylaxis has not been evaluated in randomized clinical trials in immunocompromised patients, but should be considered as adjunctive therapy in patients receiving immunoprophylaxis or in patients who were unable to receive immunoprophylaxis before 10 days after their exposure (III) (76). The value of acyclovir as postexposure prophylaxis has been shown in a study of immunocomponent children (80) and has been suggested to be effective (in addition to VZIG) in a small study of high-risk children, which included five kidney transplant recipients (81). Because of the unpredictable absorption and low bioavailability of oral acyclovir (82,83), valacyclovir, which has improved bioavailability (84), may be preferred for prophylaxis (III). Current recommendations are for patients to receive acyclovir or valacyclovir for a 7-day course of therapy beginning 7–10 days after varicella exposure (III) (39). Alternatively, some experts believe those who are highly immunosuppressed should receive longer antiviral prophylaxis from days 3 to 22 after known exposure and from days 3 to 28 if given immunoprophylaxis (III) (85,86).

Infection Control Issues

All immunosuppressed patients admitted to the hospital with varicella or HZ should be placed on airborne and contact isolation, and close contacts who are susceptible to VZV should be immunized as soon as possible (preferably within 3 days of exposure with possible efficacy as late as 5 days postexposure) or given appropriate VZV

prophylaxis (II-2) (39). Patients should be isolated until at least all lesions are crusted, which can be delayed in immuncompromised patients (39). In addition to postexposure prophylaxis, exposed susceptible patients should remain in airborne and contact precautions from day 10 to 21 while in the hospital after exposure to the index patient, and those who receive VariZIG or IVIG should remain in precautions until day 28 (39). Patients with localized zoster lesions should also have them covered as this can potentially decrease transmission risk (74).

Because secondary cases of VZV in a household setting can be more severe due to exposure to a higher titer of virus (87), vaccination of close household members is an important part of prevention. Vaccinated individuals are at least 50% less contagious when they develop varicella and secondary attack rates are much lower (88). Close contacts and family members 12 months or older should be vaccinated for VZV if they have never received the vaccination, have no history of varicella or HZ, and have no contraindications to vaccination (I). Transplant recipients should be isolated from vaccinated contacts who develop a varicella-like rash, particularly those with >50 lesions, as vaccine associated rashes can result in transmission (88).

Future Research Issues

Studies are currently underway to evaluate the safety and efficacy of pretransplant vaccination for HZ in seropositive recipients (89,90). Large randomized trials evaluating safety and efficacy of both varicella and HZ vaccines in posttransplant patients are also needed. Inactivated VZV vaccines, which are in development, may eventually provide another option for this high-risk population (91). Additional studies to assess the use of low-dose antiviral therapy as long term postexposure prophylaxis are also needed. Finally, as new immunosuppressive agents are developed, they will need to be evaluated both in terms of altering risk for HZ posttransplant as well as their effect on vaccine efficacy.

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Special Article

Viral Hepatitis in Solid Organ Transplantation

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Key words: acyclovir, posttransplant infection, shingles, valacyclovir, varicella infections, viral infection

Abbreviations: FCH, fibrosing cholestatic hepatitis; HAV, hepatitis A virus; HBIG, hepatitis B immunoglobulin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HCV-R, HCV recurrence; HDV, hepatitis D virus; HEV, hepatitis E virus; HVPG, hepatic venous pressure gradient; IgM, immunoglobulin M; LADR, low accelerated dose regimen; LAM, lamivudine; IFNα, interferon alfa; PegIFNα, pegylated interferon alfa; PI, protease inhibitor; RBV, ribavirin; SOT, solid organ transplant; SVR, sustained virologic response.

Introduction

A number of hepatotrophic viruses affect organ transplant candidates and recipients. The most important agents causing acute and chronic hepatitis are hepatitis B virus (HBV), with or without hepatitis delta virus (HDV), and hepatitis C virus (HCV). In addition, hepatitis E virus (HEV), previously thought to only cause acute, self-limited infection in the developing world, is emerging as an increasing cause of chronic hepatitis in transplant recipients in industrialized countries. Management of viral hepatitis in transplant candidates and recipients is complex and highly depends on the organ transplanted, particularly for HBV and HCV, and the donor/recipient status. This chapter will focus primarily on the epidemiology, diagnosis, treatment and prevention of the primary hepatotrophic viruses (A-E) after hepatic and nonhepatic organ transplantation.

Hepatitis A Virus (HAV)

HAV is a nonenveloped RNA virus and a member of the picornavirus family. It is largely transmitted person-to-person by the fecal–oral route, although blood borne transmission can occur (1,2). High-income regions of the world have very low HAV endemicity levels and a high proportion of nonimmune adults, whereas in low-income regions with high endemicity most adults are immune on the basis of prior infection (3). Worldwide, approximately 1.4 million cases of hepatitis A are reported each year; however, the true incidence is estimated to be 3–10 times higher. HAV vaccines have been licensed since 1992 and vaccination of susceptible, at-risk individuals (e.g. pre- and postorgan transplantation) is advised based on national guidelines (4).

Acute HAV infection is generally self-limited, but the risk of fulminant hepatic failure increases with age (5). Young children are frequently asymptomatic, whereas older children and adults may develop a range of clinical manifestations from mild anicteric infection to fulminant hepatic failure. Those with underlying chronic liver disease of any etiology are at increased risk of fulminant disease and those who are nonimmune should be vaccinated (4).

The estimated fatality rate for HAV is low (<1.5%). Among those who develop fulminant hepatic failure, 35–40% will spontaneously recover, whereas others usually survive after liver transplantation (LT) (6,7). Rarely, HAV recurs after transplantation (8,9).

Recommendations:

- All hepatic transplant candidates and all nonhepatic transplant candidates with chronic liver disease, or other known risk factors for HAV (e.g. men who have sex with men, travel to endemic region, sanitation workers and hemophiliacs), should be tested for HAV-IgG and if negative offered vaccination. Ideally, vaccination should be performed before LT, but it may also be given afterwards (II-1).
- Patients with fulminant hepatic failure due to acute HAV should be assessed for LT (II-2).

Hepatitis B Virus (HBV)

Hepatic transplantation

Epidemiology and risk factors: HBV is a DNA hepadnavirus transmitted parenterally, sexually and vertically. Worldwide, it infects ~400 million individuals and causes over one million deaths per year (10,11). The prevalence can be high (≥8%), intermediate (2–7%) or low (<2%) depending on the geographic region (12). With an increase in immigrants from endemic countries, it is now estimated that >2 million HBV-infected individuals reside in the United States, of which ~5000 per year die from

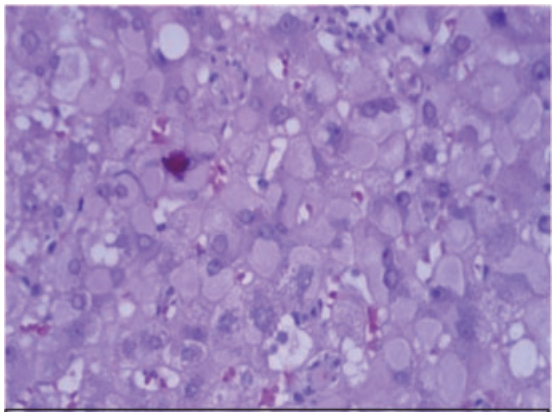

Figure 1: Ground-glass hepatocytes filled with HbsAg.

complications (13). However, the ability to effectively prevent HBV infection by immunization and treat the disease with antiviral therapy represent major advances of modern medicine. Even so, HBV infection still remains an important indication for LT.

Before the early 1990s and available HBV prophylaxis, untreatable recurrent HBV disease occurred in most recipients undergoing LT for this indication (14–16). Some developed a rapid, fibrosing cholestatic hepatitis (FCH) variant that led to poor early survival rates (17–20). A dramatic shift occurred in the mid-late 1990s with the advent of hepatitis B immunoglobulin (HBIG) and the first oral antiviral drug for HBV, lamivudine (LAM). Combination therapy (HBIG + LAM), without graft reinfection, became the rule resulting in advances in survival that now supersede other indications (21,22). More recently, nucleos(t)ide analogues (tenofovir, TDF; entecavir; ETV) with high barriers to resistance have been shown to rescue patients from liver failure and need for LT, as well as allow for excellent outcomes without recurrence post-LT (23–27). Even with risk factors, e.g. high viral load at OLT ($>2 \times 10^4$ IU/mL; HBeAg positivity), recurrence is now exceedingly rare. When recurrence occurs, the typical causes are noncompliance to antiviral therapy and/or HBIG, or resistance if an older agent (LAM; adefovir, ADV) is used as monotherapy (28–30). Patients with fulminant HBV or concurrent HDV have a low incidence of recurrence, given their characteristic low viral loads (31–34).

Diagnosis: HBV recurrence has been historically defined as the reappearance of HBsAg after LT, although patients on antiviral prophylaxis and not HBIG may develop HBsAg positivity without actual recurrence (DNA undetectable, normal biochemistry and histology). The reverse may also occur, i.e. low levels of viremia in the absence of HBsAg positivity, either spontaneously or due to HBsAg escape mutants during HBIG therapy (35–37). The histology of recurrence is similar to that of pretransplant HBV (Figure 1), with the exception of FCH. This now uncommon entity is defined as rapidly progressive cholestasis, fibrosis and multi-organ failure (38).

All patients should be followed post-LT by a clinician (hepatologist, infectious disease, other) experienced in the management of HBV infection. Although monitoring protocols for HBV recurrence vary among centers, HBsAg and DNA should be performed at least every 3 months within the first year and every 6 months thereafter even with prophylaxis. In patients receiving HBIG, it is typical to follow anti-HBs titers with a predose goal of >100 or >500 IU/L in those with low or high DNA at LT, respectively. More frequent anti-HBs titers and dosing intervals should be performed if levels remain below these thresholds.

Recommendations:

- Every 3–6 month monitoring of HBsAg and HBV DNA should be performed in HBV positive liver transplant recipients, regardless of treatment or prophylaxis regimen (III).
- Anti-HBs titers should be measured before HBIG doses, with a goal of 100 IU/L or higher depending on the risk of HBV recurrence (i.e. HBV DNA and eAg status at LT) (III).

Treatment: Central to the prevention of HBV recurrence post-LT is adequate pre-LT viral control (Table 1). Although seven drugs are licensed for HBV therapy, including interferon alfa (IFNα), pegylated interferon alfa-2a (PegIFNα-2a), telbivudine, LAM, ADV, TDF and ETV, only the latter two are advisable in patients with hepatic decompensation, due to high efficacy and low resistance. HBV DNA reduction to undetectable, or at least $<1 \times 10^5$ IU/mL, with a potent nucleos(t)ide analogue having high barrier to resistance (TDF, ETV) reduces the risk of HBV recurrence (39–42). Combination nucleoside/nucleotide [i.e. TDF/emtricitabine (FTC); TDF+ETV; LAM+ADV] therapy is often used by centers pre- and post-LT but the published data do not support any benefit of this approach over potent monotherapy. Rarely, antiviral therapy can lead to mitochondrial dysfunction and lactic acidosis requiring urgent discontinuation (43,44). Entecavir needs to specifically be taken on an empty stomach. A more detailed review of HBV therapy pre-LT can be found in current national guidelines (45).

HBV recurrence after LT is the result of failed prophylaxis (see below), either due to noncompliance or the development of drug/HBIG resistance. LAM resistance may occur in up to 50% of LT recipients and predisposes patients to ETV resistance long-term (46). Resistance to ADV is seen in up to 30% at 5 years before LT, although this has not been shown to lead to a higher rate of TDF resistance or loss of efficacy (47). However, the typical strategy is to either switch classes of drugs with high barrier to resistance or add the other class agent, resulting in combination therapy (48–54). The latter combination approach

Table 1: Suggested hepatitis B virus prophylaxis for hepatic and nonhepatic transplantation

Donor		Recipient					
HBcAb	HBsAg	HBcAb	HBsAg	HBsAb	HBIG?	Antiviral prophylaxis?	Vaccinate?[2]
Hepatic[1]							
(−)	(−)	(+)	(−)	(−) or (+)	No	No	Consider if sAb (−) or lost
(−) or (+)	(−) or (+)	(+)	(+)	(−)	Yes[3]	Yes	No
(+)	(−)	(−) or (+)	(−)	(+)	No	Yes, unless sAb persists	Consider if sAb lost
(+)	(−)	(−) or (+)	(−)	(−)	No	Yes, unless sAb persists after vaccination[4]	Yes
(+)	(+)	(−) or (+)	(−)	(−) or (+)	[5]	[5]	Consider if sAb (−) or lost
Nonhepatic[1]							
(−)	(−)	(+)	(−)	(−) or (+)	No	No	Consider if sAb (−) or lost
(−) or (+)	(−) or (+)	(+)	(+)	(−)	No	Yes	No
(+)	(−)	(−) or (+)	(−)	(+)	No	Yes, unless sAb persists	Consider if sAb (−) or lost
(+)	(−)	(−) or (+)	(−)	(−)	No	Yes, unless sAb persists after vaccination[4]	Yes
(+)	(+)	(−) or (+)	(−)	(−) or (+)	[5]	[5]	Consider if sAb (−) or lost

[1]All patients posttransplant: HBV sAg/DNA and liver function tests every 3–6 months and follow-up with an HBV provider.
[2]Typically vaccinate at 1 year posttransplant. Consider high-dose vaccine (40 mg) at 0, 7 and 28 days and assess HBsAb >1 month after.
[3]If HBV DNA (–) at transplant, consider short-term HBIG therapy; If HBV DNA (+) at transplant, consider long-term or indefinite HBIG.
[4]If donor HBV DNA is performed and negative, no prophylaxis is required, although close monitoring for HBV recurrence is recommended.
[5]Transplant typically contraindicated but may consider in select, "desparate" cases, in the setting of indefinite antiviral prophylaxis and close monitoring.

is often practiced anecdotally but again not proven to be more effective. HBIG is typically discontinued in patients with recurrent HBV. The nucleotide agents ADV and TDF may cause proximal renal tubular injury in a small percentage of patients, although this has mainly been seen in HIV infected populations (55,56). Renal function should be monitored and dose adjustments made for all agents.

Prevention/Prophylaxis: Many centers still use combination therapy with HBIG and LAM that effectively prevents HBV recurrence (Table 1; Refs.57–62). However, LAM resistance and the cost and inconvenience of intravenous HBIG have motivated a recent trend toward alternative preparations or HBIG withdrawal in conjunction with potent oral antivirals. Intramuscular HBIG is less expensive and represents an acceptable alternative to IV, particularly in patients with low HBV DNA at LT (34,59,63–67). In this group, HBIG can be safely withdrawn postoperatively (6–12 months) in conjunction with continued oral antiviral therapy (21,68). Similar low recurrence rates have also been reported with combination therapy (LAM + ADV) before and after OLT, even without HBIG therapy (48,69). Others have reported the use of newer, potent antiviral agents (TDF, ETV) ± HBIG, even in patients who are viremic at OLT (70–75). That being said, it is still currently recommended to give, at minimum, a short course of HBIG in combination with indefinite antiviral therapy with high barriers to resistance. One recent interesting study showed patients with undetectable HBV DNA at LT and no evidence of latent intrahepatic total and cccDNA may safely undergo full weaning of prophylaxis, although larger studies are needed before recommending this biopsy-driven approach (76). Vaccination as a strategy to allow discontinuation of HBIG or antivirals has yielded unreliable results and is not advisable. Antiviral prophylaxis is not necessary for anti-HBc positive "alone" recipients (i.e. sAg and HBV DNA negative), unless perhaps in situations of intense immunosuppressive therapy (i.e. lymphodepletion) (77–80).

Recommendations:

- HBsAg positive LT recipients should be treated indefinitely with nucleos(t)ide analogue therapy having high barriers to resistance + at minimum short-term HBIG (II-1).
- The choice of antiviral regimen should be based on the successful approach used pre-LT, factoring in prior exposures, resistance, potential drug interactions and side effects (II-1).

Anti-HBc positive donors and recipients: Anti-HBc positive donors have been increasingly used to expand the donor pool, although without prophylaxis they pose a 34–86% risk of transmitting HBV infection to unexposed (HBsAg negative) LT recipients (81). Oral antiviral therapy ± HBIG is effective prophylaxis for recipients who are HBsAg negative ± anti-HBc positive (Table 1). Lamivudine may be more effective than HBIG (82) and is preferred by many centers for logistical ease and cost. Although not standard of care, the available data suggest that discontinuation of prophylaxis can be considered in certain situations with careful monitoring: (1) donor serum, if available, is HBV DNA negative; (2) recipient is vaccinated or exposed pre-LT and maintains anti-HBs positivity post-LT and (3) recipient is vaccinated post-LT (~12 months) and maintains anti-HBs positivity (81,83–88). Rarely, HBV infection despite LAM or ADV has been reported in recipients of anti-HBc positive organs (82), although there are insufficient data to recommend newer agents as primary prophylaxis compared to rescue therapy for breakthrough (89). Routine HBsAg and/or HBV DNA monitoring in prophylaxed recipients of anti-HBc positive grafts may not be necessary, although transaminase elevations should prompt these investigations to exclude HBV infection.

Recommendations:

- Recipients of anti-HBc positive donors should generally receive indefinite prophylaxis with antiviral therapy ± HBIG (II-2).
- Discontinuation of prophylaxis is not standard of care but might be considered in closely monitored patients who maintain anti-HBs positivity and/or receive a donor with undetectable HBV DNA (III).
- Routine antiviral prophylaxis is not recommended for anti-HBc positive "alone" recipients (donor negative, recipient sAg and DNA negative) but may be considered in those felt to be at increased risk of reactivation (e.g. lymphodepletion therapy) (III).

Infection control issues: All HBV noninfected, nonimmune patients with cirrhosis should be vaccinated, as *de novo* HBV infection can lead to decompensated liver failure. Even with double dose regimens, the percentage who successfully seroconvert is suboptimal (16–62%), and many (37–73%) lose anti-HBs within the first year after LT (90–96). Thus, repeat or booster vaccination should be attempted at ~12 months post-LT with the goal of seroconversion. All household and sexual contacts of HBV-infected recipients should be vaccinated. HBV-infected recipients should not share with others personal items that may be contaminated with blood, such as toothbrushes, razor blades, nail clippers, etc.

Recommendation:

- All HBV noninfected, nonimmune patients with cirrhosis and transplant recipients should receive the HBV vaccine with seroconversion documented (II-2).

HBV: Nonhepatic Transplantation

Epidemiology and risk factors: The prevalence of chronic HBV infection and markers of prior HBV in nonhepatic solid organ transplant (SOT) candidates and recipients vary widely by population and geographic region (97). In Western countries, the strict institution of infection control practices and HBV vaccination in patients on dialysis has led to a decline in the prevalence of chronic HBV, which now ranges between 0% and 6.6% (97). In contrast, a registry study of dialysis patients in Asia-Pacific countries found a prevalence of HBsAg positivity ranging between 1.3% and 14.6% (98). Although incident cases of HBV acquired on dialysis are considered uncommon, particularly in the U.S. and Europe, transmissions and outbreaks are still reported and reflect a need for ongoing education, case identification and management (99,100). There are no data with regards to the prevalence of chronic HBV in thoracic organ transplant candidates/recipients. It is likely that the prevalence and risk factors for HBV mirrors that of the general background population, with mother-to-child transmission and early childhood horizontal acquisition being the major risk factors in those in or born in highly endemic regions. Parenteral and sexual transmission are the dominant modes of transmission in areas of low endemicity (101).

The risk of reactivation of HBV in HBsAg positive renal transplant recipients, in the absence of antiviral prophylaxis, ranges from 50% to 94% (102–104). Historically, before the era of effective antiviral therapy, nonhepatic SOT in recipients with chronic HBV infection was associated with substantial reductions in patient and graft survival due to rapidly progressive liver disease (105–108). Several recent studies in both renal and cardiac transplantation have showen excellent outcomes in HBsAg positive patients managed with antiviral therapy (109–114).

The prevalence of markers of prior HBV infection (HBsAg negative but anti-HBc positive, with or without positive anti-HBs) is significantly higher than the prevalence of chronic HBV in any given population. In the U.S. population, the estimated prevalence of HBsAg is 0.27%, whereas that of positive ant-HBc is 4.7% (115). In nonhepatic SOT recipients with markers of past HBV infection there is a risk of reactivation, although this is low and estimated to be at most 5% (116,117). The natural history of reactivation in this setting seems to be a loss of the protective anti-HBs (if present at baseline) followed by a rise in HBV DNA and then seroreversion to a positive HBsAg state. It generally occurs early, within the first year, after transplant. Although the overall risk of reactivation in this setting is low, when it does occur, rapid progression and death due to liver disease have been described in the absence of antiviral therapy (117).

Diagnosis: The diagnosis of HBV relies on the same serologic and virologic assays used in the nontransplant

population (39,45,118). As in all patients with chronic HBV, there is an increased risk of hepatocellular carcinoma (HCC). Nonhepatic SOT who are HBsAg positive should undergo HCC surveillance based on published guidelines (118,119).

Recommendations:

- Initial screening for HBV should be done at the time transplant candidate assessment and include HBsAg, anti-HBs and anti-HBc (III).
- Nonhepatic SOT candidates identified as HBsAg positive should undergo additional testing, including HBeAg, anti-HBe, quantitative HBV DNA, liver enzymes, alfa-fetoprotein and abdominal ultrasound (III).
- HBsAg positive nonhepatic SOT candidates and recipients should undergo risk based surveillance for HCC, in concordance with published guidelines in the nontransplant population, with an abdominal ultrasound and alpha-fetoprotein every 6 months (III).

Treatment: A nonhepatic SOT candidate identified to be HBsAg positive during assessment should be evaluated for the need for therapy before transplant. The management of HBV is complex and requires lifelong monitoring and follow-up whether or not antiviral therapy is initiated, and thus referral to a specialist with expertise in the management of HBV is recommended. Therapy should be based on guidelines published for the management of HBV in the general population (39,45,118). In those with indications for therapy before SOT, LAM is no longer recommended as first line therapy due to the high risk of resistance, unless more potent agents are unavailable. Treatment with a potent nucleos(t)ide analogue, such as ETV or TDF adjusted for renal function as needed, should be used given the need for long-term therapy and to limit the risk of future resistance. It has been suggested that ETV may be preferred over TDF in the renal transplant population due to the lack of nephrotoxicity (45). Interferon or peginterferon is not recommended due to poor tolerability, bone marrow suppression and a low rate of response in immunocompromised hosts.

The risk of HBV reactivation persists as long as patients remain on immunosuppressive therapy. Thus, once treatment is initiated pretransplant, it should be continued up to the time of transplant and indefinitely posttransplant as long as the patient remains on immunosuppressive therapy. If the recipient comes off immunosuppression (e.g. return to dialysis due to failed renal graft), the need for ongoing HBV therapy should be reviewed and any consideration of discontinuation of antiviral therapy should follow national guidelines (45).

As in the general population, nonhepatic SOT candidates initiated on therapy for chronic HBV should undergo regular follow-up and monitoring for response to antiviral therapy and continue HCC surveillance (39,45,118).

Recommendations:

- Nonhepatic SOT candidates with chronic HBV should be evaluated for the need for therapy by a specialist with expertise in HBV management before transplantation (III).
- If therapy for HBV is indicated, TDF or ETV are preferred due to their potency and high barriers to resistance (III).
- All nonhepatic SOT candidates or recipients with chronic HBV on nucleos(t)ide analogue therapy should undergo liver enzyme and HBV DNA monitoring every 3–6 months (III).
- Once therapy is started it should be continued indefinitely in the setting of immunosuppression (III).

Prevention/Prophylaxis: Nonimmune nonhepatic SOT candidates/recipients are at risk for acquisition of HBV through the usual risk factors, but also importantly via transmission from an organ donor. In many circumstances, vaccination with documented seroconversion can protect against donor-transmitted HBV (see below). Although the proportion of those with end-stage renal disease who will seroconvert, even to double dose HBV vaccine, is suboptimal, 55–67% will respond (120). Response rates are higher in those with lesser degrees of renal dysfunction and certainly better pre- than posttransplant (121). Amongst thoracic organ transplant candidates, response rates to HBV vaccine seem similarly suboptimal (45–53%) but still worthwhile (101,122).

If HBV vaccine was not given before transplant, consideration should be given to vaccination posttransplant. The rate of seroconversion to a protective titre of positive anti-HBs in the renal transplant population has been found to be 17–36% (123,124).

Recommendations:

- All HBV uninfected, nonimmune, nonhepatic SOT candidates should be vaccinated for HBV as early in the course of their disease as possible (III).
- In those not vaccinated before transplant, HBV vaccine should be considered posttransplant, once immunosuppression is at maintenance doses (generally 12 months) (III).

As described previously, the risk of reactivation of HBV in HBsAg positive renal transplant recipients, in the absence of antiviral prophylaxis, ranges from 50% to 94% (102–104). In the era before effective HBV antiviral therapy, this resulted frequently in rapidly progressive liver disease and an increased risk of graft loss and death (105–108). As LAM was the first available oral antiviral for HBV, this is the agent that has been used in most studies (110,125).

Although LAM has been shown to significantly improve patient survival after renal transplant (83% vs. 34% at 20 years), its use and impact is limited by a high (60–70%) risk of resistance over 4–5 years (125). As such, despite the improvement in overall survival, there remains an increased risk of liver-related mortality in HBsAg positive renal transplant recipients managed with LAM (125). In light of these data (126), ETV or TDF are recommended to limit the potential for resistance, with LAM or ADV reserved for those without other options (45). Interferon-based therapy is contraindicated posttransplant due to the risk of rejection.

Recommendations:

- Because of the high risk of reactivation, nonhepatic SOT recipients with chronic HBV who are not on antiviral therapy before transplant should be initiated on nucleos(t)ide analogue therapy at the time of transplant (II-2).
- Antiviral therapy should be continued indefinitely posttransplant (II-2).
- ETV or TDF is recommended as first line therapy, with LAM or ADV reserved for those without these options (III).
- Follow-up monitoring should include liver enzymes and HBV DNA every 3–6 months (III).

In those with markers of past HBV infection (HBsAg negative, anti-HBc positive ± anti-HBs positive), there is a low (~5%) risk of HBV reactivation (116,117). Data are lacking regarding the optimal approach in this situation. Given the absence of data and the low overall risk, routine antiviral prophylaxis in this group cannot be recommended. Some centers use prophylaxis in patients felt to be at increased risk (e.g. anti-HBc alone, intense immunosuppression). Alternatively, some have advocated monitoring of HBV DNA and institution of pre-emptive antiviral therapy if the DNA progressively rises (45). The challenge with this strategy is that there are no data regarding the optimal frequency of monitoring or the HBV DNA threshold at which antiviral therapy should be initiated. Given the natural history of reactivation, in those who are both anti-HBc and anti-HBs positive, some centers monitor only anti-HBs over the first 12 posttransplant months because as long as this remains above protective titres, there is a negligible risk of reactivation.

Recommendations:

- In those with markers of past HBV infection (HBsAg negative, anti-HBc positive ± anti-HBs positive), routine antiviral prophylaxis is not recommended, but may be considered in those felt to be at increased risk of reactivation (e.g. anti-HBc+ alone or intense immunosuppression) (III).
- Alternatively, HBV DNA and HBsAg should be monitored, with antiviral therapy initiated if HBsAg becomes positive or if HBV DNA progressively rises (III). With this strategy, given that antiviral therapy will be started at higher levels of HBV DNA, TDF or ETV are recommended (III).

The HBsAg or anti-HBc positive donor: Hepatitis B uninfected, nonimmune patients undergoing nonhepatic SOT may acquire donor derived HBV. The HBsAg positive donor carries a high risk of transmission to recipients although satisfactory outcomes have been described generally with the use of combined HBIG and antiviral prophylaxis (127–130). The duration of prophylaxis required is unknown, although lifelong nucleos(t)ide analogue therapy has been suggested (127). If the HBsAg and HBV DNA remain negative, consideration may be given to discontinuing HBIG 6–12 months posttransplant.

The risk of HBV transmission from an anti-HBc positive nonhepatic donor is significantly lower than that of hepatic donors, ranging from 0% to 5.2% in different studies (131,132). Renal and thoracic organs from anti-HBc positive donors have been safely used with strategies to minimize the risk of transmission (127,133,134). In recipients of a nonhepatic organ from an anti-HBc positive donor, the risk of transmission is negligible if the recipient is immune (127,133), thus highlighting the importance of pretransplant immunization. In HBV nonimmune recipients of an anti-HBc positive organ, the risk of transmission is presumed to be related to the presence of HBV DNA present in the plasma or PBMC of the organ donor. As such, assessment of HBV DNA in the donor may guide the need for prophylaxis (127). Although the optimal duration of prophylaxis is unknown, the risk period is thought to be restricted to the early posttransplant period until elimination of donor PBMC.

Recommendations:

- Consideration may be given to using organs from HBsAg positive donors, particularly for a lifesaving (i.e. nonrenal) transplant, and with HBIG/antiviral prophylaxis and informed consent (II-3).
- In HBV immune (anti-HBs positive) recipients of an anti-HBc positive nonhepatic organ, no prophylaxis is needed (II-2).
- For HBV nonimmune recipients of an anti-HBc positive nonhepatic organ:
  - If the donor is HBV DNA negative, no antiviral prophylaxis is needed.
  - If the donor HBV DNA is positive or unknown, prophylaxis with either antiviral therapy or HBIG is suggested for at minimum 6–12 months (II-2).
- Recipients of organs from HBsAg or anti-HBc positive donors should undergo monitoring with liver enzymes, HBsAg and HBV DNA:
  - Every 3 months for at least 12 months posttransplant (II-3) (127).
  - Beyond 12 months, every 6 months indefinitely, particularly in recipients of an HBsAg positive organ (III).

Infection control issues: HBsAg positive SOT recipients should not share personal items that may be contaminated with even small amounts of blood. All close contacts should be screened for HBV, vaccinated if nonimmune and have documentation of anti-HBs seroconversion.

Hepatitis C Virus

Hepatic transplantation

Epidemiology and risk factors: HCV is an RNA flavivirus closely related to hepatitis G, yellow fever and dengue viruses (135). Replication is dependent on cellular proteases and an error-prone RNA polymerase resulting in a diverse array of quasispecies that challenge immunological clearance (136). It is the cause of >4 million and >170 million cases of chronic hepatitis in the United States and world, respectively (137). Blood-screening measures have nearly eliminated the risk of transfusion-associated hepatitis in Western countries, but new cases continue to occur related to injection-drug use, repeated mucous-membrane exposures (commercial sex workers; men who have sex with men), accidental percutaneous exposures and occasionally iatrogenic transmissions (53). In the Western world, genotypes 1a and 1b are the most common, followed by genotypes 2 and 3. The other genotypes are primarily seen in the Middle East (genotype 4), southern Africa (genotype 5) and Southeast Asia (genotype 6).

Worldwide, hepatitis C is a leading indication for LT. The number of patients requiring LT for HCV is expected to peak in the next 10–15 years followed by a decline due to advances in treatment and fewer new infections (138). Recurrence after LT is universal and histological injury is accelerated in the new graft compared to the rate pretransplantation (139,140). For this reason, recipients with HCV-recurrence (HCV-R) have worse 5-year patient (65%) and graft survival (60%) compared with HCV-negative recipients (75% and 70%), both with primary or repeat transplantation (141). Early FCH due to HCV only occurs in <5% and is associated with poor outcomes (Figure 2). Fibrosis progression is best predicted by performing early (6–12 months post-OLT) liver biopsies (142,143). Once recurrent HCV cirrhosis occurs, ~50% decompensate within 1 year (140,144). Retransplantation is controversial and is often not considered in those with advanced age, deconditioning, renal failure, MELD > 25 and early (<1–2 years) aggressive recurrence (145–154).

The strongest risk factors for recurrence are high dose immunosuppressive therapy for acute rejection, concurrent HIV or CMV infection, older donor/recipient age, HCV viral load and severe preservation injury or steatosis. In contrast to HCV-negative recipients, treatment of acute rejection is associated with increased mortality and graft loss in recipients with HCV (relative risk = 2.7–2.9, p = 0.04; Ref. 155).

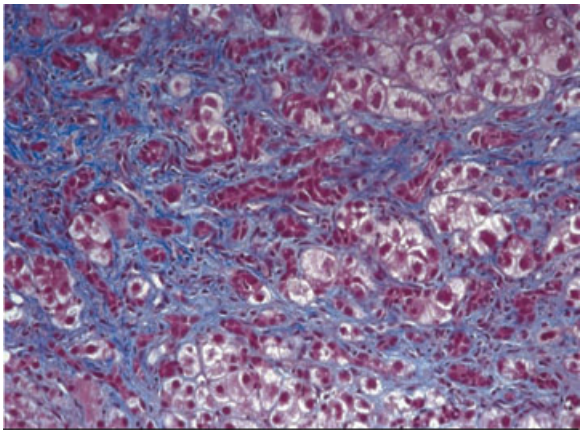

Figure 2: Fibrosing cholestatic hepatitis C.

It is not, however, conclusive that lymphocyte depleting or nondepleting antibodies increase the risk of recurrence when used primarily as induction therapy (156–159). There has been significant recent interest in donor/recipient IL-28b gene polymorphisms (either favorable C or unfavorable T alleles) being predictive of progressive HCV-R and response to IFN therapy post-LT (160–174).

Inconclusive risk factors for recurrence include genotype 1 versus non-1, HLA mismatches, the use of donors after cardiac death or live donors and the choice of maintenance IS therapy (155,157,162,168,175–204). Recent data have supported a slower steroid tapering schedule or no steroids to avoid precipitating early recurrence, although this is not universally practiced. There has been some interest in the antifibrotic properties of mTOR inhibitors in minimizing fibrosis progression in HCV+ recipients, although conflicting data suggest a higher risk of mortality in this setting (205,206). The use of nonfibrotic HCV-positive donors for HCV-positive recipients results in outcomes similar to the use of an HCV-negative donor (207,208). However, the use of genotype 1 donors for genotype 2/3 or any HCV positive donor into nonviremic recipients should be avoided. Recent studies also suggests that outcomes are inferior if HCV-positive donors over age 45 are used compared to younger HCV-positive donors (209) or if HCV-positive donors are used for HIV–HCV co-infected recipients (210).

Recommendations:

- Avoidance of older donors, acute rejection and other forms of liver injury and infectious complications may limit the progression of HCV recurrence after LT (II-2).
- Genotype 1 donors should not be allocated to recipients who are genotype 2 or 3 (III).
- No HCV positive donors should be allocated to HCV positive recipients who have had a sustained virological response (i.e. nonviremic).

Diagnosis: During the anhepatic phase of the LT procedure, HCV RNA rapidly decreases from the serum. The rate of HCV RNA decline accelerates further after reperfusion, likely due to HCV binding to its hepatocellular receptors (211). HCV RNA levels then increase rapidly after the first few weeks and, at 1 year, can reach >10–20 fold higher levels than pre-LT. In the first 6 months, acute hepatitis of varying severity occurs in approximately 75% of recipients, with <10% developing severe cholestatic forms. Fibrosis occurs in >50% at 1–2 years after LT and up to 30% progress to advanced fibrosis or cirrhosis by year 5 (212). Thus, the gold standard for diagnosis is liver biopsy, with the caveat that it may not distinguish other causes (rejection, biliary obstruction) from HCV-R early after LT and may underestimate fibrosis stage (213). Most centers routinely perform protocol liver biopsies at defined time points (i.e. yearly) to monitor for recurrence indicating the need for treatment (grade 3 or stage 1–2; Ref.214). Other adjunctive measures that are less routinely used include hepatic venous pressure gradients (HVPG), and blood tests and imaging for fibrosis. An elevated HVPG regardless of fibrosis stage has been shown to predict the development of progressive HCV-R and portal hypertension and declines with successful antiviral therapy (215–218). Noninvasive liver stiffness assessments with transient or magnetic resonance elastography can detect fibrosis and may be appropriate for fibrosis monitoring, although neither are widely available in the United States (219–221).

Recommendations:

- The current gold standard for diagnosing HCV recurrence is liver biopsy (II-2).
- Other adjunctive measures to assist in determining the progression of HCV recurrence include HVPGs and elastography (III).

Treatment:

Pretransplant: Achieving a sustained virologic response (SVR) with pre-LT antiviral therapy may delay the need for OLT and eliminate the risk of HCV-R. However, this comes at a price due to poor patient tolerability and efficacy, depending on the degree of hepatic decompensation and the genotype (222–224). A low accelerated dose regimen (LADR) approach of slowly advancing Peg-IFNα and RBV doses to target levels may improve tolerability and achieve an SVR that is usually maintained after OLT, assuming >8–12 weeks of pre-LT viral negativity is achieved (223). However, this approach is most effective in nongenotype 1 cirrhotic patients and is associated with a high risk of infectious complications with little benefit (<10% SVR) in genotype 1 patients. Thus, treatment should be limited to decompensated cirrhotic patients with CTP score ≤7, physiological MELD score <20, and primarily genotype 2 and 3 infection. In 2011, two first generation NS3/4A protease inhibitors (PIs: boceprevir, telaprevir) became available for use in combination with Peg-IFNα and ribavirin (RBV) for the treatment of genotype 1 HCV, even in compensated cirrhosis (225,226). Although there are no current published data in decompensated patients, a number of centers are starting to initiate this triple therapy regimen with the goal of viral eradication before LT. It is not currently known if the overall benefit will ultimately exceed the added risks, and it is likely that combinations of oral antiviral agents without IFN or RBV will eventually be more desirable in this high-risk population.

Posttransplant (Pre-emptive vs. Wait for Recurrence)

Preemptive HCV therapy instituted immediately or within a few weeks after OLT has not been shown to delay the onset of recurrence (227). The largest, randomized study compared the safety, tolerability and efficacy of preemptive initiation of Peg-IFNα-2a plus RBV within 26 weeks after LT versus initiation only upon established recurrence. On an intent-to-treat basis, recurrence at 120 weeks was similar in the prophylaxis (61.8%) and observation arms (65.0%, $p = 0.725$). Similar results were shown with PEG alone and other smaller trials (228–230). Given the toxicity and lack of virologic benefit, preemptive therapy is not currently advisable in clinical practice.

Most centers wait until the development of histological recurrence, typically detected by protocol or for-cause liver biopsies. Post-LT treatment of histological recurrence with PEG + RBV is only successful in 20–30% of recipients and is associated with high rates (30–50%) of discontinuation due to intolerability (228,231–245). A major limiting factor in achieving an acceptable SVR rate is the inability to reach target RBV doses due to renal insufficiency and anemia (228,237,246). Although some reports have not shown an increase in the risk of acute rejection with IFN (228), a recent multicenter case-control study reported a 7.2% rate of PEG-related immunological graft dysfunction that was associated with poor patient and graft survival regardless of SVR (247). Evidence of alloimmune injury on pretreatment biopsy, such as plasma cell hepatitis, was the main risk factor for the development of this worrisome complication. Thus, careful review of pretreatment liver biopsies for alternative diagnoses other than pure HCV-R may suggest the need to avoid IFN therapy and instead augment IS therapy in this situation.

Finally, a number of abstracts at recent meetings have revealed preliminary data of the use of triple antiviral therapy (PEG + RBV + PI) for post-LT HCV-R. These early data are inconclusive and show the potential for benefit (higher SVR than non-PI approaches) and risk (severe anemia, infection) with this approach. In addition, both telaprevir and boceprevir strongly inhibit CYP3A4 enzymes and drug (CNI therapy) metabolism and may result in CNI toxicity or graft rejection upon drug discontinuation (248,249). Therefore, no conclusive recommendations can be made at this point

and further clinical trials, particularly with IFN-free regimens, are of great need in this population.

Recommendation:

- HCV treatment should be considered at the time of histological recurrence with PEG + RBV (II-2). It is not clear if the addition of a PI to this regimen in either decompensated pre-LT candidates or post-LT recipients is safe or more effective (III).

Prevention/prophylaxis: There have been multiple failed attempts to prevent HCV reinfection in the new graft. Agents such as hepatitis C immunoglobulin and IFN do not fully eliminate blood virions, even when given in the anhepatic or immediate postoperative phase (250). Thus, no current agents are available to prevent HCV-R.

Infection control issues: HCV-infected recipients should avoid sharing potentially contaminated items with other contacts. Sexual transmission is primarily seen in HIV+ individuals engaging in high-risk behavior (251–253). Thus, the use of contraception to prevent HCV transmission in longstanding monogamous relationships is unnecessary (251). The risk of vertical transmission is low (<5%) although higher in women with HIV or high HCV RNA levels.

HCV: Nonhepatic Transplantation

Epidemiology and risk factors

The prevalence of HCV infection in candidates for nonhepatic SOT varies by organ group and geography. The prevalence of HCV in dialysis patients has declined largely due to blood product screening implemented in the early 1990s and adherence to infection control practices. In 2002, the seroprevalence of HCV in US hemodialysis units was 7.8% (254). Substantial variability in HCV prevalence however exists by country and amongst different dialysis centers within a single country (254,255). HCV infection is an independent risk factor for mortality in hemodialysis patients (267,268). The prevalence of HCV infection in thoracic organ transplant candidates has been less rigorously assessed but seems to approximate the population prevalence (256,257).

The impact of HCV on the outcomes of nonhepatic SOT has been studied most extensively in renal transplant recipients. Several studies have looked at the rate of fibrosis progression using paired liver biopsies. Some have found the rate of HCV-related fibrosis progression is accelerated (258) whereas other have documented stable or improved findings on liver biopsy postrenal transplant (259–261). With long-term follow-up, it is clear that there is an adverse impact of HCV infection on overall patient and graft survival (106,262) with the 10-year survival being approximately 15% lower in HCV-positive compared to HCV negative renal transplant recipients. In addition, there is an increased risk of posttransplant diabetes, the potential for de novo or recurrent HCV-related renal disease, and an increased risk of severe infectious complications (263–265).

On an individual basis, however, the risk of accelerated progression of fibrosis and progression to end-stage liver disease and its complications seem to be limited largely to those with advanced fibrosis or cirrhosis at the time of transplant (106,259,261). Renal transplant patients with moderate (METAVIR stage 2) or less liver disease at baseline have a low risk of progression of liver disease (106,260,266).

There are no long-term studies regarding the impact of HCV on outcomes of heart or small bowel or pancreas recipients. Studies in these populations have suggested no difference in patient and graft survival (269–271), likely due to short-term follow-up and/or the relatively small numbers studied. In lung transplant recipients, a recent analysis of the OPTN/UNOS database showed similar 5-year survival amongst HCV-seropositive and seronegative recipients (272). In a study of 14 HCV-RNA lung transplant recipients, the 5-year survival was similar to HCV negative recipients (273). Based on extrapolation from the renal transplant literature, however, there may be an increased risk of HCV-related death beyond 5 years in other nonhepatic SOT recipients. Further studies are needed to clarify the impact of HCV on the outcomes of nonrenal nonhepatic SOT.

Diagnosis

The diagnosis of hepatitis C infection relies on the same serologic and virologic investigations used in the nontransplant population. Initial screening for antibody to HCV should be done at the time of transplant assessment. In those with positive HCV serology, qualitative HCV RNA should be used to confirm current infection. In any patient considered a potential candidate for HCV therapy, HCV genotype should be determined.

In chronic HCV infection, the liver biopsy remains the "gold standard" for assessing the degree of hepatic inflammation and fibrosis and thus the prognosis of the disease. Liver biopsy results are used to guide antiviral treatment decisions, identify those who may be considered for combined (with liver) transplant and those who may be ineligible for nonhepatic SOT due to advanced liver disease (274,275). Noninvasive methods to assess hepatic fibrosis, such as FibroScan, FibroTest, transient and magnetic resonance elastography, are increasingly used but need to be validated in this population. In a small study of six renal transplant candidates, measurement of HVPG assessing for portal hypertension was shown to alter management when added to the diagnostic assessment (276).

Recommendations:

- Initial screening for antibody to HCV should be done at the time of transplant candidacy assessment, with HCV RNA used to confirm current infection (II-1).
- Liver biopsy is recommended in the assessment of all nonhepatic SOT candidates with chronic HCV to guide further management ((II-2).
- Although not recommended as routine, HVPG measurements may guide therapy and selection of candidates who may be more appropriate for combined liver-kidney transplant (III).

Treatment: The current standard of care for treatment of HCV infection in the general population is combination therapy with pegylated interferon and RBV in those with genotype 2 or 3 and peginterferon, RBV and an HCV NS3 protease inhibitor in those with genotype 1. However, treatment of HCV in nonhepatic SOT recipients is generally contraindicated due to a significant risk of acute allograft rejection. Amongst renal transplant recipients, this may occur in up to a third of patients, and is not uncommonly steroid resistant (277,278). IFN-based therapy is not recommended in life-sustaining (e.g. heart, lung) transplants (279). Clinical cure of HCV postrenal transplant has however been reported and in those with progressive HCV-related liver or renal disease, therapy may be considered (280–282). Ribavirin however is key to successful HCV therapy, but contraindicated in those with GFR <50 mL/min. There are no data on the use of HCV protease inhibitors in this population and thus they cannot be recommended. There are important interactions between telaprevir/boceprevir and calcineurin inhibitors. All of these factors severely limit the applicability of current HCV therapies to the posttransplant population. This highlights the importance of treating HCV before transplant whenever possible (see Prevention/Prophylaxis below)

Recommendations:

- In recipients of life-sustaining (e.g. heart, lung) transplants, HCV treatment with IFN-based therapy should be avoided (III).
- In renal transplant recipients, HCV therapy may be considered on a case-by-case basis in those with significant HCV-related disease and after careful review of the potential risks and benefits (II-3).

Prevention/Prophylaxis: Given the risks associated with posttransplant therapy, treatment of HCV should be considered at the time of candidacy assessment in all patients. Although this has been best studied in renal transplantation, similar principles are applied to other nonhepatic SOT patients with a few caveats noted below.

Liver biopsy is key to the management and selection of patients for HCV therapy and listing (179,282,283). Those with minimal liver disease (METAVIR stage F0-F1) have an excellent posttransplant outcome with low risk of progression of liver disease and do not generally need to undergo HCV therapy before listing (106,260,266). Those with moderate (F2) fibrosis also generally have reasonable outcomes posttransplant, although an attempt at HCV therapy is recommended but not considered necessary before listing. There are a number of factors that should be considered in those with mild to moderate fibrosis that may lead to a decision to treat on an individual basis (Table 2).

Table 2: Factors to consider in assessment for HCV treatment in nonhepatic SOT candidates with mild to moderate fibrosis

Factor	Implication
HCV genotype	Higher rates of cure for genotype 2/3 vs. 1 Greater complexity of therapy for genotype 1 (3 drugs vs. 2; more adverse effects; more drug-drug interactions)
Degree of fibrosis	Stronger consideration of therapy in F2 vs. F0/F1 fibrosis
Age	Older patients with milder disease unlikely to have significant progression
Estimated duration of infection	If estimated duration of infection is short, those with mild-moderate disease may have more rapid progression than those with similar degrees of fibrosis but longer estimated duration of infection
Comorbidities	Renal dysfunction, cardiac disease and anemia severely limit the treatment of HCV
Estimated posttransplant survival	Factoring in age, comorbidities, type of transplant to estimate survival may guide therapy as the negative impact of HCV generally not seen until 5–10 years posttransplant

Those with bridging fibrosis (F3) or compensated cirrhosis (F4) should be strongly considered for HCV therapy due to the increased risk of progressive fibrosis, cirrhosis and liver related mortality. If therapy is not otherwise contraindicated and SVR is achieved, they may then be listed for nonhepatic transplant alone. If therapy is unsuccessful, the options include nonhepatic transplant with a full discussion of the increased risk of poor outcomes, combined liver-nonliver transplant, or decline/defer nonhepatic transplant. Those with decompensated cirrhosis are not appropriate candidates for isolated renal transplant but should be considered for combined liver-kidney transplant (179,282,283). Some centers use HVPG to identify those with portal hypertension who may be particularly poor candidates for isolated renal transplant and better served by combined.

Regimens studied for the treatment of HCV in dialysis and prekidney transplant patients include IFN or peg-IFN with or without RBV (284,285). Most show response rates significantly lower than that in the general population. Standard IFN and Peg-IFN monotherapy results in SVR rates of 13–75% (179). Most studies are small and many do not report response by genotype. Prerenal transplant HCV therapy is also hampered by a higher rate of adverse events and discontinuation compared to the general population. Several small case series have documented safe use of RBV in combination with IFN in patients with chronic HCV and poor renal function, generally with measurement of plasma RBV levels (286–291). Given the limited data however, RBV remains contraindicated in patients with a GFR <50 mL/min (292). For patients with genotype 1 HCV, triple therapy with peg-IFN, RBV and PI (boceprevir or telaprevir) is the standard of care (293,294). Although the PIs are not contraindicated in renal failure there are no data on the use of triple therapy in this population.

Data regarding the management of heart and lung transplant candidates with chronic HCV are limited. Until further data are available, the principles and data from the renal transplant population may be used to guide management. A liver biopsy, or noninvasive assessment of fibrosis, should be done as part of the assessment in those infected with HCV. In heart transplant candidates, HCV therapy is contraindicated due to the adverse effect profile (i.e. worsening anemia, risk of heart failure, myocardial infarction, arrhythmia) (295,296). Those with mild to moderate disease (METAVIR stage F0–F2) may be listed for transplant, whereas those with advanced HCV-related fibrosis or cirrhosis are often not considered candidates for cardiac transplantation (297). There are limited data on the outcome of lung transplantation in HCV-positive recipients (272,273). According to international guidelines, HCV infection is a contraindication to lung transplantation (298). However, some centers do consider listing HCV-positive lung transplant candidates, using the liver biopsy to guide the decision for listing and/or consideration of therapy pretransplant (257,272,273,275). One small series has shown that selected lung transplant candidates can safely and effectively be treated for HCV before transplantation (275).

Recommendations:

- Nonhepatic HCV-infected transplant candidates should be evaluated for eligibility for HCV therapy before transplant (II-2).
- A suggested approach to the assessment and management of HCV infected nonhepatic transplant candidates is as follows (III):
  - Those with mild to moderate liver disease (METAVIR stage F0-F2) do not need to undergo HCV therapy before listing; treatment may be considered in kidney or lung transplant candidates weighing factors outlined in Table 1.
  - Kidney or possibly lung transplant candidates with bridging fibrosis (F3) or compensated cirrhosis (F4) should undergo HCV therapy; if an SVR is achieved they may then be listed for transplant. If therapy is otherwise contraindicated or unsuccessful, options include nonhepatic transplant, assessment for combined liver-nonliver transplant, or decline/defer nonhepatic transplant.
  - In heart transplant candidates, HCV therapy is contraindicated, thus those found to have bridging fibrosis (F3) or compensated cirrhosis (F4), the options include assessment for combined liver-heart transplant or decline/defer heart transplant.
  - Those with decompensated cirrhosis are not considered candidates for isolated nonhepatic transplant but may be considered for combined liver-nonhepatic transplant.

The HCV-positive donor

Transplantation of an HCV-positive organ into an HCV-negative recipient results in near universal transmission (299) and frequently an aggressive course with a high risk of death (300,301). Hence it is not recommended to transplant an HCV positive organ into an HCV negative recipient.

In those already infected with HCV, some groups have found no difference in patient and graft survival when using HCV positive kidneys into HCV positive recipients (302,303). Several other recent large studies however have shown a significant increased risk of death in HCV-positive recipients receiving an HCV-positive kidney or heart transplant (256,302,304,305). Despite this, there remains an overall survival advantage to receiving an HCV-positive kidney transplant over remaining on dialysis (306). The waiting time on the renal transplant list is also reduced significantly in the United States, by approximately 1 year. Despite the overall benefit, HCV-positive kidneys continue to be underutilized (307).

There are no data with regard to the impact of donor and recipient HCV genotype on nonhepatic transplant outcomes. Although it is desirable to avoid transplanting an organ from a genotype 1 donor into a recipient with genotype 2 or 3, data on donor genotype are rarely available at the time of transplant. The HCV genotype of the donor, whether known or unknown, should not routinely impact the decision to accept the organ for an HCV-infected recipient given the documented survival advantage and lack of data showing any negative impact of donor—recipient genotype.

Recommendations:

- Given the current era of organ shortage and risk of death on the waitlist, an HCV-positive organ should be considered for transplantation, with informed consent, into an HCV-positive recipient (II-2).
- The use of HCV-positive organs into HCV-negative recipients should be avoided due to poor outcomes;

however this may be considered with strict informed consent in critically ill patients awaiting a life-sustaining transplant (III).

Infection control issues: Nonhepatic SOT patients with HCV should not share personal items that may be contaminated with even small amounts of blood. The risk of sexual transmission in long-term heterosexual couples is minimal and routine use of barrier protection is not necessary to prevent transmission.

Hepatitis D Virus (Delta Virus)

HDV is a small, defective RNA virus that can only replicate in the presence of HBV surface antigen, either by co-infection or superinfection (308). Nearly 20 million people are infected worldwide with HDV, although the prevalence varies by location (309). Co-infection is common in the Pacific Islands, whereas other parts of the world (Japan, Europe, United States) have <10% co-infection rates. As in HBV, HDV is transmitted parentally and only requires a small inoculum. This results in the high potential for transmission in intravenous drug users and those with high-risk sexual behavior. Because of blood product screening, new infections in patients receiving blood transfusions and hemodialysis are rare. All HBsAg+ patients from endemic regions, with high- risk activities, or with unexplained elevated liver enzymes in the setting of low or undetectable HBV DNA, should be tested for HDV via sensitive real-time PCR assays or anti-HDV (IgG or IgM) antibodies (310,311).

In the transplant setting, patients with HDV infection typically have low or undetectable HBV DNA, ultimately leading to reasonable survival rates even without antiviral prophylaxis (312–314). The goal of treatment is to eradicate HDV together with HBV, although definitive resolution can only be obtained with HBsAg clearance that inhibits the potential for HDV replication. Standard treatment pretransplant is usually with interferon and has been shown to improve long-term clinical outcomes, albeit only 20–30% successful in achieving HDV RNA negativity (206,315). Oral antiviral agents for HBV have no direct efficacy against HDV (316–319). Most transplant centers use a posttransplant protocol that includes the use of HBIG and a nucleos(t)ide analogue to minimize the risk of HBV reactivation, although this will have little impact on HDV replication other than HBsAg clearance. There have been few published reports of HDV recurrence and successful IFN therapy after hepatic or nonhepatic SOT (320–322).

Hepatitis E Virus

First reported in 1980 in India, hepatitis E virus (HEV) is now a common cause of water/food-borne acute hepatitis in the developing world (323). Genotypes 1 and 2 only infect humans by fecal–oral routes, whereas genotypes 3 and 4 primarily infect other mammals (324). The typical disease is characterized by severe acute hepatitis and high mortality in pregnant women, the elderly and patients with preexisting chronic liver disease (325). However, in nonendemic regions, genotype 3 HEV via consumption of infected animal meat is now recognized as an uncommon etiology of chronic liver disease among immunosuppressed hosts and transplant recipients (326–330). Over half of SOT recipients infected with HEV will develop chronic hepatitis and ~15% will develop cirrhosis (327). The use of tacrolimus-based immunosuppression and the presence of thrombocytopenia have been associated with chronic HEV infection in such recipients (327,329). HEV IgG antibodies are present in ~ 16% of blood donors and in renal transplant recipients, although this does not specifically distinguish chronic infection versus prior exposure (326,329,331).

The diagnosis and treatment of chronic HEV infection in transplant recipients can be challenging. Most patients are asymptomatic and have a mild to moderate degree of aminotransferase elevations (ALT 100–300 IU/L) that can be elusive particularly in liver recipients with other potential causes of chronic injury. It has also been reported to cause neurological symptoms and glomerulonephritis (329,332). The diagnosis is limited by the lack of commercial assays for HEV RNA and reliance on anti-HEV immunoglobulin M (IgM) antibody testing that may be insensitive (331). It is therefore advisable to send both serological and PCR-based assays if the diagnosis is considered (i.e. unexplained hepatitis). Other than reducing immunosuppression, there is no standard antiviral therapy for chronic HEV infection. Peg-IFN may have some efficacy but carries a risk of graft rejection (328,333). There have been a few reports of successful HEV treatment with RBV in kidney and heart recipients and this can be considered in patients who remain infected despite reduction in immunosuppression. Fortunately, reactivation after clearance of HEV has not been observed to date.

Given limited treatment options, prevention is key, e.g. avoidance of uncooked meats (genotype 3 and 4) and infected water or contacts (genotype 1 and 2). There is no systematic screening for HEV infection in blood banks, as blood-borne transmission is still extremely rare. Two recombinant vaccine candidates, the rHEV vaccine and the HEV 239 vaccine, have been successfully evaluated in Phase II/III trials, although only for genotype 1 infection (334,335).

Recommendations:

- SOT recipients with unexplained chronic hepatitis should be tested for hepatitis D (if HBV+) and hepatitis E viruses, despite the lack of effective treatments in this population (III).
- Although no definitive treatment exists for HEV, reduction in immunosuppressive therapy doses or agents may be advisable (III).

- Recipients should avoid consumption of uncooked meats and potentially contaminated water, as well as contact with HEV-infected individuals (III).

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Special Article

Human Immunodeficiency Virus in Solid Organ Transplantation

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Key words: Drug resistance, drug interaction, highly active antiretroviral therapy, human immunodeficiency virus, reverse transcriptase inhibitor, viral load

Abbreviations: BMI, body mass index; CNI, calcineurin inhibitor; CMV, cytomegalovirus; HAART, highly active antiretroviral therapy; HAV, hepatitis a virus; HBV, hepatitis b virus; HCV, hepatitis c virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; LTBI, latent tuberculosis infection; MAC, mycobacterium avium complex; MELD, model for end stage liver disease; mTOR, mammalian target of rapamycin; NNRT, non nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OI, opportunistic infection; PI, protease inhibitor; TB, tuberculosis.

Epidemiology

With the advent of highly active antiretroviral therapy in the mid-1990s, the patterns of morbidity and mortality in patients with human immunodeficiency virus (HIV) infection have changed. HIV associated complications and opportunistic infections have declined and end stage renal disease and cirrhosis have become increasingly important causes of patient death (1,2). Consequently, interest in organ transplantation in HIV infected patients has increased and the number of transplants in this population as well as the regional expansion of this practice have steadily increased since 1999 (3).

Currently, the vast majority of transplant recipients with HIV are known to have HIV infection before transplant. Donor derived HIV infection has occurred rarely both before the advent of universal testing of donors for HIV and more recently due to the failure of standard testing to identify HIV infection in deceased and live donors (4–7). In an unknown number of cases, HIV has been acquired after transplantation.

Liver and kidney transplants are the most common transplant procedures performed in patients with HIV, reflecting the common occurrence of end stage renal disease and liver cirrhosis in this patient population. HIV associated nephropathy has become an important cause of end stage renal failure, especially in people of African ancestry, and people infected with HIV also have increased incidences of hepatitis associated glomerulonephritis, membranous nephropathy, IgA nephropathy, and drug related nephrotoxicity (8). Because of common infection pathways, HIV often co-exists with both hepatitis C virus and hepatitis B virus, both of which seem to have accelerated progression to cirrhosis in co-infected individuals with diminished responses and intolerance to therapy (9). Although cardiovascular disease has become an increasingly common cause of death in HIV infected patients, heart transplants are still rare in this population (1,10–13). Reports of lung transplantation and pancreas transplantation are also uncommon (12,14,15).

Historically, outcomes in HIV infected patients before HAART were generally poor when compared with patients without HIV infection (16). Recent prospective and retrospective studies both in the United States and Europe have showed improved renal transplant outcomes in the HAART era with patient and graft survival rates falling in between those of uninfected patients and transplant recipients >65 years of age (17–20). Moreover, one study involving the largest single center experience in HIV infected patients revealed superior survival when compared with maintenance on dialysis (21,22). Results in liver transplantation vary based on the underlying disease. HIV infected individuals transplanted for chronic hepatitis C have been found to have decreased survival when compared with their HIV infected counterparts transplanted for other indications, whose survival may be comparable to non-HIV infected liver transplant recipients (23–28). Information regarding transplantation of other organs has been limited to anecdotal reports and small case series. Based on limited data, successful outcomes have been noted in a limited number of HIV infected recipients of cardiac, combined kidney–pancreas transplants, and lung transplants (10–15). Combined liver and kidney transplants may be more likely to result in worse outcomes, however, especially in patients co-infected with HIV and HCV (24).

Regardless of the organ transplanted, the outcomes have been notable for the uncommon occurrence of AIDS

Table 1: Criteria for transplantation in HIV infected individuals

	Kidney transplant	Liver transplant	Heart transplant	Lung transplant	Kidney-pancreas transplant
Meet center specific inclusion criteria	X	X	X	X	X
CD4 count > 100 cells/uL, <200 cells/uL (without history of OI)	NR	X	NR	NR	NR
CD4 count > 200 cells/uL during 3 months before transplantation	X	X[1]	X	X	X
Undetectable HIV viral load while receiving antiretroviral therapy	X	X	X	X	X
Detectable HIV viral load due to intolerance of HAART, HIV can be suppressed post-tx	NR	X	NR	NR	NR
Documented compliance with a stable antiretroviral regimen	X	X	X	X	X
Absence of active opportunistic infection and malignancy[2]	X	X	X	X	X
Absence of chronic wasting or severe malnutrition	X	X[3]	X	X	X
History of hepatitis B or C with lack of evidence of advanced fibrosis or cirrhosis	X	NA	[4]	[4]	X
Acceptance of life-long *Pneumocystis* prophylaxis	X	X	X	X	X
Donor free of hepatitis C	X[5]	X[5]	X	X	X
Appropriate follow-up with providers experienced in the management of HIV	X	X	X	X	X
Ready access to immunosuppressive medication therapeutic drug monitoring	X	X	X	X	X

NA = not applicable; NR = not recommended.
[1]With a history of AIDS defining illness such as opportunistic infection or malignancy.
[2]Patients with a previous history of progressive multifocal leukoencephalopathy, chronic interstitial cryptosporidiosis, primary central nervous system lymphoma, and visceral Kaposi's sarcoma were excluded from the study.
[3]BMI > 21.
[4]Absence of data, although patients with controlled hepatitis B may be considered. Extreme caution for hepatitis C infected patients.
[5]HCV infected donors may be considered for HCV infected recipients on an individual basis.

defining occurrences when standard prophylaxis for opportunistic infections is used. Although outcomes have been generally good, rejection rates have been noted to be significantly higher in HIV infected individuals (18,20,24,29). In some studies, HIV infected liver transplant recipients have had significant recurrences of hepatitis C which have adversely affected patient outcomes (24,26,30).

Risk Factors

To limit the potential impact of HIV on transplant outcomes, most centers have required patients to have well-controlled HIV infection before transplantation. Suggested criteria for transplantation in HIV infected individuals are noted in Table 1 and mirror those used for the NIH sponsored collaborative trial of transplantation in HIV infected individuals (18,24). These criteria reflect the requirement for stable HIV infection at the time of transplant, without any evidence of active opportunistic infections or uncontrolled HIV viremia. An exception may be made for patients with end stage liver disease and intolerance of antiretrovirals related to severe liver disease but HIV genotypic and phenotypic testing that is predictive of viral suppression on resumption of HAART. Although there are no data to establish a time period for which individuals need to sustain these criteria, we recommend a minimum of 3 months (III).

Whether prolonged waiting times may affect outcomes after transplantation is debatable. Early reports suggested that pretransplant survival for liver candidates was diminished in HIV infected individuals when compared with others awaiting liver transplantation, despite equivalent MELD scores (31). Subsequent studies have not confirmed these results, instead showing that MELD was an accurate predictor of wait list mortality in HIV patients, similar to its use in HIV uninfected candidates, and a later survey suggests that hemophiliacs may be at increased risk for death due to accelerated MELD (32,33). After renal transplantation, diminished allograft survival has been noted in recipients of older donor organs and organs with prolonged ischemic time as well as delayed graft function, rejection, and receipt of antithymocyte globulin (18,34). Live kidney donor organs were associated with better outcomes (18). In liver transplant recipients, HCV positive recipients had reduced survival compared with HBV infected recipients (24,25,27). Factors associated with reduced patient and graft survival in patients coinfected with HIV and HCV included older donor age, higher donor risk index, combined liver and kidney transplant, use of an HCV infected donor, higher MELD at transplant, HCV genotype 1 and BMI < 21 (24,29). Patients whose HCV and HIV are undetectable at the time of transplant seem to have improved survival compared to those with detectable virus (25,29).

Significantly increased rejection rates (two- to threefold) have been noted throughout the posttransplant period in both kidney and liver recipients (18,20,29). The etiology of the higher rejection rates remains

unclear; innate immune system dysregulation in the HIV infected recipient and inadequate exposure to immunosuppressive agents secondary to pharmacokinetic interactions with HAART have both been considered to be contributory.

In liver transplant recipients, the biggest impact on patient survival has been the recurrence of hepatitis C infection with progression to cirrhosis (24,26,29). Older recipients and male recipients may have less severe HCV recurrence; the most significant factor associated with recurrent HCV seems to be rejection (24). Because of the rapid progression to cirrhosis, current management strategies include the earlier introduction of treatment for hepatitis C infection (30). Whether this strategy will sufficiently reduce the impact of hepatitis C on outcome to balance the potential risk of rejection associated with interferon is unknown. Of note, there have been several reports of the spontaneous clearance of hepatitis C infection after transplantation (35).

Opportunistic infections and other AIDS defining conditions have been uncommonly reported after transplantation. Instead, HIV infected recipients more commonly experience bacterial infections typically found in HIV uninfected patients (18–20,36). Patients typically experience transient declines in the CD4+ T cell counts after transplantation, but these transient declines do not seem to have an impact on infection risk (18,29,37). Moreover, T cell responses after transplantation both directed at HIV and at herpesviruses have been shown to be stable or expanded, reflecting an increase in immune reactivity (38). A major exception to this both *in vitro* and clinically has been related to the administration of anti-thymocyte globulin either for induction or treatment of rejection. This has been associated with prolonged declines in CD4+ T cell counts, loss of polyfunctional T cell antiviral cytotoxic T lymphocyte responses and the subsequent development of life-threatening bacterial infections (38,39). HIV viremia is generally well controlled with occasional transient episodes of viremia and less frequent persistent HIV viremia (18,29).

Although most reports have focused on infection and rejection, several other complications have also been noted. Malignancies have been uncommon, but those associated with human papillomavirus have been noted more frequently (18,40). Patients with hepatocellular carcinoma have been successfully transplanted with only one study suggesting a trend toward decreased survival in HIV infected recipients with hepatocellular cancer when compared with HIV negative recipients (24,41). It is unclear if there is an increased risk of vascular thrombosis; a single center reported an increased incidence of vascular complications involving arterial and venous systems (42).

Diagnostic Strategies Posttransplant in the HIV Positive Recipient

As with other transplant recipients, the cause of allograft dysfunction may not be apparent based on clinical presentation or laboratory testing. Medications, rejection, disease recurrence and superinfection may all be contributory. Consequently, allograft biopsies should be considered for persistently elevated serum creatinine (kidney transplant recipients) and liver associated enzymes (liver recipients) (II-2). Because liver enzymes may not be reflective of ongoing liver damage related to hepatitis C infection, standard protocol biopsies at 6-month intervals should be considered in liver recipients (III). Because liver enzymes may not reflect the degree of damage in renal transplant candidates co-infected with hepatitis B or C, all candidates for renal transplantation with hepatitis co-infection should undergo liver biopsy before listing (III). Patients with cirrhosis should be carefully evaluated for risk for hepatic decompensation and potentially excluded unless they could be considered for combined liver and kidney transplant (III).

To maintain virologic control of HIV infection, it is recommended that quantitative HIV RNA and CD4+ T cell counts be measured regularly, with the first assays at 1 month after transplant and subsequent studies every 2–3 months thereafter. More frequent monitoring may be necessary in patients receiving depleting antibodies to determine the need for anti-infective prophylaxis (III). If patients have persistent HIV viremia, resistance testing should be performed (genotypic and phenotypic) to determine treatment options (III).

Treatment Considerations in the HIV Positive Transplant Recipient

One of the most intriguing outcomes, seen consistently across all HIV positive transplant studies, is the surprisingly high rejection rates, which are in excess of 30% in renal recipients and nearly twice those of HIV negative liver recipients (18,24). Consequently polyclonal depleting antibodies especially antithymocyte globulin (rabbit) (rATG) have been considered for use in HIV infected kidney transplant recipients. Unfortunately, data regarding the long-term safety of such use is lacking. In addition, the use of these agents must be balanced against the increased risk of graft loss seen with anti-thymocyte globulin use in the HIV-TR study, as well as the infectious complications seen when used at higher doses for rejection (18,39). Use of rATG as an induction agent results in a similar rapid and profound depletion of CD4 + T cells compared to what is seen in the non-HIV population (18,43).

The optimal maintenance immunosuppressive regimen for the HIV-infected transplant recipient is currently unknown.

Early data suggested that cyclosporine may be the preferred calcineurin inhibitor (CNI) due to its potential antiviral activity against HIV. However, data from the large scale HIV-TR kidney study now suggest that tacrolimus is the optimal CNI as higher tacrolimus levels correlated with lower rejection rates when compared with cyclosporine (II-2) (18). Mycophenolate mofetil is the more potent antiproliferative (compared to azathioprine) and may therefore be more effective in preventing rejection in this high risk population (III). An added benefit of mycophenolate is its potential to suppress HIV replication, especially in combination with nucleoside reverse transcriptase inhibitors such as abacavir (44). Sirolimus, an mTOR inhibitor, has been shown *in vitro* to enhance the antiviral activity of enfuvirtide, efavirenz and the CCR5 inhibitors (45). Although these agents have been used in standard treatment regimens for patients with HIV, the potential benefit of using them in transplant recipients with HIV warrants further investigation.

One of the most challenging treatment issues in HIV infected transplant recipients has been managing the numerous drug interactions associated with antiretrovirals and immunosuppressive agents (46). Before transplantation, HIV infected individuals should be on a stable treatment regimen, which should be continued through the peritransplant period to limit the impact of complex drug interactions (III). Patients receiving a protease inhibitor (PI)-based ARV regimen will require significant dose adjustments of both CNI and mTOR inhibitors (47) (II-2). Tacrolimus should be initiated in patients remaining on PIs through the peritransplant period with a mini-load of 1–2 mg. Daily tacrolimus levels should be monitored and the patient should be redosed with 0.5 mg 3–5 days later when the tacrolimus level plateaus in the therapeutic range consistent with organ specific targets (III). Patients receiving boosted PI regimens typically require only 0.375–0.5 mg of tacrolimus once or twice a week to maintain therapeutic targets (46). (II-2) A similar degree of adjustment is necessary when boosted PIs are used with sirolimus (III). A sirolimus dose adjustment down to 0.5–1 mg once weekly has been reported (47). Use of cyclosporine in combination with boosted PIs is simpler because available formulations allow for administration of the substantially lower daily doses required when PIs are used. To maintain therapeutic targets, patients receiving boosted PI regimens generally require modified cyclosporine doses in the range of 15–25 mg twice daily (46). Regardless of the choice of CNI, pharmacokinetic (PK) studies evaluating the impact of boosted PIs on tacrolimus and cyclosporine exposure have shown that the peak CNI levels are blunted when these agents are used together (48,49) (II-2). Additional research is ongoing to evaluate whether this altered PK profile may be a contributing factor to the higher rejection rates seen in this population.

The potential for drug interactions also exist with the NNRTIs nevirapine, etravirine and efavirenz due to their ability to induce clearance of drugs metabolized by CYP3A; rilpivirine does not seem to have the same effect on CNI clearance. Published reports detailing the impact of efavirenz and nevirapine on CNI kinetics are conflicting. The majority of the available data implies that minimal or no dose adjustments are necessary. However, the study by Frasseto et al. reported that patients receiving efavirenz required twice the dose of cyclosporine to achieve therapeutic levels (46). Consequently, close monitoring of immunosuppressive levels is critical in all patients with HIV and should begin on the first day posttransplantation with daily follow-up until levels have stabilized (II-2).

The choice of antiretrovirals should take into account the potential for increased toxicity or diminished bioavailability after transplantation (III). To diminish the risk of mitochondrial toxicity and lactic acidosis, stavudine and didanosine should be avoided (III). Zidovudine may be associated with increased risk of anemia in patients receiving interferon. Atazanavir may have diminished absorption in transplant patients, who commonly receive gastric acid suppression and can be associated with hyperbilirubinemia which may confound posttransplant assessments; consequently it is preferable to avoid this protease inhibitor (III). Use of the integrase inhibitor raltegravir offers the advantage of having no drug interactions and minimal toxicity (50). Unfortunately, that advantage comes at the cost of a lower barrier to resistance. The recently approved once daily integrase inhibitor combination containing elvitegravir, cobicistat, emtricitabine and tenofovir has a higher barrier for resistance than raltegravir but has a significant potential for drug interactions (51). The pharmacokinetic booster cobicistat is a structural analog of ritonavir and has been shown in *in vitro* studies to inhibit CYP3A to a similar degree. Promising data exist with the use of maraviroc, which has a theoretic potential for reduction of the risk of rejection (52). Enfuvirtide also has the advantage of not having any drug interactions with the CNIs or mTOR inhibitors. However, enfuvirtide's subcutaneous administration will likely continue to limit its use (III). A summary of the potential pharmacokinetic interactions that may occur between HAART therapy and immunosuppressants is provided in Table 2.

Treatment of hepatitis B before and after transplantation is essential in transplant recipients who are co-infected with hepatitis B (53) (II-2). Numerous agents, including lamivudine, adefovir, tenofovir, emtricitabine and entecavir have all been used successfully. Standard management has also included the use of hepatitis B immune globulin to maintain titers >200 IU/mL (the goal titer may vary relative to time from transplantation). Lamivudine resistance in hepatitis B has been common in patients co-infected with hepatitis B and HIV as a result of prolonged usage of lamivudine as a component of HAART therapy. Despite the presence of lamivudine resistance in the majority of HIV-hepatitis B co-infected patients, outcomes in these patients have been excellent with the administration of antiretrovirals with appropriate hepatitis B virus coverage (27). In HIV infected

Table 2: Potential pharmacokinetic drug interactions between antiretrovirals and immunosuppressants

	Glucocorticoids	Calcineurin inhibitors	Antimetabolites	mTOR inhibitors
NNRTIs	↓	↓	NI	↓
NRTIs	NE	NI	NI[1]	NE
Unboosted protease inhibitors[2]	↑↑	↑↑	NI	↑↑
Boosted protease inhibitors[2]	↑↑	↑↑↑	NI	↑↑↑
Integrase inhibitors[3]	NE	NI	NE	NI
CCR5-antagonists	NE	NE	NE	NE
Fusion inhibitors	NE	NE	NE	NE

NE = no interaction expected based on theoretical considerations; NI = no interaction found in clinical studies. ↓ = slight potential for decreased exposure due to CYP induction; ↑↑ = known significant drug interaction resulting in increased exposure due to CYP inhibition; ↑↑↑ = known severe drug interaction resulting in increased exposure due to CYP inhibition.

[1]Use of the NRTIs lamivudine, didanosine and abacavir in combination with mycophenolate products may result in an increased risk of lactic acidosis and mitochondrial toxicity. The combination of mycophenolate with zidovudine and stavudine has been found to be antagonistic.

[2]The degree of CYP inhibition may vary across the class of protease inhibitors.

[3]Integrase inhibitors combined with drugs that inhibit the CYP3A system such as cobicistat will likely result in increased exposure of glucocorticoids, calcineurin inhibitors and mTOR inhibitors. Data are currently unavailable on these combinations.

patients who are not undergoing transplantation, combination therapy with tenofovir and lamivudine or emtricitabine has been noted to decrease the development of resistance (54). Combination therapy has been recommended in published clinical guidelines for co-infected recipients unrelated to transplant status (55); this approach is appropriate for co-infected transplant recipients as well (III). Termination of antihepatitis B therapy should be avoided as it may result in a hepatitis flare (III).

Treatment of hepatitis C infection has been more difficult. Whenever possible, hepatitis C infected patients should be assessed for potential treatment before transplant to diminish the hepatitis C viral load, thereby potentially decreasing the risk of post transplant recurrence (III). The addition of telaprevir and boceprevir to standard therapy with pegylated interferon/ribavirin has resulted in significant improvements in sustained viral response rates, even in the HCV/HIV population (56,57). Significant drug interactions exist between the HCV protease inhibitors and various HIV protease inhibitors. To avoid subtherapeutic exposure in HIV and HCV therapy, changes in the ARV regimen may be required before initiation of treatment with telaprevir or boceprevir (58). Most patients will probably not tolerate this before transplantation, however.

After transplantation, patients should be considered for treatment based on liver biopsy results revealing early fibrosis and evidence of progression of recurrent hepatitis C infection; the optimal timing for this is unknown (III). Thus far, combination therapy with interferon and ribavirin has been used sporadically in co-infected transplant recipients with variable responses; toxicity and increased rejection occurrence have been limiting factors (59–61). Data are presently lacking on the impact of the drug interaction between CNIs and telaprevir or boceprevir in HIV infected patients receiving protease inhibitors for treatment of their HIV. Use of telaprevir alone results in the need for similar dose adjustments, as seen with ritonavir boosted protease inhibitors (62). The interaction between boceprevir and the CNIs is not nearly as strong as that seen with telaprevir (63). Currently no formal recommendations exist for the use of combination therapy for HCV with protease inhibitors after transplantation, especially in HIV infected patients. Given the potential for an even greater risk for rejection in HIV patients being treated with interferon and ribavirin, patients on interferon and ribavirin should be closely monitored for rejection (III). The optimal timing and duration of hepatitis C treatment is currently unknown.

Preventative Measures in the HIV+ Transplant Population

Because HIV infected patients undergoing transplantation are presumed to potentially have an augmented risk of developing opportunistic infections due to the addition of exogenous immunosuppression, prophylactic regimens for prevention of opportunistic infections have been recommended (37) (III). Recommendations for opportunistic infection prophylaxis in the HIV infected transplant population are outlined in Table 3. These recommendations differ slightly from the 2009 MMWR publication as the cutoffs for initiation of primary prophylaxis of *Toxoplasma* and *Mycobacterium avium* were higher in the original NIH protocol for transplantation of HIV+ individuals than in the more recent MMWR guidelines (37,55). In addition, the HIV-TR protocol called for lifelong *Pneumocystis* prophylaxis. Whether HIV infected transplant recipients require this more aggressive prophylactic approach is not known; although it is notable that most studies report low incidences of opportunistic infections in recipients using this prophylaxis protocol.

Similar to HIV negative transplant candidates, vaccination status should be assessed before transplantation and

Table 3: Preventative measures in HIV+ transplant recipients—Opportunistic infection prophylaxis

Opportunistic infection	Primary prophylaxis (patients with no prior history of infection)	Regimen	Additional comments
Pneumocystis pneumonia[1]	Indicated for life (III)	Sulfamethoxazole/trimethoprim (Bactrim) 1 double strength (800/160) or single strength (400/80)PO daily	Alternatives: Bactrim DS three times a week, dapsone 100 mg QD (contraindicated if G6PD deficient). If Bactrim or dapsone allergic consider atovaquone 1500 mg PO daily or aerosolized pentamidine 300 mg via nebulizer monthly
Toxoplasma gondii[1]	Toxoplasmosis IgG+ subjects with CD4+ T cell count ≤ 200 or any recipient of an organ from a donor seropositive for toxoplasmosis (II-2)	Preferred primary px: Bactrim DS once daily Alternatives: Bactrim SS 1 tab PO QD or dapsone 100 mg PO daily + pyrimethamine 50 mg PO QD + leucovorin 25 mg PO QD or atovaquone 1500 mg PO QD Preferred secondary px: pyrimethamine 25 mg PO QD plus sulfadiazine 100 mg/kg PO QD plus leucovorin 25 mg PO QD. Separate PCP prophylaxis should be discontinued if this regimen is used.	Alternative: for patients who cannot tolerate sulfa drugs pyrimethamine 25 mg PO QD plus clindamycin 300 mg PO QID. Note that only the combination of pyrimethamine plus sulfadiazine seems to provide protection against PCP, thus PCP prophylaxis must be continued with this regimen
Mycobacterium avium Complex (MAC)[1]	Indicated when CD4+ T cell count ≤ 75. Discontinue when CD4 count is >100 cells/μL for 6 months (III)	Primary px: Preferred: azithromycin 1200 mg PO weekly Alternative: clarithromycin 500 mg PO BID or rifabutin 300 mg PO QD. Secondary px: Preferred: azithromycin 600 mg PO QD in combination with ethambutol 15 mg/kg/day. Regimen may be modified based on previous MAC treatment. Alternative: clarithromycin 500 mg PO BID plus ethambutol 15 mg/kg/day	Significant drug interactions exist with clarithromycin and rifabutin, monitor immunosuppression levels closely. Rifabutin must be administered at one-half the usual daily dose (i.e., reduce from 300 mg to 150 mg PO QD) with protease inhibitors.
Cytomegalovirus (CMV)	Indicated in CMV IgG + donors or recipients for a minimum of 3 months (III)	Preferred: valganciclovir 900 mg PO QD Alternative: ganciclovir 1 gram PO TID if available, intravenous ganciclovir 5 mg/kg daily	Although no data specific to HIV infected recipients, prophylaxis may be preferred to pre-emptive therapy for highest risk individuals
Histoplasma capsulatum infection	CD4 count < 150 and at high risk because of occupational exposure or residing in an endemic area (III)	Preferred: itraconazole 200 mg PO Daily taken with food Alternative: fluconazole 400 mg PO QD	Significant drug interactions exist with fluconazole and itraconazole, monitor immunosuppression levels closely.
Mycobacterium tuberculosis infection (TB) (treatment of latent TB or LTBI)	(+)diagnostic test for LTBI, no evidence of active TB, and no prior history of treatment for active or latent TB (I) (−)diagnostic test for LTBI but close contact with person with infectious pulmonary TB (III) A history of untreated or inadequately treated healed TB (II-2)	Preferred: Isoniazid (INH) 300 mg po daily or 900 mg po BIW for 9 months – both plus pyridoxine 50 mg po daily Alternatives: Rifampin (RIF) 600 mg po daily x 4 months or Rifabutin (RFB) dose adjusted assuming no contraindication based on concomitant HAART) x 4 months	Significant drug interactions exist with rifampin and rifabutin, monitor immunosuppression levels closely
Coccidioidomycosis	IgG or IgM (+) in a patient from an endemic area and a CD4 count < 250 (I) Lifelong for recipient of organ from donor with history of coccidioides (II-3)	Fluconazole 400 mg po daily or Itraconazole 200 mg po BID	Significant drug interactions exist with fluconazole and itraconazole, monitor immunosuppression levels closely.

[1]Secondary prophylaxis in patients with a prior history of symptomatic infection could be considered in the following circumstances based on the NIH HIV-TR protocol (III):

1. During the first month posttransplant.
2. During treatment of rejection and for 1 month after acute rejection therapy.
3. When CD4 count falls below prespecified cut-off for specific OI:
   (a) CD4 cutoffs – Toxo (200), MAC (75), CMV (100).

Lifelong secondary prophylaxis should be considered for patients with a prior history of *Pneumocystis* pneumonia, *Histoplasma capsulatum* and coccidioidomycosis.

Table 4: Preventative measures in HIV+ transplant recipients—Vaccination in the HIV+ transplant recipient

Vaccine	Population	Vaccination schedule	Recommended product	Additional concerns
Influenza A and B	All HIV+ transplant recipients	Annually (II-2)	Inactivated influenza vaccine 0.5 mL IM	Avoid use of live intranasal vaccine
Streptococcus pneumonia infection	All HIV+ transplant recipients	Every 3-5 years (III)	Pneumococcal vaccine–naive adults: 13–valent pneumococcal conjugate vaccine (PCV13) 0.5 mL IM followed by 23–valent pneumococcal polysaccharide vaccine (PPSV23) ≥ 8 weeks later. Adults previously vaccinated with PPSV23: 1 dose of PCV13 ≥ 1 year after the last PPSV23 dose. Pediatrics PCV13	
Varicella-zoster virus (VZV) infection	Pretransplant pre-exposure prevention – CD4 count ≥200 who have not been vaccinated, have no history of varicella or herpes zoster, or who are seronegative for VZV Postexposure – close contact with a person who has active varicella or herpes zoster with no history of vaccination or infection with varicella or herpes zoster, or who are seronegative for VZV	One time administration with 3 months between Varivax® doses (I)	Pre-exposure prevention – Primary varicella vaccination (Varivax®) 2 doses (0.5 mL SQ) administered 3 months apart (I) Postexposure therapy Varicella-zoster immune globulin (VariZIG®) 125 IU per 10 kg (maximum of 625 IU) IM, administered within 96 hours after exposure (II-1) Alternative: Post exposure varicella vaccination (Varivax®) 2 doses (0.5 mL SQ) administered 3 months apart if CD4 count > 200 (III) Valacyclovir or acyclovir for 7 days beginning 3–10 days postexposure (III)	ProQuad® (Measles, Mumps, Rubella and Varicella Virus Vaccine Live) and Zostavax® both contain live virus and should not be administered to HIV+ transplant recipients. If vaccination with Varivax results in disease this may be treated with acyclovir VZV susceptible household contacts should be vaccinated to prevent transmission to HIV infected contact. If contacts develop a rash due to vaccine, transplant recipient should avoid contact with vaccine recipient until rash resolved (II-3)
Hepatitis A virus (HAV) infection	HAV-susceptible patients with chronic liver disease, or who are injection drug users, or men who have sex with men. May delay vaccination until CD4+ count > 200	One time administration unless patient is considered a non-responder (I)	Hepatitis A vaccine 1 mL IM × 2 doses at 0 and 6–12 months	IgG antibody response should be assessed 1 month after vaccination; non-responders should be revaccinated
Hepatitis B virus (HBV) infection	All HBV seronegative patients	One time administration unless patient is considered a nonresponder (I)	Hepatitis B vaccine IM (Engerix-B® 20 μg/mL or Recombivax HB® 10 μg/mL) at 0, 1 and 6 months Some experts recommend vaccinating with 40 μg doses of either vaccine	Anti-HBs should be obtained 1 month after completion of vaccine series. If patient is a nonresponder (anti-HBs < 10 IU/mL) they should be revaccinated with a second series. If the first series was given with low CD4 count consideration should be given to wait for a sustained increase in CD4 count
Human Papillomavirus (HPV) infection	Men and women aged 9–26	One time administration of three vaccines over 6 months (I)	HPV quadravalent vaccine 0.5 mL IM at months 0, 2 and 6	

Whenever possible vaccines should be administered before transplantation.

vaccines updated as per regular schedules (55). Vaccination recommendations for HIV infected transplant recipients are outlined in Table 4. In addition, all candidates should be screened for latent tuberculosis using either tuberculin skin testing or interferon gamma release assay (55).

Future research

Patients with HIV can be appropriate candidates for transplantation. Because of the significant drug interactions and high risk of rejection and recurrent disease (especially hepatitis C), management of these patients can be complex. Future research will need to focus on strategies to decrease the incidence of posttransplant rejection and reduce the impact of HCV co-infection on patient outcomes. Studies to date have focused on adult populations. Whether there may be differences in the management of adult and pediatric patients is an area that will require future study. Finally recent reports from South Africa using HIV infected kidney donors have suggested that select HIV infected donors may be appropriate for some HIV infected candidates (64). Whether this approach can successfully expand the donor pool is unknown and should be considered for future study. Ultimately given the challenging issues related to patient selection and posttransplant management, an integrated multidisciplinary approach involving diverse health care providers experienced in the care of these patients is recommended for optimal long-term outcomes.

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Special Article

BK Polyomavirus in Solid Organ Transplantation

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The human BK polyomavirus (BKV) is the major cause of polyomavirus-associated nephropathy (PyVAN) putting 1–15% of kidney transplant patients at risk of premature allograft failure, but is less common in other solid organ transplants. Because effective antiviral therapies are lacking, screening kidney transplant patients for BKV replication in urine and blood has become the key recommendation to guide the reduction of immunosuppression in patients with BKV viremia. This intervention allows for expanding BKV-specific cellular immune responses, curtailing of BKV replication in the graft, and clearance of BKV viremia in 70–90% patients. Postintervention rejection episodes occur in 8–12%, most of which are corticosteroid responsive. Late diagnosis is faced with irreversible functional decline, poor treatment response, and graft loss. Adjunct therapies such as cidofovir, leflunomide and intravenous immunoglobulins have been used, but the benefit is not documented in trials. Retransplantation after PyVAN is largely successful, but requires close monitoring for recurrent BKV viremia.

Key words: BK virus, kidney, nephropathy, polyoma, transplantation

Abbreviations: BKV, BK polyomavirus; HSCT, hematopoietic stem cell transplantation; PyVHC, polyomavirus-associated hemorrhagic cystitis; PyVAN, polyomavirus-associated nephropathy; SOT, solid organ transplantation.

Introduction

The humanBK polyomavirus (BKV) is linked to two major complications in transplant recipients, polyomavirus-associated nephropathy (PyVAN) in 1–10% of kidney transplant patients (1–4) and polyomavirus-associated hemorrhagic cystitis (PyVHC) in 5–15% of allogeneic hematopoietic stem cell transplant (HSCT) patients (5–8). Both diseases occur only sporadically in patients with nonkidney solid organ transplantation (SOT) or with inherited, acquired or drug-induced immunodeficiency (9,10). Besides PyVAN and PyVHC, BKV has been implicated rarely in extrarenal pathologies such as pneumonia, encephalitis, hepatitis, retinitis, capillary-leak syndrome and cancer (9,11–13). A potential association of sustained BK viruria with acute T cell mediated rejection has been suggested (14).

Epidemiology of BKV Infection and Replication

BKV and JC polyomavirus (JCV) infections are widespread in the general population (15–17). Primary infection with BKV occurs in the first decade of life as evidenced by increases in BKV seroprevalence to 90% and more (15,16). Natural BKV transmission is not resolved, but likely occurs via the respiratory or oral route (9). Subsequently, BKV colonizes the renourinary tract as the principle site of latent infection, most likely via a primary viremia (9,18). In healthy BKV seropositive immunocompetent individuals, reactivation and asymptomatic urinary shedding of BKV is detectable in up to 10%, with urine BKV loads of 5 $\log_{10}$ genome equivalents (geq)/mL as the 75th percentile (16). BKV type I is found in 70–80%, followed by BKV type IV in 10–20% (16). In individuals with impaired immune functions, particularly after SOT or HSCT, asymptomatic high-level urinary BKV replication is observed with BKV loads of >7 $\log_{10}$ geq/mL, appearance of "decoy cells" in urine cytology and virus particles detectable by direct negative staining electron microscopy (19–21). High-level BKV viruria only rarely leads to viremia and PyVAN in nonkidney SOT (22–26). In kidney transplant recipients, however, approximately one third of patients with high-level viruria/decoy cells develop BKV viremia, and in the absence of any intervention, progress to histologically proven PyVAN (3,27–29). This progressively affects graft function and increases the risk of graft loss from <10% to more than 90% (28,30,31). Because effective and safe antiviral therapies are lacking, screening for BKV replication has become the key recommendation to initiate and guide a stepwise reduction of immunosuppression. This intervention allows for expanding BKV-specific cellular immune responses,

curtailing of BKV replication in the graft and clearance of BKV viremia (32–35).

Risk Factors of PyVAN

The preferential occurrence of PyVAN in kidney transplants compared to other nonkidney SOT or HSCT is striking and suggests that factors specific to transplanting this organ of BKV latency, are of importance. These include donor (organ) determinants (such as HLA-mismatches, deceased donation, high BKV-specific antibody titers interpreted as a marker of higher recent BKV exposure and possibly graft load, female gender), recipient determinants (such as older age, male gender, low or absent BKV-specific antibody titers) and modulating factors after transplantation (such as ureteric stents, acute rejection and antirejection treatment, steroid exposure, lymphocyte depleting antibodies, higher immunosuppressive drug levels, tacrolimus-mycophenolic acid compared to cyclosporine-mycophenolic acid or to mTOR inhibitor-combinations, and low or absent BKV-specific T cell responses) as well as retransplantation after graft loss due to PyVAN (3,36–42). There is a considerable variability of PyVAN incidence rates in different transplant centers as well as discordant results about risk factors, which may reflect differences in the immunosuppressive protocols of the respective programs.

Prevention and Prophylaxis

Kidney transplant recipients should be screened for BKV replication to identify patients at increased risk of PyVAN [II-1]

Screening for BKV replication should be performed at least every 3 months during the first 2 years posttransplant, and then annually until the fifth year posttransplant (Figure 1). Using this strategy, at least 80–90% patients at risk for PyVAN can be identified before significant functional impairment of the renal allograft occurs. More frequent screening will identify additional cases and should be performed according to the center-specific incidence (43). The following strategies have been used successfully in a larger number of adult and pediatric patients: Biweekly urine cytology for decoy cells for the first 3 months, then monthly until month 6, then every 3 months until 2 years posttransplant followed by plasma testing for BKV viremia if positive (44); or monthly plasma screening for the first 6 months, then every 3 months until 2 years posttransplant (45–47).

In a simulation model assuming an 80% efficacy to clear PyVAN and a 10% risk of precipitating acute rejection after reduced immunosuppression, screening seemed to be cost-effective for PyVAN incidence rates of more than 2.1% (48). A cost analysis suggested that preventing PyVAN by screening and reducing immunosuppression may be cost saving after the second year posttransplant (49).

Screening for BKV replication can be done either by testing urine for high-level BKV viruria/decoy cells or by testing plasma for BKV viremia (Table 1).

- Testing for BKV viruria has the following advantages (3,22,50,51): (a) a high negative predictive value to rule out BKV nephropathy; (b) a window period of 6–12 weeks before viremia and nephropathy; (c) being noninvasive and (d) lesser costs and instrumentation requirements than PCR in specialized centers with experienced cytopathologists available. Recently, identifying a subgroup of persistent urinary BKV shedders without viremia has been reported to be at risk for recurrent episodes of rejection-like graft dysfunction (14). The disadvantages of urine BKV testing are: (a) low positive predictive value for PyVAN; (b) the physiological fluctuations of urine BKV loads requiring differences to be greater than 2 $\log_{10}$ in order to be significant and (c) delayed decline of urine BKV loads (and lack of clearance) compared to plasma BKV loads in response to reduced immunosuppression. This may increase the risk of overly reduced immunosuppression and rejection. The positive predictive value for proven PyVAN increases when high-level viruria persists for more than 2 months, but at the same time increases the risk of late diagnosis and irreversible PyVAN.
- Testing for BKV viremia has a positive predictive value of 30–50% for proven PyVAN with a window period of 2–6 weeks. Because of this shorter window period, monthly plasma screening is preferred in many centers as it detects clinically more significant replication, and provides a widely accepted trigger for therapeutic intervention. The positive predictive value of BKV viremia increases to more than 90% when plasma BKV loads are very high (e.g. 6 $\log_{10}$ copies/mL), renal allograft function is impaired or when rearrangements in the BKV noncoding control region appear in the blood (28,29,52–55). In patients with sustained plasma BKV DNA and loads of >4 $\log_{10}$ cp/mL, a diagnosis of "presumptive PyVAN" should be made in absence of demonstrable BKV replication in biopsies. Increases in serum creatinine from baseline are not required for the diagnosis of presumptive PyVAN.
- Detection of three-dimensional viral aggregates in urine by electron microscopy has been reported to have high positive and negative predictive values for BKV nephropathy reaching >90% (56). However, electron microscopy is not widely available and independent prospective studies confirming the utility of this diagnostic tool are warranted.

The caveats of the different strategies reside in suboptimal performance, timing and intra- and interlaboratory

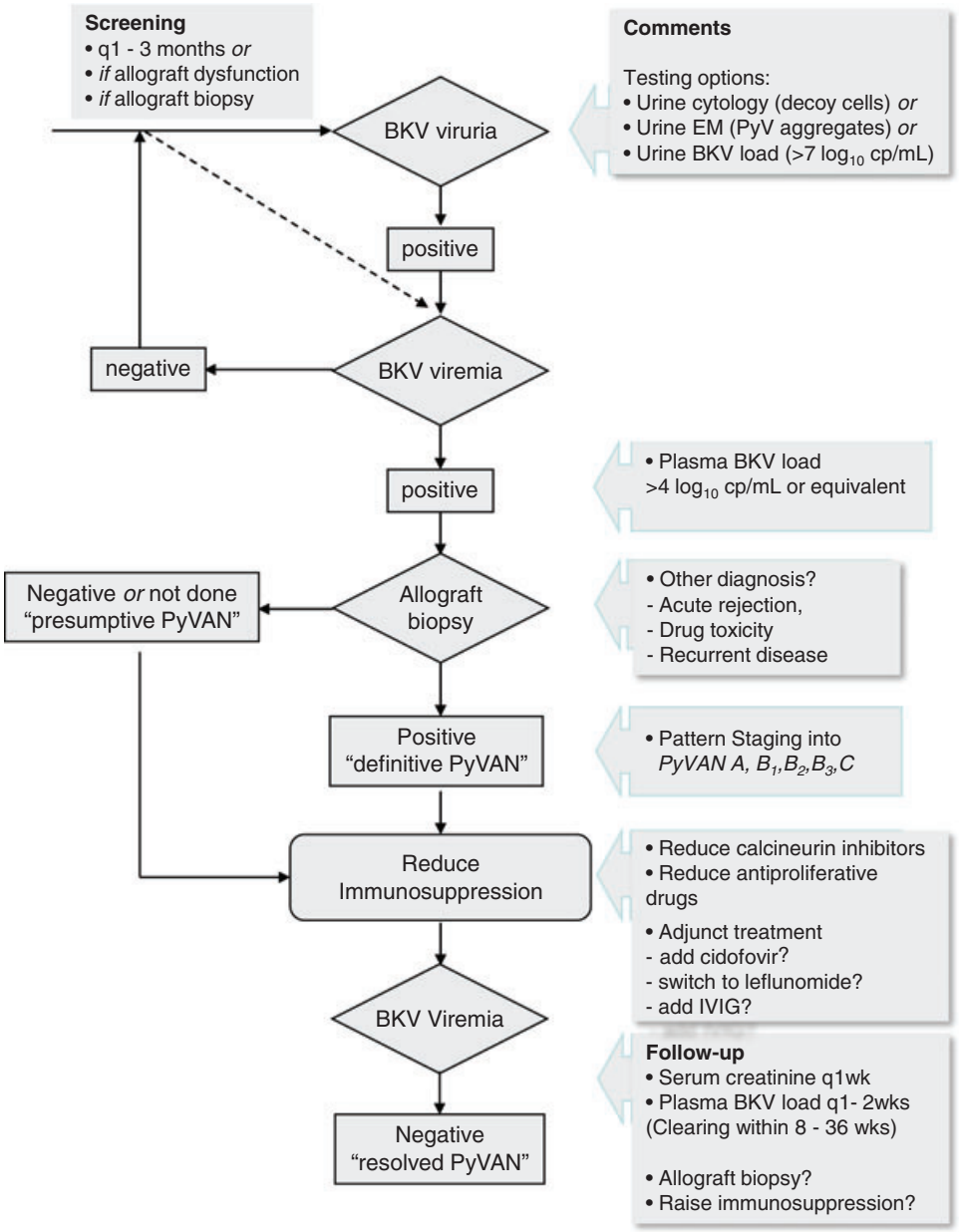

Figure 1: Screening and management of kidney transplant patients for BKV replication and polyomavirus-associated nephropathy (PyVAN).

variability of all assays including urine cytology, electron microscopy, PCR and graft histology, particularly if performed outside of dedicated expert laboratories with implemented quality assurance (29,57,58). Quantifying BKV DNA in urine and plasma specimens by PCR is key to initiating and monitoring of treatment. However, the results of different assays and laboratories cannot be considered equivalent until an international standard has become available as reference calibrator. In addition, PCR detection may be reduced by rare mutant strains to <1% compared to prototype strains indicating that target sequences and assays must be periodically re-evaluated (59–61). Similarly, performance and interpretation of PyVAN in biopsies requires quality control regarding the biopsies, the confirmatory assays (immunohistochemistry or *in situ* hybridization) and histology interpretation (62).

Reducing immunosuppression should be considered for kidney transplant patients with sustained plasma BKV loads [II-1]

The following strategies and their combinations have been reported:

Strategy 1. First dose reduction of the calcineurin inhibitor by 25–50% in one or two steps; followed by reducing the antiproliferative drug by 50%; followed by discontinuing the latter (44,46).

Table 1: Screening and intervention for BKV replication and nephropathy

		Diagnosis of PyVAN		
Testing		Possible	Presumptive	Proven
Urine	"High-level viruria"	+	+	+
	Decoy cells (check if inflammation and/or casts)			
	BKV DNA load >7 $\log_{10}$ cp/mL			
	BK VP1 mRNA load >6.5 $\log_{10}$ cp/ng RNA			
	PyV particles (check if in clusters)			
Plasma	"Viremia"	–	+	+
	BKV DNA load (check if > 4log10 cp/mL)			
Biopsy	"Nephropathy"	–	–	+
	Viral cytopathic changes			A*
	Inflammatory infiltrates / tubulitis			B1*, B2, B3
	Interstitial fibrosis/tubular atrophy			C*
Therapy		No	Yes	Yes

*A, PyVAN histology dominated by cytopathic changes; B1, B2, B3, PyVAN histology dominated by inflammatory infiltrates and tubulitis; C, PyVAN histology dominated by interstial fibrosis and tubular atrophy (see Table 2 for details).
–, not detectable (testing negative); cp, copies; +, detectable (testing positive); PyVAN, polyomavirus-associated nephropathy.

Strategy 2. First reducing the antiproliferative drug by 50% followed by reducing calcineurin inhibitors by 25–50% followed by discontinuing the antiproliferative drug (45).

Oral prednisone is typically tapered to 10 mg or less per day. Immunosuppression is further adapted according to the plasma and the course of serum creatinine concentration.

Both protocols appear safe and effective in adult and pediatric patients for preventing PyVAN and clearing BKV viremia with subsequent acute rejections ranging from 8–12% all of which responded to steroid treatment (44–46). Most centers reduce immunosuppression and monitor serum creatinine in 1–2 week intervals, and the BKV load in 2- to 4-week intervals. Detailed kinetic follow-up data are sparse (51,63). In one study, half of the patients with presumptive PyVAN cleared BKV viremia after a one-step intervention, the first ones starting after 4 weeks. The other half required two-step interventions, with overall mean clearance achieved by 4 months (44). Of note, despite preemptive BKV viremia-guided reduction of immunosuppression, proven PyVAN still occurred in one third of cases (44).

Proven PyVAN was characterized by higher plasma BKV loads, longer median time to clearance of BKV viremia and three steps of reducing immunosuppression in one-third of patients (44).

Diagnosis of PyVAN

Plasma BKV loads should be determined in all kidney transplant patients undergoing renal allograft biopsy for surveillance or for decline in function [II-3]
The presentation of PyVAN is initially inconspicuous, with no clinical or laboratory signs other than high-level viruria as defined by decoy cell shedding, urine BKV loads >7 log geq/mL, and BKV viremia (3,50). Detecting BKV viremia can guide more specific histopathology studies and impact on therapeutic management.

The definitive diagnosis of PyVAN should be sought by demonstrating PyV cytopathic changes in allograft tissue, and confirmed by immunohistochemistry or in situ hybridization ("proven PyVAN") [II-1]
For immunohistochemistry, most centers use cross-reacting antibodies raised against the large T-antigen of the Simian virus 40 (clone PAb 416, Calbiochem). There is considerable interlaboratory variation in staining intensity and assessment of percentage of infected cells, but the binary classification of biopsies into virus positive and negative is fairly reliable. In one study of 20 biopsies with PyVAN, 50% were plasma cell rich (>15% of infiltrate) with a predominance of IgM positive plasma cells which correlated with high anti-BKV antibody levels (64). In Pittsburgh, the overall incidence of PyVAN in plasma-cell rich biopsy material is less than 1% (unpublished observations) due to undefined factors such as the immunosuppressive regimen use.

A minimum of two biopsy cores should be taken, preferentially containing medullary tissues [II-2]: Because of the focal nature of PyVAN and the possibility of sampling error in at least 10–36.5% of cases (50), negative biopsy results cannot rule out early focal PyVAN with certainty.

The histological findings PyVAN should be semi-quantitatively assessed [II-3]
Standardized assessment and reporting is important to improve the comparability of case series. Classification of PyVAN into categories PyVAN-A, PyVAN-B and PyVAN-C is reasonably reproducible (kappa = 0.47; Ref. (65)), but may not be sufficient to provide sufficient statistical discriminatory power for clinical studies. Reporting of subgroups B1, B2 and B3 defined by the percentage of biopsy area affected should be considered (Table 2; Ref. 50). Extent of fibrosis and tubular atrophy may be the most important predictor of a poor outcome (Refs. 31,66; Table 2).

Table 2: Histological patterns of PyVAN modified after (36,50,113,114)

Pattern	Description	Extent of biopsy core	Graft function	Risk of graft loss
PyVAN-A				
Viral cytopathic changes	Mild	≤25%	Mostly baseline	<10%
Interstitial inflammation	Minimal	≤10%		
Tubular atrophy	Minimal	≤10%		
Interstitial fibrosis	Minimal	≤10%		
PyVAN-B*				
Viral cytopathic changes	Variable	11 – >50%	Mostly impaired	50%
Interstitial inflammation	Significant	11 – >50%		
Tubular atrophy	Moderate	<50%		
Interstitial fibrosis	Moderate	<50%		
PyVAN-B1				
Interstitial inflammation	Moderate	11–25%	Slightly above baseline	25%
PyVAN-B2				
Interstitial inflammation	Significant	26–50%	Significantly impaired	50%
PyVAN-B3				
Interstitial inflammation	Extensive	>50%	Significantly impaired	75%
PyVAN-C				
Viral cytopathic changes	Variable	Variable	Significantly impaired progressive failure	>80%
Interstitial inflammation	Variable	Variable		
Tubular atrophy	Extensive	>50%		
Interstitial fibrosis	Extensive	>50%		

Subclassification of PyVAN-B into categories B1, B2 and B3 was initially proposed by Drachenberg using both inflammation and tubular atrophy and biopsies with > 50% involvement were designated B3 (50). However, tubular atrophy > 50% usually correlates with interstitial fibrosis > 50% which is used to define PyVAN-C. For simplicity, it is suggested that subclassification PyVAN-B be based entirely on inflammation, which is an important and independent predictor of outcome (66,67). The degree of viral cytopathic effect has been included in prior staging schema (36,114), However, biopsies with the same degree of inflammation can vary widely in the frequency of viral inclusions. Moreover, an international quality assurance study indicates that immunohistochemistry techniques available in different laboratories differ substantially in their sensitivity for demonstrating virus infected tubules (62).

More recently, the 2009 Banff conference formulated a working proposal in which stage A and B were defined based exclusively on the extent of viral cytopathic effect. Thereby, an identical stage can be assigned to biopsies that differ markedly in the degree of inflammation. This may turn out to problematic, because inflammation may portend an unfavorable prognosis (14,67).

The diagnosis of acute rejection concurrent with PyVAN is only considered secure if one finds endarteritis, fibrinoid vascular necrosis, glomerulitis, or C4d deposits along peritubular capillaries [II-3]: Determining whether interstitial infiltration and tubulitis is directed against viral or tubular antigens cannot be reliably done by light microscopy. In PyVAN, C4d deposits have been observed in the tubular basement membranes, but not peritubular capillaries (56,68). However, one case of PyVAN with intimal arteritis and one with generalized polyomavirus vasculopathy in the skeletal muscle has been reported (69,70). MHC class II upregulation by the tubular epithelium has been proposed as a marker of rejection which is absent in PyVAN biopsies with acute viral tubular necrosis, but requires independent studies (71). Molecular studies attempt to identify markers in biopsies and in urine require further investigation for utility in the routine setting (67,72,73).

JCV-mediated PyVAN should be considered in kidney transplant patients with histological signs of PyVAN, declining renal function and absence of BKV in blood, urine and graft tissue [III]

In rare cases, not BKV, but the related JCV has been identified histologically proven PyVAN (28,74–76). Although high-level viruria and decoy cell shedding was common, JCV viremia was not a consistent feature of JCV-mediated PyVAN (28). In most cases, JCV-mediated PyVAN was cleared after reduced immunosuppression. No universal screening for JCV replication and nephropathy can be recommended given the rarity of this condition and the overall better outcome.

Treatment of PyVAN

Immunosuppression should be reduced in kidney transplant patients with proven PyVAN [II-1]

The mainstay of therapy for PyVAN in kidney transplant patients without concurrent acute rejection is reducing or discontinuing immunosuppressive drugs as outlined above (33,44,77,78). Although there are no randomized controlled trials, a number of observational studies have reported successful clearance of BKV viremia in >85%. More advanced disease may require more interventional steps, a longer time for recovery and result in a permanent loss of renal function (31,44,79–83).

Tacrolimus trough levels are commonly targeted to $<$6 ng/mL [II-3], cyclosporine trough levels to $<$150 ng/mL [II-3], sirolimus trough levels of $<$6 ng/mL [II-3], and mycophenolate mofetil daily dose equivalents of $\leq$1000 mg [II-3]

Further reduction may be appropriate in individual patients and more advanced disease. Recent studies suggest that lower calcineurin inhibitor levels, i.e. targeting trough levels for tacrolimus of 3 ng/mL and cyclosporine of 100 ng/mL may be appropriate (44,84).

Additional strategies have been switching from tacrolimus to low-dose cyclosporine, or switching from the calcineurin inhibitor to low-dose sirolimus, or switching from mycophenolic acid to leflunomide or to low-dose sirolimus [III]

Successful outcomes have been reported using each of these different interventions in small case series, but there is to date no randomized controlled trial recommending one over the other strategy.

In patients with sustained high-level plasma BKV load despite adequately reduced immunosuppression, the adjunctive use of antiviral agents may be considered [III]

However, there are no randomized controlled trials providing evidence that adjunctive use of these agents is superior to timely reduction of immunosuppression.

- *Cidofovir*, trade name Vistide® (Gilead), is a nucleoside analog, licensed by The Food & Drug Administration for the treatment of cytomegalovirus retinitis. Cidofovir has been administered intravenously for PyVAN in doses from 0.25 to 1.0 mg/kg at 1–3 weekly intervals, without probenecid. The patients should be followed closely by serial measurements of serum creatinine concentration, leukocyte counts, eye symptoms and vision, as well as bi-weekly plasma BKV load. Anterior uveitis was observed in 12–35% cases (85,86). Some studies report a stabilization of renal function (87,88), whereas others report no demonstrable benefit (31,85,89–92). Maximal blood levels of 5 ug/mL were reached (92), which are below the BKV IC-50 or IC-90 of 30–40 ug/mL (93,94). Clinical studies are underway in HSCT and kidney transplantation evaluating the in vitro more potent lipid-ester derivative 1-O-hexadecyloxypropyl-cidofovir (CMX001; Refs.95,96).
- *Leflunomide*, Trade name Arava® (Aventis) is orally administered as a replacement for discontinued mycophenolic acid with a loading dose of 100 mg for 5 days, followed by an initial maintenance dose of 40 mg. Regular blood counts and liver function tests are advisable once a month for all patients on leflunomide treatment, as well as plasma BKV loads once every two weeks. Significant toxic effects have been described including hepatitis, hemolysis, thrombotic microangiopathy, bone marrow suppression, and fungal pneumonia. Therapeutic response to leflunomide was correlated with blood levels between 40 ug/mL and 100 ug/mL in some studies (89,97,98), but not in others (91,99). However, in most studies, immunosuppression was also reduced by replacing mycophenolate and/or reducing calcineurin inhibitor dosing.
- *Intravenous immunoglobulin (IVIG)* preparations have been administered in doses ranging from 0.2 to 2.0 g/kg in conjunction with reduced immunosuppression (100). Commercially available IVIG preparations contain high titers of potent BKV neutralizing antibodies (101). IVIG does not penetrate the intracellular compartment, but its direct neutralizing activity and plethora of indirect immunomodulatory effects could contribute to an improved resolution of active disease.
- Fluoroquinolones can inhibit BKV replication via an effect on the helicase activity of virus encoded large T antigen but the selectivity index is low (93,102). This modest anti-viral effect has been associated with some prophylactic efficacy in both hematopoietic stem cell and kidney transplant recipients in several nonrandomized studies (103–105). Treatment of well established PyVAN may not be effective (106).

Acute rejection after reduced immunosuppression for presumptive or definitive PyVAN should be treated according to standard protocols [III]

If acute rejection is diagnosed in allograft biopsies, after clearance of plasma BKV DNA and PyVAN by histology, anti-rejection treatment is indicated and a judicious increase in maintenance immunosuppression be considered. Administration of lymphocyte depleting agents should be done after careful evaluation of the competing risks of failure to control rejection and recurrence of PyVAN.

The nature and the pathophysiology of inflammatory infiltrates after clearance of BKV viremia and PyVAN may be diverse and include a response to viral infection termed immune reconstitution inflammatory syndrome (9,13,44,107). In the setting of persistent viruria (without viremia or nephropathy) biopsies with putative episodes of acute rejection that satisfy Banff criteria for diagnosis, do not always respond well to steroids (14).

Retransplantation can be considered for patients after loss of a first kidney allograft due to PyVAN, but frequent screening for BKV replication is recommended [II-2]

Retransplantation after kidney allograft loss due to PyVAN has been successfully performed in at least 118 cases, with a 93% graft survival at 3 years (108). Therapeutic intervention for recurrent infection is needed in 17.5% of patients (108). Surgical removal of the primary transplant has been performed in approximately half of all cases, but did not protect against recurrent BKV replication and PyVAN (109). Safe retransplantation (with or without

nephrectomy) requires the expansion of BKV-specific immune effectors that is facilitated by reduced or discontinued immunosuppression (110). In case of retransplantation of patients with detectable plasma BKV loads, a significant decline of plasma BKV loads indicative of emerging BKV-specific immunity should be achieved and prior graft nephrectomy considered (109,111,112). Induction therapy is not contraindicated after clearance of BKV replication, but extended periods of intense maintenance immunosuppression should be avoided.

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Disclosure

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Special Article

Human Papillomavirus in Solid Organ Transplantation

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Key words: Anal cancer, anal intraepithelial neoplasia, cervical cancer, cervical intraepithelial neoplasia, human papillomavirus, immunocompromised host, papillomaviridae, transplantation, vaccination

Abbreviations: AIDS, acquired immunodeficiency syndrome; AIN, anal intraepithelial neoplasia; ASC-H, ASC suspicious for HSIL; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; CMI, cell mediated immunity; CMT, combined-modality therapy; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; HRA, high resolution anoscopy; HPV, human papillomavirus; HSIL, high-grade SIL; LEEP, loop electrosurgical excision procedure; LSIL, low-grade squamous intraepithelial lesions; PCR, polymerase chain reaction; SCC, squamous cell carcinoma; VLP, virus-like particle.

Introduction

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections worldwide and causes cervical and anal cancer, as well as its associated precancer lesions of cervical intraepithelial neoplasia (CIN) and anal intraepithelial neoplasia (AIN). HPV also causes a proportion of vulvar, vaginal and penile squamous cell cancers (1). There is increasing evidence that HPV plays an important role in head and neck cancer. HPV also causes cutaneous and anogenital warts, which are of low malignant potential. Cell-mediated immunity is important for the control of HPV infection. Immunosuppression for solid organ transplantation decreases the capacity to eradicate new HPV infection, and enables increased HPV replication in latently infected cells. As a result, transplant recipients have a substantially increased risk of HPV-associated malignancies compared with the general population. Transplant patients also experience an increased occurrence of extensive and treatment-refractory cutaneous and anogenital warts.

Transmission and Host Response

HPV is a double-stranded DNA virus that infects the basal epithelial cells of keratinized skin, mucous membranes and the transformation zone of the cervix. Different HPV types have tissue tropism for cutaneous versus mucosal membranes in different body sites, with varying level of malignant potential (2,3). HPV types can be broadly classified into "high risk" and "low risk" types based on their propensity to cause cancer. In a large, global epidemiological study, Munoz and others found that at least 40 HPV types were associated with neoplasms. Of these, 18 were classified as "low risk" and associated with anogenital warts, mild cervical dysplasia and recurrent respiratory papillomatosis, and 12 were considered "high risk" including types 16 and 18 (4). More frequent HPV types associated with various clinical manifestations are listed in Table 1 (5).

The vast majority of HPV acquisition occurs via direct person-to-person transmission. Indeed, anogenital HPV is estimated to be the most common sexually transmitted infection in the United States (6). HPV can also be acquired by infants during the passage through the birth canal of HPV-infected mothers—this is likely the mode of viral transmission in children who later develop recurrent respiratory papillomatosis (7,8). Most persons infected with HPV are asymptomatic so transmission of the infection from individuals without visible lesions is common. In addition, anogential HPV can be seen concurrently with cutaneous warts or oral mucosal disease, suggesting that auto-infection can occur from one site to another (9,10). To date, there have been no reports of HPV acquired through organ transplantation.

Once HPV has infected epithelial cells, it evades the host immune response by various mechanisms. These include a prolonged infection cycle, a relative lack of inflammatory response during viral replication, and downregulation of the interferon response. In addition, HPV infection rarely causes viremia. Infection is localized to the mucosal and cutaneous surfaces and away from the vascular and lymphatic systems where adaptive immune responses are initiated (11,12). Nevertheless, at least 80–90% of genital

Table 1: HPV and tissue tropism

Disease	HPV types frequently associated
Plantar and common warts	1,2,4
Flat or plane warts	3,10
Butcher's wart	7,2
Bowen's disease	
Genital	16
Extragenital	2,3,4,16
Condylomata acuminta	6,11
Bowenoid papulosis	16,34,37,42
Intraepithelial neoplasia	
Low grade	6,11
High grade	16,18
Respiratory papillomatosis	6,11

Adapted from Table 1 in epidemiology of human papillomavirus infection (Ref. 5).

HPV infections clear spontaneously over time. Histologic analysis of regressing warts show a CD4+ T cell-dominated Th1 response. Resolution of the lesions depends on a successful cell-mediated immune response against early viral proteins (12,13). Failure to develop effective cell mediated immunity (CMI) results in an inability of the host to clear or control the HPV infection, leading to persistent infection, and resulting in an increased probability of cancer.

The importance of CMI was highlighted in a recent systematic review of population-based registry studies in HIV/AIDS and in transplant recipients. The investigators demonstrated a similar pattern of significantly increased incidence of all HPV-related cancers in both populations (14). This suggests that immune deficiency likely plays the most important role in the increased risk of HPV-associated neoplasia. Among HIV-infected patients, the risk of HPV-associated cancers is increased in those with higher HIV viral load and is inversely related to CD4+ count (15–17). Similarly, in a single-center review of renal transplant recipients over 40 years, T cell depleting induction with antithymocyte globulin was an independent risk factor for the development of anogenital cancer. This again suggests an association between the degree of immunosuppression and the probability of HPV-related malignancy (18). As a result of impaired cell-mediated immunity, transplant recipients experience an increased frequency of extensive, sometimes treatment-refractory cutaneous and anogenital warts (19,20) and are at a higher risk for neoplastic transformation in cervical and anogenital HPV infections (21,22).

Epidemiology and Clinical Presentation

HPV is associated with both benign and premalignant/malignant neoplasms in a variety of sites (Table 2).

Cutaneous and anogenital warts

Cutaneous warts are skin lesions of characteristic appearance and include common warts, deep plantar and flat warts. The prevalence of warts in transplant patients corresponds with the duration of immunosuppressive therapy, increasing to 50–92% in patients who are more than 4–5 years after transplantation (23). Ultraviolet light is also believed to be an important risk factor for the development of cutaneous warts in transplant recipients, as most lesions appear in sun-exposed areas (24).

Table 2: Clinical manifestations of HPV

Localization	Benign	Premalignant/ Malignant
Skin	Cutaneous warts	Potential role in squamous cell carcinoma of the skin
Anogenital	Anogenital warts	CIN, cervical cancer, AIN, anal cancer, vulvar and penile carcinoma
Respiratory tract	Respiratory papillomatosis	No clearly established link to malignant respiratory neoplasm
Head and neck	None established	Squamous cell carcinoma of head and neck

Anogenital warts, also known as condyloma acuminata, are one of the most common sexually transmitted diseases worldwide. They are caused by low-risk HPV types, most commonly types 6 and 11. However, at least 18 other HPV types have been associated with anogenital warts, including types 16 and 18, which are more commonly associated with malignant lesions (25,26). These exophytic, typically flesh- or gray-colored lesions are frequently multifocal, involving different parts of the anogenital tract simultaneously. In women, external anogenital warts are often associated with cervical lesions (27). Patients with anogenital warts, especially those who are immunosuppressed, are often also infected with high-risk HPV types. Therefore, immunosuppressed patients with anogenital warts will require monitoring and screening for HPV-mediated malignancies. A French study of organ transplant recipients reported a prevalence of anogenital warts of 1.8% (19).

Premalignant and malignant lesions of the cervix and anal canal

The oncogenic role of HPV infection has been most firmly established in the pathogenesis of CIN and cervical cancer. Persistent HPV infection, particularly with types 16 and 18, may lead to progressive deregulation of the replication of epithelial cells and potential malignant transformation (28). HPV infection also causes AIN and anal cancer with similar high risk HPV-types as those implicated in cervical neoplasia (3).

An increasing number of studies have investigated the epidemiology of cervical and AIN among transplant recipients.

In a Scottish study in the late 1980s, CIN and high-risk HPV types 16 and 18 were present more frequently in renal transplant recipients compared to age-matched controls (29). In a more recent study from Italy, 7% of 151 transplant recipients were found to have CIN (21). One of the largest reports to date is a retrospective Dutch single center study of 1023 women who underwent renal transplants between 1968 and 2008. Of these patients, a total of 16 anogential malignancies (1.6%) were noted, including six vulvar, five cervical and six anal carcinomas (18). Investigators found detectable HPV in 22/24 malignant and precancer lesions, and 54.5% of these were HPV type 16. Using cancer registry data from the general Dutch population, the authors estimated that these kidney transplant patients had increased risks of 5-fold for cervical, 41-fold for vulvar and 122-fold for anal carcinoma. Another review of 453 women who received renal transplants from 1990 to 2008 in South Korea revealed an incidence of 58.1 cervical carcinomas per 100 000 patient-years, which was 3.5-fold higher than the general population (29).

There is also a high burden of HPV-associated anal precancer lesions among transplant recipients. Ogunbiyi et al. showed a high proportion of AIN in renal transplant recipients who presented for elective lower gastrointestinal or genitourinary surgeries compared to matched controls (20% vs. 1%; Ref.30). Patel and others collected anal cytology and performed anal HPV polymerase chain reaction (PCR) in 108 renal transplant recipients (68 men and 40 women). They reported a 5.8% prevalence of AIN, with risk factors as follows: oncogenic HPV infection, duration of immunosuppression, a previous history of genital warts and receptive anal intercourse (31). Similar results were found in a systematic review of 32 000 transplant recipients from Danish, Finnish, Swedish, Canadian and Australian cohort studies. This study demonstrated a twofold increased risk of cervical cancer compared to the general population, and a nearly fivefold excess risk for anal cancer (14). Particularly striking was the 22-fold excess risk of vulvar and vaginal cancers. Compared to the general population, carcinomas of the anogenital region occurred at an earlier average age (41 years) in transplant recipients and were frequently multifocal. Over 40% of transplant recipients with anogenital carcinomas reported a prior history of anogenital warts (32).

Nonmelanoma cancer of the skin

SCC of the skin occur 65–250 times more frequently in transplant recipients than in the general population, and are often characterized by earlier age of onset, multiple lesions, a rapid course and more frequent node metastases than in the general population (33). Using degenerate PCR, a high prevalence (65–81%) of a variety of HPV DNA types has also been consistently demonstrated in premalignant skin lesions and in skin cancers of transplant recipients (34). Another study reported high-risk HPV in 46.2% of the SCC epithelium in renal transplant recipients compared to 23.5% in the immunocompetent control group (35). HPV was highly prevalent (>94%) in DNA analysis of eyebrow hairs in renal transplant patients, both with SCC and without SCC, although the presence of HPV DNA and the corresponding antibodies for the same HPV type was associated with increased risk of SCC (36). While the results of these studies are intriguing, it is not clear to what extent HPV contributes to the development of skin cancer among transplant recipients (37). Interestingly, sirolimus, an immunosuppressant with antineoplastic and antiviral properties, may have a protective effect against skin cancers compared to other immunosuppressive agents. A cohort of 1000 renal transplant recipients on sirolimus regimens had a similar incidence of skin cancers compared with the general population (38). However, HPV infection was not examined in this cohort, and the potential benefit of the antiviral effect of sirolimus in this setting remains speculative.

HPV and cancer of head and neck

There is increasing evidence that HPV is implicated in the pathogenesis of some head and neck cancers. This is especially seen in neoplasms arising from the base of the tongue and tonsillar region, and is not typically associated with smoking or alcohol consumption as seen in other head and neck cancers. D'Souza et al. (39) conducted a case-control study and showed that seropositivity for HPV-16 (odds ratio 32.2) and the presence of an HPV oral infection (odds ratio 14.6) had strong associations with oropharyngeal cancer. Of note, HPV-associated head and neck cancer appears to have a better prognosis compared to those not associated with HPV (40). HPV infection in the oral cavity is not rare. One large cross sectional study (41) showed a prevalence of oral HPV infection in the general population in the United States of 6.9%, with more men than women infected (10.1% vs. 3.6%). There are no published studies that explicitly investigate the association between oral HPV infection and head and neck cancer in transplant recipients. However, the prevalence of oral HPV infection is known to be higher in renal transplant recipients compared to immunocompetent patients (42). In one systematic review, transplant recipients were found to have a threefold excess risk of oropharyngeal cancer (14). It is likely that the increased rate of head and neck cancer is partly attributable to more persistent HPV infection in transplant recipients. Further studies are needed to clarify this relationship.

Respiratory papillomatosis and lung cancer

HPV can also cause a benign upper airway neoplasm called recurrent respiratory papillomatosis. The most frequently affected population is young children. Babies acquire HPV (typically HPV types 6 and 11) through contact with infected secretions in the birth canal. Lesions can also be adult-onset, occurring as a sequelae of HPV infection acquired sexually (43). It has been proposed that there is a relationship between HPV and SCC or adenocarcinomas of the lungs. However, studies are conflicting (44,45). Whether there is a more substantial role of HPV in the pathogenesis of pulmonary neoplasms among transplant recipients remains to be determined.

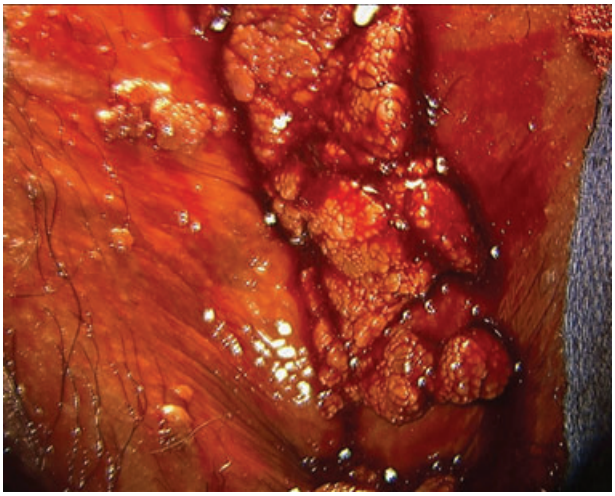

Figure 1: Extensive anal condylomata in a heterosexual male kidney transplant recipient.

Diagnosis

General principles

A thorough clinical inspection of the entire genital tract is sufficient to diagnose most external anogenital warts. Bright light and magnification with a hand lens or colposcope may assist in the diagnosis (Figure 1). All women with external anogenital warts must have a speculum examination for possible vaginal and cervical lesions. For men and women with recurrent perianal warts and/or a history of receptive anal intercourse, evaluation for intra-anal warts is recommended (46). If urinary symptoms are prominent, the distal urethra and meatus should be visually examined and a referral for urethroscopy should be considered.

Providers should have a low threshold to biopsy any genital warts that have an atypical appearance. This is because high-grade squamous epithelial neoplastic lesions are common and may be clinically indistinguishable from genital warts among immunocompromised patients (47). Using dilute (3–5%) acetic acid solutions (i.e. "acetowhite test") may be of help in delineating the extent of disease before biopsy; however, routine use of the test for screening individuals for HPV infection is not recommended due to poor sensitivity and specificity (32) in predicting active disease. Patients with anogenital warts and their sex partners should be screened for other sexually transmitted diseases including gonorrhea, chlamydia, syphilis, trichomonas, hepatitis B virus infection and HIV infection (48).

Cytology

The Papanicolau (Pap) smear is the backbone of the screening strategy for early diagnosis of HPV-related cervical atypia and cancer. The implementation of regular Pap tests has reduced the rate of invasive cervical cancer by approximately 70% since the 1950s.

Molecular-based methods

Molecular diagnostic methods to detect HPV have become more widely available in the last few years. These methods include *in situ* hybridization on cell smears or histological sections, DNA hybrid capture and PCR on clinical specimens. A high viral load of HPV 16 has been shown to be associated with development of carcinoma *in situ* (49,50). There are now multiple FDA-approved tests to detect high-risk HPV DNA. See Table 3 for recommendations for HPV co-testing in immunocompetent and the immuncompromised women. Detection and typing of HPV have no proven benefit in the diagnosis and management of anogenital warts and is therefore not recommended (50). When the diagnosis is in doubt, consider referral to a practitioner experienced in the diagnosis of anogenital warts. See below for the incorporation of molecular methods in screening.

Cervical cancer and CIN screening

A magnifying glass and a bright light source are used to examine the external genitalia. Given that genital warts may coexist with CIN, we recommend further evaluation such as colposcopy if genital warts are present on the external examination. Cervical cancer screening has been very successful where it has been established given that there is a long preinvasive state with CIN before the onset of cervical cancer, and that these precancer lesions can generally be successfully treated.

The American Cancer Society, the American Society for Colposcopy and Cervical Pathology, the American Society for Clinical Pathology, the American College of Obstetricians and Gynecologists and the U.S. Preventive Services Task Force have all issued cervical cancer screening guidelines. Many of these guidelines have been updated recently for the general population (51–54). Refer to Table 2 for a summary of the various guidelines including recommendations for immunocompromised individuals. In general, experts recommend initiating screening in women at age 21 and discontinuing screening at age 65. For women 21–29, screening should occur with cervical Pap tests only every 3 years. For women 30–65, screening can occur with cytology alone every 3 years, or by a combination of an HPV molecular test (testing for high-risk HPV types) and cytology every 5 years. However, these guidelines do not apply to immunocompromised women.

We recommend that transplant recipients be screened with the same periodicity as women who are HIV-infected (55). In solid organ transplant recipients (as in HIV-infected women), we recommend that a cervical Pap test be performed every 6 months for the first year after the transplant. If these tests are normal, then the screening interval can be increased to annual cervical Pap testing. There is little guidance from published studies, but it may be reasonable to reinstate cervical Pap tests every 6 months for 1 year after treatment for rejection, particularly if

Table 3: Guidelines for cervical cancer screening for different populations

	Immunocompetent women				
	American Cancer Society (52)	US Preventive Services Task Forces (53)	American College of Obstetricians and Gynecologists (54)	HIV positive women (55)	Solid organ transplant recipients
When to start	Age 21, recommend against screening women aged< 21	Age 21, recommend against screening women aged< 21	Age 21 regardless of the age of onset of sexual activity	Twice in the first year after the diagnosis of HIV[1]	Every 6 months in the first year posttransplant
Intervals					
Conventional or liquid based cytology	Every 3 years for women 21-65 years (Strong recommendation)	Every 3 years for women 21-65 years	Every 2 years (age 21-29); May move to 3 years for 30-65 of age after 3 negative tests	Annually if the first two tests after HIV diagnosis are normal	Annually if negative tests after every 6 month screening for a year
HPV co-test	Every 5 years for women aged 30-65 (Weak recommendation); not recommended for women < 30 years	HPV every 5 years an option for women (30–65) who want to extend the screening interval	Every 3 years if cytology normal and HPV test negative.	Insufficient evidence to use HPV to space out screening in HIV+	HPV test (if negative) as an adjunct to move on to annual screening
Primary HPV testing	For women 30-65 years, HPV test alone is not the recommended screening method in most clinical settings	Recommended against those < 30 years of age either alone or in combination with cytology	Not addressed	Not addressed	Not addressed
When to stop	Women ≥ 65 with adequate screening (Weak recommendation)	Women ≥ 65 years with negative tests, if they are at low risk for cervical cancer	Between age 65-70 if ≥ 3 negative consecutive results	Insufficient evidence; continue annually	Insufficient evidence; continue annually
Vaccinated against HPV 16/18	Continue screening per age-appropriate recommendation	Continue the same screening	Same regardless of vaccination	Same regardless of vaccination	Same as unvaccinated

[1] Routine colposcopy is recommended for HIV+ women with atypical squamous cells of undetermined significance.

antilymphocyte agents are used. There is less consensus about the incorporation of high-risk HPV testing in the algorithm in transplant recipients. However, some providers use high-risk HPV testing (if negative) for further reassurance that the Pap testing can increase from 6 months to 1 year. If high-risk HPV testing is positive, then screening can be continued every 6 months. Every visit should also be accompanied by a careful inspection of vulva, vagina and anus as well as the cervix. Women found to have abnormal cervical cytology on screening (atypical squamous cells of undetermined significance [ASC-US], ASC suspicious for HSIL [ASC-H], low-grade squamous intraepithelial lesions [LSIL] and high-grade SIL [HSIL]) should undergo colposcopy and biopsy of any suspicious-looking lesions.

Unfortunately, adherence to the minimum recommended annual cervical cancer screening appears to be very low in transplant recipients (56). Every effort should be made to encourage patients to adhere to regular screening schedule, and individuals with an abnormal screening test should be promptly referred to a qualified specialist.

Anal cancer and AIN screening

Cervical and anal cancers share many similarities. They both arise in the transformation zone, they are both caused by high-risk HPV types and they are preceded by precancer lesions (57). Cancers also have been noted to arise in the same location as antecedent precancer disease. Given the high prevalence of AIN and anal cancer in the HIV-infected population, and given the similarities between cervical and anal cancers, Chin-Hong and Palefsky have proposed an anal cancer screening algorithm (57,58). This incorporates many of the elements of cervical cancer screening above. Given the high prevalence of anal cancer in the transplant population, we recommend a similar approach for transplant recipients as in the HIV-infected population.

Like the protocol used for cervical cancer screening, the Pap test is the first step. To perform an anal Pap test, we recommend using a water-moistened polyester swab (Fisher Scientific, Pittsburgh, PA, USA). We recommend polyester swabs because cells cling to cotton and this may decrease the yield of the Pap test. The polyester swab

is inserted in the anal canal. As the swab is withdrawn, rotate the swab and maintain pressure against the anal canal. The goal is to obtain exfoliated cells from the areas most at risk for HPV-associated disease such as cells from the lower rectum, the squamocolumnar junction and the anal canal. Either a glass slide or liquid-based media can be used to collect and transport these cells for analysis.

Men and women who are found to have abnormal anal cytology (ASC-US, ASC-H, LSIH and HSIL) can be referred to the next step, which is high resolution anoscopy (HRA). HRA uses similar equipment as in cervical colposcopy (powerful light and binocular lens). As in colposcopy, we use HRA to locate and biopsy lesions that have contributed to the cytologic abnormalities seen. Lugol's (iodine) solution and 3% acetic acid are tools that we can use to increase the ability to identify abnormal lesions. We recommend this screening approach in transplant patients only if there is sufficient infrastructure to do so. This includes the availability of trained high resolution anoscopists and pathologists used to interpreting AIN.

Recommendations

(1) Perform a cervical Pap test every 6 months for the first year after the transplant. If these tests are normal, then the screening interval can be increased to annual cervical Pap testing (II-2).
(2) Women found to have abnormal cervical cytology on screening (atypical squamous cells of undetermined significance [ASC-US], ASC suspicious for HSIL [ASC-H], low-grade squamous intraepithelial lesions [LSIL] and high-grade SIL [HSIL]) should undergo colposcopy and biopsy of any suspicious-looking lesions (III).
(3) Perform an anal Pap test once yearly for transplant patients (III).
(4) Refer patients with abnormal anal cytology on screening (ASC-US, ASC-H, LSIL and HSIL) for high-resolution anoscopy for biopsy and treatment (III).

Treatment

General principles

There are several principles in the treatment of HPV-associated disease. Treatment options depend on the size, location and grade of the lesion (57,59). For cutaneous and external genital warts with very little malignant potential, goals of treatment may be for cosmesis or to relieve anxiety in general. However, in immunosuppressed patients such as transplant recipients, these low grade lesions may become quite large. In these cases, removal of warts may be needed to alleviate obstruction, itching and bleeding. CIN I and AIN I have a very low probability of progression to cancer in general. However, some providers may treat these lesions in immunocompromised patients given the observations in some clinical studies that there is progression from low-grade to high-grade cervical disease among HIV-infected individuals (60,61). CIN II, CIN III, AIN II and AIN III are treated when possible as they are considered direct precursors to cervical and anal cancers, respectively.

Although there is limited evidence, some providers will also try to reduce immunosuppression if this is possible. This is particularly if disease is refractory to treatment, or if recurrent. There is also a theoretical basis for switching from calcineurin inhibitors to mTOR inhibitors such as sirolimus, particularly if malignant transformation has already occurred (62). However, there are few published studies that specifically address the role of mTOR inhibition in HPV-associated malignancies, other than in non-melanoma skin cancer (63).

Treatment of cutaneous warts

Cure is maximized when presoaked dead skin is first pared down using a pumice stone, nail file, emery board or scalpel. Common treatment options then include products containing salicylic acid, cryotherapy and imiquimod 5% cream (64,65). A salicyclic acid preparation in combination with an occlusive dressing such as duct tape may increase the efficacy of the treatment modality. Cryotherapy can be performed using liquid nitrogen spray, a liquid nitrogen soaked swab, or a cryoprobe cooled with nitrous oxide. This can be repeated every three weeks. Imiquimod 5% cream (Aldara) is a topical immune response modifier that induces cytokines locally. We advise patients to apply the cream once daily before bedtime, three times a week for up to 16 weeks. In our experience, transplant and other immunocompromised patients may require repeat cycles of therapy, or may not respond completely. If lesions look atypical or are refractory to treatment, we recommend referral to a dermatologist to rule out nonmelanoma skin cancer and other malignancies given the high incidence in this population (Ref. 33; see Figures 3 and 4).

Treatment of CIN

Some providers may elect to treat CIN I given that the natural history may be unpredictable in transplant recipients as has been observed for HIV-infected women (66). In most immune competent patients, however, CIN I is generally not treated. CIN II and CIN III are treated in all women to prevent cancer.

A variety of excisional and ablative therapies can be used. Loop electrosurgical excision procedure (LEEP) is generally the treatment of choice for CIN II and CIN III. LEEP uses an adjustable wire loop to diathermically excise lesions of various dimensions. In general LEEP is widely used because it is easy to use and has a low complication rate. In addition, tissue is relatively well preserved and can be used to confirm the diagnosis histopathologically and to ensure that clear margins are obtained (67). Cryotherapy may also be employed with the direct application of a supercooled probe to the affected cervical area using multiple

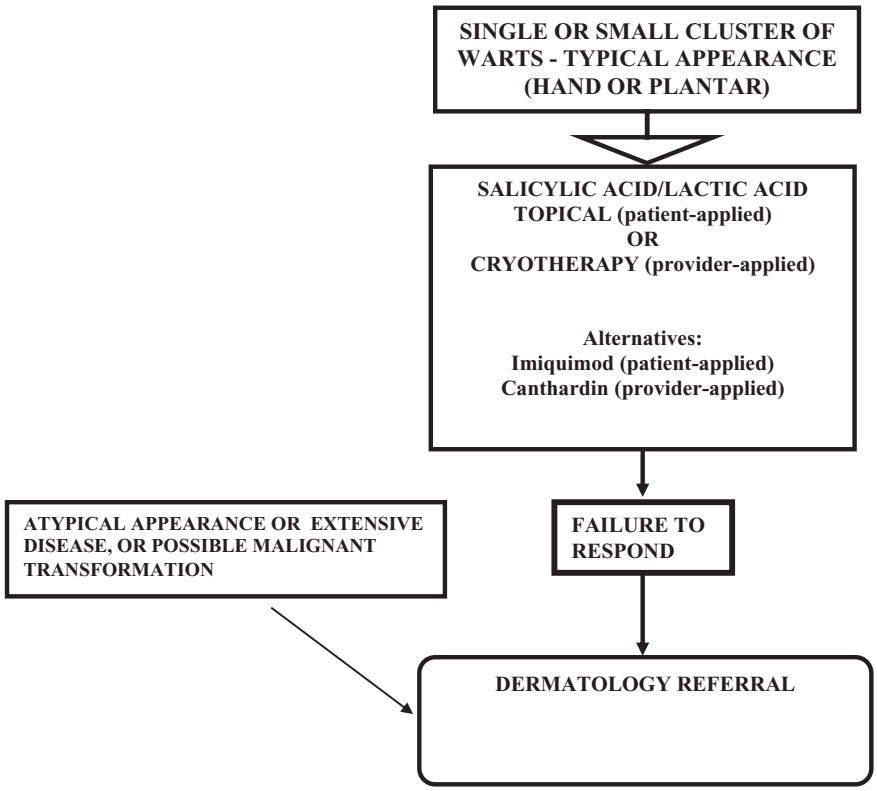

Figure 2: Management of cutaneous warts.

freeze-thaw cycles. Adverse effects are mild cramping and persistent vaginal discharge. The advantages are low cost, ease of use and the absence of major complications in general. The disadvantages are a higher failure rate compared to LEEP, and the inability to get tissue to assess whether treatment is adequate with clear margins (68). Other less used options include laser therapy (69) and cold-knife conization (70). Laser therapy uses carbon dioxide under colposcopy to precisely vaporize lesions to the adequate depth needed. Cold-knife conization utilizes a scalpel to excise a cone-shaped portion of the cervix including the entire transformation zone. General anesthesia must be used in these cases and there is a higher risk of complications (e.g. bleeding, infection and cervical incompetence) compared with the other office-based procedures.

Treatment of cervical cancer

Treatment options depend on the stage of cervical cancer diagnosed. For early stage microinvasive disease (<3 mm), conization may be offered to young women who want to maintain fertility (71). For disease up to stage IIa, a primary regimen of chemoradiation (primary radiotherapy with chemotherapy) is preferred (72). The role of surgery (radical hysterectomy with para-aortic and pelvic lymphectomy) for all cases is controversial, particularly if there is no residual disease burden (73). For patients with locally advanced disease, radiotherapy followed by chemotherapy is usually offered (74). Women with metastatic cervical cancer could have combined chemotherapy (75) or radiotherapy (if symptomatic) (76) to help alleviate symptoms.

Treatment of AIN

It may be more difficult to treat AIN compared with CIN given the anatomical challenges of the anal canal compared with the cervix (57). Treatment depends on the grade of the lesion. Patients with AIN I may elect to have lesions treated for symptomatic or psychological relief since these have low malignant potential. Some providers may elect to treat AIN I in transplant patients given the observation that there is a faster progression from AIN I to AIN II/III in HIV-infected patients when compared to HIV noninfected patients (77). We treat AIN II and AIN III to prevent anal cancer. Size and location are important considerations when deciding on the appropriate treatment strategy. Intraanal AIN I lesions <1 cm^2 at the base (including condyloma) can be treated with 80% trichloroacetic acid (59), topical 5-fluorouracil (78) or cryotherapy. Some providers may use imiquimod 5% cream for AIN given recent data to support this practice (79,80). For larger and higher grade lesions, infrared coagulation in the outpatient setting (81–83) or intraoperative fulguration (using intraoperative HRA to localize lesions) can be used (84). For very large lesions of any grade that are not causing patients symptoms, we may elect to follow patients closely rather than automatically remove disease, given the associated morbidity of these procedures (pain, anal stenosis and anal incontinence; Ref.57).

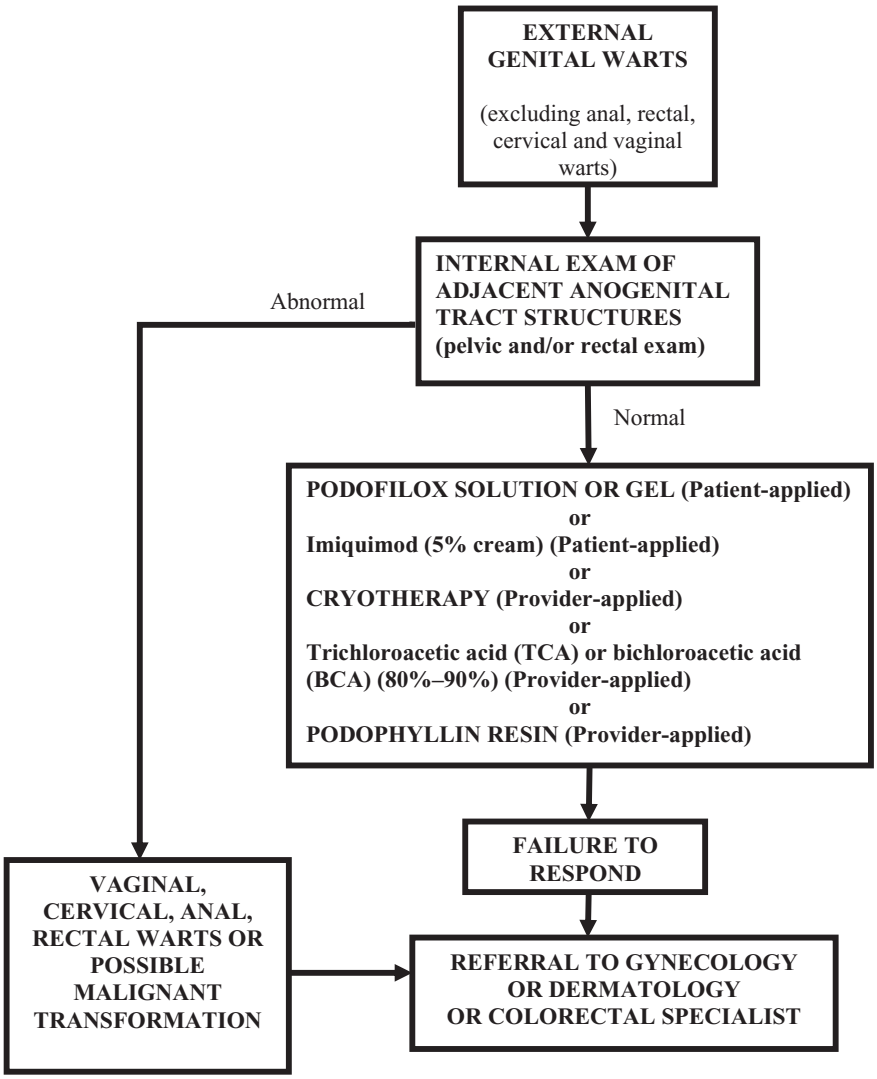

Figure 3: Management of anogenital warts.

Treatment of anal cancer

Invasive anal cancer is usually treated with a combination of radiotherapy and chemotherapy (5-fluorouracil and mitomycin; Ref.85). This combined-modality therapy (CMT) approach could avoid the morbidity of abdominoperineal resection with removal of the anorectum and creation of a permanent colostomy. Because immunosuppressed patients may experience CMT toxicity, sometimes lower doses of radiotherapy and alternative chemotherapy (e.g. cisplatin instead of mitomycin) can be offered (85).

Prevention

Trials of prophylactic HPV vaccines have been very effective in those unexposed to the HPV types included in the vaccine. The vaccines use components of the major HPV capsid proteins (L1 alone or in combination with L2) which self-assemble into virus-like particles (VLP). VLP induce neutralizing antibodies which protect the individual before exposure to HPV infection. There are two prophylactic HPV vaccines currently available. One is a quadrivalent vaccine (HPV types 6, 11, 16 and 18) (Gardasil, Merck, Whitehouse Station,NJ, USA) and the other is a bivalent (HPV types 16 and 18) vaccine (Cervarix, GlaxoSmithKline, Rixensart, Belgium). Both vaccines have demonstrated over 90% efficacy in preventing CIN II, CIN III, adenocarcinoma *in situ* and cervical cancer associated with the HPV types included in the vaccine provided that women had not been previously exposed to these types (86–88). Because the quadrivalent vaccine also includes HPV types 6 and 11 which are the major causes of genital warts, clinical trials have demonstrated over 90% efficacy in preventing warts caused by the four HPV types included in the vaccine in both women and men (86,87,89). In addition, trials of the quadrivalent vaccine have shown 78% efficacy in preventing incident AIN among men who have sex with men (90).

Given these findings, multiple expert panels have recommended HPV vaccination of girls and young women. Routine vaccination should be offered to all females 11–12 years old, and as young as 9 years old, with catch up vaccination from 13 to 26 years if not previously immunized (91). Routine HPV vaccination is also recommended for boys aged 11–12 years old, and as early as 9 years old, with catch-up vaccination between 13 and 21 years old, and permissive use for ages 22–26 (92). Only the quadrivalent vaccine has been widely studied in males, with only limited immunogenicity data for the efficacy of the bivalent vaccine in boys (93). The schedule of the quadrivalent vaccine is three doses at time 0, and at months 2 and 6. The corresponding schedule of the bivalent vaccine is three doses at time 0, and at 1 and 6 months of follow up.

There are limited safety and efficacy data specifically in the transplant population. However, given that the HPV vaccines do not contain live virus, we suggest vaccination of transplant patients using similar guidelines as above. There are also no data on whether vaccination would increase the likelihood of allograft rejection. There is some evidence in the HIV-infected population that the HPV vaccine is safe and immunogenic (94,95). Vaccination of eligible patients before transplantation would be preferred, given the higher likelihood of developing a robust neutralizing antibody response. Note that vaccination does not substitute for ongoing Pap screening in the transplant population. Not all oncogenic HPV types are included in the current generation of prophylactic vaccines.

Until the advent of HPV vaccines, there were few other options for primary prevention of HPV infection. HPV vaccines now form part of a menu of options that can be discussed with transplant candidates and patients (96,97). Limiting the number of sexual partners can help reduce the rate of HPV-related disease, as a high number of partners is associated with increased rate of HPV infection and cervical cancer. Sexual contact with anyone who has genital sores or unusual growths in the genital area or anus should be avoided. Condoms can reduce, but do not eliminate, the risk for HPV transmission to uninfected partners. Condoms should be used nonetheless, not only to reduce HPV transmission, but also to prevent other sexually transmitted diseases (97). Circumcision is also effective in decreasing the risk of HPV transmission (96). Limiting exposure to UV radiation is important to prevent skin carcinogenesis, which may be associated with HPV (98). In transplant recipients, avoidance of overimmunosuppression may reduce the probability of HPV-associated disease, although there is less evidence for this.

Recommendations

Immunize all male and female transplant patients (ideally before transplantation) ages 9–26 (target age 11–12) with the HPV quadrivalent vaccine. Females can also receive the HPV bivalent vaccine (I)

Infection Control

Some reports have indicated that intact HPV virus can be isolated from the laser generated plume used to treat human lesions (99,100). Given these observations, safety precautions are recommended during laser surgery such as gloves and gowns to cover exposed skin surfaces. Likewise use of eye protection, masks and smoke suction systems that have high flow volume and good filtration are recommended if carbon dioxide laser must be used as a treatment modality (101).

Future Research

Although there is increasing population-based data that transplant recipients have a substantial burden of HPV-associated malignancies, there have been few natural history cohorts that aim to describe the precise epidemiology of disease in this population. In contrast, there is a robust literature in the HIV-infected population that demonstrates a high proportion of HPV-associated precancer lesions and cancer, and its association with immunosuppression. We need to begin to examine knowledge and attitudes of patients and providers regarding these issues, and as knowledge becomes available, raise awareness of screening and treatment paradigms. Perhaps one of the most exciting developments in the field has been the success of the HPV prophylactic vaccines in the general population. We need targeted studies in our transplant populations to study immunogenicity and safety, as well as efficacy. Small studies are underway, but multicenter studies will provide more robust and generalizable data. As the new generation of 9-valent HPV prophylactic vaccines and HPV therapeutic vaccines continue to be developed and studied, we need to consider how they fit in to our armamentarium of cancer prevention options for transplant recipients.

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Disclosure

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Special Article

Human Parvovirus B19 in Solid Organ Transplantation

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Key words: Anemia, intravenous immunoglobulin, parvovirus B19, posttransplant infection, viral infection

Abbreviations: IVIG, intravenous immunoglobilin; PCR, polymerase chain reaction; SOT, solid organ transplantation.

Epidemiology

Virology

The *Parvoviridae* family includes the genus erythrovirus with human parvovirus B19 being the classic type member. The virus is 25 nm in diameter, nonenveloped and consists of a single-strand linear DNA that is approximately 5 kb in length. The viral genome *encodes* three main proteins, a nonstructural protein (NS1) and two structural proteins (VP1 and VP2; Ref. 1). The nonstructural protein is cytotoxic to host cells (2,3). Parvovirus B19 is classified into three different genotypes (genotype 1, 2, 3), but there is no definitive association of genotypes with specific clinical manifestations.

Parvovirus B19 was first detected in a healthy blood donor's serum in 1974 (4). It was subsequently linked to disease in children with sickle cell anemia experiencing transient aplastic crisis (5) and then in children with a contagious exanthem, called erythema infectiosum, or fifth disease (6). Parvovirus B19 has particular tropism for human erythroid progenitor cells, which is the natural host cell (7,8). The cellular receptor is globoside (also called erythrocyte P antigen; Ref. 9), which is found on erythroid cells, erythroid precursors and red cells of the placenta and fetal myocardium, fetal liver and some megakaryocytes and endothelial cells. Viral replication induces a distinctive cytopathic effect by light microscopy, represented by giant pronormoblasts (10), and productive infection has only been described in erythroid precursors (11). Although P antigen receptors are found on nonerythroid cells, there is evidence that a region of the viral genome is responsible for inhibiting viral replication in nonpermissive cells (12).

Transmission

Parvovirus B19 is ubiquitous and a common illness of childhood so that half of the population have detectable IgG antibody by 15 years of age (13,14). Most infections occur in the spring in temperate climates with small epidemics regularly occurring several years apart (15). The incidence of parvovirus infection in solid organ transplant patients is unknown because of the lack of surveillance studies. Based on detection of parvovirus DNA in peripheral blood, one study reported a single institution incidence of 12% in kidney transplant patients who had anemia (16).

Transmission of parvovirus B19 appears to be via respiratory secretions (6,17). Direct intranasal inoculation of parvovirus B19 into healthy volunteers resulted in viremia and clinical manifestations (18). Transmission can also occur to the fetus via transplacental infection and rarely through blood products (19,20). No FDA approved test is available for parvovirus B19 screening in blood donors. However, nucleic acid testing (NAT) is available for plasma units in process of being fractionated (21). There is evidence that transmission of parvovirus B19 infection may occur at the time of transplantation (22–24). Barzon et al. showed that in the majority of 10 pediatric kidney transplant patients (pretransplant parvovirus serology D+/R−), positive detection of parvovirus B19 DNA in the allograft kidney biopsy sample, preservation solution or washing solution (which contain circulating donor cells and resident kidney cells) was associated with posttransplant detection of parvovirus DNA in the blood of the recipient (22).

The incubation period ranges from 4–14 days, and individuals with erythema infectiosum are contagious before onset of rash but rarely afterwards. Individuals with aplastic crisis can be contagious before symptoms until about one week after onset of symptoms (25). Secondary infection rates are 50% for susceptible household members (26) and 20% for school and childcare personnel (27). Transmission to hospital personnel can occur.

Clinical disease

The clinical manifestations of parvovirus B19 infection in immunocompromised patients are atypical (Table 1). Among SOT recipients, fever, arthralgia and rash were observed in 25%, 7% and 6% of patients with parvovirus B19 infection, respectively. Anemia, however,

Table 1: Clinical manifestations of parvovirus B19 in immunocompromised hosts

Persistent anemia
Severe anemia
• Lack of reticulocyte response
• Lack of response to erythropoietin
Fever
• Observed in 25% of solid organ transplant patients.
Lacy skin rash
• Not always present because of lack of antigen-antibody complexes (30,33)
Arthropathy
• Not always present because of lack of antigen-antibody complexes (30,33)
Pancytopenia
• A subset of patients will manifest concomitant leukopenia or thrombocytopenia with the anemia (8,18,51).
• The pathogenesis is speculated to be non-specific cytopathic effects in the bone marrow (8) or restricted non-structural protein expression in megakaryocytes, which leads to cytotoxicity but not viral progeny (52).

was present in 99% of the patients (28). Therefore, parvovirus B19 infection should be suspected in SOT recipients with erythropoietin-resistant anemia since the reported incidence in this group of patients is relatively high (29).

Many clinical manifestations have been associated with parvovirus B19 (30). However, the association with parvovirus is predominantly based on finding DNA in tissue, which may not be proof of causation. Parvovirus DNA has been found persistently in a number of tissues including bone marrow, synovium, heart tissue and skin from individuals who are asymptomatic (31). The reason for the persistence is unclear but may be related to inhibition of viral replication in nonpermissive cells. Furthermore, normal healthy blood donors have been found to have circulating parvovirus B19 DNA in peripheral blood (32).

Immunity

Antibody response to parvovirus B19 appears to confer life-long protective immunity for the individual. Lack of an antibody response is observed in patients with persistent infection (33). "Recurrences" of parvovirus B19 infection may be more related to poor initial neutralizing antibody production in immunocompetent and immunocompromised hosts.

T cell responses to parvovirus B19 have been detected (34) but their role in protective immunity is not clear (35).

Diagnosis

Parvovirus B19 infection can be diagnosed by serology or direct viral detection in clinical specimen such as blood, bone marrow and other organs (i.e. liver, lung, kidney). In highly viremic patients following acute parvovirus B19 infection, serology might be falsely negative because antibodies could be complexed by viral particles (36). In addition, parvovirus B19 serology is not reliable in immunocompromised patients due to inadequate or delayed antibody-mediated immune response (37,38). Parvovirus B19 IgM antibody was present in only 75% of SOT recipients at the time of disease onset. The detection of parvovirus B19 IgG antibody alone is suggestive of remote infection and is uncommonly seen (7% of patients) among transplant recipients with parvovirus B19 infection (28). The current use of polymerase chain reaction (PCR) assays significantly improved the detection of viral DNA (39). However, one should keep in mind that some PCR assays are unable to detect non-B19 strains (genotypes 2 and 3; Refs. 40–42), and real-time PCR can be falsely negative in case of high-level viremia (43). Furthermore, parvovirus B19 DNA can be detected by PCR in the serum of some patients for long time after the acute phase of infection (44). Thus, a positive PCR for parvovirus B19 does not necessarily indicate acute infection. However, the positive predictive value of positive PCR in an immunocompromised host with red cell aplasia is high. Bone marrow examination associated with *in situ* hybridization or immunohistochemical staining could be very helpful in establishing the diagnosis when the clinical presentation is strongly suggestive of parvovirus B19 infection but the PCR and serology are negative (28). Typical bone marrow findings include overall hypercellularity and the presence of giant pronormoblasts with finely granulated cytoplasm and glassy intranuclear inclusions with a clear central halo (lantern cells), and absent late normoblasts.

Recommendations:

(1) Parvovirus B19 infection should be suspected in SOT recipients with:
   (a) Erythropoietin-resistant anemia or anemia with inappropriate reticulocyte response with or without:
      (i) Fever, arthralgia or rash
      (ii) Organ-invasive disease such as hepatitis, myocarditis, pneumonitis, neurological disease or vasculitis (III).
   (b) Pancytopenia
(2) The initial work-up for suspected parvovirus B19 infection should include serology (IgG and IgM) and serum/whole blood PCR for parvovirus B19 (III).
(3) If not done earlier, bone marrow examination should be performed when parvovirus B19 infection is strongly suspected and the serology and serum PCR are negative. In addition, *in situ* hybridization or immunohistochemical staining should be performed (III).

Treatment

Antiviral drugs are not available for the treatment of parvovirus B19 infection. However, intravenous immunoglobulin (IVIG) has appeared to be beneficial in a large number of SOT recipients with parvovirus B19 infection (28,45,46). The optimal dosing regimen and duration of IVIG

therapy for parvovirus B19 infection has not been established and some patients have been reported to have long-lasting resolution of the infection without IVIG therapy (28). Most patients are treated with 400 mg/kg/day for 5 days, although higher doses have been used for shorter periods of time. In one review, the rate of relapse was not different among transplant recipients who received a total dose of $\leq$2 g/kg or >2 g/kg (28). Unfortunately, in the same case series up to 28% of SOT recipients experienced relapse after receiving IVIG. The value of PCR use to monitor the response to therapy is not known, especially that persistent low grade viremia for months despite adequate clinical response to therapy is not uncommon (47). Therefore, it would be reasonable to simply follow serial hemoglobin measurement and consider obtaining parvovirus B19 PCR in case of recurrence of anemia. Patients with recurrence of parvovirus B19 infection have been successfully treated with additional courses of IVIG (47). Yet, there is a wide variation in the clinical practice in terms of dose and duration of therapy. The side effects of IVIG treatment include fever, chills, headache, myalgia, nausea, hypertension, chest pain and renal failure.

The reduction of immunosuppression is believed to contribute to the resolution of infection; however, the timing of such an intervention (i.e. before or after IVIG therapy) is a subject of debate.

Recommendations:

(1) Patients with parvovirus B19 infection may be treated with 400 mg/kg/day of IVIG for 5 consecutive days (III).
(2) Reduction of immunosuppression should be attempted if at all possible at the time of diagnosis (III).
(3) In case of nonresponse to the first IVIG course or in case of relapse another course of IVIG (400 mg/kg/day for 5 days) may be given (III).

Prevention

In the SOT population, no proven specific preventive strategy against parvovirus B19 infection is available. Routine screening of donor and recipient serostatus for parvovirus B19 is not recommended. In one study, donor and recipient serostatus and more importantly the detection of viral DNA in renal allograft tissue, preservation solution or washing solution were useful to predict the risk of posttransplant viremia (22). However, only a few patients developed clinically significant disease in this study, which raises the question of cost-effectiveness of such method. Furthermore, strategies to prevent symptomatic parvovirus B19 infection are yet to be defined. Recommendations aimed at avoiding exposure of transplant recipients to children or adults with parvovirus B19 have not been offered by any advisory group because symptomatic patients are usually no longer contagious. In addition, the relative rarity of this diagnosis in transplant recipients, particularly among pediatric transplant recipients, does not support the introduction of such a policy. To avoid nosocomial transmission, standard and droplet precautions should be implemented when a patient has an active disease. Anecdotal data in bone marrow transplant recipients have demonstrated the absence of parvovirus B19 disease in cohorts of patients who received prophylactic IVIG for other reasons (48). However, studies comparing the incidence of parvovirus infection among bone marrow transplant recipients who received IVIG and those who did not are not available. Furthermore, the lack of evidence of efficacy in the SOT population, the relative low incidence of symptomatic parvovirus B19 infection and the high cost and potential toxicity associated with IVIG therapy do not favor its prophylactic use. Finally, the development of recombinant human parvovirus B19 vaccine composed of VP1 and VP2 capsid proteins is underway. All 24 volunteers who received either 2.5 or 25 μg of parvovirus B19 recombinant vaccine (MEDI-491) formulated with the adjuvant MF59C.1 at 0, 1 and 6 months developed neutralizing antibody titers that peaked after the third immunization and were sustained through study day 364 (49). A phase I/II randomized, placebo-controlled, double-blind clinical trial of the immunogenicity and safety of 2 dose levels of a recombinant human parvovirus B19 vaccine (VAI-VP705) conducted by the National Institute of Allergy and Infectious Diseases was halted because of three unexplained cutaneous events. After the second dose of the vaccine, most vaccine recipients developed ELISA and neutralizing antibody to parvovirus B19 (50). Hopefully a vaccine will be available in the near future for clinical use in high-risk populations. However, studies will be required to specifically define its use in the SOT population.

Recommendations:

(1) To avoid nosocomial transmission, standard and droplet precautions should be implemented when a patient has an active disease.

Future Studies

Future studies should further evaluate the utility of parvovirus B19 monitoring in SOT recipients. The significance of parvovirus B19 DNA detection in the blood or tissue samples obtained from immunocompetent patients and SOT recipients should be determined. Large, prospective, multicenter studies are needed in order to investigate current and novel therapeutic options for parvovirus B19 disease. Finally, future studies are needed to investigate new parvovirus B19 vaccines and the benefit of their use among SOT candidates and recipients.

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Special Article

Adenovirus in Solid Organ Transplantation

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Key words: Adenovirus, cidofovir, cystitis, opportunistic infection, viral infection

Abbreviations: CDC, Centers for Disease Control and Prevention; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; DNA, deoxyribonucleic acid; EBV, Epstein–Barr virus; FDA, Food and Drug Administration; GALT, gut associated lymphoid tissue; GVHD, graft-versus-host disease; HICPAC, Healthcare Infection Control Practices Advisory Committee; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; OKT3, Orthoclone OKT3, muromonab CD3; PCR, polymerase chain reaction.

Epidemiology and Risk Factors

Adenoviruses are nonenveloped, lytic double-stranded DNA viruses typically associated in immunocompetent patients with self-limited respiratory, gastrointestinal or conjunctival disease throughout the year (1). Adenovirus infections are endemic in pediatric populations and people living in close quarters (such as college students and military recruits; Ref. 1). Although even in organ transplant recipients these infections may be asymptomatic, as with other infections in immunocompromised patients, adenovirus infections can be severe, prolonged, disseminated, and impact morbidity, mortality and graft survival (2,3).

Adenoviruses are classified into seven subgroups (A–G) based on hemagglutination properties, DNA homology and oncogenic potential in rodents, that can be further divided in 52 distinct serotypes on the basis of neutralization by specific animal antisera (2). There are different genotypes that can be distinguished within the same serotype (2). Several serotypes, particularly from subgroup C, are capable of inducing a latent infection. T lymphocytes from tonsils and adenoids, as well as bronchoalveolar lavage fluid from asymptomatic adults, have been found to harbor viral DNA, which may serve as the source of reactivation during immunosuppressive states. The presence of viral DNA in pediatric specimens peaks in early childhood (age 2 years) and then declines (2,4,5).

Adenovirus infections can be acquired *de novo*, or through reactivation of a latent infection of the recipient or from the transplanted organ (6). Transmission of adenovirus occurs by the respiratory route via infected aerosols, person-to-person contact, fomites or by the fecal–oral route (1). Nosocomial transmission has been suggested among hospitalized solid organ transplant recipients infected with common serotypes (6–8). Diagnosis of adenovirus disease early in the posttransplant course suggests that the infection is reactivating from the recipient or acquired from the donated organ (6,9). Detection of adenovirus by PCR in the myocardium of pediatric heart transplant recipients without evidence of acute infection is additional evidence that transplanted organs could be the source of latent virus (10).

Although there is no consensus on the definitions of adenovirus infection and disease, we propose the following definitions, as they have been used in other studies. Asymptomatic adenovirus infection is defined as detection of adenovirus in patients from stool, blood, urine, or upper airway specimens (by viral culture, antigen tests, or PCR) in the absence of signs and symptoms (11). Adenovirus disease is defined as the presence of attributable organ signs and symptoms combined with adenovirus detection in the biopsy specimens (immunohistochemical stain) or from bronchoalveolar lavage and cerebrospinal fluid (culture, antigen detection, or PCR), in the absence of another diagnosis (2,11). Adenovirus disease is considered disseminated if two or more organs are involved, not including viremia (2,12). The ability of adenovirus to establish latency may lead to challenges in the interpretation of the presence of DNA in clinical specimens.

The true incidence of adenovirus infection is unclear, mainly because asymptomatic infection and disease are not always reported separately. Adenovirus appears to be more commonly isolated in pediatric than adult solid organ transplant recipients, probably reflecting the epidemiology of adenovirus infections in children (3,13,14). Most studies demonstrate diagnosis of infection within the first few months in all posttransplantation populations. The incidence of adenovirus infections among pediatric solid organ transplant recipients was found to be at 6.25% in a retrospective study, liver transplant recipients accounting for a significant proportion of cases (57%), followed by heart (32%) and kidney (11%) transplant recipients (15). The rate

of adenovirus infections after pediatric liver transplantation has ranged from 3.5% to 38%, with the infections being diagnosed at a median of 0.85–1 months after transplantation (range 0.13–29.6 months; Refs. 8,13,16,17). The incidence in pediatric lung and heart–lung transplant recipients has been reported between 7% and 50%, infections being diagnosed mainly in the first few months after transplantation (9,18–21). Adenovirus infection has also been found at high rates (incidence of 4.3–57.1%) after pediatric intestinal or multivisceral transplantation, with median occurrence of 1.6 months after transplantation, and the majority of cases being diagnosed in the first 6 months posttransplantation (6,8,22,23).

In a recent study of adult liver, heart, kidney and kidney–pancreas transplant recipients, 7.2% of the recipients developed transient self-limited adenovirus viremia in the first year posttransplant; 79% recipients were asymptomatic, while 10.5% had gastrointestinal symptoms (predominantly diarrhea) and 10.5% respiratory symptoms (7). A single retrospective study reported a 5.8% incidence of adenovirus infection in adult liver transplant recipients, of which 36% had asymptomatic infection and 64% developed adenovirus disease (14). In this study, the mean time to the initial detection of adenovirus was 2.2 months posttransplantation (range 0.1–6 months; Ref. 14). In kidney transplant recipients, for unclear reasons, adenovirus infections are more commonly reported in the adult population, with incidence rates of 4.1%, with a median time to infection of 1.25 months (range 0.5–75 months; Ref. 24). In adult lung transplant recipients, adenovirus infection has a high incidence, 22.5% in one report, in which the majority of the infections (78%) were asymptomatic, and only a minority of patients developed self-limited flu-like illness (25). Severe and fatal adenoviral infections have been reported in adult lung transplant recipients (9,18). Very few cases of adenovirus infections have been reported in adult intestinal transplantation (26).

Data regarding the risk factors for adenovirus infection in solid organ transplant recipients are emerging. Young children, under 5 years, are at increased risk of infection, likely because they are immunologically naïve and have higher exposure (3,14,20). Age has been found to be an independent risk factor for adenovirus infection, with a 19% increase in the risk of adenovirus infection for every year decrease in age (23). The type of the transplanted organ appears to correlate with the risk of adenovirus infections. The highest rates in children have been reported in intestinal transplantation. The large amount of gut associated lymphoid tissue (GALT) in the allograft poses a higher risk of rejection requiring more intense immunosuppressive regimens, and could be also be the source of persistent latent adenovirus infections (3,6,8,22,23,26,27). The common recognition of resolution of infection with reduction in immunotherapy alone, supports the role of immunosuppression as a risk factor for adenovirus infection (3). Further, the rate of adenovirus infections is the highest in the first months after transplantation, correlating with exposure to lytic antibodies therapy (OKT3, thymoglobulin) and higher levels of maintenance immunosuppression therapy during these months (13,23,24). Use of adenovirus seromismatch has also been considered to be a risk factor for infection (2,28).

Table 1: Adenovirus serotypes and associated disease (16)

Subgroup	Serotypes	Common clinical presentation
A	12, 18, 31	Disseminated disease (31)
B1	3, 7, 16, 21, 50	Respiratory tract disease Hepatitis (3,7) Myocarditis (7,21) Hemorrhagic cystitis (7) Conjunctivitis (7) Meningoencephalitis (7)
B2	11, 14, 34, 35	Respiratory tract disease Hemorrhagic cystitis (11,34,35) Disseminated disease (11,34,35)
C	1, 2, 5, 6	Respiratory tract disease Conjunctivitis (1,2,5) Hepatitis (1,2,5) Meningoencephalitis (2,5) Disseminated disease (1,2,5)
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51	Keratoconjunctivitis (8,19,37)
E	4	Respiratory tract disease Conjunctivitis
F	40, 41	Gastroenteritis Disseminated disease (40)
G	52	Gastroenteritis

Several risk factors could increase the risk of progression of asymptomatic infection to adenovirus disease: isolation of the virus early after transplantation, persistent isolation of the virus from one site, isolation of the virus from more than one site, initial high viral load in blood and intensification of immunosuppression (6,8,24). However, in adult solid organ transplant recipients, asymptomatic viremia is common (6.5–22.5%) and the risk of progression to adenoviral disease is still to be defined (7,25); routine screening for adenovirus DNAemia is not recommended for solid organ transplant recipients (III) (28).

Diagnosis

Clinical manifestations vary with the sites affected and the type of transplanted organ; the allograft is frequently involved. Certain clinical diseases are associated with specific serotypes (Table 1). In liver transplant recipients, infection with adenovirus commonly results in hepatitis, other sites affected including gastrointestinal tract, respiratory and urinary tract (8,13,14). In lung transplant recipients, adenovirus can produce various manifestations from acute flu-like illness, diffuse alveolar damage or necrotizing pneumonia and chronic changes such as bronchiolitis obliterans, interstitial fibrosis or bronchiectasis (9,18,25). In heart

transplant recipients, detection of adenoviral genome in myocardial biopsy specimens might be predictive of coronary vasculopathy and graft loss (10,29). In transplants involving the small bowel, with isolated or multivisceral allografts, enteritis is common and a significant proportion of these patients develop disseminated adenovirus disease (6,23,27). Hemorrhagic cystitis and graft dysfunction are described more often in adult than pediatric renal transplant recipients (24,28,30).

The available diagnostic methods for adenovirus infections are viral culture, direct antigen detection, molecular methods and histopathology. Serology and electron microscopy are available, but not routinely used in clinical practice (2,28). The diagnostic methods used depend on the site of infection and the sample collected.

All adenovirus serotypes, with the exception of serotypes 40 and 41, grow well in human epithelial cells and produce a characteristic cytopathic effect after 2–28 days, which can be followed by serotyping (1,2). Expedited results can be obtained by centrifugation in shell vial assays with immunoflorescent monoclonal antibody staining, although serotyping cannot be performed. Recovery of adenovirus from urine, respiratory or stool specimens by culture does not confirm adenovirus disease since patients can asymptomatically shed for prolonged periods of time (2,12,23,28). Accordingly, recovery of adenovirus should be correlated with clinical symptoms, detection of the virus from other sites and histopathological findings. While detection of adenovirus at two or more sites has been found to be predictive of invasive disease in bone marrow transplant recipients, similar data are not available for organ transplant recipients (12,28).

Rapid antigen detection kits are commercially available, which yield rapid and specific results; their sensitivity and specificity in the solid organ transplant population is not studied. For respiratory specimens, the immunofluorescence assays are used, while for stool samples, enzyme immunoassays, immunochromatography and latex agglutination tests are common approaches (2). Most of the assays detect the common adenovirus serotypes (such as serotypes 1, 2, 3, 5, 7, 40 and 41).

Histopathologic evaluation remains the gold standard for the diagnosis of invasive adenoviral disease (1,12,28). Adenovirus-infected cells, so called "smudge cells" have large nuclei with basophilic inclusions and a thin rim of cytoplasm. The presence of the virus within tissue could be confirmed through immunoperoxidase and *in situ* hybridization staining (2). Amplification and detection of the viral genome using polymerase chain reaction (PCR) has emerged as a powerful and widely used tool for detection of adenovirus; it is highly sensitive (the lower limit of detection from 100 to 1000 copies/mL) and rapid, and sequencing can further identify serotypes when necessary. Two types of PCR methods can be used, qualitative and quantitative assays. The sensitivity of the assay depends not only on the specimen, but also on methods of sample processing, DNA extraction, the primers used and the amplificationplatform employed. To date there are several commercial and home brew PCR assays. These results should be correlated with the histopathology, when available, and clinical presentation to differentiate between asymptomatic infection and disease. No clear adenovirus viral load cut off that predicts patient outcome, progression to adenovirus disease or dissemination has been established (2). Most likely serial quantitative PCR would be useful regarding decision to initiate therapy and monitoring response to therapy (II-3). Persistently high or rising viral loads (.5–1.0 log increase) may signal the need for intervention, whereas decreasing viral loads correlate with clinical improvement (24,31–33).

Treatment

The most important component of therapy remains supportive care and decrease in immunosuppression (II-2). The role of immune recovery during the course of the infection should not be underestimated, as in many cases, reduction of immunosuppression leads to the resolution of the infection (6,15,24,26,27). In multiple cases it is unclear if the recovery can be attributed to addition of antiviral therapy versus reduction of immunosuppression or to the combination of these interventions (6,23).

Consultation with an infectious diseases expert is advisable to decide if antiviral treatment should be considered and to decide on the optimal regimen (2,28,34). The use of antiviral agents is not supported by prospective randomized clinical trials, and none of the agents has been FDA approved for the treatment of adenoviral infection or disease. There are case reports and series describing the use of cidofovir (II-3) (17,34–36), ribavirin (III) (32,37) and ganciclovir (III) (38,39) in the treatment of adenoviral infection after solid organ transplantation.

Of all proposed antiviral agents for adenovirus, cidofovir has the best evidence to support its use (3,9,24,26–29) (BII). Cidofovir has activity against all adenovirus serotypes (2), but standard dosing has been associated with significant nephrotoxicity (in up to 50% of adults) and neutropenia (in up to 20% of patients; Refs. 40,41). However, in most transplant centers, intravenous cidofovir is considered the standard practice for treatment of severe, progressive or disseminated adenovirus disease, but without clear consensus on the timing of initiation or dosage of the drug. Typically, one of two regimens of cidofovir can be used for the management of adenoviral disease, although the efficacy of the two regimens has not been directly compared: 1 mg/kg three times a week (42) or 5 mg/kg/week (Vistide, standard dosing) for 2 weeks followed by 5 mg/kg every other week until complete resolution of the symptoms and documentation of three negative adenovirus samples, 1-week apart, from the sites that were originally positive (II-3) (2,19,23,28,31). In patients with creatinine clearance <50 mL/min in adult

population or <0.3 mL/min/kg in pediatric population, the dose of cidofovir should be decreased to 0.5 mg/kg three times a week (III) (23). For patients on hemodialysis, consideration should be given in stopping the hemodialysis 1 h before and 4 h after cidofovir administration to allow intracellular distribution of the drug (III) (23). Probenecid at 0.5–1.25 g/m^2 should be administered 3 hours before, 2–3 hours and 8 hours after the administration of cidofovir to prevent nephrotoxicity (II-2) (19,23,31,42). Probenecid decreases tubular secretions and increases the plasma elimination half-life of other drugs (i.e. methotrexate, trimethoprim/sulfamethoxazole, acyclovir, ganciclovir, penicillins, cephalosporins, imipenem). Pre- and posttreatment hydration (normal saline solution at 5 mL/kg/h) should be administered along with probenecid (III-3) (2,23,28,42). Although the 1 mg/kg thrice weekly regimen might be less nephrotoxic (42), it might be associated with breakthrough cytomegalovirus and herpes simplex infections, and the emergence of antiviral resistance (43,44). Few small studies using plasma adenovirus DNA monitoring showed that virologic response to cidofovir therapy correlated with clinical improvement and survival (31,33). Limited data suggest that high adenovirus viral load before initiation of treatment and long interval between the onset of symptoms and administration of treatment might be risk factors for poor response to cidofovir (31). Failure to have at least one log decline in adenovirus viral load in the first 2 weeks of therapy could be associated with progressive clinical symptoms, persistent rises in viral load and death secondary to symptomatic disease (31).

A lipid conjugate of cidofovir (CMX001, Chimerix Inc.) has been developed. CMX001 has good oral bioavailability, has not been associated with nephrotoxicity and achieves higher intracellular levels of active drug compared to cidofovir (45). CMX001 is 5- to >2500-fold more potent against adenovirus based on IC_{50} (inhibitory concentration at which 50% inhibitory response is seen) values on *in vitro* testing, as compared to the unmodified parent compounds (46). Currently, CMX001 is being evaluated for treatment of adenovirus infection in stem cell transplant patients (study CMX001–202) and in an open label study that allowed for treatment of patients with serious and/or life-threatening infections caused by dsDNA viruses (study CMX001–350). In a retrospective study using CMX001 as salvage therapy for adenoviral disease in 13 patients, two-thirds demonstrated a sustained drop in viral load and experienced a survival advantage, which could not be explained by immune recovery alone. In addition, therapy was well tolerated in this mainly pediatric population of immunocompromised patients (47).

Ribavirin seems to have antiviral activity specific to subtype C viruses (serotypes 1, 2, 5 and 6; Ref. 48), and has not been documented to reduce significantly the viral titers in treated patients (32,34,48), thus limiting its clinical utility. The main side effect described is anemia (2,28,34). Ribavirin should not be routinely used in the treatment of adenovirus (III).

Antibody preparations have been used in a few cases of adenovirus disease (49,50). Transplant recipients who develop severe hypogammaglobulinemia (IgG levels <350 mg/dL) seem to be at higher risk of opportunistic infections compared to patients with IgG >350 mg/dL which maybe decreased with immunoglobulin administration (51) (II-3). Though antibody preparations have biologic plausibility, their benefit remains unclear.

Limited, although convincing, data showed that low absolute lymphocyte count at the time of adenovirus viremia and recovery of lymphocytes might be predictors of adenovirus disease and outcome (3,6,24,26,27). Hence, enhancement of adenovirus-specific immunity through antigen specific cytotoxic T lymphocyte (CTL) infusion, might improve outcomes in adenovirus disease in HSCT. Preliminary data suggest safety and efficacy (52–54). Several studies are ongoing to evaluate the safety, persistence and efficacy of viral antigen specific CTL infusions in HSCT for therapy and prophylaxis of adenovirus, EBV and CMV infections. CTLs are derived from original donors including cord blood and most closely HLA matched donors as well (clinicaltrials.gov). Although this intervention for adenovirus has not been studied in the solid organ transplant population, an initial study in adult solid organ transplant recipients with autologous EBV specific CTL have been published and serves as proof of concept (52–55). Theoretically this therapy might be associated with increased risk of rejection or GVHD, but preliminary data have not reported this; non-specific alloreactive T cells are selected out during CTL preparation to reduce this risk (54).

Infection Control Issues

Outbreaks of adenovirus infections have been reported in hospital or institutional settings (56). CDC/HICPAC guidelines recommend contact and droplet precautions during hospitalization for the duration of illness to prevent nosocomial transmission (I) (56). In an immunocompromised host the duration of contact and droplet precautions should be extended due to prolonged shedding of the adenovirus. A live attenuated vaccine is currently under development but is limited to a few serotypes and would likely be contraindicated in solid organ transplant recipients. Its role remains to be established in the pretransplant period.

Future Research

Additional research is needed to understand the natural history of adenovirus infection in solid organ transplantation, the role of immune recovery, and implicitly the need for antiviral treatment. The utility and standardization of adenovirus viral load monitoring in the peripheral blood for identification of patients at risk of developing disease as well as a marker of response to therapy in recipients of solid organ transplantation should be further explored. Investigation is also needed to develop antiviral agents, which will provide a therapeutic effect against adenovirus with minimal

toxicity. Randomized, multicentered, placebo controlled trials evaluating new treatment options such as CMX001 and perhaps some of the existing medications (cidofovir, immunoglobulins) for efficacy and safety in the treatment of adenovirus disease in transplant recipients are warranted. Finally, efforts towards understanding the role of adoptive immunotherapy, with antigen specific T cell infusions, in this patient population, should be considered.

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Special Article

RNA Respiratory Viruses in Solid Organ Transplantation

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Key words: Amantadine, influenza, oseltamivir, respiratory viral infections, vaccines

Abbreviations: BAL, bronchoalveolar lavage; hMPV, human metapneumovirus; IgIV, intravenous immunoglobulin; LAIV, live-attenuated influenza vaccine; PIV, parainfluenza virus; RSV, respiratory syncytial virus; SOT, solid organ transplant.

Introduction and Epidemiology

A wide range of respiratory viruses have been identified as causes of significant morbidity and mortality among transplant recipients, including: influenza, respiratory syncytial virus (RSV), parainfluenza virus (PIV), rhinovirus, human metapneumovirus (hMPV), and coronavirus (1) (Table 1). Several features are common among all of these viruses in the transplant population:

1. The seasonality of respiratory viral infections among transplant recipients usually follows that of the general population (2,3).
2. The viruses all cause a range of disease, from mild congestion and rhinorrhea to more severe tracheobronchitis, bronchiolitis and pneumonia. No one virus is exclusively associated with one clinical syndrome (i.e. influenza-like illness, croup, etc.). As such, diagnostic strategies should initially be broad, attempting to screen for all recognized viruses (3,4) with particular emphasis on ones that might be amenable to therapy.
3. Transplant recipients often present with mild or atypical symptoms and fever may be absent. Lung transplant recipients, for example, may initially only have subjective symptoms of shortness of breath or subtle changes in pulmonary function testing without more typical symptoms (5).
4. Viral shedding is usually prolonged among transplant recipients. Prolonged shedding is seen even with the use of antivirals and therefore may contribute to the increased risk of resistant variant emergence (1,6).
5. Transplant recipients are at higher risk of infectious complications compared to immunocompetent hosts. Respiratory viral infections are a significant risk factor for subsequent development of fungal and bacterial pneumonia (1).
6. Respiratory viral infections appear to be a risk factor for both acute and chronic rejection with the greatest risk in lung transplant recipients (5,7–9) (II-2), although data on this topic in the literature are conflicting (10). The pathogenesis of the link between respiratory viral infections and rejection is not clearly understood.
7. All pediatric solid organ and lung transplant recipients appear to have the greatest risk of both respiratory viral infections and more severe courses and complications (1).
8. All are potential nosocomial pathogens that can be potentially spread by staff or visitor with mild upper respiratory illness.

Diagnosis

Since one cannot clinically distinguish disease caused by any of the respiratory viruses, diagnosis using broad ranging techniques should be considered particularly in the early period after transplantation or augmented immunosuppression and during respiratory viral season. Diagnosis can be achieved by combinations of serology, viral culture, antigen detection, and nucleic acid testing. In general, all patients with presumed respiratory viral infection should have a nasopharyngeal swab, wash, or aspirate performed and sent for testing. If upper tract samples fail to document the cause of the respiratory illness or if there is clinical or radiologic evidence of lower tract involvement, bronchoalveolar lavage (BAL) should be considered and sent for the range of available tests. Testing of a wide range of pathogens is most important among lung transplant recipients.

Serology is not useful for diagnosis of acute infection, but can be used for epidemiological studies in case of influenza, although some SOT recipients might not respond and antibody can wane quickly, even after infection. Rapid antigen detection is available for influenza and RSV and has the advantage of rapid result testing (within 15′). For influenza, rapid antigen detection testing has high specificity but variable sensitivity (20–70%) as compared to other assays, making them less useful in SOT recipients (11). Some

Table 1: Common respiratory virus infections in solid organ transplant recipients

Virus	Isolation recommendations	Prophylactic interventions	Therapeutic alternatives
Influenza	Contact and droplet	Annual Injectable Vaccine Neuraminidase inhibitor[1]	Neuraminidase Inhibitor[1] M2 Inhibitor[2]
RSV	Contact	Palivizumab	Aerosolized ribavirin[3] ± Antibody-based treatment[4] ± Corticosteroids
PIV	Contact	None	Aerosolized ribavirin[3] ± IgIV
hMPV	Contact	None	Aerosolized ribavirin[3] ± IgIV
Rhinovirus	Droplet contact added if copious secretions or close contact	None	None
Coronavirus	Standard precautions except for SARS, which requires contact, droplet, and airborne precautions	None	None

[1]Oseltamivir or zanamivir.
[2]Amantadine or rimantadine. Currently not recommended due to high rate of antiviral resistance.
[3]Oral or IV ribavirin can be used as well, although patients should be monitored for hemolytic anemia; less data are available about the efficacy of these formulations in treating RSV than with aerosolized ribavirin.
[4]IgIV, palivizumab, RSV-Ig (no longer produced but may still be available in some locations).

commercial assays can distinguish between influenza A and B, but some others cannot. In the case of RSV, one study documented a sensitivity with one rapid test method of 15% for nasal wash specimens among immunocompromised patients; sensitivity is improved to 89% when BAL is used (12). Several studies of direct fluorescent antibody (DFA) testing of primary patient specimens have documented sensitivity that approached that of PCR for certain viruses (13,14). DFA testing is limited by lack of reagents for some of the viruses (hMPV, rhinovirus, coronavirus) (15). Although viral cultures previously were considered the preferred diagnostic tests, molecular tests tend to provide higher yields and can detect a wider range of viruses in a more timely fashion (1). For influenza, viral culture has the advantage of allowing the identification of the influenza strain and to test antiviral susceptibility (11).

A wide range of PCR-based assays to detect respiratory viruses are commercially available and many centers have locally developed assays that detect select viruses. Nucleic acid amplification assays appear to be the most sensitive diagnostic tools available and most allow for simultaneous detection of a broad range of respiratory pathogens from a single sample and is therefore preferred testing method for immunocompromised patients (1). Multiplex PCR assays provide the advantage of identification of viruses not routinely found by conventional methods, including rhinovirus and hMPV (16–19). Commercially available multiplex assays differ in sensitivity and specificity for different viruses most notably adenovirus (16,20–22). New assays are being developed to address these limitations (23,24) but the clinician should be aware of the performance characteristics of the assay used. For influenza, PCR can distinguish among viral subtypes and can quantify viral load, making them useful for the monitoring of viral shedding. Recently, rapid PCR-based assays allow rapid results (within 3–4 hours), although their sensitivity may vary among virus types (25).

Influenza Virus

Epidemiology and risk factors

Influenza virus is an orthomyxovirus associated with significant morbidity and mortality during the winter season. Three main viral strains are associated with human infection, namely influenza A/H1N1, influenza A/H3N2, and influenza B. In 2009 a new strain of influenza A/H1N1, coming from reassortant animal and human viruses, caused a global pandemic (26). In the last influenza seasons, the pandemic influenza A/H1N1 virus replaced prior seasonal influenza A/H1N1 virus.

Recent studies performed during the pandemic have greatly increased our knowledge of the epidemiology of influenza infection in the transplant population (27–32). The risk of complications appears to be higher in SOT recipients as compared to the general population, particularly the incidence of pneumonia (up to 22%–49% in transplant recipients). Allograft dysfunction and acute rejection have been observed after severe cases of influenza (28). Most studies have observed an excess of influenza-associated morbidity and mortality in SOT recipients as compared to the general population. Rates of reported severe influenza varied between 16 and 20%, and attributable mortality was estimated to be 4%–8% (27–32). Ascertainment biases towards inclusion of patients with more severe disease may overestimate the severity of influenza in SOT recipients.

Risk factors for severe influenza in SOT recipients include use the antilymphocyte globulins, diabetes mellitus, pneumonia, bacterial and fungal co-infection, and early infection (<3 months) after transplantation (27,28). Use of early antiviral therapy has been consistently associated with a reduced rate of influenza-associated complications (admission to ICU, use of invasive ventilation, and death) (27–31).

Prevention/prophylaxis

Patients with known or suspected influenza infection should be isolated from other patients with standard and droplet precautions. Influenza vaccination is an important measure to prevent influenza infection (33). Two types of influenza vaccine exist, the inactivated influenza vaccine and intranasal live-attenuated influenza vaccine (LAIV). LAIV is contra-indicated in SOT recipients and close contacts, due to a potential risk of dissemination of the vaccine strain. One dose of the seasonal intramuscular trivalent influenza vaccine is the standard of care in adults, and two doses 4 weeks apart is recommended for naïve children <9 years of age (33). Immunogenicity of influenza vaccine is variable in SOT recipients, depending on the type of organ, immunosuppressive regimen used, and composition of the vaccine (34). However, there is increasing data reporting on the beneficial effects of influenza vaccination in SOT recipients. In lung transplant recipients, vaccination with adjuvanted influenza A H1N1/09 vaccine was associated with a reduced incidence of subsequent influenza infection (1.3% vs. 25% in unvaccinated patients) (35). Influenza vaccination was also associated with a lower risk of graft loss and death in kidney transplant recipients (36). Even if vaccinated patients develop influenza, a reduction in the severity of the disease as compared to unvaccinated patients has been observed (37). Influenza vaccine is therefore recommended for all SOT recipients and household members (33) (Table 2).

Influenza vaccine is well tolerated in SOT recipients, and adverse events to vaccination are usually mild and short lived. Recently, a study described the development of low-level anti-HLA antibodies in kidney transplant recipients who received multiple doses of adjuvanted influenza vaccine in one season. There was no proven association between vaccination, the development of the *de novo* antibodies, and graft rejection. Further studies are required to clarify this potential association (38). The optimal timing for vaccination after transplant has not been established. It is generally recommended to vaccinate at least 3 months post transplantation (33), although in this early period post transplant is when the risk of influenza-associated complications is higher (28). Antiviral prophylaxis with oseltamivir may be an alternative to influenza vaccination in case of contra-indication or expected nonresponse to the vaccine. A randomized controlled trial in transplant recipients found an efficacy of ~80% of prophylaxis (39).

Table 2: Summary recommendations for treatment and prevention of influenza in solid organ transplant recipients

Recommendations	Grading
• Transplant recipients should receive antiviral therapy with a neuraminidase inhibitor (either oseltamivir or zanamivir) when influenza is suspected.	II-2
• Although early (<48h) administration of antivirals is associated with better outcome, all symptomatic patients should receive antiviral therapy, irrespective of symptom onset.	III
• Duration of antiviral therapy should be at least 5 days. Antiviral therapy may be prolonged in case of persistent viral shedding.	III
• Double dosing of oseltamivir may be considered in severe cases or in case of insufficient response to therapy.	III
• IV drugs (peramivir or zanamivir) can be also used in selected cases (intubated patients, concerns with oral absorption).	III
• Patients with influenza infection need to be isolated with standard and droplet measures.	II-2
• Trivalent inactivated influenza vaccine should be administered to SOT recipients and household members.	II-2
• In patients whom influenza vaccine is contraindicated or may have insufficient response (e.g. therapy for acute rejection, early after transplantation), antiviral prophylaxis with oseltamivir 75 mg OD for a duration of 12 weeks starting at the beginning of the influenza season may be proposed.	I

Treatment

Two families of drugs are approved for the treatment of influenza, namely M2 inhibitors and neuraminidase inhibitors (11). M2 inhibitors (amantadine and rimantadine) are not active against influenza B, and because of the high incidence of antiviral resistance to influenza A/H1N1 and A/H3N2, theses drugs are no longer recommended for treatment of influenza (11). Neuraminidase inhibitors include oral oseltamivir, and inhaled zanamivir (Table 3). An intravenous form of oseltamivir and zanamivir is also available as investigational drug, but not currently approved. Intravenous peramivir, another neuraminidase inhibitor, is approved for its use in South Korea and Japan. None of these drugs has been specifically tested in prospective trials in SOT recipients for the therapy of influenza. Studies performed during the influenza A/H1N1 pandemic showed that early treatment with oseltamivir was associated with decreased mortality, admission at the ICU and complicated outcomes in SOT recipients (27–31). Less data are available for zanamivir, but it appears to be equally effective. Therapy with neuraminidase inhibitors may be associated with reduced incidence of allograft dysfunction in lung transplant recipients (31,40). Given the beneficial effect of early administration of antiviral drugs, oseltamivir or zanamivir therapy should be started empirically in all patients with symptoms compatible with influenza, before microbiological confirmation.

Transplant recipients are known to have prolonged viral replication, so it is generally recommended to extend the duration of therapy beyond the approved 5 days period. Monitoring of viral replication in naso-pharyngeal swabs by PCR may be used to guide duration of antiviral therapy (41). Although early (<48h) administration of antivirals is associated with better outcome, patients may still benefit from

Table 3: Recommended dosage of neuraminidase inhibitors for treatment of influenza[1]

		Adjustment for renal failure in adults		Children (≥1 year old)	
Drug	Adults	Renal function	Dose	Weight	Dose
Oseltamivir	75 mg BID	CrCl ≥ 30 mL/min	75 mg BID	≤15 kg	30 mg BID
		CrCl < 30 mL/min	75 mg OD	16–23 kg	45 mg BID
		Hemodialysis/CAPD	30–75 mg after dialysis	24–40 kg	60 mg BID
		CRRT	75 mg BID	>40 kg	75 mg BID
				Infants (<1 year old)	
				3 mg/kg/dose BID	
Zanamivir	10 mg (2 inhalations) BID	No adjustment required		Zanamivir approved for treatment and prophylaxis of persons ≥5 years, same dose than adults	

BID = twice daily; CAPD = continuous ambulatory peritoneal dialysis; CRRT = continuous renal replacement therapy; OD = once daily.
[1]Resistance patterns may change and affect recommended antiviral strategies; consult your national health authority regularly for updated recommendations.

therapy irrespective of the duration of symptoms. In severe cases, double dosing (i.e. 150 mg of oseltamivir twice a day for normal kidney function) is recommended by some experts, with some anecdotal cases of positive outcomes in SOT recipients reported in the literature (42). Importantly, pharmacokinetic studies have not observed a clinically relevant interaction between oseltamivir and immunosuppressive drugs (tacrolimus, cyclosporine, and mycophenolate) (43). The use of peramivir or IV zanamivir can be considered in cases of life-threatening infection or concerns with oral absorption, although experience with these drugs in SOT recipients is lacking (44,45).

As mentioned, the use of M2 inhibitors for treatment of influenza is no longer recommended due to the high rate of resistance to these drugs (>95%). Rates of oseltamivir resistance were high for prepandemic influenza A/H1N1 virus, but antiviral resistance has been only occasionally described for the new influenza A/H1N1 strain (46). Immunosuppression and exposure to oseltamivir are risk factors for development of antiviral resistance (47). Most resistance in H1N1 viruses in patients exposed to oseltamivir is caused by the H275Y mutation, which results in increase IC50 for peramivir but retains activity of zanamivir (46). Resistance to neuraminidase inhibitors is uncommon in influenza A/H3N2 and influenza B viruses. Most commercially available resistance assays only detect H275Y and other mutations may occur, particularly when agents other than oseltamivir are used or influenza A/H3N2 or B are being treated. As resistance patterns may change and affect recommended antiviral strategies, it is important to regularly consult the national health authority for updated recommendations.

Respiratory Syncytial Virus

Virology and epidemiology

RSV is a paramyxovirus in the genus pneumovirus that causes seasonal annual epidemics worldwide; year round disease is seen in some tropical locations. By two years of age, virtually all children have experienced a primary infection although re-infection can occur throughout life. Risk factors for more severe disease after organ transplantation include infection in children under a year of age or with underlying lung disease (1,9). Early acquisition of RSV after transplantation or after augmented immunosuppression has been associated with increased severity of disease in some but not all studies (1,8,48–53). Transmission occurs through inhalation of infectious droplets or through contact with fomites.

Prevention

Patients with known or suspected RSV should be isolated from other patients using standard contact precautions (II-2) (54,55). Prophylaxis with the RSV-specific monoclonal antibody (palivizumab) or high titer RSV-IgIV has been shown to be effective for specific groups of high-risk infants and young children (I) (56,57). However, no studies have been conducted to evaluate their use in the transplant setting and the cost of the weight adjusted dosing of these products in adults would be extremely high. Palivizumab is recommended for children less than two years of age with chronic lung disease or with cyanotic or complicated congenital heart disease during the RSV season (58) (III), however, guidelines regarding use of this agent in older children and adults do not exist. Survey data suggest that antibody-based prophylaxis is commonly used among pediatric transplant centers (59,60). There are no approved vaccines for prevention of RSV.

Treatment

Given the limited data on treatment of RSV, supportive care is recommended (II-2) and reduction of immune suppression should be considered, particularly in those with severe disease. The role of specific antiviral treatment is controversial. Ribavirin has been shown to have *in vitro* activity against RSV and the aerosolized form of this drug has been approved for the treatment of lower respiratory tract disease due to RSV in certain at-risk populations (61). Despite its FDA approval, convincing data describing the clinical efficacy of this agent are lacking and a consensus on the treatment of RSV disease does not

currently exist (60,62). Published data on the treatment of RSV disease in SOT recipients are limited and most of the data pertains to lung transplant recipients. Experience in stem cell transplant populations suggests that the use of aerosolized ribavirin may reduce mortality associated with severe RSV infections, particularly those affecting the lower airways (51,61,63). The combination of aerosolized ribavirin and antibody-based interventions, including IgIV, RSV-Ig, and palivizumab appeared to have an even greater impact on mortality (1,64,65). Many experts, therefore, would recommend the use of the combination of ribavirin and an antibody preparation with or without corticosteroids for the treatment of severe RSV infections (II-2) (1,49,65). Based upon published experience from pediatric organ transplant recipients, patients without risk factors for severe disease and with only upper respiratory infections are unlikely to benefit from aerosolized ribavirin (II-2) (49). There are also published reports of successful treatment of RSV in lung transplant recipients with oral and IV ribavirin with and without corticosteroids (66–68). Further studies are needed to determine the clinical efficacy of these alternatives since there is a risk of adverse effects, notably hemolytic anemia.

Parainfluenza Virus

Virology and epidemiology

Parainfluenza is a pneumovirus for which there are 4 types that commonly cause disease in humans (types 1–4). PIV types 1 and 2 tend to circulate sporadically in fall and winter months in temperate areas while type 3 occurs year round; type 4 is least commonly isolated and its epidemiology is still being defined (1). Transmission occurs via person-to-person spread by direct contact with infectious secretions or fomites. Disease can be serious, particularly in pediatric transplant recipients and lung transplant recipients of any age (1,5,69). Although all respiratory viruses are associated with an increased risk of progression to obliterative bronchiolitis in lung transplant recipients, the association appears to be clearest and strongest with PIV lower tract disease (5,7,8).

Prevention

Patients with known or suspected PIV should be isolated from other patients using standard contact precautions (54,55). There are no approved vaccines nor are there recognized preventative antiviral agents.

Treatment

Although the use of IgIV and ribavirin are not associated with benefit in the management of PIV infections in stem cell transplant recipients, ribavirin has *in vitro* activity and has been used to treat lung transplant recipients with lower tract disease; some experts also consider the use of IgIV and corticosteroids as well (51,52,65,69).

Human Metapneumovirus

Human metapneumovirus discovered in 2001 is an RNA paramyxovirus that has a clinical pattern similar to RSV and is a significant cause of disease in transplant recipients (70). As with other pneumoviruses, there are no vaccines and prevention is focused on tight infection control measures, including contact precautions (55). Case reports and animal data suggest that ribavirin with or without immunoglobulin can be considered for the management of severe cases of hMPV (1,70–72) but supportive care remains the mainstay of treatment.

Rhinovirus

Human rhinoviruses are members of the *Picornaviridae* family and are the most common cause of colds in adults and children. They have been recognized to cause clinically significant disease in some transplant recipients with fatal cases described (73,74). Most of the fatalities are associated with co-infections. Prolonged shedding with minimal symptoms has been described, particularly in lung transplant recipients. The clinical importance of this prolonged shedding has not been fully defined, although could potentially pose a threat of nosocomial transmission (1,8,74,75). Currently, there are no approved preventive or therapeutic interventions.

Other Respiratory Viruses

With the use of molecular diagnostics, a wider range of respiratory viruses have been isolated. Many of these viruses, such as newly recognized variants of coronavirus (HKU1, NL63), the polyomaviruses (WU, KI viruses), and bocavirus have not been widely studied in transplant recipients and so their clinical impact has not been fully assessed (1). Severe and sometimes fatal cases of all of these viruses in immunocompromised patients have been recognized, so they should be considered in the differential diagnosis of patients presenting with severe lower tract disease. The newer agents are more challenging to diagnose since they are not included in the routine, clinically available diagnostic tests. In addition, optimal management of these agents has not been defined.

Future Studies

Although respiratory viruses are increasingly recognized as causes of morbidity and mortality in transplant recipients, there is still much to be learned about the impact of these viruses. Prospective studies using molecular diagnostics are needed to understand the true epidemiology and clinical spectrum of respiratory viral diseases. In particular, studies of the long-term consequences of infection, even when mild or asymptomatic, are needed. This is

particularly important in lung transplant recipients in whom lower tract infection has been associated with an increased risk of chronic rejection and bronchiolitis obliterans syndrome. Prospective studies, using contemporary molecular diagnostic tools including metagenomics, are also needed to define the efficacy and cost of preventative interventions, particularly in high risk pediatric populations and lung transplant recipients. Novel therapeutic agents are also under development (76) and may be useful in the SOT population. Prospective trials are needed to define the optimal timing, duration, and treatment regimen for each of the viruses.

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The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

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Special Article

Candida Infections in Solid Organ Transplantation

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Key words: Antifungals, antifungal prophylaxis, *Candida* epidemiology, invasive candidiasis, transplantation

Abbreviations: ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; AmB, amphotericin B; AmB-d, deoxycholate amphotericin; BDG, 1–3 β glucan; CNI, calcineurin inhibitor; CSF, cerebrospinal fluid; 5-FC, flucytosine; GI, gastrointestinal; IFI, invasive fungal infection; LAmB, liposomal amphotericin B; LFAmB, lipid formulation of amphotericin B; PCR, polymerase chain reaction; PNA-FISH, peptide nucleic acid fluorescent *in situ* hybridization assay; TDM, therapeutic drug monitoring.

Epidemiology and Risk Factors

Infections due to *Candida* spp are the most common invasive fungal infections (IFIs) among organ transplant recipients, accounting for over half of all IFIs in this population (1). In a large prospective study, invasive candidiasis had a 12-month cumulative incidence of 1.9%, the highest of all IFIs, and occurred more frequently in small bowel, pancreas, liver, kidney, heart and lung transplant recipients, in descending order (1). Invasive candidiasis occurs earlier than other invasive mycoses, generally within the first 3 months after transplantation, and is viewed as a classic nosocomial infection (2–6). However, a substantial number of cases of invasive candidiasis, especially among liver and small bowel transplant recipients, occur well beyond this traditional risk period (1,3,4). The most common sites of infection are bloodstream infection, intra-abdominal and urinary tract infection (1,6–8).

Candida albicans is the dominant invasive pathogen, accounting for approximately 50% of isolates. *C. glabrata* is the most common non-*albicans* isolate. *C. krusei* and *C. guilliermondii*, an important pathogen in neutropenic hosts, are more common among stem cell transplant recipients, but far less common among organ transplant recipients (9), and may vary according to institution and geographic location.

Established risk factors for invasive candidiasis in the general population include age, broad spectrum antibiotic therapy, use of central venous catheter, receipt of parenteral nutrition, prolonged neutropenia, prolonged intensive care unit stay, diabetes and renal replacement therapy. Unique risk factors for invasive candidiasis in transplant recipients include the type of transplant and the surgical anastomosis (10). For instance, among liver transplant recipients, a choledocho-jejunostomy is associated with a higher risk of invasive candidiasis compared to a choledocho-choledochostomy anastomosis (11). Similarly, among pancreas transplant recipients, enteric drainage is associated with a higher risk of invasive candidiasis than bladder drainage (12). Other well established risks in transplant recipients include acute renal failure, recent CMV infection, primary graft failure, early surgical re-exploration and early colonization with *Candida* spp (13).

Diagnosis

A definitive diagnosis of invasive candidiasis is dependent on recovery of an organism from a sterile body site. Unfortunately, blood cultures are an insensitive means of identifying patients with invasive candidiasis. Even with newer blood culture techniques, the overall sensitivity of blood cultures for the isolation of *Candida* spp is estimated at 70% (14). Therefore, the development of nonculture based diagnostic methods is important. Presently, there are several FDA-approved assays available, but their use has been very limited in clinical practice. Among these, the 1–3 β-D-glucan (BDG) assay is probably the most reliable, with the sensitivity and specificity of 70% and 87%, respectively, among patients with proven invasive candidiasis (15–17). At present, this assay is only approved as an adjunct to the diagnosis of invasive candidiasis. Other newer diagnostic assays, including PCR-based multiplex assays, are in development. In a prospective study of 55 patients with invasive candidiasis, of which 20% were organ recipients, the sensitivity of BDG with a cut-off for positivity of ≥80 pmol/mL and PCR for invasive candidiasis was 56% and 80% and the specificity was 73% and 70%, respectively (18). The sensitivity of either test was not affected by antifungal therapy. The sensitivity of blood cultures combined with BDG

Table 1: General susceptibility patterns of *Candida* species

Species	Fluconazole	Itraconazole	Voriconazole	Posaconazole	5FC	AmB	Echinocandins
C. albicans	S	S	S	S	S	S	S
C. tropicalis	S	S	S	S	S	S	S
C.parapsilosis	S	S	S	S	S	S	S to R[1]
C. glabrata	S-DD to R	S-DD to R	S-DD to R	S-DD to R	S	S to I	S
C. krusei	R	S-DD to R	S	S	I to R	S to I	S
C. lusitaniae	S	S	S	S	S	S to R	S

AmB = amphotericin B; 5-FC = flucytosine; I = intermediate susceptibility; R = resistant; S = susceptible; S-DD = susceptible dose-dependent.
[1] *C. parapsilosis* isolates resistant to echinocandins are uncommon.

or PCR among patients with invasive candidiasis was 79% and 98%, respectively.

Identification of *Candida* isolates to the species level is critically important in selecting antifungal therapy, and to a lesser extent, in predicting outcome. The germ tube test is an inexpensive and specific means of identifying *C. albicans* and *C. dubiliniensis*. The peptide nucleic acid fluorescent *in situ* hybridization assay (PNA-FISH) reliably identifies *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and *C. krusei* in positive blood cultures (19,20). Chromogenic agar, a specialized media for *Candida* isolation and identification, is easily used and readily distinguishes *C. albicans*, *C. tropicalis* and *C. krusei* based on production of distinctive pigments (21).

Susceptibility testing for all clinically significant *Candida* isolates is not practical for many centers. Generally, antifungal susceptibility can be predicted on the basis of species and local epidemiology (see Table 1).

In a prospective study of invasive candidiasis in organ and stem cell transplant recipients fluconazole resistance was observed in 1% of *C. albicans*, *C. tropicalis* and *C. parapsilosis* isolates. Overall voriconazole resistance was observed in 3% of isolates and in 8% of *C. glabrata* isolates. Isolates that were resistant to voriconazole were also resistant to fluconazole. All isolates were susceptible to caspofungin. In multivariate analysis, among organ recipients fluconazole nonsusceptibility was independently associated with any fluconazole use within 3 months before IFI, *C. glabrata*, ganciclovir use within 3 months before the IFI, diabetes acquired because the transplant and gender (22).

Antifungal susceptibility testing is recommended for clinically significant *C. glabrata* isolates, in the clinical setting where azole resistance is strongly suspected, and in case of treatment failure (22,23) (II-3).

Treatment

The treatment of invasive candidiasis among organ transplant recipients is similar to treatment of other patients based on the recently published 2009 IDSA guidelines (23). There are no randomized studies for the treatment of invasive candidiasis among organ transplant recipients; thus, the therapeutic approach is based on large randomized studies in a heterogeneous group of patients, which include only small portion of organ recipients. A summary of the treatment recommendations is described in Table 2.

Therapeutic Drug Monitoring (TDM)

All azoles show significant drug–drug interactions, especially with calcineurin inhibitors (CNI; Ref. (24). Therefore careful monitoring of (CNI) levels is done and dose reduction of CNI is made once an azole is initiated. For patients receiving prolonged courses of voriconazole or posaconazole, TDM is recommended but there is no consensus on this topic (25,26) (III).

In a prospective study of 93-lung transplant recipients receiving voriconazole prophylaxis, patients ≥60 years old and cystic fibrosis patients were associated with higher and lower initial troughs, respectively. Prophylaxis was most effective with voriconazole troughs >1.5 μg/mL, and troughs correlated directly with aspartate transferase levels (27).

In another study of 17 cardiothoracic transplant recipients, patients with posaconazole levels consistently >0.5 μg/mL were more likely to have a successful outcome (28).

The main purpose of TDM is to potentially avoid toxicity that may be observed at higher serum concentrations and to reduce the risk of treatment failure at lower concentrations (29).

Specific treatment recommendations

Candidemia: The selection of any particular agent for the treatment of candidemia should take into account azole exposure within the last 90 days, a history of intolerance to an antifungal agent, the dominant *Candida* spp cultured and current susceptibility data in a particular location (30). In addition, the severity of illness, relevant co-morbidities and evidence of metastatic involvement to other organs systems are important considerations. Early initiation of therapy is critical to the successful treatment of candidemia (31,32).

Table 2: Summary of recommendations for the treatment of candidiasis (23)

Condition	Therapy		Comments
	Primary	Alternative	
Candidemia			
Nonneutropenic	Fluconazole 800 mg (12 mg/kg) load, then 400 mg (6 mg/kg) daily[1] or an echinocandin (I)	LFAmB 3–5 mg/kg daily; or AmB-d 0.5–1 mg/kg daily; for *C. krusei* and flu-resistant, voriconazole-sensitive *C. glabrata*, voriconazole 400 mg (6 mg/kg) twice daily for 2 doses, then 200 mg (3 mg/kg) twice daily after initial therapy with an echinocandin	Choose an echinocandin for moderate to severe illness and for patients with recent azole exposure (III). Transition to fluconazole after initial echinocandin is appropriate in many cases (II-3). Remove all intravascular catheters, if possible. Treat 14 days after first negative blood culture and resolution of signs and symptoms associated with candidemia
Neutropenic	An echinocandin or LFAmB 3–5 mg/kg daily (II-2)	Fluconazole 800 mg (12 mg/kg) load, then 400 mg (6 mg/kg) daily[1]; or voriconazole 400 mg (6mg/kg) twice daily for 2 doses, then 200 mg (3 mg/kg) twice daily	An echinocandin or LFAmB is preferred for most patients. Fluconazole is recommended for patients without recent azole exposure and who are not critically ill
Urinary tract infections			
Asymptomatic	Therapy not usually indicated, unless high risk or undergoing urologic procedures		For patients undergoing urologic procedures, fluconazole, 200–400 mg (3–6 mg/kg) daily[1] or AmB-d 0.3–0.6 mg/kg daily for several days before and after the procedure is recommended
Symptomatic Cystitis	Fluconazole 200 mg (3 mg/kg) daily[1] for 2 weeks (III)	AmB-d 0.3–0.6 mg/kg for 1–7 days; or flucytosine (5-FC) 25 mg/kg four times daily[1] for 7–10 days (III). AmB-d 0.5–0.7 mg/kg daily ± 5-FC 25 mg/kg four times daily[1]	Alternative therapy is recommended for patients with fluconazole-resistant organisms. AmB-d bladder irrigation is only recommended for patients with refractory fluconazole-resistant organisms (e.g., *C. krusei*, *C. glabrata*)
Pyelonephritis	Fluconazole 200–400 mg (3–6 mg/kg) daily[1] for 2 weeks		For patients with pyelonephritis and suspected disseminated candidiasis, treat as for candidemia
Urinary fungus balls	Surgical removal strongly recommended. Fluconazole 200–400 mg (3–6 mg/kg) daily[1] or AmB-d 0.5–0.7 mg/kg daily +/–5-FC 25 mg/kg four times daily[1]		Local irrigation with AmB-d may be a useful adjunct to systemic antifungal therapy
Respiratory candidiasis	Therapy not recommended unless associated with clinical evidence of anastomotic tracheobronchitis		*Candida* lower respiratory tract infection is rare, even among lung transplant recipients, and it requires histopathologic evidence to confirm a diagnosis

[1]Doses of fluconazole and 5-FC require adjustment for renal function.

Based on data from clinical trials, fluconazole remains the standard therapy for selected patients with candidemia (33–36). Fluconazole is considered first-line among patients with mild to moderate illness, no recent azole exposure and in whom *C. glabrata* is unlikely (23) (I).

The echinocandins show rapid fungicidal activity against all *Candida* spp, and have shown approximately 75% success in randomized clinical trials (37–39). Because of their efficacy, favorable safety profile and very few drug–drug interactions, the echinocandins are favored as initial therapy for patients with a recent history of azole exposure, moderately severe to severe illness, a history of allergy or intolerance to the azoles, or high risk for infection due to *C. krusei* or *C. glabrata* (23) (III). After a short course of intravenous echinocandin therapy (3–5 days), fluconazole is a reasonable choice for step-down therapy, provided that the organism is predictably susceptible to fluconazole (*C. albicans, C. parapsilosis* and *C. tropicalis*) and the patient is clinically stable (23) (II-3). There are reports of decreased susceptibility of *C. parapsilosis* to the echinocandins, but the clinical significance of this is unknown. However, it may

be prudent to choose an alternative to an echinocandin as first line therapy for invasive infections due to this organism (40,41). The echinocandins are sufficiently similar and therefore interchangeable.

Voriconazole is approved for treatment of candidemia, but clinical trials have not shown a particular advantage compared to other agents (42). The role of voriconazole for the treatment of candidemia is limited to patients who have an infection due to a fluconazole-resistant organism, and who are ready for transition to oral therapy. Examples include infections with *C. krusei* and fluconazole-resistant but voriconazole-susceptible *C. glabrata* (23). The role for LFAmB is limited due to potential nephrotoxicity, especially in kidney transplant recipients, and is generally reserved for individuals who are intolerant or refractory to other forms of therapy.

Removal of central venous catheters, when feasible, is strongly recommended among patients with candidemia (43) (II-3). There is debate as to the necessity of removing all intravascular catheters (44), but most experts agree that removal is indicated if the source of candidemia is unclear. In addition, all patients with candidemia should have a dilated funduscopic exam to identify signs of metastatic complications to the eye, such as endophthalmitis; and repeat blood cultures at 48–72 h intervals until blood cultures are negative. The duration of therapy for treatment of candidemia without metastatic complications is generally 2 weeks after clearance of *Candida* from the bloodstream and resolution of symptoms attributable to candidemia (23). Patients with metastatic complications require longer therapy.

The treatment of candidemia in neutropenic organ transplant recipients differs somewhat from nonneutropenic patients with a greater emphasis on the use echinocandins and LFAmB (45,46) (II-2). Most clinicians prefer these agents over fluconazole based on persistent concerns that a fungicidal agent (such as echinocandin or LFAmB) is preferred over a fungistatic agent (fluconazole or voriconazole), although there are few data to support this approach.

Urinary Tract Infections

In the absence of fever or other evidence of systemic infection, candiduria in the organ transplant recipient does not generally necessitate treatment (47,48). There are no prospective and comparative trials comparing treatment versus nontreatment in this group, thus treatment in this setting is largely driven by anecdotal experience and personal preference. For purposes of determining selection of an agent and duration of therapy, it is helpful to divide organ recipients with candiduria into asymptomatic and symptomatic categories. Treatment of asymptomatic candiduria is generally discouraged unless the patient is undergoing a urologic procedure or is neutropenic (23). Imaging of the kidneys and collecting system is prudent to exclude abscess, fungus ball or urologic abnormality.

Among symptomatic patients with candiduria and suspected disseminated candidiasis, it is appropriate to treat as for candidemia (see above). For patients with cystitis due to a fluconazole-susceptible *Candida* spp, oral fluconazole 200–400 mg (pediatric dosing 3–6 mg/kg/dose) daily for 2 weeks is advisable (23) (III). For patients with fluconazole-resistant organisms, LFAmB or oral flucytosine 25 mg/kg four times daily are recommended (23) (III). Flucytosine may cause diarrhea and bone marrow suppression, especially in individuals with baseline renal insufficiency, and side effects must be monitored carefully. If prolonged use is expected, flucytosine drug level monitoring is indicated to avoid dose-related toxicity. AmB-d bladder irrigation is generally not recommended, but might be useful for patients with fluconazole-resistant *Candida* spp, especially *C. glabrata* (49). For patients with pyelonephritis, treatment with fluconazole is indicated for fluconazole-susceptible organisms. For fluconazole-resistant organisms, AmB-d possibly with flucytosine, or flucytosine alone can be offered for at least 2 weeks (23) (III). Echinocandins are normally avoided due to poor urinary concentration.

Pulmonary Candidiasis

Isolation of *Candida* spp from the respiratory tract rarely indicates invasive candidiasis and generally is not treated with antifungal therapy (50–52). An exception exists for lung transplant recipients in whom anastomotic tracheobronchitis due to *Candida* is a concern. Evidence of *Candida* tracheobronchitis is based on visual inspection and histologic confirmation, usually accompanied by a positive culture from an appropriate specimen. Selection of a specific agent could be based on the same principles as for selecting an agent for treatment of candidemia. There are no specific studies to guide duration of therapy, but it is reasonable to continue treatment until there is clinical resolution of the infection.

Prophylaxis

Identifying patients at the highest risk of infection is crucial to the development of effective approaches to antifungal prophylaxis. The major points that need to be addressed when deciding if antifungal prophylaxis is warranted include: (1) general prophylaxis versus targeted prophylaxis; (2) selection of an appropriate agent and (3) the duration of prophylaxis.

The prophylactic approach implies that an antifungal agent is administered to all transplant recipients, whereas *targeted* prophylaxis applies to the use of an antifungal agent in a subgroup of transplant recipients with predisposing conditions that place them at higher risk of developing

Table 3: Risk factors for *Candida* infection and recommended prophylactic strategies

Organ	Risk factors	Antifungal prophylaxis	Duration
Liver	Prolonged or repeat operation Retransplantation Renal failure Choledocho-jejunostomy *Candida* colonization High transfusion requirement	Fluconazole 400 mg/day LFAmB 3–5 mg/kg/day[1]	Up to 4 weeks or Until resolution of risk factors
Small bowel	Graft rejection/dysfunction Enhanced immunosuppression Anastomotic dysruption Abdominal reoperation Multivisceral transplantation	Fluconazole 400 mg/day LFAmB 3–5 mg/kg/day[1]	At least 4 weeks Until healing of anastomosis and absence of rejection
Pancreas	Enteric drainage Vascular thrombosis Postperfusion pancreatitis	Fluconazole 400 mg/day LFAmB 3–5 mg/kg/day[1]	At least 4 weeks

[1]If high rates of non-*albicans* spp or risk factors for *Aspergillus*.

invasive candidiasis. If high-risk patients can be easily identified, and if it is shown that withholding prophylaxis in patients considered low-risk is not associated with a high incidence of invasive candidiasis, then the targeted approach is preferred.

The ideal antifungal agent used for prophylaxis is one that is efficacious, safe to the allograft and other organs, with predictable or no drug interactions, ease to administer, with minimal/manageable side effects, and affordable. It is also important to determine if the patient at risk for *Candida* infection is also at risk for mold infections, particularly due to *Aspergillus*, so an agent with good anti-mold activity can be selected.

Duration of antifungal prophylaxis is not clearly defined, but as a general rule, prophylaxis should be maintained for at least 14 days posttransplantation, and longer if predisposing comorbidities persist. Because the risk factors and best choice of antifungal agent vary according to the transplanted organ, each organ will be discussed separately and recommendations are summarized on Table 3.

Liver transplantation

Antifungal prophylaxis against *Candida* should be given to all adult liver transplant recipients at high risk for development of invasive candidiasis; i.e. those with ≥2 of the following risk factors: prolonged or repeat operation; retransplantation; renal failure; high transfusion requirement, i.e., transfusion of ≥40 units of cellular blood products including platelets, packed red blood cells and auto transfusion; choledocho-jejunostomy and *Candida* colonization in the peri-operative period (1,4) (II-1). Liver transplant candidates are highly colonized with *Candida* spp in their gastrointestinal (GI) tract (53). Duration of prophylaxis is not clearly determined, and has ranged from 5 days to 10 weeks in clinical trials. Duration of up to 4 weeks, or for the duration of persistent risk factors, seems reasonable. The use of fluconazole as a prophylactic antifungal agent should be limited only to patients at high risk for invasive candidiasis. Liver transplant recipients at risk for both candidiasis and aspergillosis should receive an agent with anti-*Aspergillus* activity.

Three prospective randomized controlled trials in adults have shown the efficacy of antifungal prophylaxis of invasive candidiasis. In one study, fluconazole 100 mg/day was compared to oral nystatin in 143 liver transplant recipients. Prophylaxis was given for 4 weeks after liver transplantation. Fluconazole was associated with a reduction in *Candida* colonization and superficial infections, as well as a trend toward reduction of invasive infections (54). In the second trial, fluconazole 400 mg/day or placebo were administered for 10 weeks after liver transplantation. Antifungal prophylaxis with fluconazole compared to placebo resulted in a decreased rate of proven fungal infection (43% vs. 9%) and invasive infection (23% vs. 6%; Ref.55). Overall survival was not improved. In the third study, itraconazole was compared to placebo, and showed a decrease in the rate of candidiasis from 24% to 4% (56).

Studies with LFAmB, including LAmB and ABLC, have used different doses for variable periods of prophylaxis. Risk factors for IFI were also not uniform in these trials. These studies have shown that low dose of liposomal amphotericin B (1 mg/kg/day), administered for as few as 5 days, is associated with a significant reduction in invasive candidiasis (57–59).

Caspofungin given for at least 21 days was shown to be an efficacious and well-tolerated antifungal regimen in high-risk liver transplant recipients in a recent multicenter, non-comparative, open-label trial (60). Its use as a prophylactic agent seems promising due to lack of significant drug interactions with tacrolimus, lack of nephrotoxicity and activity against non-*albicans Candida*. A randomized controlled trial of anidulafungin versus fluconazole for the prevention of

fungal infections in liver transplant recipients is currently ongoing.

A recent meta-analysis showed that antifungal prophylaxis in liver transplant recipients significantly reduced the total episodes of superficial and IFI, as well as mortality attributable to fungal infections; however it did not affect overall mortality or the need for empirical antifungal treatment (61). Compared to controls, patients receiving antifungal prophylaxis experienced a higher proportion of non-*albicans Candida* infections.

Observing liver transplant recipients at low risk for IFIs without antifungal prophylaxis is safe, as shown by a recent multicenter, prospective, observational study, in which 200 liver transplant recipients at low risk for IFIs did not receive antifungal prophylaxis. In this trial only 7% of the 193 eligible patients developed an IFI at 100 days posttransplantation (62). Of those, only 2% were due to *Candida* spp and potentially preventable by the use of fluconazole prophylaxis. The use of nonabsorbable agents such as nystatin, clotrimazole and amphotericin B to achieve selective decontamination of the GI tract and oral cavity has shown inconsistent results and not proven to be useful (63–66).

Intestinal (small bowel) transplantation

Despite an absence of clinical trials in this patient population, antifungal prophylaxis in small bowel adult transplant recipients is routinely practiced and justified by the high rate of *Candida* infections. Rates of invasive candidiasis have been described to be as high as 28% in small case series (67,68). Patients at high risk are those with graft rejection or dysfunction, enhanced immunosuppression, anastomotic disruption, abdominal reoperation or multivisceral transplantation. Fluconazole is an acceptable agent. However, LFAmB should be utilized in patients where there is high suspicion of non-*albicans Candida* spp. Prophylaxis is usually administered for a minimum of 4 weeks, until anastomosis has completely healed, and rejection is not present (II-3).

Pancreas and kidney transplantation

The risk factors for candidiasis among pancreas transplant recipients include enteric drainage, vascular thrombosis and postperfusion pancreatitis (12). The use of prophylactic fluconazole should be considered whenever one of these risk factors is identified. LFAmB is preferred in centers with a high prevalence of non-*albicans* species. Duration of prophylaxis will depend on reduction of risk factors (II-3). The risk of invasive candidiasis is too low after isolated kidney transplantation to warrant prophylaxis.

Lung, heart–lung and heart transplantation

Candida is commonly isolated from the respiratory tract of lung and heart–lung transplant recipients. The highest risk for *Candida* infection is in the first 30 days posttransplantation, and risk factors include the use of broad spectrum antibiotics, duration of antibiotic use, presence of central venous catheters and need for renal replacement therapy (3,4). There is a wide variation in the practice of antifungal prophylaxis in lung and heart–lung transplant recipients, not only in terms of the antifungal agent, but also on its mode of administration, timing and duration. Because of the high rates of *Aspergillus* infection after lung and heart–lung transplantation, antifungal prophylaxis should be directed towards the prevention of invasive aspergillosis, and prophylaxis with an agent without adequate anti-*Aspergillus* activity is not appropriate (II-1). *Candida* infections are infrequent after heart transplantation, and antifungal prophylaxis is not routinely recommended for these patients (III).

Infection Control Issues

There are no infection control measures specifically targeted towards prevention of *Candida* infections. Measures to reduce the incidence of these infections should include adequate hand hygiene, judicious use of antibiotics and frequent assessments to determine the need for intravascular and urinary catheters.

Future Research

Invasive candidiasis has been associated with increased length of hospitalization and increased mortality. Despite recent advances in microbiology techniques, the sensitivity of blood cultures is still poor. Future research should focus on better diagnostic methods. Randomized controlled trials are also needed to determine the best agent and duration of antifungal prophylaxis in organ transplant recipients.

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Special Article

Aspergillosis in Solid Organ Transplantation

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Key words: Antifungal, aspergillosis, invasive fungal infections, opportunistic infection, pulmonary infection

Abbreviations: BAL, bronchoalveolar lavage; CMV, cytomegalovirus; EORTC/MSG, European Organization for Research and Treatment of Cancer and Mycosis Study Group; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; IA, invasive aspergillosis; IgG, immunoglobulin G; PCR, polymerase chain reaction; SOT, solid organ transplant.

Introduction

Invasive aspergillosis (IA) occurs in 1–15% of the solid organ transplant (SOT) recipients. Mortality rate in transplant recipients with IA historically has ranged from 65% to 92% (1–4). However, currently reported mortality rate in IA among SOT recipients is 22% (5). An estimated 9.3–16.9% of all deaths in transplant recipients in the first year have been considered attributable to IA (6). Although the outcomes have improved in the current era, IA remains a significant posttransplant complication in SOT recipients. The review herein discusses the epidemiologic characteristics, risk factors, diagnostic laboratory assays and the approach to antifungal prophylaxis and treatment of IA in SOT recipients.

Epidemiology and Risk Factors

The net state of immunosuppression including the intensity of immunosuppressive regimen is a major determinant of the development of IA in SOT recipients, regardless of the type of transplant. However, the incidence of IA differs and there are unique risk factors for *Aspergillus* infections for various types of organ transplant recipients as discussed herein (Table 1). IA is typically acquired by inhalation of the conidia. Less frequently local infections may result in surgical wound infections. Invasive disease may manifest as localized (pulmonary or extrapulmonary disease) or disseminated aspergillosis. In lung transplant recipients, airway disease can manifest as tracheobronchitis or bronchial anastomotic infections.

Liver transplant recipients

IA occurs in 1–9.2% of the liver transplant recipients (1,4,6–9). A number of well characterized risk factors have been described for IA after liver transplantation. Retransplantation and renal failure are amongst the most significant risk factors for IA in these patients (4,10–12). Retransplantation confers 30-fold higher risk and renal dysfunction, particularly the requirement of any form of renal replacement therapy, e.g. hemodialysis or continuous venovenous hemofiltration is associated with a 15- to 25-fold greater risk of IA in liver transplant recipients (3,11). Most Invasive fungal infections in these high-risk patients occur within the first month posttransplant; the median time to onset of IA after renal replacement therapy and retransplantation was 13 and 28 days, respectively in one study (9,13). Other factors associated with IA in liver transplant recipients include transplantation for fulminant hepatic failure, cytomegalovirus (CMV) infection and prolonged intensive unit care stay (7–9,14–16; Table 2).

Historically IA in liver transplant recipients has occurred in the early posttransplant period; the median time to onset after transplantation was 17 days in one study (2) and 16 days in another (17). More recently, however, *Aspergillus* infections have been shown to occur in the late posttransplant period, i.e. more than 90 days after transplantation. In a study that compared a cohort of patients with IA from 1998 to 2002 with those from 1990 to 1995, median onset to IA was 60 days posttransplant; 55% of the infections in the later compared with 23% in the earlier cohort occurred after 90 days of transplantation (3). Improved outcome in the early postoperative period due to technical surgical advances, and delayed onset of posttransplant risk factors such as CMV infection, allograft dysfunction due to recurrent hepatitis C virus hepatitis are proposed to have led to delayed occurrence of IA in liver transplant recipients in the current era (3). CMV and hepatitis C virus infection are

Table 1: Risk factors for invasive aspergillosis in organ transplant recipients

Liver transplant recipients
– Retransplantation
– Renal failure, particularly requiring renal replacement therapy
– Transplantation for fulminant hepatic failure
– Reoperation
Lung transplant recipients
– Single lung transplant
– Early airway ischemia
– Cytomegalovirus infection
– Rejection and augmented immunosuppression
– Pretransplant *Aspergillus* colonization
– Posttransplant *Aspergillus* colonization within a year of transplant
– Acquired hypogammaglobulinemia (IgG < 400 mg/dL)
Heart transplant recipients
– Isolation of *Aspergillus* species in respiratory tract cultures
– Reoperation
– CMV disease
– Posttransplant hemodialysis
– Existence of an episode of invasive aspergillosis in the program 2 months before or after heart transplant
Kidney transplant recipients
– Graft failure requiring hemodialysis
– High and prolonged duration of corticosteroids

independent risk factors for late-onset IA in liver transplant recipients (2,7,11).

Mortality in liver transplant recipients with IA has ranged from 83% to 88% (6,18). Requirement of dialysis and CMV infection are independent predictors of mortality in SOT recipients, including liver transplant recipients with IA (13). More recent studies have reported improved outcomes with mortality ranging from 33.3% to 65% (3,19). Mortality, however, remains high in patients who develop IA after liver retransplantation (82.4%), particularly in those undergoing retransplantation after 30 days of primary transplant (100%; Ref.13).

Renal transplant recipients

IA has been reported in approximately 0.7% and in up to 4% of the renal transplant recipients (6,7,20–25). High doses and prolonged duration of corticosteroids, graft failure requiring hemodialysis and potent immunosuppressive therapy have been shown to be risk factors for IA after renal transplantation (6,23,26). Despite a relatively lower overall incidence as compared to other organ transplant recipients, IA is a significant contributor to morbidity in renal transplant recipients. Mortality in renal transplant recipients with IA has ranged from 67% to 75% (4,6).

Lung transplant recipients

Earlier studies had reported the overall incidence of IA in lung transplant patients ranges from 4% to 23.3% (27). In a recently concluded multicenter prospective study, the first year cumulative incidence of fungal infections in lung transplant was 8.6% (28). This incidence of all fungal infections was in parallel with the reported incidence in donor mismatch allogeneic bone marrow transplant recipients (29). These data highlight the highest risk status of fungal infections in lung transplant recipients despite widespread use of antifungal prophylaxis. IA is the predominant fungal infection in lung transplant recipients (30). The median time to onset of IA in lung transplant recipients from 2002 to 2005 was 508 days posttransplant (30). In lung transplant recipients, the continuous exposure of the organ to the environment, coupled with impaired defenses due to decreased mucociliary clearance and blunted cough reflex, contributes to the vulnerability to IA (31). Other risk factors that confer an increased risk of IA in lung transplant recipients are relative ischemia at the anastomosis (32), receipt of single lung transplant (33), hypogammaglobulinemia (34), CMV infection (35) and pre/postcolonization of the airways with *Aspergillus* (36–38). The presence of bronchiolitis obliterans syndrome as a risk factor for IA is not well determined. However, one study failed to find a higher rate of IA in lung transplant recipients with bronchiolitis obliterans syndrome (39).

The mortality of IA in lung transplant recipients varies according to the clinical presentation, ranging from 23% to 29% in patients with tracheobronchitis to as high as 67–82% in patients with invasive pulmonary disease (10). Recent data would suggest that overall mortality of 20% among patients with IA (30).

Heart transplant recipients

The overall 12 months cumulative incidence of fungal infection in heart transplant recipients was 3.4% in a large prospective cohort study (28). The incidence of IA in heart transplant recipients ranges from 1% to 14% (40). The risk factors for the development of IA include the isolation of *Aspergillus fumigatus* from bronchoalveolar lavage (BAL), reoperation, CMV disease, posttransplant hemodialysis, (41–43). Overall mortality in heart transplant recipients with IA at 1 year was 66.7% in one study (40).

Diagnosis

A substantial delay in establishing an early diagnosis remains a major impediment to the successful treatment of IA. Diagnostic criteria have been established to facilitate the diagnosis of IA. The European Organization of Research and Treatment and Mycosis Study Group had put forth the criteria for the diagnosis of fungal infections in immunocompromised host (44). However, they lack complete applicability in lung transplant recipients owing to the unique clinical syndromes and lack of sensitivity of certain diagnostic tests (serum galactomannan) in lung transplants. The International Society for Heart and Lung Transplantation has developed a working formulation for the diagnosis of invasive fungal infections in lung transplant recipients. This definition excludes the "possible" category from EORTC/MSG criteria and defines the clinical syndromes

Table 2: Recommendations for prophylaxis for invasive aspergillosis in solid organ transplant recipients

Organ	Risk factors	Antifungal prophylaxis	Duration
Liver II-2	Retransplantation Renal failure, particularly requiring renal replacement therapy Reoperation involving thoracic or abdominal cavity	Lipid formulation of amphotericin B (3–5 mg/kg/day) OR an echinocandin	Initial hospital stay or for 4 weeks posttransplant
Lung	**Presence of one of these risk factors (II-2)** Pretransplant *Aspergillus* colonization Posttransplant *Aspergillus* colonization within a year of transplant **Presence of more than one of these risk factors (II-3,III)** Induction with alemtuzumab or Thymoglobulin Single lung transplant *Aspergillus* colonization following cytomegalovirus infection Rejection and augmented Immunosuppression (particularly use of monoclonal antibody posttransplant with *Aspergillus* colonization) Acquired hypogammaglobulinemia (IgG < 400 mg/dL)	Inhaled amphotericin B 6 mg/q8 or 25 mg/day OR Inhaled Abelcet 50 mg OR Inhaled Ambisome 25mg OR Voriconazole 200 mg bid OR Itraconazole 200 mg bid	Preferably guided by interval airway inspection, respiratory surveillance fungal cultures, and clinical risk factors. Once every 2 days for 2 weeks and then once per week for at least 13 weeks Three times/week for 2 months, followed by weekly administration for 6 months and twice per month afterwards 4 months or longer
Heart II-3	Isolation of *Aspergillus* species in respiratory tract cultures Reoperation CMV disease Posttransplant hemodialysis Existence of an episode of IA in program 2 months before or after heart transplant	Itraconazole 200 mg bid OR voriconazole 200 mg bid	50–150 days

of colonization, tracheobronchitis/bronchial anastomotic infection with the inclusion of *Aspergillus* PCR in the microbiological diagnostic criteria. These definitions may be more specific in the epidemiological and intervention studies in lung transplant recipients (45).

Among the diagnostic modalities, cultures of the respiratory tract secretions lack sensitivity and the *Aspergillus* may only be detected in clinical samples in late stages of the disease. On the other hand, a positive culture with *Aspergillus* from respiratory tract samples does not always indicate invasive disease. The significance of a positive culture from an airway sample also varies with the type of organ transplant. Isolation of *Aspergillus* spp. from the respiratory tract of liver transplant recipients is an infrequent event (~1.5%). However, it has a high positive predictive value, ranging from 41% to 72% for the subsequent development of IA (6). *Aspergillus* spp. can be detected in airway samples of ~25–30% of the lung transplant recipients (3,36,46). Although positive airway cultures have a low positive predictive value for the diagnosis of IA in lung transplant recipients, they portend a higher risk for subsequent invasive infection (6). Recovery of *Aspergillus* spp. from an airway sample in lung transplant recipients warrants a bronchoscopic examination to exclude the presence of tracheobronchitis because radiographic and imaging studies may be nonrevealing at this stage.

In heart transplant recipients, the positive predictive value of culturing *Aspergillus* from respiratory tract samples for the diagnosis of IA was 60–70% (43). The positive predictive value of recovering *A. fumigatus* for the diagnosis of IA was 78–91%, whereas it was 0% for other including *A. versioclor, A. terreus, A. glaucus* and *A. candidus* (43). The isolation of *A. fumigatus* from the sputum had a positive predictive value of 50–67% that increased to 88–100% when the sample was a respiratory specimen other than the sputum such as BAL and bronchial aspirate (43).

The utility of the galactomannan test for the early diagnosis of IA has been assessed in a limited number of studies in SOT recipients. In liver transplant recipients where archived sera were tested, the sensitivity of the test was 55.6% and the specificity was 93.9% (47). A prospective study in 154 liver transplant recipients documented a specificity of 98.5% (48). In lung transplant recipients, the galactomannan test had a specificity of 95%, but a

relatively low sensitivity (30%) for the diagnosis of IA (49). Although the test was able to detect the only case of systemic IA, and 29% of the cases of pulmonary IA, it detected none of the cases of *Aspergillus* tracheobronchitis (49). A meta-analysis showed that galactomannan assay may have greater utility in hematopoietic stem cell transplant recipients than in SOT recipients in whom the sensitivity and specificity of the test was 22% and 84%, respectively (50).

Sensitivity of the galactomannan assay for the diagnosis of IA in SOT recipients may be improved by testing BAL. In one study, BAL had a sensitivity of 67% and specificity of 98% at the index cutoff value of $\geq$1 for the diagnosis of IA in lung transplant recipients (51). In another study, BAL had a sensitivity of 100% and specificity of 91% at the same index cutoff value for the diagnosis of IA in SOT recipients (52). In another study which combined the data from two previously reported studies the galactomannan sensitivity was 81.8% in patients with IA, and specificity was 95.8% in lung transplant patients who underwent BAL for surveillance for infection or (53).

False positive galactomannan tests have been documented in up to 13% of the liver and 20% of the lung transplant recipients (48,49). Liver transplant recipients undergoing transplantation for autoimmune liver disease and those requiring dialysis were significantly more likely to have false-positive galactomannan tests (48). In a report of lung transplant recipients, false-reactivity of galactomannan was documented in 20% (14/70) of the patients (49). Most false-positive tests occurred in the early posttransplant period, i.e. within 3 days of lung transplantation in 43%, within 7 days in 64% and within 14 days of transplantation in 79% of the patients (49). Patients undergoing lung transplantation for cystic fibrosis and chronic obstructive pulmonary disease were more likely to have positive tests in the early posttransplant period (49). False-positive galactomannan tests in 29% of the liver transplant recipients in the first week posttransplantation were attributed to perioperative prophylaxis with β-lactam agents that included pipercillin-tazobactam and amoxicillin–clauvanic acid in serum. However, this association is not significant in the newer preparations of pipercillin-tazobactam (54). Plasma-lyte sodium gluconate-containing solution but not gluconate-free Plasma-lyte solution may result in false positive galactomannan values in the BAL fluid (43). The use of plasma-lyte sodium gluconate containing solution should be avoided during bronchoscopy for the diagnosis of IA.

1–3,β-D-Glucan is a component of fungal cell wall. It is present in most of the medical important fungi but is notably absent in *Cryptococcus* species and *Zygomycetes* species. The utility of 1–3, β-D-glucan for the diagnosis of IA has not been fully defined. The test, however, was useful for the diagnosis of IA in living-donor liver allograft recipients in one study (55). In lung transplant recipients, serum 1–3,β-D-glucan had the sensitivity of 64% for the diagnosis of invasive fungal infection (56). A panfungal PCR in the blood preceded clinical signs of invasive fungal infections in renal transplant recipients by 27 days (57). Recently two PCR-based molecular diagnostic tests for *Aspergillus* have become commercially available (Viracor-IBT Laboratories, Myconostica). In a study of viracor Pan fungal PCR in BAL of lung transplant recipients, the sensitivity and specificity for the diagnosis of invasive pulmonary aspergillosis was 100% and 88%, respectively (58). However, their precise role in the diagnosis and management of IA in SOT recipients remains to be determined.

Compatible CT findings for the diagnosis of invasive fungal Infection include the specific but poorly sensitive "halo sign" (54), or multiple nodules/masses, particularly if there is central low density as a precursor to cavitation (the air-crescent sign; Ref. 59). These finding are more prevalent in stem cell transplant recipients. The development of pulmonary nodules in the early posttransplant period is highly suggestive of invasive fungal infection in lung and heart transplant recipients (59). Clinicians should therefore have a low threshold for performing a chest CT in this patient group and should also be mindful that endobronchial fungal disease is under-recognized.

Management

Treatment

Prompt initiation of antifungal therapy is critical for achieving optimal outcomes in SOT recipients with IA. Beginning in the early 1990s and for almost a decade, lipid formulations of amphotericin B largely because of a lower potential of nephrotoxicity have been the mainstay for the treatment for IA in SOT recipients. In a study consisting of 47 SOT patients with IA who were treated with lipid formulations of amphotericin B (5–7.4 mg/kg/day), the overall 90-day mortality was 49% and the IA-associated mortality was 43% (13). Another study that compared the efficacy of amphotericin B lipid complex (median dose of 5.2 mg/kg/day) and amphotericin B deoxycholate (median does of 1.1 mg/kg/day) for the treatment of IA in SOT recipients (60), the overall and IA-related mortality rate was 33% and 25% in amphotericin B lipid complex group and 83% and 76% in amphotericin B deoxycholate group (60). In patients intolerant of or in those failing primary therapy with voriconazole, liposomal amphotericin B or amphotericin B lipid complex can be considered as alternative therapy. *Aspergillus* species such as *A. terreus* are typically resistant to the polyenes but susceptible to voriconazole. However, only 5–6% of IA in SOT recipients is due to *A. terreus* (13).

Based on a large randomized trial that compared voriconazole with amphotericin B deoxycholate for the treatment of IA mostly in hematopoietic stem cell transplant recipients and patients with hematologic malignancies, voriconazole has emerged as the preferred agent for primary therapy

Table 3: Antifungal therapy for invasive aspergillosis in adult organ transplant recipients

Drug	Dosing (Adult)	Comments
Primary therapy		
Voriconazole	6 mg/kg IV every 12 h for 1 day, followed by 4 mg/kg IV every 12 h; oral dosage is 200 mg every 12 h	Monitoring of plasma drug levels of voriconazole, hepatic aminotransferase levels and calcineurin agent levels is recommended
Alternative agents		
Liposomal amphotericin B (AmBisome®)	3–5 mg/kg/day IV	Higher dosages are not more effective
Amphotericin B Lipid Complex (Abelcet®)	5 mg/ kg/day IV	Higher dosages are not more effective
Caspofungin	70 mg day 1 IV and 50 mg/day IV thereafter	Has been evaluated only as salvage therapy. Its role as single agent therapy is controversial
Micafungin[1]	100–150 mg IV qd	May be used as alternative therapy in cases of intolerance or disease refractory to primary therapy
Posaconazole[1]	200mg qid initially and then 400mg po bid	May be used as alternative therapy in cases of intolerance or disease refractory to primary therapy
Itraconazole[2]	200–400 mg/day orally	Use should be considered only in mild cases intolerant to other therapies. Itraconazole oral solution and capsule are not bioequivalent and should not be used interchangeably. Therapeutic drug monitoring is recommended intolerance or disease refractory to primary therapy

Duration of therapy for aspergillosis has not been optimally defined. Most experts recommend continuing treatment of infection until resolution or stabilization of all clinical and radiographic manifestations. Generally, treatment is continued for a minimum of 6–12 weeks.
[1]Currently micafungin and posaconazole do not have an approved indication for the treatment of invasive aspergillosis.
[2]IDSA guidelines (2008) recommend 600 mg/day for 3 days, followed by 400 mg/day.

of IA (61). Successful outcome at 12 weeks was documented in 52.8% of the patients in the voriconazole group and in 31.6% in the amphotericin B deoxycholate group. The survival at 12 weeks was 70.8% in the voriconazole group and 57.9% in the amphotericin B group (hazard ratio, 0.59; 95% CI 0.40–0.88). Voriconazole-treated patients had significantly fewer severe drug-related adverse events, except for transient visual disturbances.

Since this study, a number of reports of employing voriconazole for the treatment of IA specifically in SOT recipients have appeared in the literature. In three studies that included SOT patients with IA, complete or partial response rates observed with voriconazole were 100%, 100% and 50% (62–64). In another report that included 11 SOT recipients with central nervous system aspergillosis treated with voriconazole, the favorable response rate was 36% and survival was 31% (65). Voriconazole was successfully used in heart transplant recipients as first-line and salvage therapy for IA (66,67). Mean hospital length of stay in SOT recipients with IA in the current era is 29.7 days and initial voriconazole use was associated with decreased length of stay (68). Intravitreal voriconazole has also been used in a lung transplant patient with *Aspergillus* endophthalmitis (69). Voriconazole is now regarded as the drug of choice for primary treatment of IA in all hosts, including SOT recipients (Table 3) and this recommendation is endorsed by the Clinical Practice Guidelines of the Infectious Diseases Society of America (IDSA) for the treatment of IA (level I recommendation; Ref. (70).

Posaconazole is another extended spectrum triazole with activity against *Aspergillus*. Although not approved by the U.S. Food and Drug Administration for the treatment of IA, it has been used as salvage therapy for patients with IA who are refractory to or intolerant of primary antifungal therapy (71) and can be considered as alternative therapy in these settings. Itraconazole is suboptimal therapy for IA in the current era. Plasma drug level monitoring of the triazoles should be considered when using these agents for the treatment of IA.

The echinocandins (caspofungin, micafungin and anidulafungin) inhibit fungal 1,3-β-D-glucan and have *in vitro* activity against *Aspergillus* species. Caspofungin and micafungin are hepatically metabolized while anidulafungin is eliminated by nonenzymatic degradation in the blood, without hepatic metabolism or renal elimination. All three echinocandins, however, have been used anecdotally as salvage therapy in IA as single agent (72) and in combination with other drugs in SOT recipients (73,74). However, only caspofungin is currently approved by the US Food and Drug Administration as salvage therapy for the treatment of IA.

For the treatment of tracheobronchial aspergillosis, current guidelines of the Infectious Disease Society of America recommend systemic voriconazole as primary therapy (70). Aerosolized amphotericin B deoxycholate or lipid formulations of amphotericin B may have some benefits; however, their use for the treatment of tracheobronchial infection has not been standardized and remains investigational (70). There is little experience with caspofungin or other echinocandins in treating tracheobronchial infections.

The role of combination antifungal therapy for IA has not been fully defined at present. Updated guidelines of the Infectious Disease Society of America suggest reserving this option for salvage therapy (70). A prospective, multicenter study in SOT recipients compared outcomes in 40 patients who received voriconazole plus caspofungin as primary therapy for IA with those in 47 patients in an earlier cohort who received a lipid formulation of amphotericin B as primary therapy (13). The two groups were well matched, including the proportion with disseminated disease (10% vs. 12.8%), proven IA (55% vs. 51.1%), or *A. fumigatus* (71.1% vs. 80.9%). Overall survival at 90 days was 67.5% in the cases and 51% in the control group. Mortality was attributable to IA in 26% of the cases and in 43% of the controls ($p = 0.11$). Combination therapy was associated with a trend towards lower mortality when controlled for CMV infection and renal failure. When 90-day mortality was analyzed in subgroups of patients, combination therapy was independently associated with reduced mortality in patients with renal failure and in those with *A. fumigatus* infection, even when adjusted for other factors predictive of mortality in the study population (13). No correlation was found between *in vitro* antifungal synergistic interactions and outcome. None of the patients required discontinuation of antifungal therapy for intolerance or adverse effects however, patients in the combination therapy arm were more likely to develop an increase in calcineurin-inhibitor agent level, or gastrointestinal intolerance (13).

A prospective, randomized, double-blind clinical trial to investigate the efficacy of the combination of voriconazole and anidulafungin for the treatment of IA in allogeneic hematopoietic stem cell transplant recipients and patients with hematologic malignancies has recently been completed (75). Patients were randomized to receive initial treatment with the combination of voriconazole and anidulafungin or voriconazole monotherapy (with placebo). Study treatment was administered for $\geq$2 weeks, followed by voriconazole maintenance to complete 6 weeks. Mortality at week 6 was 26/135 (19.3%) in patients treated with the combination of voriconazole and anidulafungin, compared to 39/142 (27.5%) for monotherapy (95% CI –18.99, 1.51, $p = 0.09$). In a posthoc analysis of 218/277 (78.7%) patients with probable IA based on detection of galactomannan in BAL or serum, mortality at week 6 was 17/108 (15.7%) for combination and 30/110 (27.3%) for monotherapy (95% CI –22.69, –0.41, $p < 0.05$). Safety parameters did not show significant differences between treatment groups. Thus, although the difference in all-cause mortality was not statistically significant, the combination was beneficial in patients with a diagnosis of probable IA based on a positive galactomannan (75).

The combination of voriconazole and caspofungin for the treatment of IA posed a lesser economic burden on institutional resources than 5 mg/kg/day of liposomal amphotericin B (76). Despite relative paucity of data regarding the efficacy, a survey of antifungal therapeutic practices for IA in liver transplant recipients documented that combination therapy is used as first-line treatment in 47% and as salvage therapy in 80% of the transplant centers in North America (77). We believe that potential benefits of combination therapy may be best realized when used as initial therapy, particularly in patients with more severe forms of the disease such as disseminated IA or with poor prognostic factors such as renal failure.

Surgical excision or debridement remains an integral part of the management of IA for both diagnostic and therapeutic purposes (78–83). Specifically, surgery is indicated for persistent, or a life-threatening hemoptysis, for lesions in the proximity of great vessels or pericardium, sinonasal infections, for single cavitary lung lesion which progress despite adequate treatment, for lesions invading the pericardium, bone, invading the subcutaneous or thoracic tissue (70). Pneumonectomy lead to successful outcome in a lung transplant recipient with progressive, refractory angioIA whose disease worsened despite conventional antifungal therapy (84). Surgical resection is also indicated for intracranial abscesses depending upon the location, accessibility of the lesion and neurologic sequelae.

The optimal duration of therapy for IA depends upon the response to therapy, and the patient's underlying disease(s) or immune status. Treatment is usually continued for 12 weeks; however, the precise duration of therapy should be guided by clinical response rather than an arbitrary total dose or duration. A reasonable course would be to continue therapy until all clinical and radiographic abnormalities have resolved, and cultures if they can be readily obtained, do not yield *Aspergillus*. Lowering of immunosuppression is an important adjuvant measure to surgical and medical treatment of IA. Close monitoring of Cyclosporine A or tacrolimus levels and of allograft function is critical.

Drug interactions of antifungal agents with immunosuppressants

Drug interactions of a number of antifungal agents with immunosuppressants must be carefully considered when treating transplant recipients with IA. The triazole agents are potent inhibitors of the CYP34A isoenzymes and have the potential to increase the levels of calcineurin-inhibitor agents and sirolimus (85). Itraconazole has been shown to increase CsA or tacrolimus levels by 40–83% (86,87).

A 50–60% reduction in the dose of calcineurin-inhibitor agents may be necessary with the concurrent use of voriconazole (85). The use of sirolimus is contraindicated in patients receiving voriconazole. In some reports, however, the two agents have been safely coadministered with sirolimus dose reduction by 75–90% (88,89). Coadministration of posaconazole increased cyclosporine exposure and necessitated dosage reductions of 14–20% for cyclosporine (90). Posaconazole increased the maximum blood concentration and the area under the concentration-time curve for tacrolimus by 121% and 357%, respectively (90).

The pharmacokinetics of caspofungin is unaltered by coadministration of tacrolimus, but caspofungin may reduce tacrolimus concentrations by up to 20% and may increase cyclosporine A plasma concentrations by 35% (91). Elevated liver function tests in healthy volunteers receiving caspofungin and cyclosporine A led to the exclusion of cyclosporine recipients from the initial phase II/III clinical studies of caspofungin (91). In the clinical setting, however, coadministration of caspofungin with cyclosporine A has been well tolerated (92–94). Nevertheless, it is prudent to monitor hepatic aminotransferase enzyme levels in cyclosporine recipients treated with caspofungin. There is no interaction between caspofungin and mycophenolate mofetil.

Anidulafungin clearance is not affected by drugs that are substrates, inducers, or inhibitor of cytochrome P450 hepatic isoenzymes (96). Further, because the drug is negligibly excreted in the urine, drug-drug interactions due to competitive renal elimination are unlikely (96,97). Coadministration with tacrolimus documented no pharmacokinetic interaction between the two agents (96). When administered with cyclosporine A, a small (22%) increase in andifulafungin concentration was observed after 4 days of dosing with cyclosporine A and was not considered to be clinically relevant (96). Micafungin is a weak substrate and a mild inhibitor of the CYP3A enzyme, but not of P-glycoproteins (97). In healthy volunteers, micafungin was shown to be a mild inhibitor of cyclosporine levels (97,98). In patients receiving sirolimus, serum concentrations of this agent was increased by 21% with concomitant use of micafungin and minimal dose adjustment may be needed (99). No drug interactions have been noted between micafungin and mycophenolate mofetil or cyclosporine (97).

Adjunctive immunotherapeutic agents

Enhancement of the host's immune status with immunomodulatory agents is a potentially attractive therapeutic adjunct in the management of IA. Evidence from *in vitro* and animal studies has shown enhanced antifungal activity with cytokine or colony stimulating factors, and modulation of cellular immune responses (100–102). Granulocyte-colony stimulating factor (G-CSF) stimulates proliferation and maturation of committed myeloid precursor cells and also augments neutrophil functions including chemotaxis, phagocytosis and oxidative responses (102,103). Granulocyte macrophage colony stimulating factor (GM-CSF) stimulates the proliferation and differentiation of multiple lineages of cells such as neutrophils, eosinophils and monocyte progenitor cells (104). G-CSF or GM-CSF has been shown to be effective for IA as adjuvant therapy for invasive fungal infections in some studies in patients with hematologic malignancies (105). Although GM-CSF use in SOT recipients appears to be safe, there are no studies that have evaluated its efficacy as adjunctive antifungal therapy specifically in these patients. Routine use of these colony stimulating factors in nonneutropenic SOT recipients with IA is not deemed necessary.

In vitro studies have also demonstrated a potential role of interferon-γ (IFN-γ) against *Aspergillus* (106–109) and case reports in hosts other than SOT recipients have documented possible beneficial effects of the adjunctive use of IFN-γ in invasive fungal infections in, including IA (110–113). Guidelines of the IDSA suggest a role for IFN-γ as adjunctive antifungal therapy for IA in immunocompromised nonneutropenic host (70). The use of this cytokine in organ transplant recipients is of concern, however, given the risk of potential graft rejection.

Prophylaxis

At present, prophylaxis against IA is not routinely recommended in all SOT recipients. Clinical trials of antifungal prophylaxis in liver transplant recipients have comprised small sample sizes in single center studies. An optimal approach to the prevention of invasive fungal infections in these patients, therefore, has not been defined.

Antifungal prophylaxis targeted toward high-risk patients is the most commonly employed approach in liver recipients. A meta-analysis of antifungal prophylactic trials in liver transplant recipients documented a beneficial effect on morbidity and attributable mortality, but an emergence of infections due to non-*albicans Candida* spp. in patients receiving antifungal prophylaxis (114). Because the risk factors and the period of susceptibility to invasive fungal infections is clearly definable, antifungal prophylaxis targeted towards these high-risk patients is also deemed a rational approach for the prevention of IA after liver transplantation. Targeted antifungal prophylaxis using the lipid formulations of amphotericin B in doses ranging from 1 to 5 mg/kg/day has been shown to be effective in observational studies (19,115–117). Currently, targeted prophylaxis in liver transplant recipients is employed most frequently during the initial hospital stay or for the first month post-transplant (77).

The availability of echinocandins with their good tolerability and safety profile has led to an expanded armamentarium of antifungal drugs with a potentially promising role

as agents for targeted prophylaxis for invasive fungal infections in high-risk liver transplant recipients (118). Caspofungin employed as antifungal prophylaxis in 71 high-risk liver transplant recipients was associated with success rate (defined as absence of breakthrough invasive fungal infection after (100) days of caspofungin and absence of premature discontinuation of prophylaxis) of 88.7% (119). However, discontinuation of caspofungin due to drug-related liver toxicity was required in six patients (119). Other studies using caspofungin and anidulafungin as prophylaxis or therapy have documented favorable safety profiles in liver transplant recipients (73). Given the potential for significant drug interactions with the immunosuppressive agents, the role of newer triazoles as antifungal prophylaxis in high-risk liver transplant recipients has not yet been fully defined. The choice of antifungal regimen should also take into consideration that a vast majority of invasive mycoses even in these high risk patients are due to invasive candidiasis for which fluconazole is an appropriate approach for preventive therapy.

An optimal antifungal prophylactic strategy in lung transplant recipients still remains to be determined. Current practices of antifungal prophylaxis in lung transplant recipients are derived from nonrandomized clinical trials of inadequate sample sizes, single center noncomparative case series or case control studies (27,120–126). Although all but one study (127) have employed universal antifungal prophylaxis, a more rational approach would be to use a risk stratification strategy for anti-fungal prophylaxis. To date, no data exist on the preemptive treatment of IA based on positive galactomannan in serum or BAL in lung transplant recipients.

Among the antifungal drugs, aerosolized amphotericin B allows the direct administration of the drug into the transplanted lung, avoiding systemic side effects and drug-drug interactions. Its use, however, is limited by tolerability. Common side effects include cough, bronchospasm and nausea. Amphotericin B deoxycholate and the lipid formulations (lipid complex and liposomal) have been shown to be safe and well tolerated (121,128); however, aerosolized amphotericin B lipid complex was associated with fewer side effects (121). A disadvantage of aerosolized amphotericin B is the fact that distribution in single lung transplant recipients occurs preferentially in the allograft, with unreliable distribution in the native lung, which could remain as a source of infection (129). It is also important to note that use of aerosolized amphotericin B may fail to prevent systemic fungal infections such as candidemia and pleural candidiasis in lung transplant recipients (130). Moreover the data on the long term safety of aerosolized preparations of amphotericin B are not available. Triazoles including itraconazole and voriconazole have been shown to decrease the rate of IA in lung transplant recipients. In one study using voriconazole prophylaxis, liver enzyme abnormalities developed in more than 40% of the patients (27). In a study, age less than 40 years, cystic fibrosis, use of azathioprine, history of liver disease and early initiation of voriconazole were associated with hepatotoxicity. In multivariable logistic regression analysis, perioperative initiation of voriconazole (within (30) days of transplantation) was independently associated with hepatotoxicity (OR 4.37, 95% CI: 1.53–12.43, $p = 0.006$) (131). Itraconazole may be less hepatotoxic than voriconazole in lung transplant recipients receiving antifungal prophylaxis (132). Moreover generic itraconazole is much cheaper than nongeneric voriconazole. Some centers have taken this into account to device the institutional prophylaxis strategy. Due to interactions with calcineurin inhibitors, levels of the immunosuppressive agents need to be measured and doses adjusted routinely when voriconazole is used concomitantly. An association between prolonged voriconazole use and development of skin cancer in lung transplant recipients has been reported (133–135). Although definite association requires further validation, it is prudent to screen these individuals for skin cancer and evaluate the necessity of continued prophylaxis periodically. Long term use of voriconazole prophylaxis may also result in the development of periostitis (136). Higher fluoride levels were reported in patients with periostitis receiving voriconazole (137). The data on posaconazole prophylaxis in lung transplant recipients remain thin but its use may be associated with lower rate of hepatotoxicity.

Pediatric Issues

Most of the data reviewed above regarding the treatment of IA are derived from studies in adults. Data from adult patients cannot be reliably extrapolated to infants and children due to differences in pharmacokinetic and toxicity profiles. For example, children have a higher capacity for elimination of voriconazole and as such higher doses are required compared with adults. Voriconazole exhibits nonlinear pharmacokinetics in most children (138,139). The recommended dose of 7 mg/kg i.v. in children 2–11 years of age provides exposure (area under the concentration-time curve) comparable to that observed in adults receiving 4 mg/kg i.v. (138). For older children (12–13 years of age), adult dosing strategies are often used.

Table 4 summarizes currently available agents for use in the treatment and prevention of *Aspergillus* infection in children. Clinicians need to be aware of data that are emerging for several newer agents, including posaconazole and anidulafungin; as such, the precise place of these agents in the management of pediatric IA is yet to be fully defined. The infectious diseases consult service should always be engaged when children are being treated for IA after organ transplantation.

Key Recommendations

These recommendations are primarily intended for the first year following the lung transplant. No definite

Table 4: Antifungal agents for potential use in children with invasive aspergillosis (listed alphabetically)

Agents	Route of administration and dosages	Comments
Amphotericin B deoxycholate	IV. 1.0–1.5 mg/kg; infuse as single dose over 2 h	
Amphotericin B lipid complex (Abelcet)	5 mg/kg; infuse over 2 h	
Anidulafungin	IV. Load 1.5–3 mg/kg once, then 0.75–1.5 mg/kg/day	Limited pediatric data; not for CNS disease
Liposomal amphotericin B (AmBisome)	IV. 3–5 mg/kg; infuse over 1–2 h	Acceptable front-line therapy
Caspofungin	IV. 70 mg/m^2 loading dose, then 50 mg/m^2 once daily	Not for CNS disease
Itraconazole	IV; PO. 5–10 mg/kg divided into 2 doses	Mild infections in selected older individuals
Micafungin	IV. 4–12 mg/kg once daily (higher doses needed for patients <8 years of age	Not for CNS disease
Posaconazole	PO. Limited data; see adult dosage for children 13 years and older	Limited pediatric data
Voriconazole	IV. 7 mg/kg IV q12h on day 1, then 7 mg/kg IV q12h. PO 10 mg/kg every 12 h for 1 day, then 7 mg/kg every 12 h.	Preferred treatment in most cases; more PK data needed for infants and young children

Adapted from: Recommended doses of parental and oral antifungal drugs. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, eds. *Red Book: 2009 Report of the Committee on Infectious Diseases*. 28th Ed. Elk Grove Village, IL: American Academy of Pediatrics; 2009, pp. 767–783.

recommendation can be made for the later years of lung transplantation due to the lack of existing data.

- Use of serum galactomannnan for the screening of invasive apsergillosis is not recommended in lung transplant recipients (II-2).
- The positive predictive value of BAL galactomannan as a screening tool for the diagnosis of IA is best in centers with higher incidences of IA (II-2).
- No recommendation can be made about reinitiating of prophylaxis after 1 year of lung transplant.
- With regards to the choice of the drug and duration of antifungal prophylaxis against *Aspergillus* in lung transplant recipients with risk factors stated in Table 2 following recommendation are made.
- Inhaled amphotericin B or lipid preparation of amphotericin B can be used post operatively in patients with a risk of developing IA. Caution should be exercised in single lung transplant recipients (II-2). The dosage of amphotericin B may vary from 20 mg tid to 25 mg q day. The duration of prophylaxis should be guided by interval airway inspection, respiratory surveillance fungal cultures and clinical risk factors.
- Nebulized ABLC can be used at a dose of 50 mg once every 2 days for 2 weeks and then once per week for at least 13 weeks (II-3).
- Nebulized ambisome can be administered as 25 mg three times/week for 2 months, followed by weekly administration for 6 months and twice per month thereafter (II-3).
- In high risk lung transplant recipients systemic antifungal agents active against *Aspergillus* such voriconazole or itraconazole can be used for prophylaxis. The recommended duration is 4 months (II-2). Liver enzymes should be monitored to assess the hepatic toxicity. Further continuation of the prophylaxis should be guided by the continued existence or emergence of a new risk factor of IA upon evaluation of transplant recipients.
- Screening for squamous cell cancer should be considered in patients receiving voriconazole prophylaxis.

Heart transplant recipients

- Targeted prophylaxis with itraconazole or voriconazole 200 mg bid for 50–150 days may be considered in recipients with one or more risk factors as stated in Table 2 (II-3).

Liver transplant recipients

- Targeted prophylaxis with a lipid formulation of amphotericin B in dosages ranging from 3 to 5 mg/kg/d (II-2) or an echinocandin (II-3) may be considered in patients with high-risk factors as stated in Table 2.

Other solid organ transplant recipients

There are insufficient data to routinely recommend anti-*Aspergillus* prophylaxis in other solid organ transplant recipients.

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Special Article

Cryptococcosis in Solid Organ Transplantation

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Abbreviations: BAL, bronchoalveolar lavage; CGB, canavanine glycine bromothymol blue; CNS, central nervous system; CSF, cerebrospinal fluid; IRIS, immune reconstitution inflammatory syndrome; MIC, minimum inhibitory concentration; SOT, solid organ transplant.

Epidemiology and Risk Factors

Cryptococcosis is the third most commonly occurring invasive fungal infection in solid organ transplant (SOT) recipients. Approximately 8% of invasive fungal infections in SOT recipients are due to cryptococcosis (1). The overall incidence of cryptococcosis in SOT recipients ranges from 0.2% to 5% (1,2). Cryptococcosis is typically a late-occurring infection; the median time to onset usually ranges from 16 to 21 months posttransplantation (1,3,4). The time to onset is earlier for liver and lung (<12 months) compared to kidney transplant recipients and this may be due to a higher intensity of immunosuppression in the former subgroups (4).

As in most other hosts, cryptococcal disease in SOT recipients is considered to represent reactivation of quiescent infection (5,6). Epidemiological investigations suggest that acquisition of primary infection following transplantation also occurs (7,8). Isolates from a pet cockatoo and a renal transplant recipient with cryptococcosis showed identical genotypic profile suggesting recent acquisition of this yeast (9). Infection with *Cryptococcus* is thought to be caused by inhalation of the organism, either in yeast form or perhaps as basidiospores, from an environmental source such as bird droppings or soil. Although rare, cases of transmission from donor organ and tissue grafts have also been recognized (10–13). Donor-derived cryptococcosis should be considered when diagnosis occurs in the recipient within 30 days of transplant, cryptococcosis is diagnosed in more than one organ recipient from a single donor or *Cryptococcus* is documented at the surgical or graft site (14).

Calcineurin-inhibitors remain the mainstay of immunosuppression in SOT recipients in the current era. These agents do not appear to influence the incidence, but may affect the manifestations of cryptococcal disease (3). Patients receiving a calcineurin-inhibitor-based regimen were less likely to have disseminated disease and more likely to have cryptococcosis limited to the lungs (4). Anticryptococcal activity of these agents that target the fungal homologs of calcineurin was considered to account for these findings (4,15). Corticosteroids are associated with an increased risk of cryptococcosis in all non-HIV infected hosts (16–19); however, the precise daily dose that confers a higher risk in SOT recipients remains unknown (20). Also, cirrhotic patients are at an increased risk for disseminated cryptococcosis rather than local pulmonary infection (16). T-cell depleting antibodies such as alemtuzumab are increasingly employed as induction therapy or as treatment of rejection in SOT recipients (21). Alemtuzumab causes profound lymphocyte depletion of CD4+T cells which may last several months. Employment of more than one dose of alemtuzumab or antithymocyte has been associated with an increase in the risk for cryptococcosis (21). The cumulative incidence of cryptococcosis was 0.3% in SOT recipients who did not receive alemtuzumab or antithymocyte globulin, 1.2% in those who received a single dose, and 3.5% in the patients who received $\geq$1 doses of these agents ($p = 0.04$) (21). Invasive fungal infections occurred more frequently in SOT recipients who received alemtuzumab as antirejection as opposed to induction therapy (22).

While *C. neoformans* var *grubii* (serotype A) has no particular geographic predilection and causes most infections in SOT recipients, (23) *C. neoformans* var *neoformans* (serotype D) is prevalent in Northern Europe (18). *Cryptococcus gattii,* previously regarded as a tropical and subtropical fungus, has emerged in the Pacific Northwest in the United States and British Columbia, Canada (24,25). *C. gattii* infects mostly nonimmunocompromised hosts, causes cryptococcomas more frequently than *C. neoformans*, and may require prolonged antifungal treatment (24,25). The organism has been characterized into four genotypes through multilocus sequence typing: VGI, VGII, VGIII, and VGIV (26). The VGII genotype has been further characterized into VGII a, b and c. The *C. gattii* that is endemic in

tropical and subtropical regions is mostly VG1 (27). The outbreaks in Oregon and Vancouver island are genetically different isolates of VGIIa (28). The incubation period of *C. gattii* disease in Vancouver Island and Pacific Northwest US has been documented to be ~6 months (29). The Oregon subtype (VGIIc) has currently been associated with 70% mortality in SOT recipients and is likely to have a high fluconazole minimum inhibitory concentration (MIC) (30,31).

Clinical findings

Cryptococcosis usually manifests as CNS disease (meningitis) or pneumonia, but can affect multiple sites, including skin and soft tissues, the prostate gland, liver, kidney, bones, and joints. Pulmonary disease ranges from asymptomatic colonization or infection to severe pneumonia with respiratory failure. Radiographic findings are nonspecific and include nodular opacities or masses and less often consolidation or effusions (32–34). Isolated pulmonary disease may occur in 33% of SOT recipients (4). Approximately 50–75% of SOT recipients with cryptococcosis have extrapulmonary disease or CNS involvement (3,4,20,35). It has been reported that 61% of SOT recipients had disseminated disease; 54% had pulmonary and 8.1% had skin, soft-tissue or osteoarticular disease (4). Liver as opposed to other types of SOT recipients had a sixfold higher risk for developing disseminated disease. Up to 33% of the SOT recipients with cryptococcosis had fungemia (3,4,36). Patients with CNS disease in one report were more likely to be fungemic than those without CNS disease (37). Cutaneous cryptococcosis may present with papular, nodular, or ulcerative lesions or as cellulitis, with the majority found on the lower extremities and associated with CNS disease (38,39). While cutaneous lesions largely represent hematogenous dissemination, the skin has also been identified as a portal of entry of *Cryptococcus* and a potential source of subsequent disseminated disease in SOT recipients (8).

Mortality in SOT recipients with cryptococcosis has ranged from 33% to 42% and may be as high as 49% in those with CNS disease (3). Overall mortality in SOT recipients with cryptococcosis in the current era is 14% (4). In a case series of 28 SOT recipients with cryptococcal meningitis, mortality was associated with altered mental status, absence of headache, and liver failure; the latter was an independent predictor for death (36). In contrast, receipt of calcineurin-inhibitor agents was independently associated with a lower mortality, but renal failure at baseline with higher mortality (4). Improved outcomes with the use of calcineurin-inhibitor agents may be attributable in part to their synergistic interactions with antifungal agents (40).

Diagnosis

An important aim of diagnosis of cryptococcosis in SOT recipients is to determine the site and extent of disease, as this will help to dictate management (41). All SOT recipients with suspected or documented cryptococcosis should undergo a thorough evaluation for extrapulmonary cryptococcosis, including lumbar puncture and blood and urine cultures (II-2). Blood cultures for *Cryptococcus* may be positive in up to 45% of patients with meningitis (37). If isolated pulmonary disease is suspected, a bronchoalveolar lavage (BAL) with or without biopsy should be considered and is important when eliminating other causes (42) (II).

A lumbar puncture should be performed in all SOT patients with suspected cryptococcosis. Opening pressure should be recorded, and large volume (>1 mL or 20 drops) cerebrospinal fluid (CSF) removal should occur and be sent for Gram's stain, cell count, protein, glucose and cryptococcal antigen testing (37,42,43) (II-1). CSF cryptococcal antigen testing is more sensitive and specific than India ink staining or fungal culture. Titers are higher with leptomeningeal than intraparenchymal brain lesions (3,4,36). Cryptococcal antigen testing from serum is also a very sensitive test (90%) for initial diagnosis of infection, but titers among SOT recipients are normally lower (usually <1:1024) than in HIV-infected patients (36,44). Cryptococcal antigen testing can identify *C. gatti* infections but has lower titers than with *C. neoformans* (30,45) and its sensitivity decreases to less than 25% with other nonneoformans species such as *C. laurentii* and *C. albidus* (46–48). Serum cryptococcal antigen titers are higher in patients with disseminated and CNS disease than in those with isolated pulmonary disease (33,34,37). Serologic tests such as *Aspergillus* galactomannan and β-D-glucan are not effective for the diagnosis of cryptococcosis.

Brain CT imaging may be performed prior to lumbar puncture to determine the presence of mass lesions or hydrocephalus, but has suboptimal sensitivity for evaluating cryptococcomas (42). Up to 33% of patients may have cryptococcomas on presentation and MRI is more sensitive than CT imaging for detecting these lesions. Cerebral cryptococcomas are more common in patients with *C. gattii* infection than in patients with *C. neoformans* infection. Mortality is higher in patients with intraparenchymal lesions than with meningeal disease alone (30,37,49).

Extraneural cryptococcosis can occur in the skin, prostate gland, liver and kidney. Biopsies with culture of tissues will confirm the diagnosis. On routine hematoxylin and eosin staining of tissues, *C. neoformans* is difficult to identify. However, Gomori-methenamine silver or periodic acid-Schiff staining allows for identification; the organism can be recognized by its oval shape, and narrow-based budding. With the use of mucicarmine staining, the cryptococcal capsule will stain rose to burgundy in color and help differentiate *C. neoformans* from other yeasts, especially *Blastomyces dermatiditis* and *Histoplasma capsulatum* (50).

Prostatic and kidney disease may present as yeast in the urine and clinical suspicion is frequently needed to make

this diagnosis (41,51). Diagnosis of pulmonary disease is frequently made by the detection of the yeast in BAL specimens. Cryptococcal antigen testing is also useful, with a recent study in SOT recipients reporting positivity in 83% of patients with any pulmonary involvement (34). Patients with cryptococcosis limited to the lungs were less likely to have a positive antigen than those with concomitant extrapulmonary disease (34).

Identification of cryptococcal species

With the emergence of *C. gattii* in the Pacific Northwest USA, Canada, and other areas, the importance of identification of the cryptococcal species has increased, as it may affect the choice of antifungal therapy (52). Use of canavanine glycine bromothymol blue (CGB) agar will help to differentiate *C. neoformans* from *C. gattii* colonies and should be considered for patients with endemic exposure or clinical findings suspicious for *C. gattii* infection (II). Presently, serotyping of isolates is being performed using agglutination test kits or immunofluorescence assays (53,54). Currently under investigation at research laboratories are the experimental use of rapid technologies such as polymerase chain reaction (PCR), loop-mediated isothermal DNA amplification, high resolution melt analysis and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) (52,55–57) (III).

Susceptibility testing

Antifungal susceptibility testing of *C. neoformans* has been standardized by the Clinical and Laboratory Standards Institute (CLSI), but to date the relationship of MIC to clinical outcomes has not been well studied (58). Presently, the routine use of antifungal susceptibility testing for cryptococcal infections is not recommended. However, several *C. gattii* genotypes may be associated with increased fluconazole MICs. In patients who have documented *C. gattii* infection, or for those with relapsed infection, testing is recommended for flucytosine and azole medications (31,42,53,59). Testing is usually available in a specialty laboratory.

Treatment

There have been no randomized controlled trials of cryptococcosis treatment in SOT recipients. Treatment recommendations have been extrapolated from clinical trials among HIV patients and from data collected retrospectively in SOT recipients. The recommendations herein are consistent with revised guidelines from the Infectious Diseases Society of America (IDSA) (42). Choice of antifungal therapy is dependent on site and extent of disease, net state of immunosuppression and severity of illness. Distinguishing between disseminated disease and localized pulmonary and asymptomatic disease is important prior to initiating therapy. This requires a thorough evaluation for CNS disease with a lumbar puncture (37,42,49,60,61) (II-2). Table 1 summarizes antifungal therapy in SOT recipients.

Table 1: Antifungal therapy for cryptococcal disease in solid organ transplant recipients

Meningoencephalitis or disseminated disease	
Induction	Duration
Preferred therapy	
Liposomal amphotericin B 3–4 mg/kg/day or Amphotericin B lipid complex 5 mg/kg/day plus flucytosine 100 mg/kg/day[1]	Minimum of 2 weeks
Alternative therapy	
Liposomal amphotericin B 3–4 mg/kg/day or Amphotericin B lipid complex 5 mg/kg/day	Minimum of 4–6 weeks
Consolidation	
Fluconazole 400–800 mg/day	8 weeks
Maintenance	
Fluconazole 200–400 mg/day	6–12 months
Pulmonary Disease	
Asymptomatic or mild-to-moderate disease	
Fluconazole 400 mg/day	6–12 months
Severe pulmonary disease, or azole use not an option	
Same as for CNS disease	

[1]Dosages of flucytosine and fluconazole outlined above are in the absence of renal insufficiency. Both require dose reduction for renal insufficiency. Monitoring of flucytosine levels is recommended (58,58).

Note: Patients with asymptomatic pulmonary disease require antifungal therapy. Disseminated disease must be excluded in all patients. Those with disseminated disease, diffuse pulmonary infiltrates, and acute respiratory failure should be treated with the same regimen as Cryptococcal meningoencephalitis. Synergistic interactions of antifungal agents with a calcineurin inhibitor may improve outcomes (4,40).

In patients with CNS disease, disseminated disease or severe respiratory disease, fungicidal therapy with a polyene and flucytosine is recommended (41,42,62) (I). A lack of flucytosine as induction therapy has been shown to be an independent risk factor for mycologic failure at week 2 in SOT patients (62). The use of a lipid formulation of amphotericin B is preferred over amphotericin B deoxycholate, as nephrotoxicity is a more common complication in patients receiving amphotericin B deoxycholate. Moreover, many transplant recipients already have baseline renal dysfunction and may be receiving other nephrotoxic agents such as calcineurin inhibitors or antibiotics such as vancomycin or aminoglycosides (37,63–65). Additionally, mortality at 90 days in SOT recipients with CNS cryptococcosis was lower with the use of lipid formulations of amphotericin B compared to amphotericin deoxycholate (65). To avoid adverse effects of flucytosine, including bone marrow suppression and nephrotoxicity, monitoring and maintenance of flucytosine levels (2-h postdose level of 30–80 μg/mL) are recommended (58,62).

The recommendations for the management of neurological disease, disseminated cryptococcosis or severe pulmonary disease is induction therapy with liposomal amphotericin B (3–4 mg/kg/day) OR amphotericin B lipid complex (5 mg/kg/day) plus flucytosine (100 mg/kg/day

in 4 equally divided doses every 6 hours based on creatinine clearance) for a minimum of 14 days followed by consolidation with fluconazole (400–800 mg/day) for 8 weeks and, finally suppression with fluconazole (200–400 mg/day) for 6 to 12 months (42) (II-2). Extended courses of fluconazole suppression may be required for patients based on clinical progress or net status of immunosuppression (III).

The recommendation for patients with focal pulmonary and incidentally detected pulmonary disease in otherwise asymptomatic patients is fluconazole 400 mg/day for 6–12 months. (II-3) Disseminated disease should always be excluded with a lumbar puncture and blood and urine cultures (3,42) (II-2). *C. neoformans*-positive cultures from sterile and nonsterile sites (i.e. sputum) warrant treatment even if the patient is asymptomatic. This is true in lung transplant recipients where *Cryptococcus* may be colonizing the donor allograft and without treatment, may become invasive disease in the presence of immunosuppression (11). Relapse of cryptococcosis after 6 months of fluconazole maintenance is very uncommon based on available data and thus the recommendations are for 6–12 months of therapy (66) (II-3). However, discontinuation of therapy must be made on the basis of signs, symptoms and level of immunosuppression. With careful monitoring of the drug interactions between fluconazole and calcineurin inhibitors, long-term fluconazole therapy in SOT recipients has proven to be very safe (67).

The use of extended-spectrum azoles, such as voriconazole, itraconazole, and posaconazole do not offer benefit over fluconazole for treatment of *C. neoformans* infection. These agents are more expensive, have more potential drug interactions with immunosuppressive agents and data in HIV-infected patients showed that itraconazole was inferior to fluconazole in the clearance and maintenance phases of cryptococcosis (68–70). In contrast, some genotypes of *C. gattii* appear to have reduced susceptibility to fluconazole. *In vitro* data suggest that the extended-spectrum azoles have excellent potency against with this species and may offer an oral alternative when transitioning from induction to maintenance therapy (31,53) (III). Close monitoring of tacrolimus levels is needed with co-administration of azoles, and dose-reduction should be considered at the time of azole initiation (see chapter 32 for specific recommendations). Voriconazole or posaconazole should preferably not be co-administered with sirolimus given potential for significant elevation of sirolimus levels (68,71,72).

Adjunctive therapies

Interferon-γ has been utilized as an adjunct to antifungal therapies in HIV-infected patients; however, other than one case report there are no large randomized clinical trial data available. Interferon-γ cannot be recommended due to concerns that it may induce organ rejection in this population (4). Heat shock protein 90 (hsp90) recombinant antibodies are in development and *in vitro* studies show that in combination with amphotericin B may increase killing of organisms. Currently here are no clinical trial data to support its use in treating SOT recipients (73).

Immunosuppression

An important factor in the management of cryptococcosis is the concurrent attention to the degree of immunosuppression. Whenever possible, a reduction in the net state of immunosuppression should occur during therapy, but this can be complicated if the patient has received profound T-cell depleting agents such as alemtuzumab or thymoglobulin (74). The aim is a gradual tapering of immunosupression, preferably with corticosteroids first, while on antifungal therapy such that there is eradication of infection with preservation of allograft function. A rapid reduction in immunosuppression may cause adverse acute organ rejection or emergence of IRIS, although no data are available to suggest the optimal methods of reduction in immunosuppression (75).

Complications

Immune reconstitution inflammatory syndrome (IRIS)

It is increasingly appreciated that restoration of host immunity, particularly if abrupt, may have adverse sequelae and when a threshold is reached, the host can become gravely ill with symptomatic disease due to immune reconstitution (76). Rapid reduction of immunosuppressive therapy in conjunction with initiation of antifungal therapy in SOT recipients may lead to the development of immune reconstitution inflammatory syndrome (IRIS), the clinical manifestations of which mimic worsening disease due to cryptococcosis (Table 2) (66,77). IRIS may present as lymphadenitis, cellulitis, aseptic meningitis, cerebral mass lesions, hydrocephalus or pulmonary nodules (66,77). Clinically, CNS IRIS appears to have less inflammation and has been found on lumbar puncture to be associated with protein levels $\leq$50 mg/dL and less than 25 white cells/μL (78). In kidney transplant patients, development of IRIS has been temporally associated with allograft loss (79). The overall probability of allograft survival following cryptococcosis in kidney transplant recipients was significantly lower in patients who developed IRIS compared to those who did not (79).

Immunosuppressive agents administered to transplant recipients such as calcineurin-inhibitors and corticosteroids exert their effect by preferentially inhibiting Th1 (IL-2 and IFN-γ) compared to Th2 (IL-10) responses (80,81). Tacrolimus inhibits Th1 to a greater extent than cyclosporine A (82,83). The biologic basis of IRIS in SOT recipients is believed to be reversal of a Th2 to Th1 proinflammatory response upon withdrawal or reduction of immunosuppression. A potential role of Tregs and Th17 regulatory pathways in the pathogenesis of IRIS in SOT

Table 2: Features of IRIS in patients with cryptococcosis (III)

1. New or worsening appearance of any of the following manifestations:
   (a) CNS: Clinical or radiographic manifestations consistent with inflammatory process, such as contrast enhancing lesions on neuroimaging studies (CT or MRI); CSF pleocytosis, defined as >5 white blood cells; or increased intracranial pressure, that is, opening pressure ≥20 mm of water (with or without hydrocephalus).
   (b) Lymph nodes, skin or soft tissue lesions, for example, cellulitis or abscesses.
   (c) Pulmonary, for example, nodular, cavitary, mass lesions, pleural effusions (detected by chest radiography or CT).
   (d) Other focal tissue involvement with histopathology showing granulomatous lesions

and

2. Symptoms occurred during receipt of appropriate antifungal therapy and could not be explained by a newly acquired infection.

and

3. Negative results of cultures for *C. neoformans* during the diagnostic workup for the inflammatory process.

Note: Table constructed from references (75,78,79).

recipients has also been proposed (84). Potent T cell lymphocyte depleting agents such as alemtuzumab have also been recognized as a risk factor for IRIS (85).

An estimated 5–11% of SOT recipients with cryptococcosis may develop IRIS, typically between 4 and 6 weeks after initiation of antifungal therapy (66,86). In one study, patients who developed IRIS were more likely to have received potent immunosuppression comprising a combination of tacrolimus, mycophenolate mofetil, and prednisone when compared to patients without IRIS ($p = 0.007$). Additionally, cases with IRIS versus those without were more likely to have disseminated cryptococcosis (79). These data are consistent with those in HIV patients where more profound immunosuppression at the onset of infection and greater severity of infection (or disseminated disease) correlated with an increased likelihood of IRIS after antiretroviral therapy. There are no laboratory markers or clinical criteria that can diagnose IRIS reliably or distinguish this entity from worsening cryptococcosis (49,75).

There is no proven therapy for IRIS. Minor manifestations may resolve spontaneously within a few weeks. Modifications in antifungal therapy are not warranted unless viable yeasts are documented in culture. Anti-inflammatory drugs such as corticosteroids have been employed anecdotally with success in *Cryptococcus*-associated IRIS in SOT recipients (77,86). Corticosteroids in doses equivalent to 0.5 to 1 mg/kg of prednisone may be considered for major complications related to inflammation in the CNS or severe manifestations of pulmonary or other sites (77) (III). The efficacy of thalidomide and other nonsteroidal anti-inflammatory agents remains unproven.

Elevated intracranial pressure (ICP) management

Cryptococcal infection of the brain causes a significant inflammatory response with the development of a film over the pial layer preventing the absorption of CSF, with subsequent elevation of intracranial pressure potentially leading to hydrocephalus, blindness, deafness or death (43). A significant factor related to patient morbidity and mortality is failing to address the raised intracranial pressure. Initial opening pressure should be recorded and if >25 mmHg, a large volume fluid removal should be performed to reduce the intracranial pressure to normal levels. If the initial opening pressure is >25 mmHg, lumbar puncture should be performed daily until opening pressure is < 25 mmHg (III). If the ICP remains high (>25 mmHg) and symptoms persist, consider temporary lumbo-peritoneal or external ventricular drains to monitor CSF pressure (III). Permanent ventriculo-peritoneal shunting should be considered in patients who have received appropriate antifungal therapy and if other conservative measures to reduce ICP have failed (42,43,61).

Antifungal prophylaxis

We do not currently recommend that SOT recipients receive routine antifungal prophylaxis against cryptococcosis, as there is no specific high-risk group that has been identified. In SOT recipients with previous cryptococcosis needing enhanced immunosuppression, consideration for resuming secondary prophylaxis can be made on an individual basis. For SOT recipients who experience graft failure after cryptococcosis, the ideal timing of retransplantation is unknown. However, in kidney transplant recipients, where there is the possibility of a hemodialysis bridge, it is reasonable to consider if they have received a year of antifungal therapy, have no signs or symptoms attributable to active cryptococcal disease and negative cultures from the original site of infection. In those SOT populations where no bridging option is available, we recommend that induction therapy is completed, all sites that yielded positive cultures have cleared and the cryptococcal antigen titer should be optimally declining. In these cases, secondary fluconazole prophylaxis should be considered for at least 1year period (34,67). For pretransplant patients with active cryptococcal disease, our recommendations are the same as for those patients with graft failure (III).

Future research directions: The emergence of *C. gattii* infections has led to many future research endeavors. These include the development of rapid diagnostic techniques such as PCR testing and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) to differentiate *C. gattii* and *C. neoformans* more rapidly and accurately from a variety of clinical specimens. These tests are currently only performed in major research laboratories. Also, additional clinical studies are underway to better define the clinical outcomes of SOT recipients with *C. gattii* versus *C. neoformans* infections. Lastly, a new water soluble azole antifungal agent, isavuconazole, is currently in

phase 3 studies (Cinicaltrials.gov identifier: NCT00634049) as salvage therapy of invasive fungal infections. This compound may have activity against fluconazole resistant cryptococcal strains, making it a potential therapeutic option in the future.

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Special Article

Endemic Fungal Infections in Solid Organ Transplantation

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Key words: Antifungal, azole, blastomycosis, coccidioidomycosis, fungal infection, histoplasmosis

Abbreviations: AIDS, acquired immune deficiency syndrome; ARDS, adult respiratory distress syndrome; BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; CMV, cytomegalovirus; CNS, central nervous system; CT, computed tomography; DNA, deoxyribonucleic acid; EIA, enzyme immunoassay; GMS, Grocott methenamine-silver; HIV, human immunodeficiency virus; HPLC, high performance liquid chromatography; IDSA, Infectious Diseases Society of America; IgG, immunoglobulin G; IgM, immunoglobulin M; KOH, potassium hydroxide; PAS, periodic acid-Schiff; PCR, polymerase chain reaction.

Introduction

The endemic mycoses, histoplasmosis, blastomycosis and coccidioidomycosis, are fungal diseases prevalent in specific geographic regions. The environment is the main source for exposure to these fungi, with the respiratory tract serving as the primary portal of entry into the human body. Although the epidemiologic and clinical features of each infection are unique, some characteristics are shared. Symptomatic disease occurs in both the immunocompetent and immunocompromised host with the severity of infection correlating with underlying immune status. Cell mediated immunity plays an important role in the susceptibility to and control of these infections. Recently reports of endemic fungal infections occurring in organ transplant recipients have been increasing (1,2). In addition, increased recognition of donor-derived fungal infections in recipients prompted the recent development of guidelines discussing the unique characteristics, evaluation and approach to their management (3). Although the true incidence of these infections among this population is unknown, estimates suggest it is <5% (4–6). The focal geographic distribution of the endemic fungi and indolent symptoms of infection frequently lead to diagnostic delays and contribute to increased morbidity and mortality (5,7). Knowledge of the epidemiology, pathogenesis, clinical manifestations, diagnostic methodologies and therapy will enable clinicians to more effectively identify and manage transplant recipients with endemic mycoses.

Blastomycosis

Epidemiology and pathogenesis

Blastomycosis refers to disease caused by the fungus, *Blastomyces dermatitidis*, which occurs more often in persons living in the midwestern, southeastern and south central United States, particularly along the Ohio-Mississippi River Valley (8). *B. dermatitidis* is also found in the soil of northern New York and Canadian provinces that border the Great Lakes and St. Lawrence Seaway. Recent studies have shown an increase in the incidence of blastomycosis in some of these endemic regions (6,9,10). The majority of reported cases of blastomycosis after organ transplantation have occurred in patients residing in endemic areas (1,11).

Historically blastomycosis has been a disease that affects immunocompetent hosts, predominantly men with outdoor occupations or recreational activities involving soil exposure, although many individuals have no apparent source for infection (8,12). In the immunocompromised host, it may be associated with severe pneumonia or disseminated infection, particularly in patients with diabetes, HIV or those receiving chronic corticosteroids or cytotoxic chemotherapy (13). Unlike coccidioidomycosis or histoplasmosis, blastomycosis has been described infrequently as an opportunistic pathogen after solid organ transplantation (1,6,11). In one review, the cumulative incidence posttransplant was only 0.14% during a 16-year period (1). Reports of blastomycosis after renal, cardiac, hepatic and lung transplantation have been published with disease onset ranging from 1 week to 20 years posttransplant (1,6,11,13,14). Blastomycosis in this population may result from primary infection, reactivation of latent disease or conversion of subclinical infection to symptomatic disease after organ transplantation (1).

To date, there are no reports of donor transmission of *B. dermatitidis*.

Infection with *B. dermatitidis* results from inhalation of fungal spores into pulmonary alveoli. Cell-mediated immunity limits progression of *B. dermatitidis* infection in the lungs. If impaired, pneumonia or extrapulmonary dissemination may develop. As such, the majority of transplant recipients who develop blastomycosis are concurrently taking two or more immunosuppressive agents (1,11,13,14). Cytomegalovirus (CMV) infection can also impair cellular immune defenses and, although its exact role is unclear, in one study one-third of patients with posttransplant blastomycosis were co-infected with CMV (1). There are no data to suggest that acute rejection increases the risk for blastomycosis (1). Though less common, blastomycosis arising from primary cutaneous inoculation is also described (15).

Clinical presentation

Pneumonia with or without extra-pulmonary dissemination is the most common presentation of blastomycosis in solid organ transplant recipients (1,6,11,13,14). Although the time from transplantation to development of blastomycosis is variable, the median time ranges from 1 to 2 years posttransplant (1,11). Median time from symptom onset to diagnosis is 14 days (range 3–90 days; Ref.11).

Though nearly all transplant associated blastomycosis infections involve the lungs, the spectrum of pulmonary infection ranges from subclinical disease to acute or chronic pneumonia (11,16). Acute pulmonary blastomycosis is a flu-like illness which develops 30–45 days after initial infection. Typical symptoms include fever, chills, arthralgias and productive cough with an accompanying alveolar or lobar infiltrate on chest radiography. In solid organ transplant recipients the most common presenting symptoms are fever and cough (1). These symptoms are not specific for blastomycosis and, not uncommonly, patients may be misdiagnosed with bacterial pneumonia. Radiographic findings in transplant patients include lobar or interstitial infiltrates, a reticulonodular pattern with mediastinal adenopathy or lung cavities (14). A subset of individuals with pulmonary blastomycosis develop fulminant multi-lobar pneumonia and rapid progression to the adult respiratory distress syndrome (ARDS) and respiratory failure (17). In patients who underwent solid organ transplantation, diffuse bilateral pneumonia was the most common radiographic finding; 78% developed respiratory failure and ARDS complicated 67% of cases. The majority of patients that developed ARDS died (1).

Chronic pulmonary blastomycosis may follow acute infection with more prolonged symptoms such as fever, night sweats, anorexia, weight loss, productive cough, pleurisy and occasional hemoptysis. Chest radiography or a computed tomography (CT) scan may show a mass-like infiltrate or cavitary pneumonia mimicking tuberculosis or malignancy (8). Although blastomycosis usually remains localized to the lungs, 25–40% of those infected will develop extra-pulmonary dissemination manifested by cutaneous, osteo-articular, genitourinary or central nervous system (CNS) disease (8). In solid organ transplant patients, disseminated disease was observed in 36–50%, with skin being the most common site of involvement outside the lungs (1,11,13,14). CNS blastomycosis is rare in the setting of organ transplantation; though has been reported (11,13). Fungemia is rare.

Diagnosis

A presumptive diagnosis of blastomycosis is made by identifying the organism in sputum, bronchoalveolar lavage (BAL) fluid or tissue specimens; growth in culture confirms the diagnosis (16). In one study of solid organ transplant patients, culture of sputum or BAL fluid was 100% sensitive for diagnosing pulmonary blastomycosis (1). Alternatively other sites of involvement, such as skin, bone, synovial fluid, brain tissue or cerebrospinal fluid (CSF) may be sampled for histopathologic examination and culture. Gastric lavage cultures may also be a useful diagnostic technique, particularly in pediatric patients, as it may avert the need for more invasive diagnostic techniques (18). The characteristic fungal forms seen on direct examination are large (8–15 μm), broad-based budding yeast. A potassium hydroxide (KOH) wet mount or special fungal stains, may enhance visualization of *B. dermatitidis* in body fluids or tissue. Micro-abscesses and noncaseating granulomas are often observed on histopathology since the initial inflammatory response to *B. dermatitidis* is both neutrophilic and cell-mediated.

A second generation assay for detection of *Blastomyces* antigen in urine, blood and BAL fluid is available and can often lead to more rapid diagnosis than culture (19–21). In patients with blastomycosis, sensitivity of this assay is over 90%. Specificity is 99% in individuals with nonfungal infections and healthy subjects, however, cross-reactivity occurs in 96% of patients with histoplasmosis (19–21). The utility of this test has not been well established in solid organ transplant recipients. Limited data suggest sera from patients with proven blastomycosis tests negative for (1–3)-β-D-glucan (Fungitell®; Ref.22). Currently available serologic tests lack sensitivity and are not useful for diagnosis of blastomycosis.

Treatment

The management of blastomycosis in solid organ transplant recipients follows published guidelines (23). All immunocompromised individuals require treatment and since these patients are more likely to present with severe pulmonary or disseminated infection, amphotericin B is

recommended as first line therapy (III). A lipid formulation, such as liposomal amphotericin B or amphotericin B lipid complex, is preferred because of the reduced potential for nephrotoxicity (23). Amphotericin B is administered for the first 1–2 weeks until clinical improvement is demonstrated at which time transition to oral itraconazole may be acceptable (III) (23). Liposomal amphotericin B is recommended for infection involving the CNS but more prolonged therapy is given, generally 4–6 weeks, before transitioning to azole therapy (III) (23). In some patients with mild pulmonary infection, oral itraconazole may be given as initial therapy but close clinical monitoring is warranted (III). Corticosteroids may be considered as adjunctive therapy in severe blastomycosis-induced ARDS (24).

Fluconazole appears to be less effective for blastomycosis (II-1) and should only be used as second line therapy or in high doses for prolonged treatment of CNS infection (III) (23,25). Oral voriconazole has good CNS penetration and excellent *in vitro* activity against *B. dermatitidis*, thus another option for prolonged therapy of CNS blastomycosis (1,23,26–28). Voriconazole and fluconazole are preferred over itraconazole for CNS infection, given the limited CNS penetration (<1%) and *in vitro* susceptibilities of itraconazole. Data are lacking for posaconazole use in CNS infection. Echinocandins have intermediate to poor *in vitro* activity against *B. dermatitidis* and should not be prescribed (27,28).

The duration of treatment is generally 12 months with resolution of symptoms and signs of infection (III). Consideration may be given to more prolonged treatment courses for organ transplant recipients, although conclusive data are lacking (III) (23). As the *Blastomyces* antigen assay is quantitative, serial measurements can be used to follow treatment response over time both for adult and pediatric patients (11,29). However, using the antigen assay to guide treatment duration, is not well established. In a recent series of 8 transplant associated blastomycosis cases, median time to urine antigen negativity was 22 months (range 10–48 months; Ref.11). Data suggest that relapse of blastomycosis is uncommon after therapy and evidence of cure (1,11).

Pretransplant evaluation

There is no sensitive or specific serologic assay available to diagnose previous exposure to *Blastomyces* or active disease. Careful screening for active infection, including symptom assessment and chest radiography, should be a part of the pretransplant evaluation of patients who live in *Blastomyces* endemic areas. There have been no trials of targeted antifungal prophylaxis for prevention of blastomycosis in organ transplant recipients who reside in endemic regions. At this time primary or secondary antifungal prophylaxis for blastomycosis after solid organ transplantation is not recommended (III).

Coccidioidomycosis

Epidemiology and pathogenesis

Coccidioides species are fungi that thrive in the arid, desert soil of the southwestern United States, particularly the San Joaquin Valley and Sonoran desert of southern California, Arizona and northern Mexico (30,31). Other regions of endemicity include New Mexico, western Texas and parts of Central and South America. Coccidioidomycosis, whether primary or reactivation disease, may also develop in individuals after return from an endemic location or in those without a history of travel to an endemic area. In some cases, exposure to *Coccidioides* occurs when spores are carried to distant locations on fomites or on the surfaces of produce or textiles exported from endemic regions (32). Two species of *Coccidioides* have been identified: *C. immitis* is associated with infection acquired in California and *C. posadasii* with infection acquired outside of California, such as Arizona and New Mexico (33).

Coccidioides spores gain entry into the body when aerosolized from soil and inhaled into the lungs. Increased infection rates have been observed after rainy seasons, dust storms or earthquakes which disrupt soil and enhance the spread of spores. *Coccidioides* is highly infectious; a single inhaled spore may produce infection. Resolution of infection depends ultimately on T cell immune responses (30,34).

Coccidioidomycosis has been described after lung, kidney, heart and liver transplantation with an incidence of 1.4–6.9% in endemic regions (35–40). The majority of these infections are diagnosed within the first year posttransplant, and in most cases, result from primary or reactivation infection. Other risk factors for *Coccidioides* infection in the transplant population include treatment of acute rejection, prior history of coccidioidomycosis and/or positive pretransplant serologies and African American race (37,41). It is unclear whether concomitant immunosuppressing conditions such as diabetes or CMV infection further increase the risk for posttransplant coccidioidomycosis. Donor transmission of *Coccidioides*, has also been described (42–45). In these cases, recipients presented with symptoms within 1 month after transplantation, most with severe infections. Prompt identification of recipient infection and initiation of antifungal prophylaxis in other common donor recipients has led to more favorable outcomes in recent transmission events (41,42).

Clinical presentation

Coccidioidomycosis should be considered in the differential diagnosis of any solid organ transplant recipient with a febrile illness who has traveled to or resides in an endemic area. Clinical manifestations of *Coccidioides* infection in solid organ transplant recipients range from asymptomatic seroconversion to widespread dissemination with multi-organ failure and shock (39). However, unlike

immunocompetent hosts in whom infection is often mild and self-limited, organ transplant patients are more likely to develop severe pneumonia and disseminated infection (39,41). The most common symptoms of pulmonary coccidioidomycosis are fever, chills, night sweats, cough, dyspnea and pleurisy (39). Radiographic findings are varied and may consist of lobar consolidation, pulmonary nodules, mass-like lesions, interstitial infiltrates or cavitary disease (39,41). Pulmonary coccidioidomycosis can progress to severe pneumonia with multilobar involvement, diffuse nodularity, ARDS and respiratory failure, particularly in the setting of immunosuppression (39).

In individuals with coccidioidomycosis, extrapulmonary infection occurs in 1–5%. Risk factors include male gender, African, Filipino or Native American ancestry, pregnancy and other forms of immunosuppression (46). It is unclear whether these factors pose any additional risk for dissemination of *Coccidioides* in solid organ transplant recipients. Extrapulmonary infection usually manifests as cutaneous, osteo-articular or meningeal disease. Widespread dissemination with multi-organ involvement, including graft infection, is common in patients with coccidioidomycosis after organ transplantation (38–41). CNS *Coccidioides* infection, usually presenting as meningitis with headache and/or altered mentation, has been reported in organ transplant recipients and may be fatal (40,47). *Coccidioides* fungemia is an uncommon manifestation of disseminated infection, but is associated with 30 day mortality of 62% (48). Coccidioidomycosis in children presents similarly as in adults, though reactive rashes, including erythema multiforme are more common (49).

Diagnosis

Culture of sputum, BAL fluid or tissue is the gold standard for diagnosis of coccidioidomycosis. Blood, CSF and pleural or peritoneal fluids are less likely to be culture positive. *Coccidioides* may also be diagnosed by histopathologic examination, although this is less sensitive than culture. On direct examination, visualization of the characteristic spherule containing endospores is diagnostic of infection (45). Spherules are not detected by Gram stain, but microscopic identification may be aided by a variety of fungal stains. *Coccidioides* reverts back to the highly infectious mould form when cultured and care must be taken to prevent aerosolization and accidental inhalation in the laboratory. Thus it is imperative to notify laboratory personnel when *Coccidioides* is suspected.

Serologic testing can be useful for diagnosing *Coccidioides* infection when histopathology or cultures are negative. Serologic testing is based on the identification of IgM or IgG antibodies. IgM appears first and can be detected in serum by a tube precipitin method, immunodiffusion, latex agglutination and enzyme immunoassay (EIA) within 1–3 weeks of acute *Coccidioides* infection. IgG follows the IgM response and can also be detected by several methods. Complement-fixing IgG antibodies, which typically appear 2 weeks after infection, can be quantitated to assess the severity of infection; high or rising IgG antibody levels may be seen with worsening pulmonary infection or disseminated disease (46). Conversely, IgG antibody titers should decrease with effective therapy. Diagnosis and management of meningeal coccidioidomycosis requires lumbar puncture for CSF analysis. Because CSF cultures are positive in only 15% of patients with coccidioidal meningitis (50), CSF complement-fixing IgG antibodies are the primary method for diagnosis (47). Immunosuppression can lead to diminished immunoglobulin responses in serum and CSF, and false negative serologic results have been observed in solid organ transplant recipients, complicating test interpretation and diagnosis (40,41,50,51).

Other nonculture based diagnostic methods for detecting coccidioidomycosis include a *Coccidioides* antigen EIA and *Coccidioides* polymerase chain reaction (PCR) testing. The *Coccidioides* antigen EIA (available for urine, serum, BAL and CSF) can be useful in the rapid diagnosis of more severe forms of coccidioidomycosis. Like the *Blastomyces* and *Histoplasma* antigen assays (discussed in other sections), this assay lacks specificity among individuals with other endemic mycoses (52). *Coccidioides* PCR testing of respiratory specimens and CSF is available in some centers and recent reports indicate its promise as a rapid diagnostic method (53,54). The utility of these assays has not been studied extensively in organ transplant recipients.

Treatment

Acute pulmonary coccidioidomycosis may be mild and self-limited in the immunocompetent host and antifungal therapy may be withheld with close clinical monitoring (III) (55). However all patients with underlying immune impairment, including organ transplant recipients, must be treated regardless of the severity of infection (III).

As for blastomycosis, treatment of coccidioidomycosis in the setting of solid organ transplantation follows published guidelines (55). Treatment options for mild to moderate coccidioidomycosis include oral fluconazole or itraconazole (I) (55,56). Amphotericin B, or preferably a less toxic lipid formulation, is generally reserved for severe pneumonia or disseminated infection (III). The decision to treat with oral versus intravenous therapy must be individualized, but symptom severity, respiratory status, extent of infection and the ability to take enteral therapy must be considered.

Alternatively, meningeal coccidioidomycosis may be treated with high dose fluconazole (II-1), which has excellent CSF penetration, but lifelong therapy is necessary to prevent relapse (III). Repeat lumbar puncture during therapy to document improvement in CSF parameters and

a decline in CSF complement-fixing antibodies is recommended (III).

Favorable clinical responses have been demonstrated with voriconazole and posaconazole for treatment of refractory coccidioidomycosis or when toxicity develops to standard therapies (57–59). The echinocandins have variable *in vitro* activity against *Coccidioides* and sufficient clinical data are limited (28,60,61). Lifelong antifungal prophylaxis is recommended for organ transplant recipients once active coccidioidomycosis has been controlled to prevent relapse (46).

Pretransplant evaluation and posttransplant interventions

Preventing *Coccidioides* infection in solid organ transplant recipients is imperative because infection is frequently severe and mortality is high (39,41). The risk of developing coccidioidomycosis after organ transplantation is greater in persons with a past history of infection or positive antibodies for *Coccidioides* before surgery (46,62). During the pretransplant evaluation, clinicians must determine if transplant candidates have a history, even remote, of residence in or travel to an endemic area given the risk for reactivation of latent infection posttransplant. The evaluation should include an assessment of previous or current symptoms consistent with coccidioidomycosis, a chest x-ray and serologic testing. Any evidence of prior or active infection requires evaluation by an infectious diseases specialist, with ultimate clearance for transplant listing determined on a case by case base (III) (46). When possible, organ transplantation should be deferred in patients with active coccidioidomycosis until the infection is clinically, serologically and radiographically quiescent (III) (46,63).

Prophylactic antifungal therapy with fluconazole is recommended for all transplant recipients with a past or recent history of coccidioidomycosis or positive *Coccidioides* serologies before surgery (II-1) (38,46,51). The recommended fluconazole dose (200–400 mg) and duration (6–12 months or lifelong) varies based on the extent of prior/current infection and serology results (38,46). Based on a large retrospective review, universal antifungal prophylaxis for liver transplant recipients who reside in endemic areas for 6–12 months posttransplant is recommended (38). Lifelong antifungal prophylaxis is also recommended for recipients who receive organs from donors with active coccidioidomycosis or positive serologies (III) (46,62). For recommendations specifically addressing donor-derived coccidioidomycosis, we refer the reader to recently published guidelines (3). Though antifungal prophylaxis reduces the risk for posttransplant coccidioidomycosis, it does not eliminate it. Among 100 patients in an endemic area who underwent solid organ transplantation with prior coccidioidomycosis, 94% received antifungal prophylaxis, of whom five experienced reactivated infection. Conversely, of the six patients who did not receive antifungal prophylaxis, none developed reactivation infection (37). Further characterization of risk factors for recrudescent infection requires additional study.

Posttransplant clinical and serologic monitoring of at-risk patients should be performed periodically to assess for evidence of reactivation infection. Because reactivation infection occurs most commonly in the first year after transplantation, an evaluation should be performed every 3–4 months initially, then once or twice yearly thereafter (III) (46).

Histoplasmosis

Epidemiology and pathogenesis

Histoplasmosis is an opportunistic fungal infection caused by the dimorphic fungus, *Histoplasma capsulatum.* Although found in many areas of the world such as South America, India and Bangladesh (64–66), the organism is endemic in the Ohio and the Mississippi River valleys in the United States. The clinical spectrum of infection ranges from a self limited febrile illness to severe multi-organ dysfunction, depending on the size of the host inoculum and immune status of the infected individual. Posttransplantation histoplasmosis is rare, with an estimated incidence of $<1\%$, even in endemic areas (2,11,66,67).

Primary infection occurs via inhalation of *H. capsulatum* mycelia, typically found in high concentrations in excavated soil, avian or bat droppings in endemic areas. Exposure to disrupted soil around construction or agricultural areas, caves where bats reside or buildings inhabited by birds or bats pose particular risk. Intact cellular immunity is critical to containing and eradicating *Histoplasma* infection, thus solid organ transplant recipients are at particular risk for significant infection. Histoplasmosis in transplant recipients can result from a primary infection, reactivation of previous infection, or rarely, transmitted via an infected allograft (11,68–70). Human to human transmission has not been reported.

Clinical presentation

Histoplasmosis was initially described among liver and kidney transplant recipients (71–73), however more recent case series also include heart, lung and kidney-pancreas transplant recipients (2,11,66,67). The illness most commonly presents in an occult manner among transplant recipients, with the burden of disease often out of proportion to the severity of symptoms at initial presentation. Although a spectrum of clinical manifestations have been reported in solid organ transplant recipients, the most common form is progressive disseminated infection, characterized as a subacute febrile illness with radiographic and/or laboratory evidence of extrapulmonary infection. The typical period from onset of symptoms to diagnosis is 2–4 weeks (2,11,66,67). As the infection progresses,

associated clinical findings include hepatosplenomegaly, pneumonia, gastrointestinal involvement, pancytopenia, weight loss, hepatic enzyme elevations, mucosal/skin findings and increased lactate dehydrogenase levels. Any organ can be involved with *Histoplasma* as cases of septic arthritis and prostatitis have been described in transplant recipients (64,74). Unusual presentations in more severely ill patients have also been reported as part of the clinical picture, such as thrombotic microangiopathy and hemophagocytic lymphohistiocytosis (75–77). Most infections occur within the first 1–2 years after transplantation, though patients can present over a broad time range from months to several years posttransplant (2,11,66,67). Reports of histoplasmosis in transplanted children are few. However, in nonimmunosuppressed children, symptoms of histoplasmosis are similar to those that occur in adults, though meningitis accompanying progressive disseminated infection is more commonly seen in infants <2 years (78).

Diagnosis

Confirmation of the diagnosis rests on direct visualization of *H. capsulatum* yeast forms with or without granulomas in involved tissues, culture growth of *H. capsulatum* and/or antigenuria/antigenemia. The availability of newer generation antigen assays has improved early detection through increased sensitivity and specificity, as blood and tissue cultures may take up to 4 weeks to demonstrate growth (79,80). The sensitivity of antigen detection in disseminated histoplasmosis is higher in immunocompromised patients (92%) and in patients with more severe illness than in immunocompetent patients (73%). Though not specifically studied in organ transplant recipients, recent case series suggest the sensitivity is comparable for patients with disseminated disease (2,11,67,81). The sensitivity for detection of antigenemia is similar to that for antigenuria (100% vs. 97%) in disseminated infection (81). The specificity of antigen detection is 99%, however, cross-reactive antigen is detected in 90% of patients with blastomycosis, and has also been reported in the setting of other endemic fungal infections such as sporotrichosis (79,81–83). The degree of antigenuria correlates with the severity of disseminated infection: concentrations of ≥19 ng/mL occurs in 73% of severe cases, 39% of moderately severe cases and 17% of mild cases (81). Antigen detection is similarly useful in children.

For patients with pulmonary histoplasmosis, the diagnostic utility of *Histoplasma* antigen detection in BAL fluid carries a sensitivity of 93%, specificity 97%, a positive predictive value 69%, and negative predictive value 99% (84). False-positive results approximate 10% in cases of pulmonary aspergillosis. Cross reactions can be expected in most cases of pulmonary blastomycosis and a lower proportion of those with pulmonary coccidioidomycosis (85). Conversely, the *Aspergillus* galactomannan test (Platelia™, Bio-Rad Laboratories Inc., Hercules, CA, USA, *Aspergillus* enzyme immunoassay [EIA]) is positive in 50% of serum and BAL samples from patients with histo-plasmosis, which could lead to a false diagnosis of aspergillosis (86,87).

Detection of *Histoplasma* capsulatum DNA in human samples by real-time PCR is under investigation (88,89). Case reports have detailed the use of PCR on whole blood and synovial fluid for detection of histoplasmosis (74,90,91). The use of the (1–3)-β-D-glucan (Fungitell®) test in the diagnosis of histoplasmosis is still under investigation. Limited data suggests a sensitivity of the test is 87–89% in disseminated histoplasmosis cases and a specificity of 68% with controls (22,92). Values also correlated with *Histoplasma* antigenuria levels (22).

Histopathologic examination of biopsy specimens from suspected sites of involvement, including liver, lung, skin, lymph nodes and bone marrow can also expedite diagnosis. Special stains such as hematoxylin and eosin and Wright-Giemsa may aid in visualization of *Histoplasma* in blood or bone marrow while GMS or PAS may enhance visualization in tissue.

Although serologic testing is beneficial for the diagnosis of histoplasmosis in the normal host, the diagnostic utility of serologic testing is variable in organ transplant recipients (80,93). For both immunosuppressed and nonimmunosuppressed individuals from endemic areas, potential background seropositivity confounds test interpretation. In healthy individuals with acute histoplasmosis, *Histoplasma* serology by immunodiffusion and complement fixation become positive in the majority of patients by 6 weeks. Seroconversion or fourfold increase in titers strongly suggests the diagnosis of histoplasmosis. However, the effects of immunosuppressive agents on the humoral immune response may blunt the serologic response to infection, decreasing the sensitivity of the test in this setting (94). Among disseminated cases, antibodies are detected in up to 89% of immunocompetent patients but only 18–30% of solid organ transplant recipients (67,81).

Treatment

As the most common manifestation of histoplasmosis in solid organ transplant recipients is progressive disseminated infection, treatment recommendations will be limited to this form. For more detailed treatment recommendations for other forms of histoplasmosis, the reader is referred to the published 2007 IDSA clinical practice guidelines (95). Antifungal agents with proven efficacy in the treatment of progressive disseminated histoplasmosis include amphotericin B deoxycholate, liposomal amphotericin B (96), amphotericin B lipid complex (96) and itraconazole (97). Echinocandins have no established efficacy (28,98,99). Mild to moderate infection may be treated effectively with itraconazole monotherapy (200 mg twice daily for at least 12 months), (II-2). For moderately severe and severe infection, initial therapy with amphotericin is

recommended, (I) (95). As there are no randomized studies of comparative efficacy in organ transplant recipients, the choice of amphotericin formulation is usually dictated by availability, cost and potential for nephrotoxicity. Amphotericin therapy should be continued for 1–2 weeks or until there is stabilization of the infection, followed by "step-down" therapy with itraconazole (200 mg twice daily) to complete a 12 month total treatment course (95,97). In most instances antigen levels correlate with response to therapy over time, though the use of antigen levels to guide duration of therapy has not been established. Case series suggest antifungal therapy can be successfully discontinued after a prolonged course in some individuals despite a persistently positive antigen assay (11,59,67). Concomitant reduction of immunosuppression, especially calcineurin inhibitors, is also an important treatment adjuvant if possible. Criteria for characterizing mild, moderate and severe illness is not well defined in the literature, but rather rest on clinical impression based on factors such as need for hospitalization, hemodynamic stability, respiratory status, extent of infection and ability to take oral medication. Mortality in solid organ transplant recipients with histoplasmosis ranges from 0% to 13% (11,59,67).

Treatment recommendations for children with progressive disseminated histoplasmosis are similar to adults, though longer initial courses of amphotericin are recommended based on published treatment experience (95). Amphotericin-associated nephrotoxicity is generally less severe in infants and children than adults (100).

Other azole agents, specifically voriconazole (59), posaconazole (101,102), fluconazole (103) and ketoconazole, all demonstrate *in vitro* susceptibility against *H. capsulatum*. Clinical efficacy data are limited to small series and case reports, thus inadequate to establish treatment recommendations. Consequently, these agents are considered second line treatment options for those individuals intolerant of itraconazole (III) (95).

Urine and serum antigen levels typically fall with effective therapy and can be used to follow treatment response and assess for relapse. Antigen levels should be measured before treatment is initiated, at 2 weeks and 1 month, then every 3 months during therapy (II-2). In AIDS patients with disseminated histoplasmosis receiving amphotericin B, antigen levels decline most rapidly during the first 2 weeks of treatment. Whereas in similar AIDS patients treated with itraconazole alone, the decline in antigenuria is slower, occurring later during treatment compared to those treated with amphotericin B. With effective therapy, *Histoplasma* antigenemia decreases more rapidly than antigenuria, providing a more sensitive early laboratory marker for response to treatment (104). In a recent series it was observed that 70% of solid organ recipients with positive *Histoplasma* antigen assays had a negative test by 10 months of treatment (11). Monitoring should continue at least 6 months after therapy is discontinued (80). Persistent low level antigenuria may be observed in organ transplant recipients treated for histoplasmosis, despite complete clinical response and an appropriate duration of therapy. Limited experience suggests that antifungal therapy can be safely withdrawn in this situation with careful monitoring for relapse (2,11,62,67,95). Despite the severity of illness upon presentation, treatment efficacy among infected solid organ transplant recipients in the post-azole era ranges from 80–100% (2,11,67). Mortality in one transplant series was 30%, with mortality attributable to histoplasmosis of 13% (11). Immune reconstitution syndrome has also been described in transplant recipients with disseminated histoplasmosis, mainly related to concomitant reduction of immunosuppression (105,106; Table 1).

Pretransplant evaluation

Pretransplant serologic and/or radiologic screening for prior histoplasmosis infection in endemic areas is not recommended based on the low likelihood of subsequent infection (107). Patients who have recovered from active histoplasmosis infection, with or without treatment, during the 2 years before the initiation of immunosuppression may be considered for itraconazole prophylaxis (200 mg daily), although the efficacy and appropriate duration of prophylaxis is unknown. Serial monitoring of urinary antigen levels in individuals with previous infection should also be performed during periods of intensive immunosuppression to monitor for relapse (III) (95). Management of individuals with incidental *H. capsulatum* detection in the explanted organ or donor tissue is not well established. This scenario occurs primarily in lung transplant recipients, and based on one center's experience, antifungal prophylaxis could be considered (67). For additional recommendations regarding donor-derived histoplasmosis we refer the reader to recently published guidelines (3).

Specific issues related to azole therapy

Drug–drug interactions are an important consideration when prescribing azole antifungal agents to organ transplant recipients. Azoles inhibit hepatic cytochrome P450 enzymes and modify the pharmacokinetics of the many drugs metabolized by this route. Azoles increase serum concentrations of cyclosporine, tacrolimus and sirolimus (108–110), thus drug levels of these immunosuppressive agents must be closely monitored in individuals during the initiation and discontinuation of azole therapy to prevent inadvertent drug toxicity or allograft rejection (Table 2). Preemptive dose adjustment is recommended (I). Other immunosuppressive drugs such as mycophenolate, antithymocyte globulin, prednisone and alemtuzumab have no known drug–drug interactions with azoles (108). Pharmacokinetics of azole agents differ between adults and children in that children have more rapid drug clearance, necessitating more frequent and higher dose administration (100). Because of the potential hepatotoxic effects of azole use, hepatic enzymes should be

Table 1: Summary of recommendations

Infection	Geographic distribution	Diagnosis	Treatment	Suggested duration	Strength of recommendation
Blastomycosis	Midwest, Southeast & South central US	Culture, direct visualization, urine/serum antigen	Mild to Moderate: itraconazole 200 mg BID	Minimum of 6–12 months.	II-1
			Moderately severe or severe: AMB[1]	Minimum of 2 weeks of AMB until clinical improvement, then transition to oral azole.	
Coccidioidomycosis	Southwest US	Culture, direct visualization, serology (serum & CSF), urine/serum/BAL/CSF antigen, PCR	Mild to Moderate: fluconazole 400–800 mg daily (preferred) OR itraconazole 200 mg BID	Minimum of 6–12 months followed by chronic suppressive therapy.	I
			Meningeal disease: AMB[1] or fluconazole 800 mg daily	Lifelong suppression for meningitis	II-1
			Moderately severe or severe: AMB[1]	Minimum of 2 weeks of AMB until clinical improvement then transition to oral azole.	
			Pretransplant or donor infection: fluconazole 200–400 mg daily	Minimum of 6–12 months	II-1 III II-1
Histoplasmosis	Mississippi & Ohio River valleys	Culture, direct visualization, urine/serum/BAL antigen, PCR	Mild to Moderate: itraconazole 200 mg BID	Minimum of 12 months.	II-2
			Moderately severe or severe: AMB[1] for 1–2 weeks or until favorable response, followed by itraconazole 200 mg BID		I

[1]There are no established data to recommend a specific amphotericin B (43) preparation. Lipid formulations are generally preferred for patients at high risk for nephrotoxicity.

Table 2: Summary of azole-immunosuppressant drug interactions

Antifungal	Immunosuppressant	Severity of interaction	Interaction	Suggested actions	Evidence
Ketoconazole	CsA, Tac, Sir	+++	↑ Imm level	Avoid	A
Voriconazole	CsA, Tac, **Sir**	+++	↑ Imm level	↓ CsA by 1/2, ↓ Tac by 2/3	A
Itraconazole	CsA, Tac, Sir	++	↑ Imm level	Monitor Imm level	A
Posaconazole	CsA, Tac, Sir	+++	↑ Imm level	↓ CsA by 1/4, ↓ Tac by 2/3	A
Fluconazole	CsA, Tac, **Sir**	++	↑ Imm level	Dose dependent↓ CsA and Tac by 1/2	A

Drugs in bold are contraindicated.
CsA = cyclosporine; Tac = tacrolimus; Imm = immunosuppressant; Sir = sirolimus. +++ = severe interaction, use alternative drug if possible, otherwise monitor levels of immunosuppressant or potential toxic effects and modify dose accordingly; ++ = moderate interaction, requires monitoring levels or potential toxicity, and may require modification of immunosuppressant dosing.

monitored in all individuals before therapy is started, at 1, 2 and 4 weeks, followed by every 3 months during therapy (95).

Issues related to itraconazole therapy deserve special consideration given the variable absorption among patients and among available drug formulations. The lipophilic composition of itraconazole limits its solubility and consequent gastrointestinal absorption. The bioavailability of oral itraconazole is dependent on the dosage formulation and the presence or absence of food. Food enhances the dissolution and absorption of itraconazole capsules, thus the dose should be taken with a full meal. As absorption is reduced with decreased gastric acidity, itraconazole capsules should not be co-administered with medications that lower gastric pH, such as antacids, H2 blockers or proton pump

inhibitors (111–113). Conversely, capsule absorption can be enhanced when taken with an acidic or carbonated beverage such as Coca Cola (114). Itraconazole suspension is preferred over the capsule formulation owing to enhanced gastric absorption (115). Blood concentrations are ~30% higher using the suspension rather than the capsule formulation (115). Itraconazole suspension does not require food or gastric acidity for absorption and is best taken on an empty stomach but the higher cost might be prohibitive in some patients.

Because of the marked intra- and interpatient variability in the pharmacokinetics and absorption of itraconazole, therapeutic monitoring of serum drug levels is strongly recommended to optimize therapy once steady-state has been reached (~2 weeks) (III) (23,116). Random itraconazole serum concentrations of at least 1.0 ug/mL (by HPLC) are recommended and correlate with clinical efficacy. Therapeutic drug monitoring of itraconazole may also be useful for assessing a poor treatment response, managing drug–drug interactions or interpreting an adverse effect (117). Monitoring of voriconazole levels is also suggested in certain clinical scenarios such as in patients with poor clinical response, or with the addition of an interacting medication. Levels of 0.5–2.0 g/mL are to be achieved for efficacy. For posaconazole, conditions that might hinder gastrointestinal absorption would also prompt measurement of drug concentration. The trough goal should be 0.5–1.5 ug/mL for patients with invasive fungal infection (118).

Additional information regarding drug–drug interactions relevant to treating transplant-associated infections can be found in the Drug Interactions section of these guidelines.

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Special Article

Emerging Fungal Infections in Solid Organ Transplantation

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Key words: fungal, transplant, emerging, zygomycosis, Scedosporium, Fusarium, dematiaceous

Abbreviations: AmBd, amphotericin B deoxycholate; L-AmB, lipid formulation of amphotericin B.

Introduction

Infections due to a variety of generally innocuous fungi are increasingly recognized as a problem in solid organ transplant (SOT) recipients (1–5). These organisms include filamentous fungi such as members of the Zygomycetes class (order Mucorales), *Fusarium*, *Scedosporium,* yeast-like organisms such as *Trichosporon*, *Malessezia* and *Rhodotorula* and the dematiaceous fungi (a collective term referring to a variety of darkly pigmented fungi) (6–13). Diseases caused by these diverse fungi are collectively known as "emerging" or "rare" fungal infections. The clinical manifestations and diagnosis of emerging and rare fungal infections are summarized in Table 1. Since these fungi cause a minority of infections in SOT, data regarding treatment options are limited. All of the treatment recommendations in these guidelines are derived from small case series, anecdotal experiences, and joint center reviews and are summarized in Table 2 (evidence grade III). Data gleaned from non-SOT populations, such as patients with hematological malignancies and/or HSCT, further inform decisions regarding these infections (6,14–18). Since data are quite limited, distinctions between adult and pediatric patients are not addressed in these guidelines.

Epidemiology and Risk Factors

Emerging fungal infections represent approximately 7–10% of fungal infections in SOT recipients and should be considered whenever invasive aspergillosis is suspected (12,13). These infections are rare and their incidence depends upon type of transplant. Lung and liver transplant recipients are at greatest risk. For example, mucormycosis, which is caused by Zygomycetes (order Mucorales) and is the best characterized of these infections, accounts for approximately 2% of fungal infections in SOT recipients. The overall incidence is 0.07% at 1 year after transplant, but twice that rate for liver and lung recipients (12,13,19).

Exposure to the emerging fungi is generally presumed to be from direct contact or inhalation from environmental sources, such as soil, vegetation, water, sewage or air. These organisms are encountered worldwide, but rates of infection can vary by geographic locale and intensity of environmental exposure (20). Most infections start in the respiratory tract or skin, but can then disseminate to multiple organs including the central nervous system. The size of airborne fungal propagules often dictates modes of transmission and clinical manifestations. For example *Aspergillus fumigatus* conidia are ideally suited by size for deposition in the alveoli, and tend to cause pneumonitis. On the other hand, the larger conidia of *Fusarium* and the Zygomycetes can get trapped in the upper airways and sinuses and are more likely to cause sinus infections or infections at sites of direct inoculation.

As a general statement, major host risk factors for infection with these emerging fungi in organ transplant recipients include prolonged and profound immunodeficiency, breaks in skin integrity, and chronic respiratory disease (e.g. cystic fibrosis and bronchiectasis) (14,16,18,21). In the latter group, the underlying pulmonary architectural distortion and mucosal defects predispose patients to chronic colonization and infection with *Scedosporium*, Zygomycetes, and dematiaceous molds before and after lung transplantation (6,7,22). Exposure to selective antifungal agents, specifically azole prophylaxis or therapy, may select for less common fungi and contribute to shifts in incidence of infection with the emerging fungi. For example, voriconazole usage has been associated with mucormycosis in hematopoietic stem-cell transplant (HSCT) recipients in some medical centers (23–25). Much less commonly, infection may be transmitted during the transplantation process via contamination of the preservation fluid or from the organ itself. Transmission of *Scedosporium* and Zygomycetes has been reported in such circumstance and is associated with high rates of graft loss and mortality (26,27). Donors exposed to contaminated water such as near-drowning may be at particular risk for transmitting mucormycosis.

Table 1: Clinical manifestations and diagnosis of emerging and rare fungal infections in solid organ transplant recipients

Fungal pathogen (references)	Clinical manifestations	Diagnosis
Zygomycetes (order Mucorales) (30,31)	• Pulmonary disease (most common) • Rhino-orbital cerebral • Disseminated disease (most common in liver transplant recipients) • Primary cutaneous disease (frequently at sites of medical or surgical interventions) • Bronchial anastomosis infections in lung transplant recipients • Gastrointestinal	• Broad, ribbonlike, nonseptate hyphae in tissue with calcofluor, PAS, or GMS silver staining • Growth in culture usually within 24–48 hours • Molecular techniques in development
Fusarium	• Pulmonary disease • Superficial and deep cutaneous disease • Disseminated (usually involves lung and skin) • Fungemia • Sinusitis • Osteomyelitis • Keratitis • Peritonitis • Endocarditis	• Blood cultures may be positive • Histopathologic appearance is similar to *Aspergillus* (77)
Scedosporium (32,38,71,76)	• Lung infection • Disseminated infection • CNS infection • Skin (less common) • Bone and joint infection • Ocular infection • Hepatosplenic infection • Peritonitis • Endovascular infection	• Histopathologic appearance is similar to *Aspergillus*

Paecilomyces	• Insidious cutaneous and subcutaneous infections:
	  ○ Cellulitis in areas of trauma
	  ○ Erythematous, violaceous, or crusted ulcerations, plaques, nodules, and papulopustular lesions
	  ○ Sporotrichoid pattern (78–81)
	• Sternal wound infection in a lung transplant recipient with pretransplant respiratory colonization (82)
	• Other manifestations: sinus disease, osteomyelitis, keratitis, endophthalmitis, and disseminated infection (78)
		• Reference laboratory should be consulted for confirmation
		• Irregular septate hyphae in tissue with PAS or GMS silver stain can be confused with other molds.
		• Suppurative and/or granulomatous inflammation in tissue
		• Molecular techniques in development

Trichoderma (Hyalohyphomycosis) (83)	• Perihepatic abscesses in liver transplant recipients • Pleuropulmonary disease in lung transplant recipients • Disseminated infection involving brain in kidney transplant recipients	• Fine hyaline septate hyphae in tissues with positive cultures • Molecular techniques in development
Scopulariopsis	• Disseminated infection with cutaneous, pleuropulmonary, cardiac, and brain involvement with nearly universal mortality (84–87)	• Branched and septate hyphae with GMS silver stain
Acremonium	• Mycetoma (88)	
Dematiaceous fungi (*Exophiala*, *Alternaria* and *Bipolaris* species are most common) (89–91)	• Subcutaneous nodules and less commonly as skin abscesses, pustular lesions, or purulent ulcerations	• Septate hyphae with GMS silver stain • Fontana-Masson staining highlights the presence of melanin in the dematiaceous fungi
Chromoblastomycosis	• Chronic cutaneous disease most frequently in the tropical and subtropical areas (92)	• Pigmented sclerotic bodies with H&E stain • Septate hyphae with lactophenol alanine blue stain
Trichosporon	• Disseminated infection (93–96)	• Blood cultures are typically positive • Budding yeast in tissue

Continued

Table 1: Continued

Fungal pathogen (references)	Clinical manifestations	Diagnosis
Malassezia	• Pityriasis or tineaversicolor, folliculitis (97) • Oncychomycosis • Groin abscess (98)	• KOH preparation and/or culture
Rhodotorula	• Peritonitis in a liver transplant recipient (99) • Fungemia in a liver–kidney transplant recipient (100)	• Budding yeast
Penicilliummarneffei	• Endemic in Southeast Asia, southern China, Taiwan, and Hong Kong • Disseminated infections (101–105)	• Dimorphic fungus
Paracoccidioides	• Endemic to Latin America • Pulmonary involvement with or without disseminated disease (106–108)	• Dimorphic fungus
Sporothrix	• Infection is primarily initiated by trauma to the skin resulting in cutaneous infections marked by suppurative and granulomatous nodules that spread along lymphatic channels • Disseminated infection (109) • Pulmonary infection in a heart transplant patient (110)	• Dimorphic fungus

Invasive mucormycosis is a potentially devastating complication in SOT recipients with an overall case fatality rate of 40–50% (28–30). Like invasive aspergillosis, infection may be associated with hemorrhagic necrosis, vascular thrombosis, and tissue infarction and can extend locally to infect adjacent structures or disseminate to other sites. Traditional risk factors for mucormycosis include uncontrolled diabetes mellitus, corticosteroids and neutropenia. In addition to diabetes mellitus, risk factors uniquely described in SOT recipients include renal failure and prior voriconazole and/or caspofungin use (31). Cases typically develop within 3–6 months of transplant but may occur much later except in liver transplant recipients where disease frequently occurs in the first month after transplant (31).

Approximately 25% of non-*Aspergillus* mold infections are caused by *Scedosporium*, which represent 1% of all fungal infections in SOT recipients (13,19). The genus *Scedosporium* includes several potentially pathogenic species including *S. apiospermum* (and the related *Pseudallescheria boydii*), *S. prolificans* and the newly described *S. aurantiacum* (32). The evolving taxonomy of these fungi can confuse the reader when reviewing the literature, and some authors refer to *Scedosporium* species as dematiaceous fungi. Acquisition of *Scedosporium* occurs via direct or inhalational contact with contaminated water, soil, or from unknown sources in many cases (33). Risk factors for infection in SOT include pretransplant colonization (frequently encountered in cystic fibrosis), prior receipt of amphotericin B (to which *Scedosporium* species are generally resistant) and enhanced immunosuppression with treatment for organ rejection (34,35). The most common infecting species is *S. apiospermum* and the majority of infections are in lung transplant recipients (13). Median time to infection is approximately 3–4 months after transplantation but can vary, particularly in lung transplant recipients (2,36–38).

Fusariosis accounts for approximately 13% of non-*Aspergillus* mold infections and 0.6% of all fungal infections in SOT recipients (13,19). *Fusarium solani*, *F. proliferatum*, *F. oxysporum*, *F. moniliforme*, and *F. sacchari* have been implicated as causative agents of infections in SOT recipients (39,40). *Fusarium* is acquired from environmental sources, air, tap water, sinks and showerheads (41). The portal of entry is usually the skin or respiratory tract. Risk factors include persistent neutropenia, profound T-cell depletion and previous fungal infections.

The dematiaceous fungi are less commonly described in SOT recipients but transplant ID clinicians should be aware of them. This group of diverse fungi includes *Alternaria, Bipolaris, Cladosporium, Cladophialophora bantiana, Curvularia, Exophiala, Ochroconis* and *Rhinocladiella mackenziei* (42). In the context of the transplant patient, the most important conditions caused by these fungi are allergic respiratory tract and sinus diseases and invasive infections, such as cutaneous, subcutaneous, respiratory tract, CNS and disseminated infections. Collectively, these diseases are termed phaeohyphomycoses. Presence of such fungi in respiratory tract or sinus culture does not necessarily indicate invasive disease. For example, *Cladosporium* is rarely pathogenic and *Curvularia* and *Bipolaris* are frequently associated with allergic, rather than invasive sinusitis. However, identification of a dematiaceous fungus from a clinical specimen should not be carelessly dismissed as such fungi are increasingly recognized as important causes of infections in SOT recipients.

Diagnosis

Colonization with one of the emerging fungi may occur in the recipient before or after transplantation. The presence of an emerging fungal species in cultures obtained from nonsterile sources does not necessarily indicate infection. This is a particularly relevant issue in lung transplant recipients in whom a variety of emerging fungi including the

Table 2: Recommended treatment of emerging and rare fungal infections in solid organ transplant recipients

Fungal Pathogen (references)	Treatment
Zygomycetes (order Mucorales)	• Surgical excision or debridement is recommended whenever feasible • Induction antifungal therapy (1) L-AmB is the treatment of choice (2) Combination of an echinocandin + L-AmB may be considered based on animal studies and retrospective reports (111–113). (3) Posaconazole may be considered for salvage therapy in patients intolerant to or failing AmB (114–116) • Maintenance antifungal therapy: (1) Posaconazole (2) L-AmB in patients who are clinically unstable or unable to tolerate oral intake) • AmB deoxycholate was historically the drug of choice and remains the only approved agent in the United States but is associated with substantial nephrotoxicity and generally avoided in the current era (67,117,118)
Fusarium	• Surgical excision or debridement is recommended whenever feasible • Antifungal susceptibility is necessary to guide therapy • Combination therapy (AmB + voriconazole) may be considered pending final identification and susceptibility data • *F. solani* and *F. verticillioides* (119) ◦ High doseAmB • Other *Fusarium* species ◦ Either AmB or voriconazole[1] (119)
Scedosporium	• Surgical excision or debridement is recommended whenever feasible • *S. apiospermum* ◦ Voriconazole[1] (71,72) ◦ Combination of an echinocandin + voriconazole may be considered ◦ AmB, 5-flucytosine and terbinafine should not be used (72) • *S. prolificans* ◦ Surgical debridement should be considered primary therapy (resistant to virtually all of the available antifungal agents (72) • Combination antifungal options: ◦ Echinocandin + AmB or voriconazole (73) ◦ Voriconazole + terbinafine (74,75)
Paecilomyces (79,120)	• Surgical excision or debridement is recommended whenever feasible and may be sufficient for isolated cutaneous disease • Voriconazole (or posaconazole) for more extensive disease
Trichoderma (83,121)	• Surgical excision or debridement is recommended whenever feasible • Antifungal susceptibility is necessary to guide therapy • Combination of AmB + voriconazole or posaconazole may be considered until susceptibility data available
Scopulariopsis	• Surgical excision or debridement is recommended whenever feasible • Antifungal susceptibility is necessary to guide therapy • Combination therapeutic options based on *in vitro* synergy data ◦ Terbinafine + voriconazole or posaconazole ◦ Caspofungin + voriconazole or posaconazole
Acremonium	• Surgical excision or debridement is recommended
Phaeohyphomycosis *Exophiala* (89,91)	• Surgical excision or debridement is recommended whenever feasible and may be sufficient for isolated cutaneous disease • Antifungal susceptibility is necessary to guide therapy • Voriconazole, posaconazole or itraconazole are first line agents • Echinocandins may be considered based on *in vitro* data • Potential combination therapeutic options based on *in vitro* synergy data ◦ AmB + flucytosine ◦ Itraconazole + flucytosine

Continued

Table 2: Continued

Fungal Pathogen (references)	Treatment
Chromoblastomycosis	• Surgical excision or debridement is recommended whenever feasible and may be sufficient for isolated cutaneous disease • Antifungal susceptibility is necessary to guide therapy • Itraconazole, voriconazole, or posaconazole are first line agents • Echinocandins may be considered based on *in vitro* data
Trichosporon	• Identification in urine in kidney transplant recipients generally does not required treatment • Antifungal susceptibility is necessary to guide therapy • Azoles are recommended for treatment • AmB may be considered if susceptibility is confirmed • Echinocandins lack activity and should be avoided
Malassezia	• Topical preparation of clotrimazole 1% and selenium sulfide lotion or oral fluconazole for superficial infections • Catheter removal and fluconazole are recommended for disseminated infections
Rhodotorula	• AmB or posaconazole are treatments of choice based on available susceptibility data (122)
Penicillium	• Induction treatment with L-AmB followed by maintenance treatment with itraconazole is recommended.
Paracoccidioides	• Induction treatment with L-AmB for severe disease followed by maintenance treatment with itraconazole or voriconazole is recommended • Itraconazole or voriconazole may be considered for less severe disease • May be prevented with trimethoprim-sulfamethoxazole
Sporothrix (123)	• Induction treatment with AmB[2] for severe disease followed by maintenance treatment with itraconazole • Itraconazole may be considered for less severe disease

Note: None of these treatments are FDA-approved except where noted. L-AmB, lipid formulation of amphotericin B; AmBd, amphotericin B deoxycholate.
[1]Voriconazole is FDA-approved for *Scedosporium apiospermum* and *Fusarium* infections when intolerant or refractory to other agents.
[2]Amphotericin B deoxycholate is FDA-approved for *Sporothrix* infections.
Standard antifungal dosing is recommended (L-AmB 5 mg/kg daily; AmBd 1–1.5 mg/kg daily; voriconazole 6 mg/kg intravenous q12h × 2 loading dose followed by 4 mg/kg intravenous q12h or 200–300 mg orally twice daily; itraconazole 200 mg twice daily; posaconazole 200 mg four times daily or 400 mg twice daily; caspofungin 70 mg loading dose followed by 50 mg daily; micafungin 100 mg daily; anidulafungin 200 mg loading dose followed by 100 mg daily).

dematiaceous molds (e.g. *Exophiala*), *Scedosporium*, the Basidiomycetes, the Zygomycetes, *Cladosporium*, *Fusarium*, *Paecilomyces* and *Penicillium* can colonize the respiratory tract before or after transplantation (43–46). Both the colonizing species and rate of colonization are dependent on the underlying condition and geographic locale. Evidence demonstrating a direct correlation to posttransplant fungal infection has varied among transplant centers (39,47–50). Likewise, pulmonary colonization after lung transplantation does not necessarily lead to invasive infection, even in the absence of antifungal therapy. In the clinical setting, distinguishing colonization from invasive infection can be challenging and often requires tissue examination. In renal transplant recipients, isolation of *Trichosporon* species in urine cultures is usually a benign finding and rarely associated with invasive or deep-seated infection (51,52).

Infection due to emerging molds and yeasts can be difficult to diagnose. Clinical signs and symptoms can be nonspecific and indistinguishable from more common fungal infections. A comprehensive diagnostic approach that includes invasive procedures (e.g. bronchoalveolar lavage, biopsies), careful specimen collection and processing, utilization of specific culture media, and select histological staining techniques is usually necessary for establishing the diagnosis (8). Histopathologic analysis of biopsy specimens that demonstrate septate hyphae on hematoxylin and eosin (H&E) staining may be seen with *Aspergillus*, *Fusarium*, and *Scedosporium* (17). In contrast, Zygomycetes typically appear as broad, nonseptate, ribbonlike hyphae by H&E staining. Fontana-Masson staining can be valuable for identifying dematiaceous fungi in tissue. Delayed or incorrect identification may lead to the initiation of incorrect treatment and result in further tissue destruction and/or dissemination of disease. Close communication between the transplant team and the mycology and pathology laboratory is essential. Final identification and susceptibly testing frequently requires referral to a reference laboratory. Once the fungus has been identified, distinguishing colonization from active infection is a potentially challenging but essential component of the pre- and posttransplant evaluation (3,36). Radiographic studies can demonstrate pathologic changes in tissue, particularly the lung, and help distinguish disease from colonization; however, imaging findings are not specific.

Although currently available molecular fungal diagnostic assays are unlikely to be of significant value in the specific diagnosis of emerging and rare fungal infections, it should be noted that assays that detect galactomannan are reported to be positive in cases of *Penicillium*, mucormycosis, *Fusarium*; and miscellaneous hyaline molds and yeast (53–58). Furthermore, assays that detect beta-glucan may be effective at early detection of *Fusarium* and *Trichosporon* although the assay lacks specificity for any fungal pathogen (59,60).

The clinical manifestations and diagnosis of the emerging fungal infections are summarized in Table 1.

Treatment

Treatment of emerging fungal infections can be very challenging. Data regarding the optimal type and dosage of antifungal therapy are limited due to the absence of randomized controlled trials. Duration of treatment tends to be very prolonged, and many of the antifungal agents are extraordinarily costly and have the potential for drug interactions and/or substantial toxicity. In general, we recommend the following approach (III except where noted):

1. Therapy with an agent that has proven activity against the fungus should be administered as early as possible (II-3).
2. Immunosuppression should be reduced when clinically feasible. To date, immune reconstitution inflammatory syndrome has not been described with emerging or rare fungal pathogens and should not be a concern.
3. Surgical debridement (sometimes repeatedly) of the affected areas should be performed whenever feasible (II-2).
4. Antifungal therapy should be adjusted based on susceptibility testing at a reference laboratory. Although clinically validated antifungal susceptibility breakpoints are lacking, it is reasonable for clinicians to apply knowledge of general antifungal susceptibility patterns in guiding therapy.
5. Clinicians should closely monitor for renal toxicity with amphotericin B (AmB) products.
6. Clinicians should closely monitor for Q-T interval prolongation, drug interactions, hepatotoxicity and neuropsychiatric side effects with azoles (61,62). Therapeutic drug monitoring of voriconazole and posaconazole should be considered to guide dose adjustments although data for emerging fungal infections are lacking. Target voriconazole trough levels between 1.5–4.5 μg/mL are associated with the optimal balance of maximizing efficacy and minimizing toxicity (63). Based on very limited data in the prophylactic setting the target posaconazole trough levels should be at least 0.5 μg/mL (64).
7. Adjuvant therapy with interferon-gamma and/or granulocyte-macrophage colony stimulating factor is not routinely recommended but may be considered with caution in cases refractory to standard antifungal therapy based on case reports (65,66).

The recommended treatment of mucormycosis, *Fusarium*, *Scedosporium* and the other emerging fungal infections is summarized in Table 2. Although clinical data to guide assessing the response to therapy are lacking, antifungal therapy should be continued at least until all clinical and radiographic signs of infection have resolved.

Mucormycosis

Surgical resection or debridement is associated with treatment success in SOT recipients and its significance in the management of most cases of mucormycosis cannot be overemphasized (67). For bronchial anastomotic infections in lung transplant recipients, bronchoscopic or surgical debridement is essential (68). Medical therapy alone can be attempted in patients with pulmonary mucormycosis unless there is extensive necrosis or disease threatening major vascular structures. Lipid formulations of AmB are the drugs of choice for mucormycosis. Posaconazole may be considered for maintenance therapy once clinical stability has been achieved.

Fusarium

Surgical resection alone may be effective for limited cutaneous or sinus disease. Antifungal therapy is recommended for deeper sites of infection (e.g. lungs) or disseminated disease (69). Antifungal susceptibility can vary by species and *in vitro* testing should guide choice of antifungal therapy. *In vitro*, *Fusarium* species are often resistant to AmB and have a wide range of susceptibilities to voriconazole (70).

Scedosporium

Response to therapy is highly dependent on site of infection, extent of dissemination, and host factors (38). Outcomes are better when the infection is localized to the skin or lung and substantially worse with disseminated disease. Surgical excision is typically required. Outcomes tend to be better with S. *apiospermum* infection, which may be related to better response to antifungal agents (71,72). *In vitro*, voriconazole has the most potent activity against *S. apiospermum.* The echinocandins are also active, but AmB, 5-flucytosine and terbinafine have limited to no activity against *S. apiospermum* (72). Medical management of *S. prolificans* is extremely challenging and surgery is typically required to control infection. This species is resistant to virtually all of the available antifungal agents (72). Based on animal studies, combination therapy including an echinocandin and either AmB or voriconazole may be effective (73). *In vitro* and anecdotal reports suggest that combining voriconazole with terbinafine may be effective (74,75).

Prevention and Prophylaxis

The most common mechanism for colonization or infection is via environmental exposure. Patients should be instructed to avoid visiting construction sites and poultry farms, manipulating air-conditioning filters, and contact with sewage or decaying material. To reduce the risk of invasive fungal infection due to transmission during the transplantation process, care should be taken in accepting organs from near drowning victims. Organ procurement agencies should report all fungal isolates from a donor to the recipient center. Not all patients with fungal colonization require prophylaxis. Certain colonizing fungi are very rarely pathogenic (e.g. *Cladosporium*, *Paecilomyces* and *Penicillium* species other than *P. marneffei*) and their presence generally does not require prophylaxis. By contrast, the Zygomycetes and *Scedosporium* have been associated with disseminated infection in highly immunocompromised patients. Prophylaxis may be considered in such patients and in recipients of donor lungs that are colonized with these fungi (34,50,76).

Future Directions

Although it may be challenging to conduct randomized controlled trials, collaborative prospective studies should be performed to gain more information regarding the epidemiology, clinical manifestations, treatment strategies, and outcomes associated with mucormycosis, *Fusarium* and *Scedosporium* in SOT recipients. Furthermore, clinicians are encouraged to publish case reports and case series of the other emerging fungal infections.

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Special Article

Pneumocystis Pneumonia in Solid Organ Transplantation

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Abbreviation: PCP, *Pneumocystis* pneumonia.

Epidemiology and Risk Factors

Pneumocystis jiroveci, previously *P. carinii*, is the quintessential opportunistic infection among immunocompromised patients (1). Despite the availability of effective prophylaxis, *P. jiroveci* remains an important pathogen among solid organ transplant recipients. *Pneumocystis* spp. are thought to be ubiquitous in nature with serologic studies suggesting exposure occurs commonly in childhood (2). The existence and degree of respiratory tract colonization by *Pneumocystis* is a topic of great interest (3,4). Symptomatic *Pneumocystis* pneumonia (PCP) is generally limited, however, to individuals with immune deficits. Animal models suggest that *de novo* infection via airborne transmission and reactivation of previously established infection can occur (5). Clusters of infection have been described in medical facilities among solid organ transplant recipients as well, suggesting the possibility of direct or indirect person-to-person transmission (6–11).

Based on studies prior to routine implementation of prophylaxis, the overall incidence of infection among solid organ transplant recipients varied in the range of 5–15% depending on organ type, transplant center and immunosuppressive regimens. The attack rate appeared highest in lung and combined heart–lung transplant recipients with an overall incidence ranging from less than 10% to just over 40% (12). Infection has decreased with reduction in the routine use of corticosteroids in organ transplantation and the adoption of effective prophylactic measures. As with most infections, the overall net state of immunosuppression is the main contributor to risk rather than any specific immunosuppressive agent. Inconsistent use of prophylaxis is linked to a number of the published outbreaks in recent years (9). Risk factors for disease are outlined in Table 1.

Diagnosis

Symptomatic progression of PCP in HIV-negative patients is variable but classically more acute than in HIV-infected individuals. In the setting of transplantation, symptoms often develop over the course of a few days, though evolution over 1–2 weeks may also occur. The signs and symptoms of infection are outlined in Table 2 and are based on studies from the 1980s when the AIDS epidemic in the United States was just underway (27). As PCP may present as part of concomitant viral infection, symptoms may be masked by coinfection or by other causes of respiratory distress (e.g. congestive heart failure). In general, patients present with marked hypoxemia out of proportion to physical findings. Some common signs (e.g. fever) may be absent while others (e.g. dyspnea) should be expected.

Chest radiography may be normal or reveal diffuse bilateral interstitial pulmonary infiltrates. Computed tomographic (CT) scans are more sensitive than routine chest radiography. No specific radiological diagnostic pattern exists, however (28). Direct demonstration of the organism in the respiratory tract or secretions is the diagnostic method of choice. Diagnosis can be accomplished using noninvasive or invasive methods. The diagnosis of PCP has been markedly improved by the use of immunofluorescent monoclonal antibody stains against the organism (29,30). Direct staining of samples from respiratory tract secretions, or from transbronchial or open lung biopsies, bind to both the cyst and trophozoite forms of *Pneumocystis*, increasing the sensitivity of detection of the organism. Without antibody staining, routine stains such as Gomori methenamine-silver (GMS) can stain the cyst form only while Giemsa and Wright's stains also can stain trophozoites, the most common form of the organism in the alveolus. Diff-Quick staining (a modified Wright stain) may be the least sensitive method in identifying organisms from respiratory samples when used alone, and Calcoflour white and GMS staining may have the best overall predictive values for routine clinical use when monoclonal antibody staining is not available (31). For successfully treated patients, the organism may persist in sputa; this should not be considered a failure of therapy as relapse is uncommon with completion of therapy (32). Use of molecular techniques such as PCR has been increasingly

Table 1: Risk factors for the development of *Pneumocystis* pneumonia expected or observed in solid organ transplant recipients

Risk factors	Comments
Immunosuppressive therapies	
Corticosteroids	• Retrospective case series in non-HIV patients identified corticosteroids in up to 90% • Median dose and duration of therapy in one series of non-HIV patients with PCP was 30 mg/day of prednisone for 12 weeks (13)
Antilymphocyte therapy	• Antilymphocyte antibodies are linked to the highest risk for PCP in the 1–6 month posttransplant period (14) • Alemtuzumab, a monoclonal antibody with activity against B-, T-, and NK cells may confer the highest risk (15)
Mycophenolate mofetil	• The anti-*Pneumocystis* effects of mycophenolate mofetil *in vitro* and in animal models have not been confirmed in prospective clinical trials (16)
Calcineurin inhibitors	• At a single institution where cyclosporine A replaced azathioprine in renal transplantation, the incidence of PCP increased from 3% to 9% (17) • One retrospective study suggested a higher incidence of PCP among renal transplant recipients on tacrolimus-based regimens compared to cyclosporine A (18)
Other clinical factors	
CMV disease	• CMV may be an independent risk factor for PCP (19) • Coinfection with CMV and PCP may be observed in solid organ transplantation (20–22)
Allograft rejection	• PCP has been related to the intensity of immunosuppression in transplant recipients (18) • PCP has been linked to treatment and number of episodes of acute rejection (21)
Low CD4+ T cell counts	• In HIV infection, the risk for PCP is linked to CD4+T cell counts <200 cells/mL, or <20% of the total circulating lymphocytes (23) • PCP has been linked to decreased CD4+T cell counts in HSCT recipients (24), solid tumor patients receiving chemotherapy (25), autoimmune disease and hematological malignancy patients (26) • Transplant patients with CD4+T cell lymphopenia are expected to be at risk for PCP, though clinical data to support this are lacking (19)
Neutropenia	• Prolonged neutropenia is a potential risk factor for PCP in transplant recipients (19)
Exposure	• In solid organ transplant recipients not taking effective prophylaxis, being in close proximity to other transplant recipients with PCP may increase the risk for developing infection (6–11)

CMV = cytomegalovirus; GVHD = graft vs. host disease; HIV = human immunodeficiency virus; HSCT = hematopoietic stem cell transplant; PCP = *Pneumocystis* pneumonia.

Table 2: Signs and symptoms of *Pneumocystis* pneumonia as originally described in HIV-infected patients

Sign or symptom of PCP	Incidence
Fever	81–87%
Dyspnea	66–68%
Cough	71–81%
Chest pain	23–24%
Abnormal lung auscultation on exam	30–34%
Abnormal chest radiography	92–96%
Hypoxemia	78–91%

studied as a diagnostic tool for PCP (33–37). Concerns about lack of specificity linger, though quantification-based assays may increase the specificity of the approach. Application in bronchoalveolar lavage (BAL) fluid may have an increased sensitivity in detecting *P. jiroveci* compared to routine staining and antigen detection (38).

Coinfection with CMV is common and other respiratory viral infections may precede or coincide with PCP (1). Infection with *Pneumocystis* has also been observed in concert with abnormal lung changes due to sirolimus. Diagnostic tests are outlined in Table 3.

Practice recommendations for the diagnosis of PCP in transplant recipients include:

(1) Patients should undergo initial screening via multiple induced sputum samples (Grade II-2). All respiratory secretions should be stained using antibodies for PCP (immunoflourescent, immunoperoxidase, or similar) as well as routine stains for *Pneumocystis* and other organisms (Giemsa, Silver, and others) (Grade II-1). Use of PCR-based diagnostics on respiratory secretions can be considered (Grade III). Samples should also be assayed for routine bacterial, fungal, mycobacterial, and other organisms to rule out concomitant infections (Grade II-2). Evaluation for CMV or other respiratory viral coinfection, in particular, should be considered (Grade II-2).

(2) Clinicians should have a low threshold for bronchoscopy with BAL to obtain diagnostic samples (Grade II-2). This may have the dual advantage of increasing the yield and helping expedite the diagnosis of other and/or concomitant infections.

(3) Patients undergoing bronchoscopy should be considered for transbronchial biopsies. Increased yield is likely obtained by multiple samples (Grade II).

(4) Measurement of plasma (1→3) β-D-glucan levels can be considered and may suggest the diagnosis (Grade II-2). This assay lacks specificity for *Pneumocystis*, however, and can be positive in the setting of other invasive fungal infections.

Table 3: Diagnostic approaches to *Pneumocystis* pneumonia in transplantation

Test	Estimated yield	Comments
Routine sputum smears	Generally poor	• Organ transplant patients with PCP may have smaller burden of infecting organisms than AIDS patients (39) • Use of fluorescent monoclonal antibody staining may increase the sensitivity of finding the organism over other stains
Induced sputum smears	Improved over routine sputum exam when coupled with antibody staining; yield ≥50% (29)	• Yield from induced sputum in transplant patients may not reflect that found in HIV-infected patients • Sensitivity and specificity in transplant patients unknown • Repeat testing may improve yield (30)
Bronchoalveolar	Generally ≥70% in non-AIDS immunocompromised hosts when coupled with antibody staining	• Older data involving immunosuppressed patients with PCP suggested a yield close to 80% (40)
Transbronchial biopsy	Increases yield of routine BAL (1)	• Multiple biopsies preferred to increase sensitivity with some increased procedural risk
Open lung biopsy	Often considered to be a gold standard, but early patchy disease may decrease yield	• Case reports highlight PCP infections missed on BAL that were subsequently identified from open lung biopsies (41,42) • Cases of missed infection in open lung biopsy also reported (30)
PCR testing of samples	Sensitivity and specificity vary depending on manner of sampling (sputum vs. BAL) and assay employed	• Multiple assays are not standardized. Generally target genes for conserved surface glycoproteins or rRNAs • Specificity unknown
Plasma (1→3) β-D-glucan	Some reports in transplant and HIV patients (43–46). Meta-analysis suggests a sensitivity of almost 95%, but with a specificity in the mid-80% (47)	• (1→3) β-D-glucan is produced in the cyst cell wall and detection in the serum has been associated with underlying infection (also positive in other invasive fungal infections) (48) • Clinical trials data lacking

AIDS = acquired immunodeficiency syndrome; BAL = bronchoalveolar lavage; HIV = human immunodeficiency virus; PCP = *Pneumocystis* pneumonia; PCR = polymerase chain reaction; rRNA = ribosomal ribonucleic acid.

(5) Open lung biopsies can be obtained when other diagnostic approaches have been unrevealing or where other concomitant diseases may be a concern (Grade II). Video-assisted thoracoscopic (VATS) biopsies may be appropriate for some patients in this regard.

Treatment

For the established or presumed diagnosis of PCP, therapeutic options are outlined in Table 4.

Practice recommendations regarding the treatment of PCP in transplant recipients include:

(1) Trimethoprim-sulfamethoxazole (TMP-SMX) is the first-line agent and drug of choice (Grade I). No agent has been shown to have outcomes superior to TMP-SMX.
(2) In severe infections, intravenous pentamidine probably remains the second-line agent after TMP-SMX (Grade II-1). Although pentamidine is effective, use can be complicated by numerous toxicities. Most experts recommend alternative therapies in pancreas or islet transplant recipients due to the potential for islet cell necrosis (Grade III).
(3) In patients with hypoxemia ($pAO_2 < 70$ mmHg on room air), adjunctive corticosteroids should be administered with antimicrobial therapy, ideally within 72 hours of initiating antimicrobial therapy for maximum benefit (Grade II-1). Though the optimal dose of corticosteroids has not been well-established, recommendations of 40–60 mg of prednisone (or equivalent) given twice daily for 5–7 days before being tapered over a period of at least 7–14 days is often recommended (Grade III).
(4) Duration of antimicrobial therapy should be extended for at least 14 days, although clinicians treat for 21 days total in severe infection (Grade III).

Prophylaxis

Routine anti-*Pneumocystis* prophylaxis is recommended for most centers with an incidence of PCP of at least 3–5% among transplant recipients (19). With widespread use of prophylaxis and diverse immunosuppressive regimens, the true incidence of posttransplant PCP is unknown. For those patients who have risk factors such as the need

Table 4: Therapeutic options for treating *Pneumocystis* pneumonia

Agents	Dosing	Comments
Trimethoprim-sulfamethoxazole (TMP-SMX)	15–20 mg/kg/day of the TMP component given IV in divided doses every 6–8 hours often in combination with corticosteroids (see below); for milder disease, two double-strength tablets can be given po bid-tid	• TMP-SMX is the **drug of choice** and is considered to be the most effective systemic therapy for PCP. Hydration should be maintained • Patients on high-dose TMP-SMX should have regular monitoring of cell counts, creatinine and potassium
Pentamidine isesthionate	4 mg/kg/day IV initially over 1–2 hours; dose reduction to 2–3 mg/kg/day if needed	• Pentamidine side effects include pancreatitis, hypoglycemia, hyperglycemia, bone marrow suppression, renal failure and electrolyte disturbances • Pancreatic dysfunction may suggest the need for avoidance in pancreas transplantation
Atovaquone	750 mg po bid (optimal dose uncertain; 1500 bid used anecdotally)	• Atovaquone is available in an oral suspension only • Atovaquone has variable oral absorption (best with fatty foods) • Atovaquone is approved only for mild and moderate PCP
Primaquine and clindamycin	Primaquine 15–30 mg po qd in combination with clindamycin 600–900 mg IV or po q6–8 hours	• This combination has been studied in mild to moderate PCP in AIDS • Long-term use of clindamycin can predispose to infection with *Clostridium difficile* • Primaquine should be avoided in G6PD deficiency
Dapsone and trimethoprim	Dapsone 100 mg po qd used in combination with trimethoprim 15 mg/kg/day po divided tid	• This combination has been used with sulfa allergy, though dapsone may elicit sulfa allergies as well
Trimetrexate with folinic acid	Trimetrexate 45 mg/m^2/day IV (or 1.5 mg/kg/day IV in patients <50 kg) with folinic acid 20 mg/m^2 po or IV every 6 hours (80 mg/m^2 total daily); Folinic acid therapy extends ≥ 3 days beyond trimetrexate therapy	• Trimetrexate causes bone marrow suppression and must be used with folinic acid, 10 mg po qd • Outcomes are inferior to TMP-SMX in AIDS • Trimetrexate is no longer commercially available in the United States
Pyrimethamine and sulfadiazine	Pyrimethamine load of 100–200 mg po, followed by 50–100 mg po qd in combination with sulfadiazine 4 g po qd in divided doses	• Limited data available on this regimen • Usually with folinic acid 10mg po qd to reduce bone marrow toxicity
Macrolide and SMX	Macrolides such as clarithromycin or azithromycin in combination with sulfamethoxazole may be synergistic *in vivo* (49)	• Few clinical data to support the use of this combination. No recommendations available for dosing or duration of therapy
Caspofungin and TMP-SMX	70 mg IV loading dose of caspofungin on day one, followed by 50 mg IV daily after in combination with TMP-SMX (dose reduced in the setting of moderate to severe hepatic dysfunction)	• Echinocandins have activity against *Pneumocystis* in animal models (50,51) • Case reports exist of caspofungin use in combination with TMP-SMX and other drugs for PCP (52–55) • Clinical efficacy compared to TMP-SMX alone remains unknown
Adjunctive agents		
Corticosteroids	40 mg–60 mg of prednisone (or equivalent) po bid with taper after 5–7 days over a period of 1–2 weeks	• Corticosteroids are best administered within 72 hours in the setting of hypoxia (pAO_2 < 70 mmHg) • Commonly used but not well studied in transplantation • May require prolonged taper to avoid immune reconstitution pneumonitis
Colony-stimulating factors	Ideal dosing unknown	• Use of GM-CSF as an adjuvant has been studied in animal models (56) • No clinical data in humans

AIDS = acquired immunodeficiency syndrome; G6PD = glucose-6-phosphate dehydrogenase; GM-CSF = granulocyte/macrophage colony stimulating factor; PCP = *Pneumocystis* pneumonia; TMP-SMX = trimethoprim-sulfamethoxazole.

Table 5: Specific prophylactic agents for prevention of *Pneumocystis* listed by preference

Agents	Dosing	Comments
Trimethoprim-sulfamethoxazole (TMP-SMX, cotrimoxazole)	Can be given at 80 mg TMP/400 mg SMX or 160 mg TMP/800 mg SMX po (single or double strength) daily or three times weekly	• TMP-SMX remains the **drug of choice** for PCP prophylaxis (59) • Daily regimens may be required to have efficacy for other forms of posttransplant infections
Dapsone(4,4′-diaminodiphenylsulfone)	50–100 mg po qd	• Dapsone is considered a second-line agent for the prophylaxis of PCP (60) • Side effects may be more common among solid organ transplant recipients (61) • Avoid in G6PD deficiency, methemoglobin reductase deficiency • Uncommon allergy to sulfone or sulfa-containing agents • Generally not recommended in with history of severe sulfa reactions (desquamation, neutropenia, interstitial nephritis or hepatitis).
Atovaquone	1500 mg po qd (as single dose)	• Clinical trial data in HIV patients who could not tolerate TMP-SMX showed atovaquone to be equivalent to dapsone in preventing PCP (62) • Data in solid organ transplant recipients show it to be well-tolerated (19,63) • Failures of atovaquone have been reported at doses of 1000 mg or less daily (19,64)
Pentamidine	300 mg administered through aerosolized nebulizer q 3–4 weeks	• Pentamidine requires administration by experienced personnel with a nebulizer producing droplets of 1–3μ • Pentamidine is well-tolerated with minimal side effects other than cough and bronchospasm • There is a higher incidence of breakthrough infection compared to TMP-SMX or dapsone • Reports of disseminated infection involving the thyroid in HIV cases receiving inhaled pentamidine as prophylaxis (65)
Clindamycin and pyrimethamine	Up to 300 mg of clindamycin po qd with 15 mg of pyrimethamine po qd (some clinicians have administered this regimen 3 times weekly instead of daily)	• Somewhat efficacious in AIDS, though less effective than TMP-SMX or dapsone (66) • Failure rate higher than for aerosolized pentamidine • Gastrointestinal intolerance may be limiting

AIDS = acquired immunodeficiency syndrome; HIV = human immunodeficiency virus; PCP = *Pneumocystis* pneumonia; TMP-SMX = trimethoprim-sulfamethoxazole.

for increasing immunosuppression in the face of graft rejection, recurrent or chronic active infection with CMV, prolonged courses of corticosteroid therapy (e.g. >20 mg daily of prednisone for at least 2 weeks), prolonged neutropenia, or flares of autoimmune disease, prophylaxis is generally indicated. Lung transplant recipients are always considered at high risk for PCP (57). In any transplant population, the risk has always been considered highest within the first 6 months posttransplant, though features outlined above may prolong that risk. A more recent single center study found that most PCP cases occurring among transplant recipients now occur several years after the procedure and involve patients no longer taking effective prophylaxis (58).

In general, anti-*Pneumocystis* prophylaxis is recommended for all solid organ transplant recipients for at least 6–12 months posttransplant, though longer durations should be considered (Grade III). For lung and small bowel transplant recipients, as well as any transplant patient with a history of prior PCP infection or chronic CMV disease, lifelong prophylaxis may be indicated (Grade III). Agents used for prophylaxis are outlined in Table 5.

Practice recommendations regarding prophylaxis include TMP-SMX as the drug of choice for prophylaxis of PCP (Grade I). All other prophylactic agents should be considered second-line agents due to breadth of coverage, drug intolerances, cost, and efficacy issues that are not favorable compared to TMP-SMX.

The side effects of TMP-SMX dosing in prophylaxis are less common than with therapy, and rarely necessitate cessation of treatment. Bone marrow suppression may be potentiated by concomitant administration of other myelosuppressive agents. Rash may occur, spanning the gamut of benign reactions to Stevens-Johnson syndrome. Other

potential adverse effects include hepatitis, interstitial nephritis, aseptic meninigits, and pancreatitis. Trimethoprim has the capacity to inhibit potassium and creatinine secretion in the renal tubules, resulting in hyperkalemia and an elevation of serum creatinine that does not necessarily reflect true renal function. Patients on TMP-SMX may need laboratory monitoring of renal function and electrolytes including potassium levels. Other lab testing may be indicated in select cases.

Dapsone is often used as a second-line agent for PCP prophylaxis. Some reports of daily dapsone use have included it in combination with pyrimethamine at 25–50 mg once weekly. Although it may be tolerated in transplant patients who cannot receive TMP-SMX, it is generally not recommended in those who suffer severe TMP-SMX or sulfa reactions such as desquamation, neutropenia, interstitial nephritis, or hepatitis. It is also generally contraindicated in those patients with documented glucose-6-phosphate dehydrogenase (G6PD) deficiencies. The most commonly associated side effects of dapsone include hemolytic anemia and methemoglobinemia. Classically these symptoms are associated with G6PD enzyme deficiency, though G6PD deficiency is not a prerequisite (61).

Atovaquone is well-studied in the HIV population and has also been studied in small prospective trials of stem cell and solid organ transplant recipients (19). Available only in a suspension, atovaquone acts by inhibiting mitochondrial electron transport in susceptible *Pneumocystis*. Absorption is enhanced by fatty foods and decreased in the setting of diarrhea. Rash and gastrointestinal complaints are the most common side effects. Increased hepatic transaminases are rarely noted. Although ideal dosing may be unclear, breakthrough infections have been documented in patients taking 1000 mg or less daily (19,64).

Inhaled pentamidine should be considered a third-line agent. It is less effective overall compared to TMP-SMX, dapsone or atovaquone. Use of inhaled pentamidinehas been associated with breakthrough infections, notably in the upper lung zones. There is also some concern that inhaled pentamidine may negatively affect the sensitivity of diagnostic assays using respiratory secretions in patients with PCP (67).

Infection control issues

Pneumocystis jiroveci has traditionally not been thought of as a healthcare associated infection. Outbreaks among susceptible transplant recipients have been documented (6–11,68). A possible explanation for clustered infections could be person-to-person transmission—a hypothesis supported by some molecular typing studies of *Pneumocystis* from infected cases (6,7,9) and animal studies. Older studies have also shown that *Pneumocystis* can be detected in air samples from hospital patient care rooms using PCR techniques (69,70). The debate for a role of person-to-person transmission versus an unidentified environmental common source exposure is unresolved in these outbreaks. Some authors recommend strict hospital segregation of immunocompromised patients with PCP and the use of facemask filtering to prevent transmission among infected individuals (7). However, prophylaxis in susceptible patients is effective at preventing infection. Without definitive data, formal recommendations regarding infection control in the hospital or healthcare clinic cannot yet be made.

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation.* Dr. Fishman is a consultant for Primera Dx.

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Special Article

Parasitic Infections in Solid Organ Transplantation

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Key words: Antiparasitics, donor-transmitted infections, gastrointestinal parasites, parasites, posttransplant infection, protozoal infections

Abbreviations: BAL, bronchoalveolar lavage; CDC, Centers for Disease Control and Prevention; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebral spinal fluid; DD, disseminated disease; DS, double strength; EIA, Enzyme Immunoassay; ELISA, Enzyme-linked immunosorbent assay; FDA, Food and Drug Administration; FIA, Fluorescence Immunoassay; HIS, hyperinfection syndrome; SOT, solid organ transplant; TMP/SMX, trimethoprim/sulfamethoxazole; WHO, World Health Organization.

Introduction

As organ transplantation is carried out more regularly around the world and immigration and travel to and from developing countries becomes more common, infections with parasitic diseases are more frequently identified in recipients of organ transplantation. This increase in identification of parasitic infection and recognition of new infections being transmitted through organ transplantation presents the transplant community with new issues regarding donor and recipient screening, as well as, management of infections posttransplantation. Although recent years show an increase in the number of published papers on parasitic infections in transplant recipients it still remains the most understudied of all infections related to organ transplantation with very few prospective trials and no randomized studies that can be accounted for in this field. Recommendations are based primarily on expert opinion (III) unless otherwise stated.

Since publication of the last update of these guidelines in 2009, Balamuthia has been identified as a protozoa transmitted through organ transplantation. Guidelines from several groups have provided recommendations on the management of *Trypanosoma cruzi* infection in the transplant setting. Some helpful new insights on the risks of strongyloides infection and updates on treatment have been published. Slowly, some new treatment options for parasitic diseases are being developed (most are not currently FDA approved) and newer diagnostic assays for parasitic infections are being developed.

Common features of parasitic infection in the transplant recipient

Parasitic diseases may affect transplant recipients as a result of either recrudescence of latent infections in the previously infected recipient or "*de novo*" infection by means of natural infection or transmission by transplanted organ into a naïve recipient. The incidence of parasitic infection is expected to grow in solid organ transplant (SOT) recipients due to multiple factors:

- Many geographic areas where parasitic infections are prevalent now have active organ transplant programs.
- Donors and recipients from endemic areas, with latent or asymptomatic infections, are sometimes referred to transplant centers in Western countries.
- Some patients from developed countries undergo transplantation in endemic areas ("transplant tourism") and return home with either donor derived or naturally acquired infection(s).
- Immigrants to Western countries, unaware of their infectious status, are accepted for organ donation without further evaluation for diseases that are prevalent in their countries of origin.
- With the recent increase in leisure tourism, transplant recipients travel to endemic areas and enhance their risk of exposure.
- The decrease in cyclosporine-based immunosuppressive regimens and the increased use of newer drugs that lack the antiparasitic effects of cyclosporine metabolites may result in higher rates of parasitic infection.

Tissue and Blood Protozoa

Toxoplasmosis

Epidemiology and risk factors: Toxoplasmosis is a zoonotic illness due to infection with the protozoa *Toxoplasma gondii*. Infection in transplant recipients can occur

through ingestion of contaminated food or water, after receiving an infected allograft, or by reactivation of latent infection. Cardiac transplant recipients who are seronegative for toxoplasmosis and receive an organ from a seropositive donor have a 57–75% risk of developing symptomatic infection without prophylaxis, usually within 3 months after transplantation (1,2). Latent infection in the donor myocardium during cardiac transplantation is the most common method of donor transmission, although it has been transmitted through transplantation of other organs (3,4). However, among noncardiac, SOT-related cases of toxoplasmosis are more varied in origin (4).

Toxoplasmosis gondii infection occurs worldwide but it is more common in patients from endemic regions, including France and the moist tropical areas of Latin America and sub-Saharan Africa, when the prevalence may approach 90%. In the United States, 10–40% of people are seropositive for *T. gondii* (5,6). Risk factors for primary infection include ingestion of cysts in under cooked meat or contaminated soil, contact with oocysts in feline feces, maternal-fetal transmission, or via blood or SOT (7). Water-borne transmission of *T. gondii* has been considered uncommon but has been reported (8). A large review of 15 800 SOT recipients at one center found 22 cases of toxoplasmosis disease. Notably, 90% of recipients were seronegative at the time of transplant. Morbidity was high, and the crude mortality rate was 3/22 (13.6%; Ref.3).

Diagnosis: Transplant patients with toxoplasmosis can present with fever, myocarditis, lymphadenopathy, hepatosplenomegaly and meningitis, brain abscess, chorioretinitis, pneumonitis, hepatitis, pancytopenia or disseminated disease. Symptoms often present within 3 months posttransplant, however, later presentations can be seen, particularly after discontinuation of chemoprophylaxis (3,4,9). Definitive diagnosis requires the identification of tachyzoites on histopatholoy of tissue, seroconversion or amplification of toxoplasma DNA by PCR of infected tissues (10).

The presence of multiple ring-enhancing lesions in the basal ganglia or cerebrum on neuro-imaging, especially in the presence of anti-*Toxoplasma* IgG seropositivty, is suggestive of CNS toxoplasmosis and is sufficient to start presumptive treatment. Stem cell transplant recipients often show a variable enhancement pattern, with the lesion enhancement inversely correlated with the severity of immunosuppression; the radiographic appearance in SOT recipients has not been well described (11). Brain biopsy should be considered in nonresponding patients, as the radiographic differences with other infections or malignancies are neither sufficiently specific nor sensitive. Cerebrospinal fluid (CSF) may have a mild mononuclear pleocytosis and/or an elevated protein. Identification of toxoplasma DNA by PCR in the CSF of patients with HIV/AIDS has a high specificity (96–100%) but the sensitivity is more limited (52–98%; Refs.12–14). Rarely tachyzoites can be seen on centrifuged CSF samples after Giemsa staining (10).

Myocarditis may present with heart failure; the diagnosis is made by seeing tachyzoites on myocardial biopsy. Chorioretinitis often presents with scotoma, blurred vision, pain or photophobia. On fundoscopic examination raised, yellow-white, cottony lesions in a nonvascular distribution (unlike the perivascular exudates of CMV retinitis) are seen and vitreal inflammation may be present. Pulmonary disease often presents with fever, dyspnea and nonproductive cough, and reticulonodular infiltrates on chest imaging. This pattern of disease may be indistinguishable from *Pneumocystis jiroveci* pneumonia but toxoplasma tachyzoites are identified in bronchoalveolar lavage (BAL) fluid. Although rare, cutaneous toxoplasmosis has been seen after hematopoietic stem cell transplantation (15).

Treatment: Optimal treatment after SOT has not been well studied. However, an extensive literature exists on treatment of toxoplasmosis in patients with HIV/AIDS, which serves as a guide for treatment of the transplant population. The drugs routinely used in the treatment of toxoplasmosis treat the proliferative form (tachyzoites) found during the acute phase of infection but do not eradicate the encysted form of the parasite. Treatment for active toxoplasmosis includes induction therapy with pyrimethamine (plus leucovorin) and sulfadiazine to combat the tachyzoites, followed by chronic suppressive therapy (secondary prophylaxis) to prevent recrudescence of disease (16). Induction therapy is usually given for at least six weeks depending on response to therapy. Chronic suppressive therapy in HIV/AIDS patients is usually provided as reduced doses of the induction therapy until reconstitution of the patient's immune system. A similar strategy is appropriate in transplant recipients, however because the transplant population requires life-long immune suppression, chronic suppressive therapy with reduced toxicity medication, such as trimethoprim/sulfamethoxazole (TMP/SMX) may be considered (Refs.17,18; see Table 1).

Prevention/prophylaxis:

Screening: Pretransplant screening for prior toxoplasmosis exposure is generally done before heart transplant, and is less frequently done before other organ transplants. One retrospective cohort study of 1006 SOT recipients at a single center identified a pretransplant *Toxoplasma* seroprevalence rate of 13% in donors and 18% in recipients, with an incidence of *Toxoplasma* donor-recipient mismatch of 10%, of whom only 39% of mismatched recipients received TMP/SMX prophylaxis. Only four patients seroconverted, of whom two had received prophylaxis, and there were no cases of clinical disease (19). These data suggest that in transplant centers with low *Toxoplasma* seroprevalence, routine screening in SOT donors and recipients might not be necessary, particularly in the era of routine TMP/SMX prophylaxis. In areas of high seroprevalence, routine screening may be indicated.

Table 1: Therapy for common parasitic infections in SOT recipients

Organism	Preferred therapy	Alternative therapy
Blood and tissue protozoa		
Babesia	Atovaquone 750 mg (pediatric: 20 mg/kg/dose) po bid plus azithromycin 600 mg (pediatric 12 mg/kg) a day (if able to take oral medications) to ≥2 weeks beyond clearance of parasitemia (≥6 weeks minimum total treatment; Ref. 123)	Clindamycin 600 mg (pediatric: 20–40 mg/kg/day divided) po tid or 1.2 g IV q12 hours plus quinine 650 mg (pediatric: 30 mg/kg/day divided) po tid (or quinidine IV) to ≥2 weeks beyond clearance of parasitemia (≥6 weeks minimum total treatment; Ref. 123)
Leishmania		
Visceral disease	Liposomal amphotericin B given 3 mg/kg IV on days 1 through 5, 14, and 21. Consider secondary prophylaxis with intermittent dosing in patients at high-risk for relapse	Amphotericin B deoxycholate 1.0 mg/kg daily for 15–20 days **OR** pentavalent antimony compound Consider secondary prophylaxis with intermittent dosing in patients at high-risk for relapse
Cutaneous or mucocutaneous disease	A pentavalent antimony compound (stibogluconate or meglumine antimoniate) at 20 mg/kg IV/IM daily. Duration: cutaneous disease, 21 days and mucocutaneous disease, 28 days	Liposomal amphotericin B, amphotericin B deoxycholate, miltefosine, paromomycin, pentamidine, and fluconazole can be considered based on species and availability
Toxoplasma gondii	*Induction therapy*: Pyrimethamine 200 mg po x1 then 50 mg (<60 kg) to 75 mg (≥60 kg) (pediatric 2 mg/kg/day) PO daily plus sulfadiazine 1.0 (<60 kg) to 1.5 gm (≥ 60 kg) (pediatric 100–200 mg/kg/day divided) PO q6h plus leucovorin 10–25 mg PO daily for at least 6 weeks *Chronic suppressive therapy:* Pyrimethamine 25 mg (< 60 kg) to 50 mg (≥ 60 kg) PO daily plus sulfadiazine 2.0 gm (< 60 kg) to 4.0 gm (≥ 60 kg) PO daily (in 2–4 divided doses) plus leucovorin 10–25 mg PO daily	*Induction therapy*: Pyrimethamine (same dosing as preferred therapy) plus clindamycin 600 mg IV/PO q6h **OR** TMP-SMX (10 mg/kg TMP-50 mg/kg SMX) IV/PO divided BID **OR** Atovaquone 1500 mg PO BID plus either pyrimethamine and leucovorin (same dosing as preferred therapy) or sulfadiazine (same dosing as preferred therapy) **OR** azithromycin 900–1200 mg PO daily plus pyrimethamine and leucovorin (same dosing as preferred therapy) *Chronic suppressive therapy:* Pyrimethamine (same dosing as preferred therapy) plus clindamycin 600 mg PO q8h **OR** TMP-SMX 1 DS tab q12h **OR** atovaquone 750 mg PO q6–12h +/- either pyrimethamine and leucovorin (same dosing as preferred therapy) or sulfadiazine (same dosing as preferred therapy) **OR** azithromycin 900–1200 mg PO daily plus pyrimethamine and leucovorin (same dosing as preferred therapy)
Trypanosoma cruzi	Benznidazole∗ 5–7 mg/kg/day (pediatric <12 years: 10 mg/kg) divided bid for 60 days	Nifurtimox∗ 8–10 mg/kg/day divided three times daily for 90 days (pediatric: 1–10 years: 15–20 mg/kg /day divided qid; 11–16 years: 12.5–15 mg /kg/day divided qid)
Intestinal protozoa		
Blastocystis hominis	Nitazoxanide 500 mg po bid for 3 days (pediatric: 12–47 months 100 mg/dose bid)	Metronidazole 1.5 grams x 1 daily for 10 days **OR** Iodoquinol 650 g po tid x 20 days, **OR** TMP/SMX DS bid x 7 days
Cryptosporidium	Nitazoxanide 500 mg po bid x 14 days (same as for HIV+) (pediatric: 12–47 months of age, 100 mg PO bid 4–11 years of age, 200 mg PO bid ≥12 years of age – see adult dosing) Reduce immunosuppression if possible	Paromomycin or azithromycin; consider combination therapy
Cyclospora	TMP/SMX DS qid x 10 days then tid (pediatric: TMP 5 mg/kg/SMX 25 mg/kg/day divided bid)	Ciprofloxacin 500 mg po bid x 7 days, then three times a week x 2 weeks
Entamoeba histolytica	Metronidazole 750 mg (pediatric 35–50 mg/kg/day divided) po tid x 10 days **OR** Tinidazole 2 gram (pediatric >3 years 50 mg/kg) po once daily x 3 days followed by	Nitazoxanide: *intestinal amoebiasis*: 500 mg po bid x 3 days and for *extraintestinal (hepatic) amoebiasis* 500 mg po bid x 10 days followed by paromomycin or iodoquinol as per preferred therapy

Continue

Table 1: Continued

Organism	Preferred therapy	Alternative therapy
	Paromomycin 500 mg (pediatric 25–35 mg/kg/day divided) potid x 7 days **OR** Iodoquinol 650 mg po (pediatric 30–40 mg/kg/day divided) tid x 20 days to eliminate cysts.	
Giardia	Tinidazole 2 gram x 1, or Nitazoxanide 500 mg po bid x 3 days (pediatric: 12–47 months 100 mg PO bid 4–11 years 200 mg PO bid ≥12 years—see adult dosing)	Metronidazole 500–750 mg po (pediatric 15–30 mg/kg/day divided) tid x 5 days; **OR** Paromomycin 500 mg po qid x 7 days; *refractory disease:* Metronidazole 750 mg tid plus quinacrine 100 mg tid both for 3 weeks
Cystoisospora belli	*Immunocompromised host*: TMP/SMX DS qid x 10 days then bid x 3 weeks (same as for HIV+) (pediatric: TMP 5 mg/kg/SMX 25 mg/kg/day divided bid)	Ciprofloxacin 500 mg po bid x 7 days **OR** pyrimethamine 75 mg po a day with folinic acid 10 mg a day for 14 days
Microsporidia	Albendazole 400 mg (Pediatric 15 mg/kg/day divided) po bid x 3 weeks or Fumagillin 200 mg po tid	
Helminths		
Strongyloides	Ivermectin 200 microgram/kg/day x 2 days; repeat in 2 weeks (3 mg tablets) (longer for hyperinfection)	Albendazole 400 mg po bid x 10–14 days (longer for hyperinfection)
	Hyperinfection: Treat until document clearance – then 7–14 days longer	Off-Label alternatives if oral therapy not an option:
	HTLV-1 co-infection: Treat until document clearance – then 7–14 days longer. Expect persistent infection. Monitor and retreat as needed.	(a) Per rectum ivermectin (b) Subcutaneous ivermectin
Schistosoma	Praziquantel 20 mg/kg/dose po bid x 1 day if *S. hematobium* or *S. mansoni*) Praziquantel 20 mg/kg/dose po tid x 1 day if *S. japonicum* or *S. mekongi*	Oxamniquine and artemether (anti-malarial)
Echinococcus	Albendazole 400 mg po bid (pediatric 15 mg/kg/day divided bid) for 1–6 months plus possible surgery or PAIR procedure)	Off-Label preprocedure or presurgical use:albendazole (+/- praziquantel in combination) to reduce the chance of secondary seeding

Therapy for Common Parasites.
Note:There are no prospective trials for any regimen in transplantation. Very few drugs interactions with standard transplant-related medications have been reported, and may be underappreciated.
- In the United States, these drugs must be obtained from the Centers for Disease Control at 404–639-3670 (emergency after hours 404–639-2888).
- Pediatric doses are included where available.

Primary prophylaxis: The routine use of TMP/SMX for post-SOT prophylaxis has decreased the risk of toxoplasmosis (20–23) and is currently the most common prophylaxis against this parasite. Multiple studies support the efficacy of primary prophylaxis with TMP/SMX, although the optimal dose and duration of TMP/SMX remains unclear. Many studies showed successful prophylaxis using TMP/SMX (160 mg of TMP, 800 mg of SMX) thrice weekly for varying durations (range 3 months to lifelong; Refs.21–23). In patients with HIV/AIDS, TMP/SMX (160 mg of TMP, 800 mg of SMX) one tablet daily is recommended as first line prophylaxis (16). Reports of toxoplasmosis in high-risk patients after stopping prophylaxis have been described (24). An alternative to TMP-SMX that has been well studied in patients with HIV/AIDS is dapsone plus pyrimethamine (plus leucovorin; Refs.25–27). Atovaquone with or without pyrimethamine (plus leucovorin) has not been well studied but is considered a likely effective alternative regimen as well (16). Some transplant centers have reported using pyrimethamine with or without sulfadiazine for prophylaxis of toxoplasmosis infection in high-risk cardiac recipients (22,23,28).

To avoid primary infection, transplant recipients should avoid contact with undercooked meat, soil, water or animal feces that might contain toxoplasmosis cysts.

Recommendations (iii)
Pretransplant screening:

- All heart transplant candidates and donors should be tested for *Toxoplasma* IgG pretransplant.

- The benefit of screening nonheart transplant recipients and donors is not well established but could be considered in high prevalence areas.

Diagnosis:

- Acute toxoplasma infection can be identified by histopathological results, seroconversion, or molecular testing (PCR).
- Initiation of empiric therapy (particularly of CNS infection) should be considered based on clinical and radiographic findings manifestations while awaiting results.

Treatment:

- Pyrimethamine plus sulfadiazine is recommended as first-line therapy of acute toxoplasmosis infection.
- Chronic suppressive therapy after induction therapy is recommended.

Prevention:

- Among toxoplasma seropositive heart transplant recipients and seronegative heart transplant recipients receiving organs from seropositive donors, prophylaxis against toxoplasma infection is recommended with TMP/SMX. The optimal dose and duration of prophylaxis posttransplant has not been determined, but many transplant centers give lifelong prophylaxis with TMP/SMX double strength (160 mg of TMP, 800 mg of SMX) one tab three times weekly or TMP/SMX single strength (80 mg of TMP, 400 mg of SMX) one tab daily.

Chagas Disease (American Trypanosomiasis)

Epidemiology and risk factors

Chagas disease is caused by the protozoan parasite, *T. cruzi* and infection is transmitted to humans primarily by contaminated feces of a triatomine insect vector (29). However, *T. cruzi* infection has also been transmitted by blood transfusion, infected mother to fetus, oral ingestion, and organ transplantation. Chagas disease is endemic in most Latin-American countries where 8–9 million people are currently living with infection and 2–5 million people have Chagasic cardiomyopathy. Because of recent immigration it is estimated that between 0.3 and 1 million *T. cruzi* infected people are living in the United States (30–32).

Human disease has two distinct phases: the acute phase and the chronic infection. In the normal host, the acute disease usually resolves spontaneously even if untreated; but without specific treatment the infection persists in spite of strong evidence of immunity and patients become chronically infected with the parasite (29). The indeterminate phase (clinical latency) can last 10–30 years or lifelong. In approximately 30% of patients the chronic phase will evolve into irreversible disease of the heart (27%), the esophagus and the colon (6%) and the peripheral nervous system (3%; Ref.33). In transplant recipients there are three distinct scenarios that will be focused on in this section (1) heart transplant recipients with chronic *T. cruzi* infection who are at risk of reactivation posttransplantation, (2) noncardiac transplant recipients with chronic *T. cruzi* infection who are at risk of reactivation posttransplantation and (3) uninfected organ transplant recipients who received organs or blood from *T. cruzi* infected donors.

Patients with chagasic cardiomyopathy: Chagasic cardiomyopathy is the third leading cause for heart transplantation in Brazil (21.9% of all heart transplants; Ref.34). Posttransplant outcomes do not differ significantly from heart transplant for other causes (34–36). Reactivation after transplantation has been reported to occur in 27% (34) to 43% (37) of recipients and risk factors may include treatment of rejection, mycophenolate mofetil use and development of neoplasms (34) although other studies have not found the same associations (37). Clinical manifestations range from asymptomatic parasitemia to fevers, cutaneous manifestations and myocarditis (that may both clinically and histologically appear similar to rejection; Ref.38). Skin manifestations include a rash, which may look more like panniculitis rather than a macular drug rash; a skin biopsy may show trypanosomes. Early diagnosis, careful monitoring and good response to treatment allow for an adequate survival (37).

Patients with chronic T. cruzi infection undergoing nonheart organ transplant: Most of the experience outside of heart transplantation is related to kidney transplantation (39). Reactivation has been described mainly within the first year posttransplant. The most frequent reactivation feature is asymptomatic parasitemia, but fever, panniculitis or other cutaneous involvement, myocarditis, and encephalitis have also been reported (38–40).

Uninfected organ recipients receiving organs or blood from T. cruzi infected donors: *Trypanosoma cruzi* seronegative recipients of seropositive donors may develop acute *T. cruzi* infection posttransplantation (41–43). Transmission rates from seropositive donors to seronegative recipients are approximately 20% for kidney transplants (39,44) and 22–29% of liver transplants (44,45). Because of the tropism of *T. cruzi* for cardiac tissue, rates would likely be higher for heart transplants and several cases of severe acute *T. cruzi* infection in heart transplant recipients have been described (41–43). Transmission rates for other organs (lung, pancreas and intestine) are not well defined. Clinical manifestations of infection can include fever, malaise, anorexia, hepatosplenomegaly and acute

myocarditis with a mean time to symptom onset of 112 days (range 23–240 days; Refs.39,41–43,46–51).

Diagnosis

Diagnosis of chronic T. cruzi infection in organ recipients and donors: Diagnosis of chronic infection is made by detection of antibodies to *T. cruzi* antigens, most commonly by the EIA or IFA methods. Different countries have unique approved testing assays. No single test has sufficient sensitivity or specificity to be relied on alone for clinical diagnosis. Therefore, two serological tests based on different antigens and/or techniques should be used to increase the accuracy of the diagnosis (52). When discordant testing occurs, a third test should be used.

Diagnosis of acute T. cruzi infection posttransplantation: In organ transplant recipients with acute infection and those with chronic *T. cruzi* infection where there is a concern for reactivation, serological testing has limited utility. Direct parasitological test methods for diagnosis include microscopy of the fresh buffy coat preparations, Giemsa-stained peripheral blood smears, and PCR of whole blood or tissue from a biopsy. PCR techniques provide the most sensitive testing and can often identify positive results days to weeks before circulating trypomastigotes are detectable by microscopy of peripheral blood smear and buffy coat preparations (53). Patients with chronic *T. cruzi* infection may have positive PCR in the absence of disease reactivation and may not be helpful. PCR is not commercially available in the United States but can be obtained through the Centers for Disease Control and Prevention (CDC, see contact details below). Hemoculture is of limited utility because of its prolonged turn around time (2–8 weeks).

Monitoring for infection after transplant of an organ from a seropositive donor to a seronegative recipient and after transplantation in a recipient with chronic *T. cruzi* infection is recommended so that treatment can be initiated before the development of clinically significant disease. Monitoring can be accomplished by checking PCR of blood for *T. cruzi* DNA (when available) and review of peripheral blood for parasitemia weekly for 2 months posttransplant, every two weeks for the third month, then monthly afterwards for a period to be determined by the specific clinical scenario. Additional testing is recommended in the setting of intensified immunosuppression, unexplained febrile illness, or episodes of suspected graft rejection (44).

Treatment

Treatment is recommended for patients with evidence of reactivation of chronic infection or acute infection posttransplantation. Two drugs are available for treatment of Chagas disease, nifurtimox and benznidazole (Refs.54,55); see Table 1). Neither drug is approved by the FDA, but they are both available in the United States via the CDC through their investigational drugs protocols. Both drugs have significant side effect profiles; benznidazole is frequently associated with rash and a dose-dependent peripheral neuropathy while nifurtimox is associated with gastrointestinal symptoms (anorexia, weight loss and nausea) and central nervous system symptoms (irritability, insomnia and tremors). Benznidazole is better tolerated among transplant recipients and has fewer drug interactions when compared to nifurtimox and therefore, benznidazole is generally preferred for first-line treatment.

Prevention

Screening of organ donors for T. cruzi infection: Donor and recipient screening should be considered in Latin America (South America, Central America or Mexico). In lower prevalence areas (e.g. United States), universal screening of at-risk populations should be considered based on local epidemiology and targeted screening in all populations is recommended (44). Targeted screening may be accomplished for individuals who answer yes to the following question, "Was the potential donor or recipient born in Latin America (South America, Central America or Mexico)?" In a recent survey of all United States organ procurement organizations, 19% were performing either universal or targeted donor screening for *T. cruzi* infection (56).

Transplantation of organs from T. cruzi seropositive donors to seronegative recipients: Donor-derived *T. cruzi* infection has been described and organ specific rates of transmission are limited but several sets of guidelines have recently been published (44,57,58). Transplantation of kidneys and livers from *T. cruzi* infected donors can be considered with close monitoring posttransplant. However, transplantation of hearts from *T. cruzi* infected donors is not recommended given the tropism of *T. cruzi* for cardiac tissue. Limited data are available on transplantation of other organs (lung, pancreas and small bowel) and can be considered with caution based on anticipated degree of immunosuppression.

Prophylactic treatment to prevent T. cruzi transmission or reactivation of chronic infection: Systematic data are lacking for the efficacy of prophylactic treatment and may mask signs of transmission. Confirmation of infection (or its absence) has important implications for long-term management of the patient. Taking these considerations and potential for drug toxicity, most experts would prefer careful monitoring posttransplant to the use of and not recommend prophylactic treatment.

Consultation for suspected cases of T. cruzi infection

In the United States consultation about known or suspected *T. cruzi* infections, confirmatory testing, monitoring and treatment of transplant recipients should be directed to the Division of Parasitic Diseases and Malaria,

CDC. Phone 770–488-7775. E-mail: parasites@cdc.gov. CDC Emergency Operator (after business hours and weekends): 770–488-7100.

Recommendations
Pretransplant screening:

- Screening for *T. cruzi* infection should be performed in heart transplant candidates.
- Universal organ donor and recipient screening for *T. cruzi* infection should be considered in Latin America (South America, Central America, and Mexico).
- Targeted donor screening in all populations is recommended but universal donor screening for *T. cruzi* in lower prevalence areas should be considered based on local epidemiology.

Diagnosis:

- Chronic infection: Because of insufficient sensitivity or specificity of a single assay, two serological tests based on different antigens and/or techniques should be used to increase the accuracy of the diagnosis of chronic *T. cruzi* infection.
- Acute infection or reactivation of chronic infection: PCR of blood or tissue and review of peripheral blood smear for parasitemia are the preferred techniques for diagnosing active T. cruzi infection posttransplantation.

Treatment:

- In the setting of active *T. cruzi* infection, benznidazole is the preferred treatment in organ transplant recipients with nifurtimox as an alternative.

Prevention:

- Posttransplant anti-*T. cruzi* prophylaxis is not recommend in recipients of organs from seropositive donors or previously infected recipients at risk for reactivation. A strategy of preemptive monitoring and treatment following evidence of active infection is preferred.
- In organ transplant recipients with acute *T. cruzi* infection and those with chronic *T. cruzi* infection where there is a concern for reactivation screening for active infection by serum PCR and peripheral blood for parasitemia is recommended.

Leishmaniasis

Epidemiology and risk factors

Leishmaniasis is caused by a heterogeneous group of protozoan parasites, belonging to the genus *Leishmania* and presents with a variety of different clinical syndromes. The infection is acquired primarily through the bite of an infected female sandfly. It is estimated that 350 million people are at risk of acquiring the infection and that 12 million may be infected (59). Leishmaniasis is found in tropical and subtropical climates and is endemic in the Mediterranean countries in Europe. More than 90% of the world's cases of visceral leishmaniasis occur in India, Bangladesh, Nepal, Sudan and Brazil (60). The disease may appear as late as 30 years after the initial infection, therefore, even distant exposure needs to be considered for differential diagnosis. Leishmaniasis can be classified three ways, (1) geographically into New World and Old World disease; (2) clinically by syndrome into visceral, cutaneous, or mucocutaneous disease and (3) by subgenus, complexes and species based upon taxonomy (59,61).

Derangement of host cellular immunity is a significant risk factor for the development of symptomatic and severe infections and for increased mortality in patients infected with leishmaniasis (61). In most immunocompetent hosts, infection with *Leishmania* spp. is asymptomatic; however, viable organisms remain latent for life of the host (62). Therefore, it is not surprising that severe disease has become more frequently reported in organ transplant recipients who have lived or visited endemic regions. The three main potential mechanisms of acquisition of leishmaniasis in organ transplant recipients are (1) primary infection after transplantation, (2) reactivation of latent infection after transplantation or (3) receipt of an infected organ during transplantation (63,64). Most cases of leishmaniasis in organ transplant recipients have occurred in kidney transplant recipients (65–92) although reports have included liver (88,100), heart (88,102), lung (93) and kidney-pancreas (94). Diagnosis is often encountered late posttransplant with a median time of 18 months (95).

Clinical manifestations of disease vary based on the infecting organism and host immune response. Visceral leishmaniasis is caused by *L. donovani* complex (*L. donovani*, *L. infantum* and *L. chagasi*) and the clinical features are similar to what is seen in immunocompetent patients. Patients suffer from fever, hepatosplenomegaly and pancytopenia (96). Median time to onset was 30 days posttransplant (range of 7 days to 5 months) in one systematic review (95) but other reports have described reactivation as far out as 55 and 96 months posttransplantation (97,98). Cutaneous and mucocutaneous presentations are most often due to species of the *L. mexicana* complex and subgenus Viannia in the New World and *L. major*, *L. tropica* and *L. aetheopica* in the Old World. Cutaneous and mucocutaneous leishmaniasis are less commonly reported on in organ transplant recipients and have a protracted time interval between transplantation and disease manifestations (95,99).

Diagnosis

Visceral leishmaniasis: Direct visualization of amastigotes on histopathology or culture revealing promastigotes remain the gold standards for diagnosis of visceral leishmaniasis. This is most frequently accomplished by bone

marrow or splenic aspiration. In an immunocompetent cohort, splenic biopsies had a greater sensitivity than bone marrow aspirate (96% vs. 70%) for diagnosing visceral leishmaniasis (100). However, in organ transplant patients, bone marrow biopsy has been reported to have a sensitivity of 98% (95). Occasionally the diagnosis can be made from biopsy of other tissues such as lymph node or intestine. Serological testing for visceral leishmaniasis is highly sensitive in organ transplant recipients with 45/49 (92%) of patients testing positive in one systematic review (95). However, serology cannot distinguish between prior exposure and active infection and may cross-react with other protozoa. A urinary antigen test and serum PCR show high sensitivity for the diagnosis of visceral leishmaniasis and may be useful where available (101,102).

Cutaneous and mucocutaneous leishmaniasis: When cutaneous or mucosal leishmaniasis is suspected, a biopsy specimen for histopathological examination and culture should be obtained. After a parasite has been identified, speciation can be performed through isoenzyme analysis or species-specific monoclonal antibodies. Quantitative or semiquantitative PCR assays have shown a high diagnostic sensitivity when applied to histopathological specimens (103). Antileishmanial antibodies can be detected in the serum of patients with cutaneous and mucocutaneous disease but is not used routinely for diagnosis.

Contacting the CDC for diagnostic assistance with leishmania

In the US, information about CDC leishmania serology and PCR, or to obtain NNN (Novy-MacNeal-Nicolle) culture media can be made by contacting the Division of Parasitic Disease, CDC. Phone 770-488-4475. Additional helpful information can be found at www.dpd.cdc.gov.

Treatment

Visceral leishmaniasis: Drugs with efficacy in the treatment of visceral leishmaniasis include amphotericin B, pentavalent antimony, paromomycin, and miltefosine. Pentavalent antimony had previously been the primary treatment for visceral leishmaniasis but resistance rates have been increasing in some regions and there is significant toxicity associated with treatment. Liposomal amphotericin B has been shown to be the most efficacious drug for treatment of this disease and is the only drug licensed for the treatment of visceral leishmaniasis in the United States (104). Cure rates with amphotericin B in immunocompromised patients approach the same success seen in immune competent hosts. However relapsed disease was diagnosed in 24% of cases in organ transplant recipients as early as 1 month and as late as 5 years (77,95,105). Secondary prophylaxis with intermittent dosing of amphotericin may be useful for preventing relapse and is supported by a randomized control trial performed on patients with both HIV/AIDS and visceral leishmaniasis (106). Successful use of secondary prophylaxis has been reported in three cases of visceral leishmaniasis in organ transplant recipients using different regimens including weekly amphotericin B (107), daily fluconazole (63) and monthly meglumine antimoniate (105).

Cutaneous and mucocutaneous leshmaniasis: Pentavalent antimony compounds are the recommended therapy for most cases of cutaneous and mucocutaneous leshmaniasis. Varying quality studies have also evaluated the efficacy amphotericin B, pentamidine, miltefosine and many other intravenous, oral and topical preparations. In transplant recipients with cutaneous and mucocutaneous disease, treatment with both amphotericin B and pentavalent antimony compounds have been described with mixed results (99,108).

Prevention

Data are lacking to determine if screening potential organ transplant recipients for visceral leishmaniasis would be beneficial. However, those known to be seropositive at the time of transplant should be monitored closely for signs and symptoms of reactivation of infection. Given the limited data on potential donor-derived infection, donor screening cannot be recommended (109).

Recommendations (iii)

Pretransplant screening:

- Serologic screening of recipients with a history of potential exposure to *Leishmania* may be considered pretransplant in patients who have spent time in endemic regions.

Diagnosis:

- Bone marrow biopsy should be used over splenic biopsy as first line diagnostic method to obtain histopathology and/or culture to confirm the diagnosis in suspected cases after organ transplantation. Serological testing also has a high sensitivity and may be a useful test in certain cases.
- In organ transplant recipients with cutaneous or mucocutaneous leishmaniasis, skin or mucosal biopsy for histopathology and/or culture remains the gold standard. Serological testing has little role in evaluation of cutaneous disease.

Treatment:

- Liposomal amphotericin B should be considered first line therapy for patients with visceral leishmaniasis when available and secondary prophylaxis may be of benefit in select cases to prevent relapse.
- Pentavalent antimony compounds should be considered first line therapy for most patients with severe cutaneous or mucocutaneous.

Prevention:

- Patients with known prior visceral leishmaniasis or recipients of organs form donors with visceral leishmaniasis should be clinically monitored for evidence of infection after organ transplantation.

Malaria

Epidemiology and risk factors

Malaria poses an immense health problem in developing countries where it is the cause of more than 300 million acute cases and over 1 million deaths per year. It is transmitted to humans mostly through the bite of the female *Anopheles* mosquito; blood transfusions and organ transplantation are responsible for some cases in endemic areas and occasionally in countries with large immigrant populations (110). The disease does not produce protective immunity, but some degree of resistance to clinically severe hyperinfection is achieved through successive exposure and through persistence of plasmodia in the liver, the microvasculature and the blood stream. This incomplete acquired immunity is unable to completely eradicate the infection but explains the lack of detectable parasitemia and the higher incidence of asymptomatic disease in adults from endemic regions. This poses a problem at the time of blood or organ donation when the epidemiological background is not thoroughly investigated.

Many cases of malaria have been described in transplant recipients. It is not always possible to determine the mode of infection but transmission via the graft has been reported (111), although ultimately, some cases were traced to blood or blood products transfused to the recipient, even well before transplantation (112). In developed countries the disease is seldom seen but it should be considered when caring for a transplanted patient who has resided or visited areas where the disease is endemic (or has received an organ from a donor who has been in endemic areas) and presents with an unexplained febrile illness. The four different main plasmodia species that infect humans, *Plasmodium ovale*, *P. vivax, P. malariae* and *P. falciparum*, have all been diagnosed in SOT. Clinical manifestations have occurred in the early posttransplant period and have been described in kidney, liver and heart recipients (111–113). Fever has been reported as the most frequent presenting symptom, but it did not always have the typical paroxysmal or cyclic pattern (114,115).

Diagnosis

Malaria is classically diagnosed by microscopic observation of thick or thin blood smears. Rapid diagnostic tests are available by using dipsticks and allow the detection of specific plasmodia antigens in clinically significant malarial infections (116). Alternative diagnostic techniques that are recommended in some circumstances to screen blood donors include enzyme-linked immunoabsorbent assay for *P. falciparum* antigens; immuno-fluorescent-assay techniques for species-specific enzymes, DNA hybridization and DNA and mRNA amplification using PCR. In most posttransplant cases, the diagnosis was made by the identification of the parasite in blood smears in febrile patients with unexplained hemolysis and thrombocytopenia (117).

Treatment

Specific treatment of malaria relies on the use of anti-plasmodium drugs. The identification of plasmodia species, the knowledge of their geographical distribution and of their sensitivity patterns is essential. *P. vivax*, *P. malariae*, *P. ovale* and uncomplicated *P. falciparum* infection in chloroquine-susceptible regions should be treated with chloroquine. However, resistance to chloroquine has been described from Oceania for *P. vivax.* Uncomplicated *P. falciparum* infection acquired in a chloroquine resistant region can be treated with an artemisinin combination therapy, atovaquone-proguanil, quininine-based regimen, or mefloquine. Severe cases of *P. falciparum* infection should be treated with intravenous artesunate (available as an investigational new drug in the U.S. via the CDC Malaria Hotline: (770) 488-7788 or (855) 856-4713 toll-free Monday–Friday 9 am to 5 pm EST – (770) 488-7100 after hours, weekends and holidays) followed by doxycycline, atovaquone-proguanil or mefloquine. When artesunate is not available, intravenous quinine or quinidine plus doxycycline, tetracycline or clindamycin should be given. Primaquine should be used to prevent relapse of *P. vivax* and *P. ovale* (after checking for G6PD deficiency).

Malaria is potentially fatal in the transplant recipient. Early diagnosis and conventional specific treatment usually results in prompt and uneventful recovery. *P. falciparum* infection (111), drug toxicity and other infections may hamper the outcome. Special attention is needed when quinine is used for treatment because it may interfere with cyclosporine metabolism, decreasing its blood levels (118).

Prevention

Screening of donors who have recently spent time (preceding 3 years) in malarious regions should be considered. Potential screening methods should include thick and thin smear stained with Geimsa, Wright or Field stains. Rapid diagnostic tests detecting the HRP2 antigen can also be considered when expert review of thick and thin smears is not possible. Recipients traveling to malarious regions should be given appropriate chemoprophylaxis to prevent infection during travel. Chloroquine can potentiate levels of cyclosporine and appropriate dose adjustments should be made.

Recommendation (iii)
Pretransplant screening:

- Consider both donor and recipient testing for malaria with thick and thin smear if epidemiologically at high-risk for infection.

Diagnosis:

- Microscopic observation of thick or thin blood smears remains the gold standard for diagnosing malaria.
- Rapid diagnostic tests can be considered when microscopic evaluation by trained personal is not available.

Treatment:

- Treatment of patients should be performed via standard guidelines provided by the CDC (http://www.cdc.gov/malaria/resources/pdf/treatmenttable.pdf) and WHO (http://whqlibdoc.who.int/publications/2010/9789241547925_eng.pdf) based on species and severity of disease.

Prevention

- Organ transplant recipients traveling to malarious regions should be given appropriate chemoprophylaxis and instructed to perform other risk-reducing measures to prevent infection.
- Consultation with a travel medicine expert prior to international travel is highly recommended and valuable to reduce risks of illness. Providers with expertise can be found at www.istm.org and www.astmh.org.

Babesia

Epidemiology and risk factors

Babesiosis is a tick-borne, zoonotic protozoal illness that occurs after infection with *Babesia* spp., which invade and lyse red blood cells. Several species of babesia cause human disease and include *B. microti* primarily in the northeastern United States, *B. divergens* in Europe, *B. duncani* in the western United States, and an unnamed strain, designated MO-1, in Missouri. *B. divergens*, appears to be more virulent than others. Transmission to humans occurs via ticks of the *Ixodes* genus or rarely through blood transfusion. *Ixodes scapularis* is responsible for transmission of *B. microti* in northeastern United States and also may carry *Borrelia burgdorferi* and *Anaplasma phagocytophilum*. All three reports of babesiosis in transplant recipients were transfusion related and include two kidney transplant recipients and one heart transplant recipient (119–121). No current FDA licensed *Babesia* test is available for screening donated blood products.

Risk factors for severe babesiosis include asplenia, immunocompromised state and older age. Clinical manifestations range from asymptomatic to life threatening disease. Early symptoms may be fever and malaise, which can progress to severe hemolytic anemia (potentially manifesting as a posttransplant hemolytic-uremic or hemophagocytic syndromes), adult respiratory distress syndrome, multi-organ system failure and even death. Blood tests may show hemolytic anemia, thrombocytopenia and conjugated hyperbilirubinemia. Disease severity correlates with degree of parasitemia.

Diagnosis

Babesiosis can be diagnosed by microscopic visual review of peripheral blood smear or by PCR of blood. Diagnostic confusion between *Plasmodia* spp. (malaria) and *Babesia* spp. can occur due to similarity in morphology on microscopy when infecting red blood cells. Specific epidemiologic exposures and DNA testing (PCR) can aid in differentiating the diseases. For babesiosis bone marrow biopsy may reveal hemophagocytosis and marrow histiocytosis.

Treatment

Babesiosis is a potentially life threatening infection in immunocompromised hosts and antimicrobial treatment should begin immediately. There are no studies of babesiosis treatment in transplant recipients. Exchange transfusion should be considered in cases of greater than 10% parasitemia, severe hemolysis, severe renal and/or hepatic and/or pulmonary compromise (122). Reduction in immunosuppressive regimen should be considered. Atovaquone plus azithromycin can be used in those able to take oral medications. Clindamycin plus quinine is alternative regimen. In a prospective, nonblinded, randomized trial of the two regimens in 58 normal hosts, atovaquone and azithromycin was as effective as clindamycin and quinine with fewer adverse reactions (15% vs. 72%). The most common adverse effects with atovaquone and azithromycin were diarrhea and rash (8% each), while clindamycin and quinine common adverse effects were tinnitus (39%), diarrhea (33%) and decreased hearing (28%; Ref.123. Azithromycin may increase the serum concentration of tacrolimus and patients should be monitored for toxicity. Sirolimus and tacrolimus metabolism may be slowed by the CYP3A4 inhibitor quinidine.

The optimal antimicrobial or combination therapy in transplant recipients is not clear; persistent relapsing illness has been well described in other immunocompromised hosts. In one series of 14 immunocompromised subjects, most of whom had B-cell lymphoma and were asplenic or had received rituximab, antibabesial treatment was required for at least 6 weeks to achieve cure. Resolution of persistent infection occurred in 11 patients when antibiotic treatment was continued ≥2 weeks after documenting negative blood smears (124). Three (21%) subjects died, highlighting the severity of disease in this population and the need for longer treatment and prolonged monitoring compared to normal hosts (124). Though rare, resistance to the atovaquone/azithromycin regimen can occur, more commonly in immunocompromised hosts (125). Blood smears should be used for monitoring response to therapy and for relapse after completion of treatment, PCR can be considered when available.

Prevention
When visiting endemic areas, transplant recipients should avoid tick exposure by using permethrin repellants on clothing, DEET or Picaridin repellants on skin, and general protective clothing (126). Frequent tick checks and prompt removal is valuable, because early removal decreases the chance of transmission.

Recommendations (iii)
Pretransplant screening:

- Prospective living organ donors should avoid high-risk exposures in endemic regions in the weeks prior to donation.
- Living donors with a prior diagnosis of *Babesia* infection should report this and document they are clear of infection prior to donation.
- No *Babesia* tests are currently licensed for screening U.S. blood and organ donors.

Diagnosis:

- Direct visual microscopy is the most common diagnostic method. Rapid nucleic acid based tests are available through the CDC, and can aid in differentiation from malaria, as well as between various *Babesia* spp.
- Screen or monitor for tick-borne co-pathogens *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in cases of babesiosis, because all three can co-infect an *Ixodes* spp tick.

Treatment:

- Atovaquone/azithromycin is preferred over clindamycin/quinine for babesiosis.
- Consider treatment of infection for 6 weeks, or at least 2 weeks after smear negative, to achieve full eradication. Relapse is common and posttreatment monitoring is recommended.

Prevention:

- Transplant recipients should be educated on how to reduce tick exposure when traveling to endemic regions.

Balamuthia

Epidemiology and risk factors
Balamuthia mandrillaris is a free-living amoeba that has been identified as a cause of human disease in the last two decades (127,128) and more immediately recognized as a cause of donor-derived infection through organ transplantation (129,130). Balamuthia infection is relatively rare (<200 cases reported) and has been identified to cause disease in both immunocompetent and immunocompromised hosts. Unlike the other two free-living amoeba commonly associated with meningoencephalitis, *Naegleria* and *Acanthamoeba*, that are associated with fresh water exposure, *Balamuthia* is found in soil. Reported clinical manifestations of infection include chronic granulomatous skin lesions and a chronic granulomatous meningoencephalitis (127,128).

Two episodes of donor-derived transmissions of Balamuthia have been reported. The first transmissions occurred from a single donor in 2009 after a previously healthy 4-year-old with a slowly progressive (3 week) neurological decline died from presumed acute disseminated encephalomyelitis and became an organ donor (129). The two kidney recipients developed central nervous system abnormalities 20 days posttransplant and one died and the other had significant neurological deficits despite treatment. The liver and heart transplant recipients were given prophylactic antimicrobials and have remained asymptomatic. The second report of transmission occurred in 2010 when a 27-year-old male died of an apparent stroke (130). He had a chronic skin lesion for 6 months before his death. The recipients of the liver and kidney–pancreas both developed central nervous system symptoms 17 days posttransplant and ultimately died of *Balamuthia* infection. A kidney and a heart transplant recipient were treated with prophylactic antimicrobials and have remained asymptomatic. To date, no infections have been identified in organ transplant recipients from environmental exposure posttransplant or progression of subclinical pretransplant infection.

Diagnosis
Diagnosis of *Balamuthia* infection is unfortunately often made postmortem after histopathological examination of infected tissue. Most frequently brain or skin tissue are identified to have trophozoites or cysts present among granulomatous inflammation and necrosis. An indirect immunofluorescent assay is used to stain the tissue to confirm the diagnosis. Neither serum nor CSF studies are helpful for the diagnosis of *Balamuthia* infection at this time.

Treatment
Given the rarity of the disease, there is very little data on treatment of Balamuthia infection. Reported treatments have included a number of different antimicrobials, often in combination with often unsuccessful results, including amphotericin B, azoles, paromomycin, albendazole, pentamidine, macrolides, metronidazole, sulfadiazine, and miltefosine (127,128,131,132). Experts agree that treatment should include a multidrug regimen for a prolonged period of time yet the best combination of drugs is unclear at this time. Consultation with experts is strongly recommended (131).

Prevention
Because *Balamuthia* is thought to be ubiquitous in the environment, mechanisms for prevention of infection are not known. Early diagnosis and treatment of infection may improve prognosis. Caution should be made when considering transplanting organs from a donor with unexplained meningoencephalitis or unexplained chronic granulomatous skin infections.

Recommendation (iii)
Pretransplant screening:

- Not routinely recommended in asymptomatic patients.

Diagnosis:

- Diagnosis is most often made by identifying cysts or trophozoites in infected tissue. An indirect immunofluorescent assay is used to stain the tissue to confirm the diagnosis.

Treatment:

- Combination therapy is used for treatment and consultation with experts is strongly recommended.

Prevention:

- Caution should be made when considering transplanting organs from a donor with unexplained meningoencephalitis or unexplained chronic granulomatous skin infections.

Acanthamoeba and Naegleria

Epidemiology and risk factors
Acanthamoeba are protozoan parasites found in dust, soil, water, contact lens fluid, air conditioners, sewage, and may colonize the nose and throats of healthy individuals. A recent seroprevalence study found more than 80% of 55 healthy volunteers in Texas had antibodies to *Acanthamoeba* antigens, suggesting that exposure and undiagnosed infections are common (133). This disease can be seen in a variety of solid organ transplant recipient types—kidney, liver, lung and others (134). *Acanthamoeba* can cause either focal disease (usually keratitis, granulomatous amoebic encephalitis, brain abscess, pulmonary lesions, cutaneous lesions, or sinusitis) or disseminated acanthamebiasis which is often fatal in transplant recipients (135).

Naegleria are amoeba found in warm fresh water, heated contaminated tap-water, and soil. It grows best at higher temperatures (~46°C). Infection can occur from swimming in contaminated water and recently has been contracted by using nasal sinus irrigation using neti pots with contaminated water (136). Use of organs from donors unknown to be infected with *Naegleria fowleri* at the time of transplantation has occurred on at least five occasions without infection of the recipients (137).

Diagnosis
Cutaneous lesions may be the initial manifestation of infection and should be biopsied as early diagnosis diagnosis is imperative to optimize the chance of survival. A direct examination of CSF should also be performed. *Acanthamoeba* can be cultured on agar plates coated with Gram-negative bacteria; it may take up to two weeks of culture before, the amoeba appear as track marks within the bacterial growth. Immunofluorescent tests may be used for species confirmation; DNA and RNA probes can also be used, but are not widely available. Serology is only useful for seroprevalence studies but not for diagnosis.

Treatment
Optimal treatment regimens for *Acanthamoeba* infections remain unknown. Drug sensitivities of free-living amebic infections differ between genera, species, and strains. Combinations of amphotericin B products with rifampin or imidazoles have been tried, as have combinations of sulfonamide antibiotics, azithromycin, caspofungin and flucytosine. Pentamadine has some *in vitro* activity. Central nervous system disease, diagnosed in a liver transplant recipient was cured after partial lobectomy, reduced immunosuppression, and 3 months of trimethoprim-sulfamethoxazole and rifampin (138). Another case of *Acanthamoeba* sinusitis with concomitant *Aspergillus* in a lung transplant recipient was successfully treated with surgical debridement and initial intravenous amphotericin, followed voriconazole and caspofungin (139). Others have reported successful treatment of *Acanthamoeba* after transplantation using amphotericin B and miltefosine in combination with other drugs (134,140). While some drugs are effective *in vitro* against *Naegleria*; nearly all infections are fatal. Prevention is the most important defense against this infection at the moment.

Prevention
How best to prevent the rare infections due to *Acanthamoeba* is not clear, as the amoeba are fairly ubiquitous and seroprevalance rates are high. Trimethoprim-sulfamethoxazole has been used in treatment regimens; it is not known whether its common use in prophylaxis may be able to prevent infections. *Naegleria* prevention includes avoiding exposure. If nasal sinus irrigation is important, use boiled water, filtered (≤1 μm) water, or distilled/sterile water.

Recommendations (iii)
Pretransplant screening:

- Not routinely recommended in asymptomatic patients.

Diagnosis:

- Diagnosis is made by identifying cysts or trophozoites in infected tissue. An indirect immunofluorescent assay is used to stain the tissue to confirm the diagnosis.

Treatment:

- Combination therapy is used for treatment and consultation with experts is strongly recommended.

Prevention:

- If nasal sinus irrigation is important, use boiled water, filtered (≤ 1 m) water, or distilled/sterile water.

Intestinal Parasites

Intestinal parasitic infections are prevalent in developing regions of the world. Accordingly, with increasing travel to and from endemic regions, intestinal parasites may have an increasingly significant role in transplant candidates and recipients. Moreover, relevant parasites including *Strongyloides*, *Giardia*, *Cryptosporidium* and *Entamoeba* have a worldwide distribution. A careful pretransplant social history can identify at-risk individuals who may benefit from focused screening for persistent parasitic infection (141). Parasitic infections are often asymptomatic before transplantation but flourish under immunosuppression, becoming clinically evident. Eosinophilia, gastroenteritis and other clinical manifestations of parasite infections prior to transplant should also trigger an appropriate workup.

Intestinal Protozoa

Cryptosporidium/Cystoisospora belli/Cyclospora, Microsporidia/Blastocystis hominis/Giardia

Epidemiology and risk factors: *Cryptosporidium, Cystoisospora belli*, *Cyclospora*, Microsporidia, *Blastocystis hominis* and *Giardia* can all cause significant, and sometimes protracted, gastroenteritis in transplant recipients. While the use of mycophenolate mofetil is the most common cause of chronic diarrhea in transplant recipients, these fastidious organisms can mimic such colitis. *Cryptosporidium* and *Giardia* are among the most common parasitic pathogens seen in transplant recipients, given worldwide distribution. Transmission is more common in the developing world, with rates of infection as high as 20%, and can occur from contaminated food and water, person-to-person spread and zoonotic exposures (142). *Cryptosporidium* transmission in the developed world is facilitated by chlorine resistant oocytes and the 3–7 μm diameter of *Cryptosporidium* that can bypass many municipal water filtration and treatment systems. Moreover, infected individuals produce up to 100 million oocysts per day, while as few as 10–30 oocysts may cause infection in healthy persons.

Intestinal protozoa have also been reported as donor-derived infections with intestinal transplantation. Most reports of intestinal protozoa in transplant recipients have been in case reports or small series from individual institutions. Biliary disease occurs in 10–15% of HIV-positive patients with cryptosporidiosis (143) and could occur in transplant recipients as well. Extra-intestinal disease is very rare but can occur in the brain or kidney (especially with Microsporidia).

Diagnosis

Standard examination for ova and parasites may be helpful but are time consuming. Concentration of stool and subsequent special stains may be more sensitive for certain pathogens; many laboratories use a trichrome stain to diagnose microsporidial infections or Safranin stain for *Cyclospora*. ELISA of stool may help rapidly diagnose *Cryptosporidium* and *Giardia*. Direct immunofluorescence tests for Giardia and *Cryptosporidium* are also available. Nucleic acid detection studies may also be helpful when available. Electron microscopy of bowel biopsies may also be helpful in diagnosing these infections.

Treatment

Cryptosporidium can be treated with nitazoxanide, paromomycin, azithromycin, or potentially with combinations of these drugs. *Cyclospora* and *Cystoisospora belli* are usually treated with trimethoprim/sulfamethoxazole (DS tablets BID), potentially using the higher doses (DS tablets QID) as recommended for HIV patients (122). Ciprofloxacin or nitazoxanide are potential alternatives in the setting of significant sulfa allergy. *Cystoisospora belli* can also be treated with pyrimethamine combined with folinic acid. Microsporidia treatment depends on the site of infection; albendazole and fumagillin can be effective. *Blastocystis hominis* can be treated with nitazoxanide, metronidazole, iodoquinol, or TMP/SMX. In a cases series of two transplant recipients with microsporidiosis due to *Enterocytozoon bieneusi*, fumagillin was effective but resulted in drug-induced thrombocytopenia (144). *Giardia* can be treated with tinidazole, nitazoxanide, metronidazole, or paromomycin; refractory disease can be treated with metronidazole plus quinacrine (145).

Intestinal protozoa can be difficult to eradicate. Reduction in immunosuppressive regimen may hasten clearance of these durable pathogens. Tacrolimus levels may rise in the setting of diarrhea and should be carefully monitored. Diarrhea may be augmented and/or prolonged by the concomitant use of mycophenolate mofetil. There are no comparison studies of various treatments in transplant recipients.

Prevention
Intestinal protozoa infections are primarily acquired from contaminated food and water. Transplant recipients should avoid untreated well or lake water, and preferentially drink treated municipal water or bottled water. There are no data to support the use of bottled water over treated municipal water for transplant recipients. Person-to-person and zoonotic transmission can occur; transplant recipients should be aware of the potential risks.

Recommendations (iii)
Pretransplant screening:

- Not routinely recommended in asymptomatic patients.

Diagnosis:

- Stool microscopy for ova and parasite is the mainstay for diagnosis and may require special staining such as modified acid-fast stains. Stool ELISA testing enhances sensitivity for the diagnosis of *Giardia* and *Cryptosporidium* infection.

Treatment:

- Conventional therapies should be used as first-line although relapse rate for many infections may be high and repeated, high-dose, and alternative treatment strategies may be required.

Prevention:

- Transplant recipients should avoid untreated well or lake water. They should avoid inadvertent swallowing of water when swimming in lakes.
- Chlorination does not sterilize *Cryptosporidium* making prevention more difficult.
- If concerns of ongoing *Cryptosporidium* exposure exist, instillation and proper maintenance of 1-μm secondary household water filters can reduce exposure.

Entamoeba histolytica

Epidemiology and risk factors
Entamoeba histolytica infection can result in asymptomatic carriage, amebic colitis, liver abscess and more rare manifestations including pulmonary, cardiac or brain involvement. It is unknown if the clinical presentations are altered in transplant recipients. *Entamoeba histolytica* tends to occur in regions with limited sanitation. Sexual transmission, especially among men who have sex with men, is more common in industrialized countries.

Diagnosis
Entamoeba histolytica can be diagnosed via stool examination for ova and parasites, although this is less sensitive than stool assays using *Entamoeba* antigen testing or PCR; the latter two methods are species-specific, which can help distinguish between *E. histolytica* and *E. dispar* or *E. moshkovskii*. Only *E. histolytica* is considered pathogenic. Serology may be positive with extra-intestinal disease and can be helpful for screening and diagnosis in low prevalence, nonendemic regions.

Treatment
Treatment of amoebiasis generally involves the use of metronidazole or tinidazole against the active trophozoite stage (tissue amoebicide), followed by the use of paromomycin or iodoquinol to eliminate cysts (luminal agent). There is one case report of successful treatment of amoebiasis with metronidazole in a liver transplant recipient (146). Asymptomatic persons infected with *Entamoeba histolytica* can be treated with a luminal agent alone to prevent transmission and invasive disease (122). Nitazoxanide has shown cure rates of greater than 90% in some studies (147).

Prevention/prophylaxis and infection control issues
These infections are primarily acquired from contaminated food and water. Transplant recipients should avoid untreated well or lake water, and preferentially drink treated municipal water or bottled water. Sexual transmission can occur; transplant recipients should be aware of the potential risks.

Recommendations
Pretransplant screening:

- Not routinely recommended in asymptomatic patients.

Diagnosis:

- Direct microscopy is the most common diagnostic method but does not differentiate *Entamoeba histolytica* from nonpathogenic species.
- Antigen testing, or nucleic acid testing of stool samples can be used to identify active infection while serology can identify present or prior infection.

Treatment:

- Except for asymptomatic carriers, it is important to treat with both a tissue amoebicide (metronidazole or tinidazole) and a luminal agent (paromomycin) to fully eradicate the organism.
- Hepatic amoebiasis typically requires more prolonged treatment.

Prevention:

- Avoidance of contaminated food and water is the best method of prevention.

Intestinal Nematodes

Strongyloides

Epidemiology and risk factors: *Strongyloides stercoralis* infects approximately 100 million persons worldwide (148). The parasite is endemic in the tropics and subtropics, and has been reported from temperate areas such as southern and Eastern Europe, the Caucasus, Belgium, the United Kingdom and southeastern United States (149). *Strongyloides stercoralis* is able to complete its life cycle both in the environment and in the human host. As a consequence, the parasite has an "auto-infective" cycle that produces long-term persistent infections. The rate of autoinfection is regulated by the immune response of the host; the severity of the disease correlates with worm burden. The major reservoir of the parasite is soil contaminated with human feces that harbor *Strongyloides* larvae. The filariform larvae penetrate the intact skin, enter the circulatory system, migrate to the lung, penetrate alveolar spaces, and move to the pharynx/trachea where swallowing allows access to the duodenal mucosa where they become adult parasites. Significant tissue phases of the life cycle accentuate blood eosinophilia. Adult females reproduce asexually (parthenogenesis) and sexually, laying eggs that become either rhabditiform larvae—which are eliminated with the stools completing the parasite life cycle—or filariform larvae that penetrate intestinal mucosa and perpetuate the infection. The molting of rhabditiform larvae into filariform larvae is accelerated under immunosuppression, allowing a massive number of larvae from the intestinal lumen or the perianal skin to autoreinfect the host. As a result, a great number of adult worms are found in the intestinal lumen. This can lead to lung involvement or the disseminated form of the disease.

Clinical syndromes include acute infection; chronic infection with parasite persistence and autoinfection; hyperinfection syndrome (HIS) and disseminated disease (DD). Hyperinfection syndrome is characterized by accelerated larvae production, migration and elevated parasite burden with evident clinical manifestations; but the larvae are restricted to pulmonary and gastrointestinal systems. DD includes the components of HIS with additional larva spread to other organs (150). Risk factors for HIS and DD have been linked to the immune status of the host and are mainly related to corticosteroids or other immunosuppressive agents. HTLV-I co-infection is also a known risk factor for progression to HIS/DD.

Strongyloidiasis has been well described in organ transplant recipients and has been attributed both to reactivation of latent disease as well as donor-derived infection (151,152). The common use of high-dose corticosteroid preconditioning of deceased donors can increase rates and intensity of strongyloides transmission (153).

Strongyloidiasis can be a devastating disease in transplant recipients; the mortality rate approaches 50% in hyperinfection syndrome and 70% in disseminated infection (151). The clinical disease may present with pulmonary involvement, bacterial sepsis or bacterial meningitis with Gram-negative rods from intestinal flora carried on the surface of the parasite during tissue migration. Gastrointestinal presentations include acute and severe abdominal disease, bloody diarrhea, adynamic ileus, intestinal obstruction, and gastrointestinal hemorrhage, caused by larval damage inflicted as they penetrate through the gut wall. This is most likely to occur in the initial months after transplantation when immunosuppression is most intense. Yet, diagnoses associated with SOT at varied stages in some individuals (hypogammaglobulinemia, malnutrition and lymphoma) may facilitate progression to hyperinfection/disseminated disease at later dates (154).

Diagnosis: Eosinophilia can be found in patients with *Strongyloides* acute infection. However, patients with chronic infection, hyperinfection syndrome, disseminated disease and immunocompromised patients may have normal eosinophil counts. Absence of eosinophilia does not rule out disease in recipient or donors (153,155). Definitive diagnosis is achieved by identification of larvae in clinical specimens mainly in stool (typically only HIS/DD have enough larva to allow detection consistently) and duodenal aspirate samples (156). However, in the course of the disseminated disease larvae can be found in respiratory secretions, CSF, peritoneal fluid, urine, pleural effusion, blood and other tissue specimens. Larvae are often accidentally found when searching for other pathogens as causes of the severe disease. In uncomplicated cases, stool larvae density is low and elimination intermittent (direct observation sensitivity 0–14%). Duodenal fluid aspirate, while more sensitive than direct stool examination has only 76% sensitivity and involves an invasive procedure.

Serological testing is often more sensitive for diagnosis of infection, although cannot distinguish active and prior infection, and may not be available worldwide. Enzyme-linked immunosorbent assay (ELISA) is highly sensitive (80–95%) and specific (90%) in normal hosts (157). In immunocompromised patients sensitivity is reduced to 68%, with retained specificity at 89% (158,159). Small series using a combination of methods in immunocompromised patients, improved the sensitivity back toward 90% (160,161). False-positive results are mostly related to the presence of other helminthic infections; thus, local epidemiology is important when considering the positive predictive value. A gelatin particle indirect agglutination (GPIA) has a published 98.2% sensitivity and 100% specificity (155).

Though donor serotesting (when historical factors warrant) may have delayed results, these data remain useful to focus recipient evaluation and treatment. Living donors with potential exposure profiles should be screened well ahead of donation.

Treatment: Ivermectin is the treatment of choice for strongyloidiasis (145,155) and is effective at eradicating adult parasites and larvae from the intestine in normal hosts (122,145,162,163). A repeated dose at two weeks is designed to treat the less susceptible forms by life cycle stage, when they have progressed to a more susceptible phase. Adverse effects are infrequent and usually mild. Albendazole has a primary cure rate of only 45–75% making it a second-line therapy (163,164). Thiabendazole, is the agent with the most clinical experience, although the least satisfactory of all available drugs, due to frequent relapses and toxicities (149).

The experience with ivermectin for the treatment of hyperinfection or disseminated disease in transplant recipients is limited and reports describing clinical failure have been published (165). Cases with heavy parasitic burden require daily doses until clearance; with additional doses for 7–14 days to reduce the risk of relapse (154). Anecdotal experiences lead some to advocate combination or sequential ivermectin and albendazole treatment. Severe strongyloidiasis with concomitant malabsorption is a serious challenge to oral treatment. Off-label rectal ivermectin can be effective in patients unable to tolerate or absorb oral therapy (166). A parenteral veterinary formulation of ivermectin has been used subcutaneously with some success (153,167). In the United States the veterinary formulations require emergency investigational new drug approval from the Food and Drug Administration (Division of Special Pathogens, 301-796-1600).

S. stercoralis and HTLV-1 co-infection typically requires protracted therapy because no treatment reliably cures strongyloidiasis. Treatment recommendation is (typically daily ivermectin) until visible organisms are cleared and then for 7–14 additional days, followed by retreatment if significant symptoms or eosinophilia return–often at weekly to monthly intervals (144).

Prevention: Organ transplant recipients should be educated to wear closed footwear in endemic environments to reduce risk of primary infection. To reduce the risk of disseminated strongyloidiasis in asymptomatic or paucisymptomatic patients; expanded screening with detailed history, parasitological studies and serology facilitate necessary treatment of infection before transplantation is needed (141). In addition to asking about international travel, it is important to inquire about work, volunteer, or military service abroad which many patients do not consider "travel." If not feasible, consider empiric treatment before initiation of immunosuppressive therapy for transplant candidates with unexplained eosinophilia, a history of parasitic infection and/or residence in or travel to, endemic areas even in the remote past (168). Because strongyloidiasis can be transmitted via the graft, information about a donor's epidemiologic risk might trigger further serologic evaluation, or even initiation of pre-emptive treatment for the recipient (151–153,169,170). All infected living donors should be adequately treated prior to transplant. Recipients of untreated infected donors should receive empiric therapy posttransplant as well as monitoring for posttransplant infection.

Recommendations (iii)
Pretransplant screening:

- Evaluation for strongyloidiasis should be strongly considered in transplant candidates with epidemiological risk factors or unexplained eosinophilia during pretransplant evaluation.
- Evaluation for strongyloidiasis should be strongly considered in living donors, and where feasible, deceased donors with epidemiological risk factors or unexplained eosinophilia during pretransplant evaluation.

Diagnosis:

- When evaluating potential living donors and recipients pretransplant at risk for strongylodiasis, a combination of serology and stool examination is recommended.
- In the setting of hyperinfection and disseminated disease, in addition to stool, larvae may be identified in respiratory fluids, skin biopsies and many other fluids and tissues.
- Patients with strongyloidiasis should be tested for HTLV-1, because co-infection affects approaches to treatment, duration of treatment, and clinical monitoring.

Treatment:

- Ivermectin is the drug of choice for treatment of strongylodiasis. Hyperinfection and disseminated disease may require protracted therapy. Nonoral routes of administration can be considered when absorption is poor. Treatment is recommended until visible organisms are cleared and then for 7–14 additional days with close monitoring for relapse.
- *S. stercoralis* and HTLV-1 co-infection typically requires protracted therapy because no treatment reliably cures strongylodiasis.

Prevention:

- All organ transplant recipients and living donors with strongylodiasis should be adequately treated prior to transplant. Organ recipients of untreated infected

donors should receive empiric therapy posttransplant as well as monitoring for posttransplant infection.
- Organ transplant recipients should be educated to wear closed footwear in endemic environments to reduce risk of primary infection.

Trematodes

Schistosomiasis

Epidemiology and risk factors: *Schistosoma* species are found throughout much of the warmer climates; species vary by region, and specific clinical disease varies by species. Schistosomiasis is primarily a fresh-water-borne infection in endemic rural regions. *S. mansoni* and *S. japonicum* can lead to intestinal and hepatic complications, while *S. haematobium* predominantly leads to renal and bladder sequelae. Less common, *S. mekongi* and *S. intercalatum* can lead to intestinal and/or liver disease. At 700 million infected individuals, schistosomiasis is the second most prevalent tropical disease (171).

Chronic, heavy infection with *S. mansoni* can lead to pipestem fibrosis, a characteristic pipe-shaped fibrosis around the hepatic portal veins, associated with large numbers of schistosome eggs in the hepatic tissues and can lead to portal hypertension. Studies are mixed on whether schistosomiasis worsens clinical outcomes with hepatitis C infection. Intestinal schistosomal disease usually presents with chronic or intermittent abdominal pain, anorexia and diarrhea. Urinary schistosomiasis may cause hematuria (microscopic or macroscopic), dysuria and urinary frequency. Chronic infection may result in fibrosis and calcification of the bladder and ureters, with ensuing hydroureter and hydronephrosis. Schistosomal nephropathy eventually leads to end-stage renal failure. Mahmoud et al. showed treated *Schistosoma* infection had no significant impact on patient or graft outcomes but they did have a higher incidence of acute and chronic cyclosporine nephrotoxicity, a higher rates of urinary tract infection and urological complications, with no evidence of schistosomal re-infection (172). It is not clear whether the SOT and accompanying immunosuppression alter the clinical course of schistosomiasis. Recurrence of schistosomiasis after liver transplant is rare but several cases have been reported, possibly resulting from reactivation of previous infection as a consequence of immunosuppressive therapy (173,174).

While schistosomes can be transmitted by organ transplant, adult schistosomes do not replicate within the host so only transmission of nonreplicating adult worms occurs. Acute schistosomiasis is often asymptomatic. Chronic schistosomiasis is seen in up to 60% of infected individuals, yet extensive liver disease is only seen in 4–8% of cases (171). Hence both donors and recipients may be unaware of background infection. Adult worms tend to die after 3–5 years.

There are several case-reports describing the successful use of *Schistosoma*-infected donors in SOT (172–177). It is not clear whether transplant recipients with donor-derived infections are at risk for the systemic hypersensitivity reaction associated with primary infection (Katayama fever). Immunosuppression may mask these symptoms, or they may be confused with other clinical entities such as acute graft rejection.

Diagnosis

Schistosomiasis may be diagnosed by tissue biopsy, serology (of serum or CSF), or examination of stool or urine for ova and parasites. Many serologic assays are based primarily on *S. mansoni* antigens and may cross-react with other species. Antibody levels do not correlate with intensity of infection and should not be monitored for response to therapy. Seroconversion may not occur for several months after primary infection and may be delayed in organ transplant recipients.

Treatment

Praziquantel is the usual treatment for schistosomiasis. Oxamniquine and the anti-malarial artemether may be available outside the United States. Case reports of several transplant patients who were treated with praziquantel with good outcomes have been published (173,174) *S. japonicum* and *S. mekongi* require higher doses (Table 1). Altered efficacy or toxicity with treatment has not been well-studied or documented in transplant recipients. Cyclosporine may decrease the metabolism of praziquantel, resulting in higher drug levels and great potential for toxicity; potential interactions with other immunosuppressive agents have not been noted. Cyclosporine has been shown *in vitro* and in animals to have anti-schistosomal properties, especially with *S. mansoni*; similar effects with other immunosuppressive agents have not been reported, and this effect has never been confirmed in humans.

Prevention

Primary schistosomiasis infection can be prevented by avoiding contact with fresh water in endemic regions. Donor-derived and relapsing infections could be prevented by screening donors and recipients from endemic regions, and treating those with positive results.

Recommendations (iii)

Pretransplant screening:

- Consider screening and treatment of living organ donors and potential recipients with epidemiological risk factor for schistosomiasis.

Diagnosis:

- Direct visual microscopy of stool or urine for eggs is an important testing method but can be falsely negative in light infection.

- Serological testing has enhanced sensitivity but can be falsely negative early postinfection and cannot distinguish past and prior infection.

Treatment:

- Praziquantel is the drug of choice, dosing varies some by species.

Prevention:

- Organ transplant recipients should avoid contact with freshwater in areas of endemicity.

Cestodes

Echinococcosis (hydatid-alveolar cyst disease)

Epidemiology and risk factors: Echinococcosis is caused by the ingestion of eggs of either the cestode *Echinococcus granulosus* or *E. multilocularis*. *Echinococcus granulosus* is a parasite of domestic dogs that causes hydatid or unilocular cyst disease, while *E. multilocularis* is a parasite of wild canines that causes alveolar cyst disease. Humans are intermediate hosts. Hydatid cysts are usually asymptomatic. Symptoms can occur, however, from the mass effect of the enlarging cyst or from the leakage, rupture, or bacterial superinfection of the cyst. Liver failure can result from hydatid cyst growth or from treatment-related complications. Liver transplantation has been performed in terminal liver failure related to hydatid disease, and, although the patients did not receive antiparasitic drugs or intracystic scolicidal agents, no recurrences or deaths related to hydatid disease were reported (178,179). One report in a heart transplant recipient noted the growth rate of the hydadid liver cysts was not enhanced by immunosuppression suggesting that the detection of hydatid cysts in a candidate is not necessarily a contraindication to transplantation (180).

In *E. multilocularis* infection, larvae proliferate making alveolar cysts grow indefinitely and mimic a slow-growing cancer that requires wide surgical resections. Alveolar echinococcosis is similar to hepatobiliary cancer in its clinical behavior. It is lethal in approximately 10 years from diagnosis unless it is promptly identified and radically excised by surgery (181). Liver transplantation should be considered early on for patients with hilar involvement, recurrent biliary infections, secondary biliary cirrhosis and ascites, variceal bleeding caused by portal hypertension and for those with lesions that are invading the hepatic veins and the inferior vena cava. Avoidance of multiple abdominal surgeries favors better results after liver transplantation. Because the disease may spread to the lung and to the brain, patients should be evaluated for extrahepatic involvement before transplantation. Only central nervous system involvement should be considered as an exclusion criteria for transplantation (182). In the 45 cases reported by a collaborative study from 16 European transplant centers the main indications for transplant were biliary disease related to parasitic involvement of the hilum and a huge parasitic lesion (182). Survival without recurrence was 77% at 1 year and 45% at 10 years. In a series of five liver transplant recipients in China with alveolar echinococcosis of the liver, major technical difficulties were noted, but liver transplantation for otherwise incurable disease was felt to be feasible (183). Best results were achieved if transplantation was performed before blood vessel involvement occurred (184). Immunosuppression can enhance the parasitic growth and the risk of recurrence; therefore, immunosuppression should be reduced to a minimum as early as possible.

Diagnosis

E. granulosus infection (hydatid disease) is often an incidental finding on routine imaging. Radiographic studies such as X-ray, CT scan, ultrasonography and MRI often reveal characteristic cystic lesions. These findings together with a positive epidemiological exposure lead to presumptive diagnosis. Serology may be used to help confirm diagnosis. Available serologic tests have a sensitivity of 60–95%. *E. multilocularis* infection needs to be differentiated from hepatic malignancy. Liver biopsy is considered the gold standard for diagnosis. However, its use is limited due to the high risk of spreading infection. Diagnosis is therefore best achieved by imaging and antibody detection using recombinant antigens. These diagnostic tests are not widely available at all medical facilities. ELISA to measure anti-*E. granulosus* immunoglobulin G titers is considered useful for predicting recurrence (185).

Treatment

The presence of hydatid disease in a potential organ recipient should be recognized and treated with the surgical removal of the cysts and albendazole therapy before transplantation (186). Presurgical administration of albendazole for 7–10 days may reduce the risk of secondary seeding in the event of any cyst contents spillage at the time of surgery. In the event of intra-operative spillage, many experts would prescribe a course of praziquantel as well.

Donors from endemic areas may have unrecognized hydatid cysts that are found at the time of organ procurement. In an effort to reduce the organ shortage, some have suggested that livers with hydatid cysts be used for transplantation provided that the cyst is single and calcified (187), that it does not communicate with the biliary tree, and that a closed resection of the cyst is feasible without damaging the main vascular and biliary structures (188). Treatment with albendazole is recommended for a minimum of 2 years after transplantation even in cases of apparently curative surgery (182). The use of PAIR (percutaneous puncture, aspiration, injection and re-aspiration) technique can be used to obliterate a cyst before full surgical removal.

Although radical surgical excision is necessary for the treatment of *E. multilocularis* infection, recent reports provide evidence that long-term treatment with benzimidazole may slow the progression of the disease (181).

Prevention

Primary prevention involves avoiding contact with dog fecal material that may be contaminated with echinococcal eggs. Dogs involved in the care of sheep, or dogs fed sheep offal, are at greatest risk to be infected with *E. granulosus*. Good hand hygiene after sheep dog grooming reduces the risk of exposure. Because wild animals are the definitive hosts for *E. multilocularis* direct contact and acquisition of disease is rare.

Recommendations (iii)
Pretransplant screening:

- Not routinely recommended in asymptomatic patients.

Diagnosis:

- Preliminary diagnosis is often made by identifying the characteristic appearance of echinococcal cyst(s) on radiographic imaging and can be confirmed with serological testing.
- Serum echinococcal antibody sensitivity varies (60–95%), hence is not definitive for screening donors and evaluating suspicious cysts in livers of potential donors.
- Definite diagnosis can be made through microscopic evaluation of aspirated cyst contents or removed cyst wall.

Treatment:

- Before transplantation in a potential recipient, complete surgical resection of echinococcal cyst, followed by a prolonged course of albendazole is preferred.
- Preprocedure albendazole may reduce the risk of secondary seeding in the event of any cyst contents spillage at the time of surgery or PAIR.

Prevention:

- Avoiding infected sheep dog fecal matter minimizes the risk of acquiring echinococcus.
- Prolonged treatment after surgery or percutaneous treatment is commonly used in SOT recipients due to a concern of higher risk of relapse.

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Disclosure

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Special Article

Strategies for Safe Living After Solid Organ Transplantation

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Abbreviations: CMV, cytomegalovirus; DEET, N,N-Diethyl-meta-toluamide; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HSV, herpes simplex virus.

Introduction

Infections remain a risk to the recipients of solid organ transplantation, long after the initial posttransplant period. Factors that affect risk include the recipient's net state of immunosuppression, epidemiologic exposures and the consequences of the invasive procedures to which the recipient has been subjected (1–4). Infections can be due to endogenous organisms that reactivate during periods of excess immunosuppression, donor-acquired organisms which are discussed in section 3 of these Guidelines, or from the environment, whether it be in the hospital setting or the community after discharge. They may also develop opportunistic infections with exogenously acquired organisms if exposed to a high inoculum or particularly virulent microbes, even during periods of minimal or maintenance immunosuppression. A major goal of transplantation is to be able to lead as healthy and normal a life as possible; accordingly the risk of exposure to infectious agents will always be present. However, various measures can be taken to reduce high-risk epidemiologic exposures in the hospital and in the community, and transplant recipients should be counseled in ways to minimize the risk of infection. Furthermore, strategies for safe living must be carefully woven with the transplant recipient's attempts to regain normal function and return to an active and productive life.

Information on specific infections is available in other sections of these Guidelines, whereas this section will deal with infectious exposures that are encountered in daily life. Unfortunately, "hard data" and controlled studies regarding safe living practices after solid organ transplantation are lacking. Guidelines for preventing opportunistic infections in other immunocompromised populations such as hematopoietic stem cell transplant recipients (4) and in persons infected with human immunodeficiency virus (5) have been drafted by various working groups that include the Centers for Disease Control and Prevention, Infectious Diseases Society of America, United States Public Health Service and American Society of Blood and Marrow Transplantation, and can be extrapolated to the solid organ transplant population. In addition, published guidelines on isolation precautions (6), hand hygiene (7) and environmental control of infection (8) provide valuable insights, although they focus primarily on health care settings. The following recommendations are based on anecdotal clinical experience, available knowledge of the mode in which various infectious agents are transmitted, the opinions of respected authorities (9), and common sense [III]. They take into account the general recognition that solid organ transplant recipients are at greatest risk of infection during the first 6 months after transplantation or when their immunosuppression is augmented for episodes of rejection. Guidelines for the prevention of infection in solid organ transplant recipients should be tailored to the individual recipient by their health care providers with special consideration of the patient's degree of immunosuppression and personal circumstances.

Prevention of Infections Transmitted by Direct Contact

Most organisms are acquired from direct contact (particularly on hands or from fomites), ingestion, or inhalation. Frequent and thorough hand washing is imperative as a means of preventing infections that are transmitted by direct contact [II-3, III]. Hands should be washed with soap and water. Hygienic hand rubs are an acceptable alternative for maintaining clean hands, except when there is visible soiling of the hands or when contact is made with organisms that are known to have a spore stage (e.g. *C. difficile*; Ref.7). Gloves should be worn whenever handling heavily contaminated materials such as soil, moss or manure. Going barefooted outside should be avoided. Shoes, socks, long pants and long sleeved shirts should be worn while doing gardening, yard work, farming or being in parks or wooded areas [III].

Hands should be washed [including after gloves are used]:

- before preparing food and before eating,
- before and after touching wounds (whether or not gloves are used),

- before touching mucous membranes,
- after touching or cleaning up after pets and animals,
- after gardening or touching plants or soil,
- after changing diapers (though ideally other family members should change diapers rather than the transplant recipient),
- after touching secretions and excretions, including nose-blowing, and
- after touching items that have had contact with human or animal feces, (e.g., bedpans, bedding, toilets, litter boxes).

In addition, there is considerable potential for transmission of infections via percutaneous exposures. Transplant recipients should avoid intravenous or intradermal drug use not only due to the health consequences of using illicit drugs but likewise the risk of acquiring blood-transmitted infections such as HCV and HBV. Body piercings, and tattoos represent a break in the skin, which can lead to infection as well (9). If body piercing or tattoos are to be obtained, reputable centers should be used and close attention to sterile technique used. Self-piercing or tattooing or sharing of needles should be avoided.

Prevention of Respiratory Infections

Microbes that cause respiratory infections are transmitted by either inhalation of aerosolized organisms or direct contact from contaminated hands to mucous membranes. Accordingly, transmission of respiratory pathogens can be reduced by:

- Frequent and thorough hand washing, particularly before touching mucous membranes [II-3].
- Avoiding close contact with persons with respiratory illnesses [II-2]. If contact is unavoidable, ideally both the infected person and the transplant recipient should wear a standard surgical mask [III].
- Avoiding crowded areas, such as shopping malls, subways, elevators, where close contact with persons with respiratory illness is likely [III]. Though continually avoiding these areas is unrealistic, caution is advised during periods of enhanced immunosuppression. Likewise, caution should be increased when viruses are circulating in the community such as epidemic influenza.
- Avoiding tobacco smoke. Smoking and exposure to environmental tobacco smoke are risk factors for bacterial and community-acquired viral infections [III]. Marijuana smoking should also be avoided because of its association with exposure to fungal spores from *Aspergillus* spp and other organisms [III].
- Avoiding exposure to persons with known active tuberculosis and avoiding activities and occupational settings that increase the risk of exposure to tuberculosis, e.g. working in prisons, jails, homeless shelters, and certain health care settings [III].
- Avoiding, if possible, other occupational risks including working in certain animal care settings, construction, gardening, landscaping, and farming. Decisions to work in high risk areas should be made jointly by the patient, transplant team and primary care physicians so that the risks and benefits can be appropriately discussed, and precautions implemented if the patient chooses to accept these risks.
- Avoiding construction sites, excavations, or other dust-laden environments where there may be a high concentration of spores from molds (e.g., *Aspergillus, Histoplasma*).
  - Home remodeling projects which may lead to increased risk of *Aspergillus* in the environment need to be planned cautiously. Although data are not available on specific risk, it would be prudent for the transplant recipient to avoid exposure particularly early after transplantation or rejection treatment or after lung transplantation. Although clinicians may counsel patients to temporarily move out of their homes when visible mold is detected and during mold-abatement procedures, the level of infectious risk is not known.
- Avoiding exposure to fungal spores (*Cryptococcus*, *Histoplasma,* etc.) by avoiding plant and soil aerosols (such as mulching), pigeon and other bird droppings, chicken coops, and caves.
- Consideration for wearing a mask if exposure to above high risk areas is unavoidable [III].

Water Safety/Exposure to *Cryptosporidium*

Waterborne infections most often occur from consumption of contaminated drinking water or inadvertent water ingestion during recreational activities such as swimming, diving, or boating. Less frequently, infection can result from inhalation or direct contamination of the eye or a wound. In particular, cryptosporidiosis has been increasingly recognized in both healthy and immunocompromised hosts. Although only a few studies have focused on solid organ transplant recipients (10), *Cryptosporidium* can cause severe, chronic diarrheal disease in immunocompromised hosts, particularly those receiving corticosteroids (11). *Cryptosporidium* is resistant to chlorine and other chemicals and can be problematic even with treated water sources. Therefore, it is prudent for solid organ transplant recipients to decrease their exposure to this pathogen as well as others that might be found in water sources. Even treated municipal tap water may not be completely free of *Cryptosporidium*; however, there are no data to support a recommendation that all tap water be avoided unless a "boil water" advisory is issued by local authorities. To completely eliminate the risk of *Cryptosporidium* contamination, one should only drink water that has come to a rolling boil for at least one minute [I]. Persons avoiding untreated tap water should be aware that ice, and fountain beverages served at restaurants, bars,

theaters, sporting events, etc., are prepared with tap water. Personal-use filters and/or bottled water may serve as alternatives to boiling water to eradicate *Cryptosporidium* and other water-borne pathogens, but careful attention must be paid to selecting effective filters and high-quality bottled water. A list of filters certified under NSF Standard 053 for cryptosporidial cyst removal may be obtained by contacting the NSF International consumer line at 800–673-8010 or http://www.nsf.org/consumer/drinking_water/contaminant_cryptosporidium.asp. Information regarding bottled water can be obtained from the International Bottled Water Association at 703–683-5213 or (http://www.bottledwater.org). For individuals who have treated water supplies, the expense of buying bottled water is usually not warranted.

Specific recommendations for water safety include:

- Close attention should be paid to directions given during local governmental recommendations for "boil water" advisories for any waterborne pathogen.
- Well water from private or public wells in areas that are not screened frequently for bacterial pathogens should be avoided if possible because of potential risk of *Cryptosporidium*, *Giardia* and bacterial coliform contamination.
- Transplant recipients should not drink water directly from lakes or rivers because of the risk of *Cryptosporidium*, *Giardia* and bacterial coliform contamination [III].
- Waterborne infection might also arise from inadvertent swallowing of water during recreational activities such as swimming in lakes, rivers or pools, or going on water rides at amusement parks [II-2]. Transplant recipients should avoid swimming in water that is likely to be contaminated with human or animal waste, and should avoid swallowing water during swimming [II-2].
- To avoid spreading infection to others, transplant recipients who have had diarrhea should not use public recreational water facilities for 2 weeks after symptoms have resolved [III].
- Hot tubs have been associated with several infection risks, including *Pseudomonas* folliculitis, legionellosis (12) and mycobacterial infections (13); and should be avoided.
- Standing water in the home or basement, such as may occur with flooding, should be promptly cleaned up to avoid growth of mold, *Legionella* and other pathogens. Ideally someone other than the transplant recipient should perform the cleaning. If the transplant recipient cannot avoid exposure then waterproof boots and gloves should be worn during the cleaning process.
- When traveling to countries with poor sanitation, drinking tap water as well as inadvertent consumption from ice cubes or during showering should be avoided.
- Abrasions incurred during bathing in ocean or fresh water should be thoroughly cleaned with an uncontaminated water source due to risk of infection with organisms such as *Vibrio* species, *M. marinum* or *Aeromonas*.

Food Safety

Many of the following recommendations also apply to healthy individuals. Transplant recipients should avoid:

- Drinking unpasteurized milk, fruit or vegetable juice/cider in order to decrease their risk of infection with *E. coli* 0157:H7, *Salmonella*, *Brucella*, *Listeria*, *Yersinia* and *Cryptosporidium* [II-2].
- Eating cheeses made with unpasteurized milk (such as the soft cheeses such as brie, camembert, feta) to decrease the risk of *Listeria.*
- Eating raw or undercooked eggs including foods containing raw eggs (e.g. uncooked cake and cookie batter and some preparations of Caesar salad dressing, mayonnaise, or hollandaise sauce) particularly a risk for *Salmonella* infection [II-2].
- Eating raw or undercooked meat, poultry or fish with particular risk not only for bacterial contamination but also for parasitic infections such as *T. gondii*, and Tapeworms.
- All raw or undercooked seafood (oysters, clams, mussels) to prevent exposure to *Vibrio* species, viruses that cause gastroenteritis or hepatitis, and parasitic infections including *Cryptosporidium.*
- Ingesting raw seed sprouts (alfalfa sprouts, mung beans).
- Cross-contamination when preparing food (e.g. keep cooked and raw foods separate; use cleaned or separate cutting boards).
- Uncooked pate, meat spreads, cold cuts and smoked seafood.
- This website on food safety is a very user friendly resource to review current outbreaks as well as general food safety recommendations: http://www.foodsafety.gov/~dms/lmrisks5.html.

In addition to the above recommendations recent outbreaks of *Listeria*, *Salmonella spp*, toxigenic *E. coli* and *Campylobacter jejuni* show that it is prudent for transplant recipients to carefully wash lettuce and vegetable products even when they come bagged labeled as "prewashed."

Although not all outbreaks can be anticipated, transplant recipients and their families should pay particular attention to local recommendations when outbreaks occur to avoid exposure to contaminated foods such as occurred with the widespread *Listeria* outbreak associated with cantaloupe in the United States in 2012 and the outbreak of *E. coli* 0104:H4 in Germany in 2011.

Vaccination against hepatitis A should be sought before transplant if possible to offer the best protection against hepatitis A as a foodborne virus.

Animal Contact and Pet Safety

Occupational risk

Transplant recipients who work with animals (veterinarians, pet store employees, farmers, slaughterhouse or laboratory workers) should, if possible, avoid working during periods of maximal immunosupppression [III]. When returning to work, transplant recipients should minimize their exposure to potential pathogens by using proper precautions, including hand hygiene and the use of gloves and masks as indicated.

Pet ownership

Health care providers must balance the psychological benefits of pet ownership with potential risks for transmission of infection when counseling solid organ transplant recipients on the safety of maintaining pets. There are a variety of zoonoses that can be transmitted to the transplant recipient from pet animals (14). The veterinarian should be viewed as a colleague, both to the transplant clinician and the transplant recipient, because maintenance of pet health can help reduce human risk (15).

In general, transplant recipients should:

- Avoid contact with animals that have diarrhea [III].
- Keep their pets healthy by feeding them food that is not contaminated or spoiled, and seeking veterinary help at the first signs of illness.
- Wash hands carefully after handling pets.
- Avoid cleaning bird cages, bird feeders, litter boxes, and handling animal feces. If this is not possible, the use of disposable gloves and a standard surgical mask should be used.
- Avoid stray animals.
- Avoid animal bites and scratches (do not pet stray animals).
- Ensure that areas near the home are free of raccoon latrines.
- Avoid contact with non-human primates (monkeys).
- Wear gloves to clean aquariums or have someone else in household do the cleaning.
- Consider waiting to acquire a new pet until a period when the patient is on stable immune suppression (at least 6–12 months after transplantation).
- Consider the type of pet and specific risks for infections.
  - Reptiles (snakes, iguanas, lizards and turtles) have a high risk of *Salmonella* infection and should be avoided.
  - Chicks and ducklings have a risk of transmitting *Salmonella* infections.
  - Rodents have a risk of transmitting lymphocytic choriomeningitis virus.
  - Young cats have risk of transmitting *Bartonella henselae.*
  - Cats have a risk of transmitting *Toxoplasma gondii.*
  - Puppies, kittens and chicks have a risk of transmitting *Campylobacter* infections.

Cats can spread *Toxoplasma*, *Cryptosporidium*, *Salmonella*, *Campylobacter* (contaminated feces) and *Bartonella* (fleas and scratches). Young cats carry the highest risk for transmitting *Bartonella* (cat scratch). Cat litter boxes should be changed daily (preferably not by transplant recipients), because it takes longer than 24 h for *Toxoplasma* oocysts to become infectious. Although dogs are generally considered safer pets than cats, birds, and reptiles, there are documented instances of infections transmitted by dogs without a bite, such as cases of *Bordetella bronchoseptica* (the agent of "kennel cough") in lung transplant recipients (16). Although dogs classically have this disease, kittens likewise can be infected and transmit the bacteria. Puppies can transmit *Campylobacter* infections. Birds can transmit infections such as psittacosis or cryptococcosis, which may be a particular risk for lung transplant recipients. Despite the risk of infection from animals, many benefits of pet ownership have been shown and transplant recipients have often had family pets without transmission of infection. Published literature is biased toward reports of infection without denominator data on the number of transplant patients who safely maintain pets in their household. Attention to hand hygiene after contact and ensuring that pets are in good health should be emphasized.

Safer Sexual Practices

Many infections can be transmitted during sexual contact. Some of these can be reduced by having a long-term monogamous relationship or decreasing the number of sexual partners.

Sexually active transplant recipients should:

- Always use latex condoms during sexual contact outside of long term monogamous relationships to reduce exposure to CMV, hepatitis B and C, HIV, HPV, HSV and other sexually transmitted infections [II-2].
- Consider using latex condoms during sexual activity with long-term monogamous partners during periods of increased immunosuppression [III].
- Avoid exposure to feces during sexual activity [II-2].
- Immunize against HBV and HPV at appropriate ages, and when possible before transplantation to achieve greatest efficacy (see section 31, Immunizations).

Education in safer sex practices is an important component of medical care, particularly for adolescents with recent or imminent sexual debut (17). Immunization against HPV is

particularly warranted in adolescents and young adults and has been shown in immunocompetent individuals to be most efficacious when administered before initiation of sexual intercourse. In addition, transplant recipients are at increased risk for malignancy from HPV accordingly prevention is prudent.

Travel Safety

Travel to developing countries poses substantial risk to transplant recipients, particularly during periods of maximal immunosuppression [III]. Expanded recommendations can be found in the comprehensive review by Kotton et al. (18) and the sections on Travel Medicine (section 33) and Parasitic Infections (section 28) in the current Guidelines. Plans to travel should be discussed with the transplant recipient's physician at least 2 months before the planned departure date. All items discussed above are applicable for safe living during travel. Updated travel advisories should be obtained from the Centers for Disease Control and Prevention website, www.cdc.gov.

Particular attention should be paid to access to handwashing facilities, food and water consumption, updating vaccinations and potential interactions that might occur between prophylaxis medications and their routine medicines.

Travelers should take with them a sufficient supply of medications that they may require. A copy of their medication list, signed by a physician, should also be taken in case they are questioned about their medications, particularly if their medications are no longer in the original prescription bottles. Plans for evacuation in the event of medical emergency and should be formulated.

Transplant recipients should be advised about all preventive measures that pertain to their anticipated exposures (e.g. protection against arthropod vectors, swimming precautions, etc.). The use of effective insect repellents and mosquito netting may be crucial in certain areas.

Work- and School-Related Issues

The above sections have touched on many topics relevant to potential infection hazards in the workplace and in school for children. Individualized occupational counseling is important for transplant recipients contemplating return to work in such areas as health care, construction, outdoors work and other fields. Whereas some recipients may be willing to consider a career change (e.g. leaving a temporary job in a pet store), others may be strongly attached to their line of work for multiple reasons such as psychological, financial, or social. In some cases, a return to work is necessary for the transplant recipient to maintain family financial stability and their health insurance. Few guidelines exist for decisions of this nature, but the vast majority of jobs can be made safer by simple measures. These include restricting patient contact for the initial phase of returning to work in a health care environment, wearing masks when there are potential exposures to fungal spores, respiratory viruses, or other transmissible illnesses, and sometimes reassignment to other duties particularly during periods of intensified immunosuppression. Often co-workers can be encouraged to receive influenza vaccinations to help protect the recipient as well. The clinician can help by adopting an attitude of working with the transplant recipient to make the proposed work situation safer, rather than issuing an unconditional order to change jobs (with occasional exceptions).

School attendance is of major importance for children who have received transplants and often is a concern for the family. For this reason it is imperative that pediatric transplant teams discuss this issue with parents early during the pretransplant evaluation and again well in advance of discharge from the hospital so that plans can made with the schools. The timing of return to school is impacted upon by the type of transplant, the level of immunosuppression and the age of the child. In most cases, children are able to return to school several months after the transplant. It may be prudent however, to avoid returning to school during influenza season. In general a close working relationship with the school nurse is needed so that they are aware of any medical issues about the child as well as to have them inform families about infectious disease outbreaks and to remind classmates about the importance of receiving all of their required vaccinations.

Sports and Recreation

The risks of hobbies such as hunting, fishing, scuba diving, or spelunking should be discussed with the transplant recipient (9).

In general, athletic activities have been noted to be both safe and beneficial for many transplant recipients (20), with some notable exceptions. In addition to the psychological and health benefits to the individual of sporting activities, the existence of the World Transplant Games has been reported to have increased the public's knowledge and favorable opinion of transplantation (20). Returning to an appropriate level of recreational and athletic activity can help the transplant recipient's self-esteem and guard against depression. Transplant centers often offer specific restrictions related to the outdoors, as mentioned above, and in addition may choose to advise against certain activities due to risk of physical injury, such as rugby and boxing (21). In addition, the overall physical state and level of immunosuppression of the particular recipient should be considered. Occasionally unexpected consequences might occur, such as the physical effects from direct contact of climbing

harnesses with kidney allografts, leading to concern about the possible effects of rock climbing, rappelling and challenge courses (22).

Precautions to Prevent West Nile Virus and Other Mosquito-borne Infections

West Nile virus (WNV) can cause severe disease in transplant recipients, who have a much higher risk of central nervous system involvement than the general population (23). Other arthropod transmitted infections can also be severe in immunocompromised hosts. Several simple measures can help to prevent infection with these pathogens. Transplant recipients should avoid going out at dawn or dusk, during peak mosquito feeding and should use effective insect repellents that contain DEET. On average, the duration of protection offered by DEET at different concentrations is as follows: 5, 4, 2 and 1.5 h for products with DEET concentrations of 23.8%, 20%, 6.7% and 4.5%, respectively (24). In addition, transplant recipients should wear protective clothing during the high-risk season and in areas where transmission is occurring. Sources of standing water, such as old tires, should be removed from yards and property belonging to transplant recipients. Specific prophylaxis when traveling to areas with endemic mosquito borne infections such as malaria is discussed in the chapters on Parasitic Infections and Travel Medicine (sections 28 and 33).

Patient Contacts: Family, Friends and Healthcare Workers

Although prevention of infection is often aimed at interventions applied directly to the recipient (host-related interventions) it is also important to recognize that close contacts can transmit infections that can be particularly harmful to the transplant recipient. Accordingly, it is worth educating close contacts about ways to maintain their own health. All healthcare workers should receive ongoing education about hand hygiene and standard precautions when caring for people in the health care environment. Vaccination against influenza is encouraged for everyone but particularly for those involved in the care of transplant recipients. Many institutions have developed mandatory immunization policies against this virus. Vaccines for other infectious agents are also routinely offered by health care systems particularly against Hepatitis B virus, measles, mumps, rubella, varicella and more recently acellular pertussis vaccine as part of diphtheria and tetanus vaccination (see section 31, Immunizations). All household contacts should be instructed on good hygiene precautions including, handwashing, cough and sneezing etiquette and covering open wounds. They should all receive yearly influenza vaccination and to ensure that their other standard immunizations are up to date including vaccinations against pertussis, measles and varicella. Contacts at work and school should also be encouraged to receive their immunizations.

Conclusion

With the increasing longevity of transplant recipients, more and more recipients are returning to active lives, to work and to recreational activities. Inevitably potential infection risks are present with the expansion of permissible activities. Careful thought and detailed patient education can prevent many of these risks. Occupational counseling can enable transplant recipients to find safer ways to do the jobs that they love, and that they need to maintain financial stability and insurance coverage. Knowledge of the risks of food, animal exposures and other environmental exposures can help transplant recipients stay out of the hospital and lead healthy, meaningful and long lives.

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Special Article

Vaccination in Solid Organ Transplantation

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Key words: Immunizations, influenza, live virus vaccines, prevention, vaccines

Abbreviations: HCW, healthcare worker; HPV, human papilloma virus; LAIV, live-attenuated influenza vaccine; MMR, measles, mumps rubella vaccine; TST, tuberculin skin test; VZV, varicella zoster virus.

General Principles

Transplant candidates and recipients are at increased risk of infectious complications of vaccine-preventable diseases. Every effort should be made to ensure that transplant candidates, their household members and healthcare workers have completed the full complement of recommended vaccinations prior to transplantation. Since the response to many vaccines is diminished in organ failure, transplant candidates should be immunized early in the course of their disease.

It is recommended that vaccination status ideally be documented at the pretransplant clinic visit and the patient referred for the appropriate vaccines at the time of listing. Many transplant centers will do routine pretransplant serology for vaccine-preventable diseases such as Hepatitis B, Varicella, measles, mumps and rubella to guide individual vaccine recommendations (Tables 1 and 2).

While every effort should be made to vaccinate prior to transplantation, inactivated vaccines are generally safe after solid organ transplantation. For inactivated vaccines where data are lacking specifically for transplant candidates or recipients, recommendations made by national immunization advisory committees (e.g. the Advisory Committee on Immunization Practices [ACIP] in the United States) for the general population should be followed. There is no evidence to link clinical rejection episodes to vaccination (II-2). This topic is discussed in detail for influenza vaccine, in the specific vaccination section on Influenza (1).

In general live vaccines are not administered after transplantation. Therefore, when possible it is recommended to administer live vaccines such as measles, mumps, rubella (MMR), Varicella vaccine and Zoster vaccine prior to transplantation. For patients who are incompletely or unvaccinated prior to transplant, consultation with an infectious diseases specialist is recommended. If possible, this should be done at the time of pretransplant assessment to allow for sufficient time for vaccine administration.

While the optimal time to give vaccines after transplantation is not known, most centers restart vaccinations at approximately 3–6 months after transplantation when baseline immunosuppression levels are attained. The ability to mount an immune response will be impacted by the type and amount of immunosuppression after organ transplantation. It is unknown whether the type-of-transplant impacts response as this is closely linked with degree of immunosuppression. Accordingly, seroconversion should be documented by serologic assays for those specific vaccines where serologic assays are available and protective titers are known. A minimum of 4 weeks should elapse between vaccine administration and evaluation for seroconversion based on protective titers established in the literature. However, given that serology may not be an accurate measure of immunity in the posttransplant period, developing assays for cellular immunity is an area of research that needs further study in this population (III).

Healthcare Workers, Close Contacts Including Pets

Healthcare workers and close contacts, such as family members, of transplant recipients should be immunized fully, and in particular should receive influenza vaccine yearly. In general, if inactivated vaccine options are available for household members they are preferred. Influenza vaccination is especially important. It is preferable that HCW and close contacts receive inactivated influenza vaccine; however, if live-attenuated influenza vaccine (LAIV) is the only option, then it can be given with good use of infection prevention precautions such as frequent handwashing for a 2-week period after vaccination. Viral shedding has been reported to be rare more than 11 days after LAIV administration (2). With the exception of small pox and oral polio vaccines there is little to no risk from the family members or close contacts receiving live vaccines. In fact, it is preferred that household and close contacts be vaccinated

Table 1: Recommendations for immunization of pediatric patients

Vaccine	Inactivated/ live attentuated (I/LA)	Recommended before transplant[1]	Recommended after transplant	Monitor vaccine titers	Quality evidence
Influenza (17–21)	I	Yes	Yes	No	II-1
	LA	See text	No	No	III
Hepatitis B[2] (22–28)	I	Yes	Yes	Yes	II-1
Hepatitis A[3] (29,30)	I	Yes	Yes	Yes (see footnote)	II-1
Pertussis	I	Yes	Yes	No	III
Diphtheria (31–34)	I	Yes	Yes	No	II
Tetanus (31–34)	I	Yes	Yes	Yes	II-1
Inactivated Polio vaccine (31)	I	Yes	Yes	No	II-2
H. influenzae[4] (35)	I	Yes	Yes	Yes	II-1
S. pneumoniae[5] (conjugate vaccine) (1,13–15,36,37)	I	Yes	Yes	Yes	II-1
S. pneumoniae[5] (polysaccharide vaccine) (1,13–15,36,37)	I	Yes	Yes	Yes	II-1
N. meningitidis[6] (1,38)(MCV4)	I	Yes	Yes	No	III
Human papillomavirus (HPV)[7,]	I	Yes	Yes	No	III
Rabies[8]	I	Yes	Yes	Yes (see footnote)	III
Varicella (live-attenuated)[9] (39–42)	LA	Yes	No	Yes	II-1
Rotavirus	LA	Yes	No	No	III
Measles[9] (43–46)	LA	Yes	No	Yes	II-1
Mumps[9] (43,46)	LA	Yes	No	Yes	II-1
Rubella[9] (32,43,46)	LA	Yes	No	Yes	II-1
BCG[10]	LA	Yes	No	No	III
Smallpox[11] (47)	LA	No	No	No	III
Anthrax	I	No	No	No	III

[1]Whenever possible, the complete complement of vaccines should be administered before transplantation. Vaccines noted to be safe for administration after transplantation may not be sufficiently immunogenic after transplantation.
[2]Routine vaccine schedule recommended prior to transplant and as early in the course of disease as possible; vaccine poorly immunogenic after transplantation, and accelerated schedules may be less immunogenic. Serial hepatitis B surface antibody titers should be assessed both before and every 6–12 months after transplantation to assess ongoing immunity (28).
[3]For children, routine recommendation for all transplant candidates and recipients. In adults, routinely recommended for liver transplant candidates and recipients. Other adults pre-or posttransplant should receive if high risk of exposure (e.g. travel or residence in high-risk areas, occupational or lifestyle risk of exposure). Monitoring indicated only if ongoing risk for exposure, for example with planned travel to high-risk areas.
[4]Serologic assessment recommended if available. *Haemophilus influenzae* type B titer greater than 0.15 mg/L is considered protective in the general population.
[5]Serologic assessment recommended if available, see text for additional information.
[6]All patients 11–18 years of age, and adults or patients as young as 9 months of age who meet the following criteria: members of the military, travelers to high risk areas, properdin deficient, terminal complement component deficient (including acquired complement deficiency such as prior to starting eculizumab), those with functional or anatomic asplenia, college freshman living on campus. There are no immunogenicity studies in posttransplant patients. For infants and young children, newer vaccination recommendations may become available. Please check local and national recommendations for most up-to-date information.
[7]HPV vaccine, see text.
[8]Not routinely administered. Recommended for exposures or potential exposures due to vocation.
[9]MMR pretransplant, see text. Varicella vaccine should be administered after 12 months of age, and the second vaccine may be given as early as 3 months later. Although not routinely recommended after transplant, live-virus vaccines (MMR and Varivax) have been administered to selected organ transplant recipients on minimal immunosuppression (48). Vaccination is at the discretion of the individual transplant center with the understanding of the potential risks for live-virus vaccination in this population. In adults, there are reports of disseminated vaccine-strain disease occurring with inadvertent varicella vaccination (49); also see text.
[10]The indications for BCG administration in the United States are limited to instances in which exposure to tuberculosis is unavoidable and where measures to prevent its spread have failed or are not possible.
[11]Transplant recipients who are face-to-face contacts of a patient with smallpox should be vaccinated; Vaccinia immune globulin may be administered concurrently if available. Those who have less intimate contact should not be vaccinated.

against measles, mumps, rubella and varicella to prevent the transplanted patient from having contact with wild type viruses (III). Rotavirus vaccines also pose a theoretical risk of transmission and viral antigen can be detected in stool in 50–90% of infants up to 2 weeks after the first dose (3). Therefore, good handwashing practices should be used after diaper changes. Pets should also be fully immunized. There is little or no risk of transmission following immunization of pets with live vaccines (e.g. Canine *Bordetella bronchiseptica* intranasal vaccine; Table 3).

Table 2: Recommendations for immunization of adult patients

Vaccine	Inactivated/ live attentuated (I/LA)	Recommended before transplant[1]	Recommended after transplant	Monitor vaccine titers	Quality of evidence
Influenza[2] (17–21)	I	Yes	Yes	No	II-2
	LA	See text	No	No	III
Hepatitis B[3] (22,23,26–28)	I	Yes	Yes	Yes (see footnote)	II-2
Hepatitis A[4] (29,30)	I	Yes	Yes	Yes	II-1
Tetanus (31–34)	I	Yes	Yes	No	II-2
Pertussis (Tdap)[5]	I	Yes	Yes	No	III
Inactivated Polio vaccine	I	Yes	Yes	No	III
S. pneumoniae[6] (13–15,36)	I	Yes	Yes	Yes	I
N. meningitidis[7] (MCV4)	I	Yes	Yes	No	III
Rabies[8]	I	Yes	Yes	Yes (see footnote)	III
Human papilloma virus (HPV)[9]	I	Yes	Yes	No	III
MMR[9]	LA	Yes	No	No	II-2
Varicella (live-attenuated; Varivax)[10]	LA	Yes	No	Yes	II-2
Varicella (live-attenuated; Zostavax)[11]	LA	Yes	No	No	III
BCG[12]	LA	Yes	No	No	III
Smallpox[13] (47)	LA	No	No	No	III
Anthrax	I	No	No	No	III

[1]Whenever possible, the complete complement of vaccines should be administered before transplantation. Vaccines noted to be safe for administration after transplantation may not be sufficiently immunogenic after transplantation.
[2]Influenza, see text.
[3]Routine vaccine schedule recommended prior to transplant and as early in the course of disease as possible; vaccine poorly immunogenic after transplantation, and accelerated schedules may be less immunogenic. Serial hepatitis B surface antibody titers should be assessed both before and every 6–12 months after transplantation to assess ongoing immunity (28).
[4]For children, routine recommendation for all transplant candidates and recipients. In adults, routinely recommended for liver transplant candidates and recipients. Other adults pre-or posttransplant should receive if high risk of exposure (e.g. travel or residence in high-risk areas, occupational or lifestyle risk of exposure). Monitoring indicated only if ongoing risk for exposure, for example with planned travel to high-risk areas.
[5]If no tetanus booster in the past 10 years, Tdap should be administered. At least one dose of acellular pertussis should be given in adulthood, with particular attention to women of child-bearing age and individuals with in contact with infants.
[6]Serologic assessment recommended if available, see text for additional information.
[7]All patients 11–18 years of age, and adults or patients as young as 9 months of age who meet the following criteria: members of the military, travelers to high risk areas, properdin deficient, terminal complement component deficient (including acquired complement deficiency such as prior to starting eculizumab), those with functional or anatomic asplenia, college freshman living on campus. There are no immunogenicity studies in posttransplant patients. For infants and young children, newer vaccination recommendations may become available. Please check local and national recommendations for most up-to-date information.
[8]Not routinely administered. Recommended for exposures or potential exposures due to vocation.
[9]HPV vaccine, see text.
[10]MMR pretransplant, see text. Varicella vaccine should be administered after 12 months of age, and the second vaccine may be given as early as 3 months later. Although not routinely recommended after transplant, live-virus vaccines (MMR and Varivax) have been administered to selected organ transplant recipients on minimal immunosuppression (48). Vaccination is at the discretion of the individual transplant center with the understanding of the potential risks for live-virus vaccination in this population. In adults, there are reports of disseminated vaccine-strain disease occurring with inadvertent varicella vaccination (49); also see text.
[11]Zoster, see text.
[12]The indications for BCG administration in the United States are limited to instances in which exposure to tuberculosis is unavoidable and where measures to prevent its spread have failed or are not possible.
[13]Transplant recipients who are face-to-face contacts of a patient with smallpox should be vaccinated; Vaccinia immune globulin may be administered concurrently if available. Those who have less intimate contact should not be vaccinated.

Specific Vaccines

MMR

Outbreaks of measles continue to occur and disease may be acquired during a local outbreak or while travelling. Since MMR vaccine contains live attenuated virus, it is contraindicated posttransplant. Therefore, when possible, MMR serology should be checked prior to transplant and the transplant candidate immunized. In very young infants, the presence of maternal antibody interferes with response to live vaccines. Therefore, MMR is most effective after 1 year of age when maternal antibody has waned. MMR can be administered as early as 6 months of age for pediatric patients who may require transplantation. If transplantation has still not occurred by the time the infant is a year of age and transplant is not anticipated within

Table 3: Immunizations for health care workers and other close contacts/household members of transplant candidates/recipients

Vaccine	Inactivated/ live attentuated (I/LA)	Recommended	Quality of evidence
Influenza (17–21)	I	Yes	II-2
	LA	Yes (see text)	III
Hepatitis B (22–28)	I	Yes	II-2
Hepatitis A (29,30)	I	Yes	II-1
H. influenzae (35)	I	Yes	II-2
Pertussis[1] (Tdap)	I	Yes	II-2
Varicella (39–42)	LA	Yes	II-2
Measles (43–46)	LA	Yes	II-2
Mumps (43,45,46)	LA	Yes	II-2
Rubella (43,45,46)	LA	Yes	II-2

[1]If no tetanus booster in the past 10 years, Tdap should be administered. At least one dose of acellular pertussis should be given in adulthood, with particular attention to women of child-bearing age and individuals with in contact with infants.

4 weeks, MMR should be repeated. The second dose of MMR can be administered as soon as 4 weeks after the first MMR. All children should complete a two-dose MMR series with at least 4 weeks between doses (4). Seronegative adults should receive one dose of MMR with serologic testing postvaccination. If seroconversion does not occur, the dose can be repeated once if time permits. Of note, blood products such as intravenous immune globulin can interfere with the response to live vaccines. Ideally, MMR (and varicella) vaccine should be delayed for 3 months after the receipt of blood products. In addition, two live vaccines (e.g. MMR and Varicella) can be administered on the same day; however, if not done on the same day, the second live vaccine should be administered ≥28 days later. Since tuberculin skin test (TST) is also part of the pretransplant workup, it should be noted that live vaccines can interfere with the TST response. The TST can be done on the same day as the live vaccine injection; however, if not done on the same day, it should be done 4–6 weeks later.

Varicella Vaccine

Primary varicella can lead to severe complications in the posttransplant setting. Varicella vaccine is a live attenuated viral vaccine that is indicated prior to transplant in seronegative persons. Therefore, when possible, VZV serology should be checked prior to transplant and the transplant candidate immunized. Similar to MMR vaccine, maternal antibody interferes with response to varicella vaccine and the vaccine is most effective after 1 year of age when maternal antibody has waned. Varicella vaccine can be administered as early as 9 months of age for pediatric patients requiring transplantation. Two doses should be given 4 weeks apart. Seronegative adults should receive one dose of varicella vaccine with serologic testing postvaccination. If seroconversion does not occur, the dose can be repeated once if time permits. Those who do not seroconvert are candidates for postexposure prophylaxis should this occur after transplantation. As with MMR, the same exceptions regarding timing of varicella vaccine with blood products, spacing of two live vaccines, and timing of TST, applies to varicella vaccine. These should be reviewed in the MMR section above. Posttransplant administration of varicella vaccine in pediatric transplant patients has been attempted in a research setting. Accumulating evidence in pediatric transplant recipients suggests that varicella vaccine is safe and immunogenic after transplantation (5). However, these studies are relatively small in size. In light of these studies, we recommend that at this time, vaccination should be performed only in a carefully controlled setting.

Herpes Zoster Vaccine

Herpes zoster vaccine is a live-attenuated vaccine that is shown in large randomized trials to prevent shingles and postherpetic neuralgia. It is indicated for persons over age 50 years. It should not be given posttransplant whether or not the transplant recipient is VZV seropositive. Disseminated disease may occur due to poor cellular immunity against the virus. In the pretransplant setting, some centers recommend vaccination; however, there are no data yet to suggest that this will reduce the risk of VZV reactivation posttransplant or whether it will be effective in persons younger than 50 years of age. This is an area for further study. In addition, for patients that have had an episode of shingles, vaccine can be given after the active episode has resolved although the vaccine has been studied for prevention of 1st shingles episodes only. Vaccine effect on prevention of subsequent episodes when given after the first episode of shingles is unknown.

Influenza Vaccine

Several formulations are now available, including standard-dose intramuscular, high-dose intramuscular, intradermal, adjuvanted and live attenuated (6). Practitioners should review national guidelines for specific indications for each of these formulations. Not all vaccine formulations have been formally studied in the organ transplant population; most immunogenicity and safety data available are with the standard-dose intramuscular vaccine. However, a recent randomized controlled trial shows similar immunogenicity with high-dose intradermal vaccine compared to standard-dose intramuscular injection in healthy adults (7). Data using adjuvanted vaccines (ASO3 adjuvant) are primarily derived from univalent A(H1N1)pdm09 vaccine and very limited data are available for other adjuvants (e.g. MF59). Studies using AS03 adjuvanted vaccines show minor

Table 4: Travel vaccine recommendations

Vaccine	Inactivated/ live attenuated (I/LA)	Recommended before transplant/	Recommended after transplant/	Monitor vaccine titers	Quality of evidence
Yellow fever[1] (50)	LA	Yes	No	No	III
Japanese encephalitis (51,52)	I	Yes	Yes	No	III
Salmonella typhi (53) (Typhim Vi, intramuscular)	I	Yes	Yes	No	III
Salmonella typhi (Vivotif, oral)	LA	Yes	No	No	III
Traveler's diarrhea and cholera vaccine (Dukoral)[2] (54)	I	Yes	Yes	No	III

[1]Yellow fever vaccination may be required for travel to some countries of Africa and South America, but should be waived if travelers are immunosuppressed. Severely immunosuppressed travelers should be strongly discouraged from travel to destinations that present true risk of yellow fever (37).
[2]Oral inactivated vaccine against cholera and Enterotoxigenic *E. coli* provides short term protection. Not available in the United States.

increases in HLA alloantibody postvaccination but no increases in rejection rates (8,9). These studies are difficult to interpret due to lack of control groups and because they were performed during the 2009 pandemic when infection rates were also high. Live-attenuated vaccines are cold adapted and should not replicate at normal body temperature; however, due to the small theoretical risk of replication, LAIV is not recommended posttransplant (6). The vaccine has been shown to be safe in HIV and cancer patients but no study has been conducted in organ transplant patients (10,11). If a live-attenuated vaccine was to be administered inadvertently to a transplant recipient, antiviral therapy and subsequent revaccination with an inactivated influenza vaccine can be considered (III). LAIV could be given to persons awaiting transplant; however, at least 2 weeks should elapse before transplant.

Since influenza vaccine is recommended annually, timing of vaccination is of particular concern. Studies have shown that vaccine given in the first 6 months posttransplant is poorly immunogenic but is unlikely to pose an increased safety risk. Not vaccinating may leave a transplant recipient vulnerable to infection potentially for an entire influenza season. In the United States, the CMS (Medicare/Medicaid) has recommended that all patients including transplant recipients be immunized prior to discharge from hospital. This may lead to some patients being immunized very early posttransplant and decreased immune response to vaccine. Revaccination 3–6 months after transplant could be considered if still within the seasonal time period for influenza.

HPV Vaccine

Two formulations of HPV vaccine are available: quadrivalent vaccine and an AS04-adjuvanted bivalent vaccine. Quadrivalent vaccine is recommended for use in males and females 9-26 years and bivalent vaccine in females 9-26 years; however, the quadrivalent vaccine can also be used in women up to the age of 45 years. The vaccine can be given regardless of history of sexual activity. However, since these vaccines are prophylactic and not therapeutic, there is limited or no effect on existing HPV-related lesions. Limited data are available for the immunogenicity of these vaccines in the posttransplant setting. A three-dose vaccine schedule should be given prior to transplant in those who meet the indications. However, if all doses are not completed pretransplant, the additional doses can be resumed starting 3–6 months posttransplant. A small study in adult posttransplant patients suggests suboptimal immunogenicity with quadrivalent vaccine (12). No data in the transplant setting are available for the bivalent vaccine. This is an area for further study.

Pneumococcal Vaccine

Two main formulations are available: a 23-valent polysaccharide vaccine and a 13-valent protein-conjugated vaccine. Protein-conjugated vaccines may produce antibodies of higher avidity and also lead to formation of memory B cells. Therefore, conjugate vaccines are widely studied and are recommended in routine childhood immunization programs including for pediatric transplant recipients. In adults posttransplant, conjugate vaccines produce a similar immunogenicity profile to polysaccharide vaccines (13). In addition, studies in which the conjugate vaccine is used for priming followed 8 weeks later by a polysaccharide pneumococcal vaccine did not show any additional benefit of polysaccharide vaccine (for the serotypes contained in the conjugate vaccine) and titers were similar with both strategies (14). Pneumococcal vaccine recommendations for immunocompromised adults are under review by national advisory bodies; recently the ACIP has recommended a prime-boost strategy (conjugate followed by polysaccharide vaccine 8 weeks later). The absolute protective titer for Pneumococcus is unknown and may vary by serotype. Pneumococcal titers should be monitored yearly as they have been reported to decline posttransplant (15).

For children older than 5 years, Pneumovax should be given. Children less than 2 years of age should receive 13-valent conjugate vaccine (Prevnar-13) according to

national guidelines. Those 2–5 years (24–71 months) of age should receive pneumococcal vaccine as follows:

Previous dose	Recommendations (16)
Unvaccinated or any incomplete schedule (less then three doses)	Two doses PCV13 First dose ≥8 weeks after most recent dose Second dose ≥8 weeks later
Any incomplete schedule of three doses	One dose, ≥8 weeks after most recent dose
Four doses of PCV7 or other age-appropriate complete	One dose PCV13, ≥8 weeks after most recent dose

In addition, children who are transplant candidates and recipients 24–71 months should receive PPV23 at least 8 weeks after completing PCV13 dosing

Vaccines for Travel

For transplant recipients who intend to travel to areas of increased risk for infection, immunization status should be reviewed. Both routine vaccinations (e.g. Hepatitis B) and travel-specific vaccinations such as typhoid vaccine should be addressed (see Table 4 for travel vaccinations).

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Special Article

Interactions Between Anti-Infective Agents and Immunosuppressants in Solid Organ Transplantation

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Abbreviations: AUC, area under the curve; AZA, azathioprine; CSA, cyclosporine; CYP, cytochrome P450; ECMS, enteric coated mycophenolate sodium; EVR, everolimus; HCV, hepatitis C; HIV-1, human immunodeficiency virus; mTOR, mammalian target of rapamycin; MMF, mycophenolate mofetil; SRL, sirolimus; TAC, tacrolimus.

Introduction

Infectious diseases are among the leading complications of immunosuppression for solid organ transplantation (1). The first few months posttransplant are particularly critical because immunosuppression is usually at high levels, acute rejection episodes are most likely to occur during this time frame requiring further increases in immunosuppression, and patients are receiving anti-infective prophylaxis (2). However, it is essential for the clinician to consider drug interactions and infectious complications for the life of the recipient. Although the risk of infection is highest in the first year posttransplant, the risk of infection may increase at any time during the posttransplant course when the patient's cumulative immunosuppressive state is enhanced (e.g. during treatment of rejection episodes when immunosuppression is intensified; Ref. 3). In addition, infectious diagnosis may be complicated by lack of signs and symptoms of inflammation, alterations in anatomy as a result of transplant surgery, denervation of the transplanted graft and preexisting diseases (4). Optimal treatment of specific infections, therefore, should be guided not only by knowledge of the pathogen's susceptibility to antimicrobial agents but also by the effects the agents will have on the pharmacokinetics and/or pharmacodynamics of the immunosuppressants that the patient is receiving.

Drug–drug interactions with immunosuppressants and anti-infective agents can be divided into two categories: pharmacokinetic and pharmacodynamic interactions. Pharmacokinetic drug–drug interactions may lead to altered drug concentrations of immunosuppressants, anti-infective agents or their metabolites through interactions in stages of absorption, distribution, metabolism or elimination. Some of the more frequently used immunosuppressive agents used in organ transplantation are metabolized via the cytochrome (CYP) 3A4 system. For example, immunosuppressive drug interactions can be caused by an anti-infective agent directly inhibiting CYP3A4 or via drug competition for CYP3A4 substrate sites. Both of these mechanisms may result in increased immunosuppressive concentrations. In contrast, CYP3A4 induction via increased synthesis or decreased breakdown of CYP isoenzymes may result in decreased immunosuppressive concentrations (5) Another type of interaction is through the membrane transporter P-glycoprotein (6). Drugs that inhibit or induce P-glycoprotein activity can ultimately result in increased or decreased bioavailability in the intestine. Moreover, due to genetic polymorphisms, patients may express variation in CYP3A4 enzymes and P-glycoprotein, which also can influence drug levels. The reader is referred to the following published reviews for a more detailed review of the pharmacokinetic principles of immunosuppressive agents and mechanisms of drug–drug interactions (5,6).

Pharmacodynamic interactions may occur as a result of drugs increasing or decreasing the efficacy or toxicity of each other. This may lead to detrimental or beneficial drug interactions. For example, the administration of calcineurin inhibitors with aminoglycosides, amphotericin, cidofovir, foscarnet, intravenous acyclovir or higher dose sulfamethoxazole-trimethoprim may result in the detrimental effect of additive nephrotoxicity. Alternative therapies without nephrotoxicity should be used whenever possible. When nephrotoxic therapies are essential to treatment, calcineurin inhibitors should be minimized whenever possible. Renal function must be carefully monitored and changes in renal function may necessitate decreasing or discontinuing anti-infective therapies.

Administration of mycophenolate mofetil (MMF), enteric coated mycophenolate sodium (ECMS) or azathioprine (AZA) with leflunomide for BK virus nephropathy may result in additive myelosuppression. Therefore, these antiproliferative agents should be discontinued upon initiation of leflunomide. Antiviral agents such as ganciclovir and valganciclovir and antibacterials such as linezolid and sulfonamides may result in myelosuppression when combined with MMF, ECMS or AZA too. Careful monitoring of white blood cells, platelets and hematocrit is necessary as well as consideration of dose adjustments of immunosuppressive and anti-infective agents as applicable. Pharmacodynamic interactions may also work in synergy. For example, CSA, (but not TAC) has been shown to inhibit hepatitis C virus *in vitro*, and may have a beneficial effect when combined with antiviral therapy (7). In addition, the mTOR inhibitors may decrease the incidence and severity of cytomegalovirus posttransplant (8) and MMF has been reported to potentially be associated with a decreased incidence of *Pneumocystis jiroveci pneumonia* (9,10).

Another consideration in dosing anti-infective agents is that many are renal-eliminated. Doses may need to be continually adjusted to maximize efficacy and limit toxicity, particularly in patients with fluctuating renal function. It is important to note that although MDRD is available to estimate GFR in transplant recipients, renal drug dosing recommendations are still based on Cockcroft-Gault calculations unless otherwise specified in the product information (11).

Table 1 provides summary information on interactions between anti-infectives and immunosuppressants, an indication of their severity, suggested actions by the clinician, the weight of evidence supporting these effects and suggested actions. For completeness, we have also included anti-infective agents in the table that may not always result in significant interactions in clinical practice, but have either *in vitro* data showing an interaction with immunosuppressants, or case reports showing evidence of an interaction. The following discussion describes these interactions in more detail focusing on the most severe and suggests approaches to alternative treatment.

Interactions That Significantly Raise Calcineurin Inhibitor and mTOR Inhibitor Plasma Levels

Macrolide antibiotics

All macrolide antibiotics, with the exception of azithromycin, are moderate to strong inhibitors of CYP3A4 and thus decrease the metabolism of calcinerin inhibitors: cyclosporine (CSA) and tacrolimus (TAC), and mammalian target of rapamycin (mTOR) inhibitors: sirolimus (SRL) and everolimus (EVR; Ref. 12). The magnitude of this effect varies between the macrolides, with erythromycin and clarithromycin having the greatest impact. Variations in immunosuppressive agent drug half-life can also impact the duration of the interaction. For example, SRL is dosed once daily and has a longer half live than EVR or calcineurin inhibitors, which are often dosed twice daily. In all cases, however, these combinations result in very significant (3- to 10-fold) increases in immunosuppressant concentration or area under the curve (AUC). The availability of clarithromycin and azithromycin, which have fewer gastrointestinal side effects, has resulted in diminished use of erythromycin in the community, thereby diminishing the chance of inadvertent co-administration. However, some surgical and medical intensive care units have adopted the use of erythromycin for gastrointestinal motility in patients with poor gastric emptying or ileus. The co-administration of erythromycin with calcineurin inhibitors, and especially mTOR inhibitors should be avoided in this situation when feasible; however, if the combination is used, at least a 50% reduction of calcineurin inhibitors should be considered early, because the effect is rapid. Daily drug level monitoring with calcineurin inhibitors is recommended and every third day level monitoring with mTOR inhibitors is recommended. Similar considerations apply to clarithromycin, which is widely used in the community and may be required for the treatment of transplant recipients with nontuberculous mycobacterial infections. However, this agent should be avoided whenever possible due to the interactions previously noted. The majority of *in vitro* and *in vivo* data indicate there is no pharmacokinetic interaction between azithromycin and calcineurin inhibitors or mTOR inhibitors. However, two case reports describe elevation of CSA concentrations with several days of concomitant administration of azithromycin (13,14). Elevation of TAC levels have also been reported with co-administered azithromycin (15). Therefore, monitoring drug levels may be appropriate.

Antifungal agents

All of the azole derivative antifungal agents decrease the metabolism of calcineurin inhibitors and mTOR inhibitors resulting in modest to profound increases in serum concentration and AUC. The potency of the interaction is different for each agent. For example, itraconazole and posaconazole have been shown to be more-potent inhibitors of CYP3A4 than are fluconazole or voriconazole (16).

Ketoconazole has also been shown to be the most potent inhibitor of CYP3A4 and has been co-administered with calcineurin inhibitor or mTOR inhibitor based immunosuppression in an effort to decrease immunosuppressive dose requirements and cost to transplant recipients (17–20). If undertaken, this drug combination must be carefully monitored as inadvertent discontinuation of ketoconazole by the patient or an outside health provider will dramatically decrease immunosuppressive levels.

The interaction of fluconazole with calcineurin inhibitors is both dose-dependent and drug-dependent (21). At modest doses (100–200 mg/day) of fluconazole used for nonsystemic candidiasis, effects on CSA are minor, whereas moderate to significant increases are seen with TAC. At doses of fluconazole required for systemic fungal infection (e.g. 400 mg for cryptococcosis or candidemia) significant dose reductions of immunosuppressants are required.

Voriconazole prescribing information recommends empiric dose reduction of TAC by two-thirds and CSA by 50% of the original maintenance dose when voriconazole is initiated (21–23). The combination of voriconazole and SRL is contraindicated as SRL levels may rapidly rise 10-fold (23). However, a small case series reported that voriconazole and SRL could potentially be used together if low doses of SRL are used (0.5–1.0 mg/day; Ref. 24). Similarly, EVR prescribing information recommends that it not be administered with voriconazole (25), but case reports have detailed their concurrent use with EVR dose reduction (26,27). Like voriconazole, posaconazole prescribing information also recommends empiric dose reduction of TAC by two-thirds and to decrease CSA dose by one-fourth of the original maintenance dose when posaconazole is initiated (28,29). The combination of posaconazole and SRL is contraindicated as SRL rise 9-fold (28,30). No information is available on the use of EVR and posaconazole in prescribing information of either agent (25,28), but one case report in a renal transplant recipient found a 3.8-fold increase in everolimus levels with posaconazole (26). Close drug monitoring is recommended at initiation, during and after discontinuation of voriconazole or posaconazole.

In addition, oral clotrimazole troches used for oral mucocutaneous candidiasis prophylaxis or treatment has also been shown to increase TAC blood levels significantly, doubling levels in some studies (31–34). The mechanism of this interaction is thought to be related to cytochrome (CYP) 3A4. Calcineurin inhibitor and m TOR inhibitor levels should all be monitored during initiation or discontinuation of clotrimazole.

The new echinocandin antifungal agents provide alternatives to the use of azole derivatives. None of the echinochandins are significantly metabolized by CYP3A and are available only in intravenous formulations. Although the original studies that resulted in approval of caspofungin suggested increased hepatotoxicity when used in conjunction with CSA, and noted that CSA increase caspofungin AUC by 35% (35), subsequent studies found no significant increase in hepatotoxicity nor a major change in CSA or TAC pharmacokinetics (36,37). A previous review article also noted that TAC AUC, peak and 12 hour concentrations are decreased by 20% in presence of caspofungin (38), but current product information for caspofungin recommends to follow standard TAC dosing and level monitoring and has no interaction with the active metabolites of MMF either (35). No data are available on interactions with mTOR inhibitors. Micafungin has not been shown to interact with TAC (39). The micafungin product information notes that there was no effect of a single dose or multiple doses of micafungin on MMF, prednisolone, TAC or CSA (40). However, one study has shown micafungin to decrease CSA concentrations by 16% in one study and hence monitoring of CSA levels is recommended (41). Micafungin product information also notes that SRL AUC was increased by 21% with no effect on maximum concentration, in the presence of steady-state and recommends SRL dose monitoring with dose adjustment as needed (40). A 22% increase in anidulafungin concentrations and drug exposure has been observed with CSA, but is not considered to be clinically relevant, and dose adjustments are not recommended for either agent (42). Of note, this interaction has not been observed with anidulafungin and tacrolimus (43). No data are available regarding interactions with mTOR inhibitors.

Antiretroviral agents

Patients chronically infected with human immunodeficiency virus-1 (HIV-1) are increasingly being transplanted for end organ disease (44). Many of the antiretroviral medications are substrates of CYP3A4; therefore, the interactions between these medications can be severe, particularly when used with calcineurin inhibitors or mTOR inhibitors in combination with protease inhibitors (45). All HIV-1 infected transplant recipients need close monitoring of immunosuppression levels to avoid underimmunosuppression or toxicity related to these medications. A summary of these interactions is provided in Table 1, but the reader is referred to the chapter in these guidelines on *Solid Organ Transplant in the HIV-Infected Patient* for further specific information on outcomes and drug interactions.

HCV protease inhibitors

The hepatitis C virus (HCV) protease inhibitors, boceprevir and telaprevir, are important new therapies for HCV treatment and are likely to be increasingly used in the liver transplant population. Both drugs are substrates and inhibitors of CYP3A4, with the main effect being elevated blood levels of the calcineurin inhibitors in healthy volunteers (46–48). Empiric CSA dose reduction of 75% or holding calcineurin inhibitors when boceprevir or telaprevir are introduced with drug level monitoring has been recommended by some authors (47). However, in three liver transplant recipients receiving co-administered CSA and boceprevir, only a minor increase in CSA blood levels were noted (49). A series of six liver transplant patients treated with telepravir required dosing of TAC once weekly or EVR every three days with close monitoring of drug levels (50). It seems based on several small case series in liver transplant recipients that boceprevir and telaprevir can be safely given with concomitant calcineurin inhibitors with close monitoring of drug levels (49–53). No data are yet available on the use of SRL with HCV protease inhibitors, but drug interactions are expected to be similar to those with TAC (47).

Table 1: Antiinfective drug interactions

Antimicrobial	Immunosuppressant[1]	Severity of interaction[2]	Interaction	Suggested actions	Weight of evidence[3]
Antibacterials					
Fluoroquinolones					
Ofloxacin	CSA, TAC	++	↑ Imm levels	Choose alternate	B
Ciprofloxacin	CSA, TAC	+/–	May ↑ Imm levels	No adjustment/consider monitoring Imm levels	B
Levofloxacin	CSA	+/–	May ↑CsA	No adjustment/consider monitoring Imm levels	A
Moxifloxacin	CSA, TAC, SRL, EVR	–	None	No adjustment	B
Macrolides					
Erythromycin	CSA, TAC, **SRL**, EVR	+++	↑ Imm levels	Avoid	A
Clarithromycin	CSA, TAC, **SRL, EVR**	+++	↑ Imm levels	Avoid/↓ Imm by1/2	A
Telithromycin	CSA, TAC, **SRL, EVR**	+++	↑ Imm levels	Avoid	A
Azithromycin[4]	CSA, TAC, SRL, EVR	+/–	↑ Imm levels	No adjustment/consider monitoring Imm levels	A
Rifamycins					
Rifabutin	CSA TAC, **SRL, EVR**	++	↓ Imm levels	Monitor Imm Levels	A
Rifapentine[5]	CSA, TAC, SRL, EVR, Prednisone	++	↓ Imm levels	Monitor Imm Levels	N/A
Rifampin	CSA, TAC, **SRL**, EVR, MMF, ECMS	+++	↓ Imm levels	Avoid/Monitor Imm Levels	A
Aminoglycosides					
Gentamicin Tobramycin Amikacin Streptomycin	CSA, TAC	+++	Enhanced nephrotoxicity	Avoid/Monitor Imm Levels and renal function	A
Other antibacterials					
Nafcillin	CSA, TAC, SRL, EVR	+	↓Imm levels	Monitor Imm Levels	B
Quinupristin/Dalfopristin	CSA	+++	↑ CsA	Monitor Imm Levels	B
Linezolid	MMF, ECMS, AZA	++	Myelosuppression	Monitor WBC and platelets	B
Sulfonamides	MMF, ECMS, AZA	++	Myelosuppression	Monitor WBC, hematocrit, platelets and renal function	B
	CSA, TAC	++	Nephrotoxicity		
Tetracycline[6]	CSA, TAC, SRL, EVR	+	↑Imm Levels	Monitor Imm Levels	B
Tigecycline	CSA	+	↑Imm Levels	Monitor Imm Levels	C
Metronidazole	CSA, TAC, SRL, EVR	+/–	May ↑Imm Levels	No adjustment/consider monitor levels	B
Chloramphenicol (intravenous)	CSA, TAC, SRL, EVR	++	↑Imm Levels	↓CSA or TAC by 25%	B
Clindamycin	CSA, TAC, SRL, EVR	+/–	May ↓Imm levels	No adjustment/consider monitor levels	C
Antimalarial					
Artemether[5]/Lumefantrine	CSA, TAC, SRL, EVR	++	↓Imm levels	Monitor Imm Levels	N/A
Antifungals					
Azoles					
Ketoconazole	CSA, TAC, **SRL, EVR**	+++	↑ Imm levels	Avoid/↓Imm by 1/2	A

Continued

Table 1: Continued

Antimicrobial	Immunosuppressant[1]	Severity of interaction[2]	Interaction	Suggested actions	Weight of evidence[3]
Voriconazole	CSA, TAC, **SRL, EVR**	+++	↑ Imm levels	↓CsA by 1/2, ↓Tac by 2/3	A
Itraconazole	CSA, TAC, **SRL, EVR**	++	↑ Imm levels	Monitor Imm Levels	A
Posaconazole	CSA, TAC, **SRL**, EVR	+++	↑ Imm levels	↓CsA by 1/4, ↓Tac by 2/3	A
Fluconazole	CSA, TAC, SRL, EVR	++	↑ Imm levels	Dose dependent ↓CsA and Tac by 1/3	A
Clotrimazole	CSA, TAC, SRL, EVR	++	↑ Imm levels	Monitor Imm Levels	A
Echinocandins					
Caspofungin[7]	TAC	+/–	May ↓TAC levels	None	B
	CSA	++	↑ Caspofungin Levels	Monitor AST/ALT	B
	MMF (no data on ECMS) No data on SRL, EVR	–	None	None	N/A-product info
Micafungin	TAC, MMF, Prednisone (no data on ECMS)	–	None	None	N/A-product info
	CSA	++	↓ CSA levels	Monitor Imm Levels	A
	SRL (no data on EVR)	++	↑SRL levels	Monitor Imm Levels	N/A product info
Anidulafungin	CSA	+	↑ Anidulafungin levels	None	A
	TAC No data on SRL, EVR	–	None	None	A
Polyenes					
Amphotericin Lipid formulation Amphotericins	CSA, TAC	++	Nephrotoxicity	Monitor Imm Levels and renal function	A
Antiviral agents					
Antiviral agents (non HIV)					
Acyclovir	MMF, ECMS	+/–	↑ACV, ↓MPA	None	C
Intravenous acyclovir	CSA, TAC	+++	Nephrotoxicity	Monitor renal function	A
Valacyclovir	MMF, ECMS	+/–	↓MPA	None	C
Ganciclovir	MMF, ECMS, AZA	++	Neutropenia	Monitor WBC	B
Valganciclovir	MMF, ECMS, AZA	++	Neutropenia	Monitor WBC	B
Foscarnet	CSA, TAC	+++	Nephrotoxicity ↓Ca ↓Mg	Monitor renal function, Ca, Mg, CNI levels, decrease foscarnet	A
Cidofovir	CSA, TAC	+++	Nephrotoxicity	Monitor renal function	A
Boceprevir	CSA, TAC, SRL, EVR	+++	↑ Imm levels	Imm dose reductions vary (see text)	A
Telaprevir	CSA, TAC, SRL, EVR, **systemic prednisone and methylprednisolone**	+++	↑ Imm levels	Imm dose reductions vary (see text)	A
Leflunomide	MMF, ECMS, AZA, SRL, EVR	+++	Myelosuppression	Hold MMF, ECMS, AZA and monitor WBC, hematocrit and platelets	A

Continued

Table 1: Continued

Antimicrobial	Immunosuppressant[1]	Severity of interaction[2]	Interaction	Suggested actions	Weight of evidence[3]
Oseltamivir	CSA, TAC, MMF	+/–	13% increase in TAC trough only	Monitor Imm Levels	A
	SRL	–	None		C
	No data with ECMS, EVR				
Zanamivir[4]	CSA, TAC, SRL, EVR MMF, ECMS	–	None	None	N/A-product info
Antiretroviral agents					
NNRTIs[8]					
EFV	CSA, TAC, SRL, EVR	++	↓CSA, ↓ TAC	Monitor Imm Levels	A
NVP	CSA, TAC, SRL, EVR	+/–	May ↓ Imm levels	Monitor Imm Levels	N/A
ETR[5]	CSA, TAC, SRL, EVR	+/–	May ↓ Imm levels	Monitor Imm Levels	N/A
DLV[5]	CSA, TAC, SRL, EVR	++	↑ Imm levels	Monitor Imm Levels	N/A
PIs[9]					
(ATV, DRV, FPV, IDV, LPVr, NFV[10], RTV, SQV, TPVr)	CSA, TAC, SRL, **EVR not recommended for use with RTV-regimens**	+++	↑CSA	CSA 25-50 mg daily	A
		+++	↑TAC/SRL/EVR	TAC 1 mg once or twice a week	A
				SRL 1 mg once or twice a week	A
				When using RTV-PI boosted regimen	B
				TPVr interaction unpredictable	
NRTIs[11]					
AZT	MMF/ECMS	+	*In vitro* antagonism	None	C
D4T	MMF/ECMS	+	*In vitro* antagonism	None	C

Drugs in bold are contraindicated.

[1]CSA = cyclosporine; TAC = tacrolimus; SRL = sirolimus; EVR = everolimus; Imm = immunosuppressant; MMF = mycophenolate mofetil; ECMS = enteric-coated mycophenolate sodium; AZA = azathioprine. Note: Data on EVR not always present, but included in table on basis of similar route of metabolism to other immunosuppressants involved in drug–drug interactions.

[2]Severity of interaction: +++, severe interaction, use alternative drug if possible, otherwise monitor levels of immunosuppressant or potential toxic effects and modify dose accordingly; ++, moderate interaction, requires monitoring levels or potential toxicity, and may require modification of immunosuppressant dosing; +, minor interaction or does not have major toxicity; +/–, *in vitro* data and/or case reports have reported an interaction, but changes in immunosuppressive levels have not been consistently seen in clinical practice.

[3]Weight of evidence: A, very good evidence such as clinical trial, pharmacokinetic studies in volunteers or patients, many well-documented case reports, or biochemical evidence confirming interaction; B, good evidence such as several well-documented case reports; C, anecdotal experience, one or two case reports.

[4]Majority of *in vitro* and *in vivo* data show no interaction.

[5]No patient data reported. Manufacturer's data indicate interaction with CYP3A4 metabolism, which could affect CSA, TAC, SRL, EVR.

[6]Doxycycline and minocycline have no reported interaction with CSA, TAC, SRL or EVR.

[7]Product information for caspofungin recommends standard tacrolimus dose monitoring and appropriate tacrolimus dosage adjustments but a subsequent study in heart and lung transplant recipients did not find any significant changes in tacrolimus troughs or doses Ref. (37).

[8]NNRTIs = nonnucleoside reverse transcriptase inhibitors (DLV = delaviridine; EFV = efavirenz; ETR = etravirine; NVP = nevirapine). If NNRTI used in combination with protease inhibitor, will need to monitor drug levels closely as TAC and CSA as well as SRL and EVR dosing interval may need to be reduced.

[9]PIs = protease inhibitors (ATV = atazanavir; DRV = darunavir; FPV = fosamprenavir; IDV = indinavir; LPVr = lopinavir + ritonavir; NFV = nelfinavir; RTV = ritonavir; SQV = saquinavir; TPVr = tipranavir + ritonavir. All protease inhibitors inhibit metabolism of CSA, TAC, SRL, EVR. PIs "boosted" with RTV often require further reduction in immunosuppression dosage.

[10]Product information for tacrolimus notes contraindicated use with NFV.

[11]NRTIs = nucleoside reverse transcriptase inhibitors (AZT = zidovudine; D4T = stavudine). None of the NRTIs are expected to interact with CSA, TAC, SRL, EVR.

Interactions That Significantly Decrease Calcineurin Inhibitor and mTOR Inhibitor Plasma Levels

Rifamycins

All of the rifamycins are strong inducers of CYP3A4. For rifampin and rifabutin, clinical data confirm the dramatic increases in clearance and resultant decreases in plasma levels of the calcineurin inhibitors and mTOR inhibitors (54–60). This effect has been reported to remain even in the presence of multiple CYP3A4 inhibiting medications (61). No data are available for rifapentine but a similar effect is likely. This combination should be avoided if at all possible because of the severe difficulty of maintaining therapeutic levels of calcineurin inhibitors, mTOR inhibitors. In those situations where a rifamycin derivative must be used, as in patients with tuberculosis, increased doses of calcineurin inhibitors or mTOR inhibitors should be initiated with onset of combined therapy. A twofold dose increase is recommended at the initiation of therapy, with rapid subsequent increases (up to 10-fold reported) and frequent drug level monitoring until stable dosing is achieved. Similar vigilance is required when rifamycin therapy is discontinued.

A much less dramatic effect on MMF pharmacokinetics has been reported with rifampin (62). Although the increase in MMF dosing requirements seems to be moderate compared with those for calcineurin inhibitors, the manufacturer's prescribing information recommends avoiding this combination when possible and monitoring drug levels closely if it is used (63). There is no information available for use with ECMS (64), but based on the mechanism of interaction, we recommend closely monitoring drug levels if used. Of note, ethambutol and isoniazid, which are sometimes used in combination with these agents, do not seem to interact with immunosuppressive agents.

Artemether/lumefantrine

The artemether component of the new antimalarial artemether/lumefantrine is an inducer of CYP3A4 (65). Although there is no currently available data on drug–drug interactions with immunosuppressants, it would be expected to potentially decrease calcineurin levels, as well as mTOR inhibitor levels (Table 1).

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Special Article

Urinary Tract Infections in Solid Organ Transplantation

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Key words: Asymptomatic bacteriuria, candiduria, cystitis, pyelonephritis, transplantation, urinary tract infection, uropathogen

Abbreviations: CT-PET, computed tomography-positron emission spectography; ICU, intensive care unit; KT, kidney transplant; MSU, mid-stream urine; SOT, solid organ transplant; USD, United States dollar; UTI, urinary tract infection.

Introduction

Infections remain a major cause of morbidity and mortality in transplant recipients. Since the pattern of infections change continually due to evolving donor–recipient characteristics, surgical techniques and immunosuppression regimens, a periodic review of this subject and recommendation for its evaluation and treatment is essential. Infections account for 16% of patient deaths and 7.7% (14% in patients >65 years of age) of death censored graft failure in kidney transplant (KT) recipients (1,2). Urinary tract infection (UTI) is the most common infectious complication accounting for 45–72% of all infections, and 30% of all hospitalizations for sepsis in KT recipients (3–7). Occurrence of UTI early after kidney transplantation significantly increases the cost of hospitalization by an additional 5131 USD per patient (8).

The recommendations provided in this guideline are mostly expert opinion. However, when grading is mentioned, it is based on the following rating system.

Rating System:

I Randomized controlled trials
II-1 Controlled trials without randomization
II-2 Cohort or case-control analytic studies
II-3 Multiple time series, dramatic uncontrolled experiments
III Opinions of respected authorities, descriptive epidemiology

Epidemiology

The incidence of UTI differs among solid organ transplant (SOT) recipients. Among 2405 SOT recipients followed for 3 years in the Spanish Network for the Study of Infections in Transplantation (RESITRA), the incidence of cystitis per 100 recipient-years were 13.84 for renal, 3.09 for liver, 2.41 for heart and 1.36 for lung transplant recipients (9). The incidence of pyelonephritis per 100 recipient-years was 3.66 for renal, 0.8 for liver, 0.3 for heart and 0.6 for lung transplant recipients. In the same study, UTI-associated bacteremia was seen in 39% of renal, 3% of liver, 3% of heart and 0% of lung transplant recipients. Similarly, Silva et al. reported bacteremia of 37.8% secondary to UTI in KT recipients (10). Since UTI is more prevalent among KT recipients, the guideline will focus on the diagnosis and treatment of UTI in this patient population. This guideline applies to pediatric and adults.

The prevalence of UTI among KT recipients varies widely from 23% to 75%. Differences in reported incidence of UTI is likely due to lack of uniform diagnostic criteria, use of varying antibiotic prophylaxis regimens, and uneven duration of follow-up (3,4,6). While UTI can occur at any time after transplantation, the highest incidence has been reported in the first 3–6 months after KT (4,11). In a RESITRA Spanish registry study of >2000 renal transplant recipients with at least 1 year follow-up, 84% of symptomatic UTI cases were distributed over the first 6 months posttransplant (12). In a study of Medicare claims for 28 942 renal transplant recipients, the cumulative incidence of UTI during the first 6 months posttransplant was 17% in both men and women. However, by 3 years posttransplant, there was a significantly higher incidence in women (60%) compared to men (47%, $p < 0.001$; Ref.13). Recently, a UTI incidence of 26% has been reported during the first 12 months of posttransplantation in KT recipients who received the most commonly used immunosuppressive regimen (tacrolimus, mycophenolate mofetil and corticosteroids) in a Phase III randomized, multicenter, prospective study (14). The prevalence of recurrent UTI ranges from 2.9% to 27% in KT recipients (3,15–17).

Table 1: Diagnostic criteria for UTI (18,19)

Category	Description	Clinical features	Laboratory investigations
1	Acute uncomplicated UTI in women; acute uncomplicated cystitis in women	Dysuria, urgency, frequency, suprapubic pain, no urinary symptoms in 4 weeks before this episode	>10 WBC/mm^3 >10^3 cfu/mL[1]
2	Acute uncomplicated pyelonephritis	Fever, chills, flank/allograft pain; other diagnoses excluded; no history or clinical evidence of urological abnormalities (ultrasonography, radiography)	>10 WBC/mm^3 >10^4 cfu/mL[1]
3	Complicated UTI	Any combination of symptoms from categories 1 and 2 above and one or more factors associated with a complicated UTI (see text for definition)	>10 WBC/mm^3 >10^5 cfu /mL[1] in women >10^4 cfu/mL[1] in men, or in straight catheter urine in women
4	Asymptomatic bacteriuria	No urinary symptoms	>10 WBC/mm^3 >10^5 cfu/mL[1] in two consecutive MSU cultures >24 h apart
5	Recurrent UTI	At least three episodes of uncomplicated infection documented by culture in past 12 months: women only; no structural/functional abnormalities	>10^3 cfu/mL[1]

Modified according to IDSA/European Society of Clinical Microbiology and Infectious Diseases guidelines. All pyuria counts refer to unspun urine.
[1]Uropathogen in MSU culture.

Definitions and Diagnostic Criteria for UTI

Although the definitions and diagnostic criteria of UTI mentioned in Table 1 are for the general population, the same could be applied for recipients of SOT except that all symptomatic UTI in transplant recipients would be considered as complicated UTI whether it is lower or upper urinary tract involvement (18,19).

Symptomatic urinary tract infection

UTI classically presents with dysuria, urinary frequency/urgency and suprapubic pain. Pain and tenderness over the renal allograft or costo-vertebral region may indicate upper urinary tract involvement. Some patients may primarily present with fever, malaise or a nonspecific sepsis syndrome without symptoms localized to the urinary tract.

Asymptomatic bacteriuria

Patients with a urine culture yielding significant growth of uropathogens in the absence of any symptoms attributable to infection are defined as having asymptomatic bacteriuria. Asymptomatic bacteriuria in women is commonly defined as two consecutive clean-catch voided urine specimens >24 h apart with isolation of the same organism in quantitative counts of $\geq 10^5$ cfu/mL. In men, a single clean-catch voided urine specimen with isolation of a single organism in quantitative counts of $\geq 10^5$ cfu/mL is sufficient since the risk of contamination is rare. Bacteriuria is also defined as isolation of a single organism in quantitative counts of $\geq 10^2$ cfu/mL in a single specimen obtained through urethral catheterization (20).

Recurrent and relapsed UTI

Recurrent UTI is commonly defined as three or more episodes of symptomatic UTIs over a 12-month period, or two episodes in the previous 6 months (21). A relapsed UTI is defined as prompt recurrence of the same organism following treatment.

Complicated UTI

A complicated UTI is defined as an infection that is associated with structural or functional abnormalities of the genitourinary tract, or presence of an underlying disease that increases the risk for acquiring an infection or of failing therapy (22–24). By this definition, a symptomatic UTI in any transplant recipient is considered complicated whether it involves the lower or upper urinary tracts since immunocompromised status may increase the risk for infection and/or failure of therapy.

Classification and Severity Assessment of UTI

Conventionally, UTIs have been classified as lower or upper, uncomplicated or complicated and urosepsis. In order to provide clinicians and researchers with a standardized tool and nomenclature for UTI, an improvised system of classification based on four characteristics consisting of anatomical levels of infection, grades of severity, presence of underlying risk factors, and the microbiological findings of UTI as suggested by European Association of Urology is shown in Table 2. The basis of this classification and its interpretation are also shown in the same table as an appendix (25). Application of this

Table 2: Classification and severity assessment of UTI (25)

Anatomical level of infection	Grade of severity	Risk factors (RF)	Pathogens
UR: Urethritis			
CY: Cystitis	1: Low, cystitis		
PN: Pyelonephritis	2: PN, moderate	O; no risk Factor[1]	
US: Urosepsis	3: PN, Severe, established	R: Recurrent UTI RF	Species
MA: Male genital glands	4: US: SIRS	E: Extra urogenital RF[2] N: Nephropathic RF	Susceptibility grade
	5: US: Organ dysfunction	U: Urological RF	a. Susceptible
	6: US: Organ failure	C: Catheter RF	b. Reduced susceptibility
			c. Multi resistant

Appendix: Examples of UTI classification based on above characteristics:

1. CY– 1R: *E. coli* (a): Simple cystitis but recurrent with susceptability to standard antibiotics.
2. PN-3U: *K. pneumoniae* (b): Severe pyelonephritis (with high fever and vomiting) with underlying urological disease (e.g. stones or obstruction) due to *Klebsiella* sp., with moderate antibiotic resistance profile.
3. US-5C *Enterococcus* sp (a). Severe urosepsis with antibiotic sensitive *Enterococcus* sp in a patient with indwelling catheter.

[1]Modification required for transplant recipients: "No risk factor" does not apply to transplant recipients.
[2]Immunosuppression is included in this category.

classification to transplant recipients will require minor modification in the risk factor category since all immunocompromised recipients are at increased risk for UTI, and the absence of risk factors will not apply to recipients of SOT. This new classification system would facilitate standardization of the diagnosis and treatment of UTI, and will also help to assess the impact of UTI on outcomes in transplant recipients.

Risk Factors for UTI in Renal Transplant Recipients (Table 3) (6,15,26–30)

The risk factors for the development of posttransplant UTI are multifactorial and are determined by the interaction between host factors, pathologic agents and anatomical abnormalities. The potential risk factors with their odds ratio for risk of UTI in KT recipients are shown in Table 3. Even though the risk of UTI in transplant recipients is determined by the net state of immunosuppression, certain immunosuppressants have a greater effect on the risk for UTI. Antimetabolite (azathioprine or mycophenolate mofetil) based regimens that predispose to bone marrow suppression, and induction therapy with cell depleting antibodies such as antithymocyte globulin have been reported to have higher incidence of UTI (3,30,31). Any instrumentation and its duration is an important risk factor for UTI. Fayek et al. reported a higher UTI rate of 14.2% in KT recipients with stents as compared to 7.9% without stents ($p = 0.003$) despite lower ureteral complications (32). Since astent *in-situ* for more than 30 days has shown to increase the risk for UTI significantly, removal of the stent within 4 weeks of transplantation has been suggested (33). Diabetes mellitus has also been shown to increase the risk of bacterial UTI in some studies but the data are conflicting. However, diabetes mellitus has strong association with fungal UTI typically caused by *Candida albicans* (3). Risk factors for late UTI (>6 months posttransplantation) include serum creatinine levels >2 mg/dL and prednisone dose >20 mg/day in addition to history of therapy for multiple rejections and chronic viral infections (e.g. CMV) which increase the net state of immunosuppression (11).

Table 3: Risk factors for UTI in renal transplant recipients (6,15, 26–30)

Risk factors	OR (95% CI)
Bacterial urinary tract infection	
Female gender	5.8 (3.79–8.89)
Age (per year)	0.02 (1.01–1.04)
Reflux kidney disease prior to transplantation	3.0 (1.05–8.31)
Deceased donor	3.64 (1.0–12.7)
Duration of bladder catheterization	1.50 (1.1–1.9)
Length of hospitalization prior to UTI	0.92 (0.88–0.96)
Increase in immunosuppression	17.04 (4.0–71.5)
Candiduria	
Female gender	12.5 (6.70–23.0)
ICU care	8.8 (2.3–35.0)
Prior antibiotic use	3.8 (1.7–8.3)
Indwelling urethral catheter	4.4 (2.1–9.4)
Neurogenic bladder	7.6 (2.1–27)
Malnutrition	2.4 (1.3–4.4)
Acute pyelonephritis	
Female gender	5.14 (1.86–14.20)
Acute rejection episodes	3.84 (1.37–10.79)
Number of UTIs	1.17 (1.06–1.30)
Mycophenolate mofetil	1.9 (1.2–2.3)

Microbiology (Figure 1) (6,15,34,35)

In KT recipients, Gram-negative bacteria account for more than 70% of UTI; *E. coli* is the most common uropathogen (36). Other frequent uropathogens include Enterobacteriaceae, enterococci, *Pseudomonas* species, and coagulase-negative staphylococci (e.g. *Staphylococcus saprophyticus*). *Corynebacterium urealyticum* is a potentially important pathogen that requires longer incubation (48–72 h) for detection than is routine for urine cultures, may be

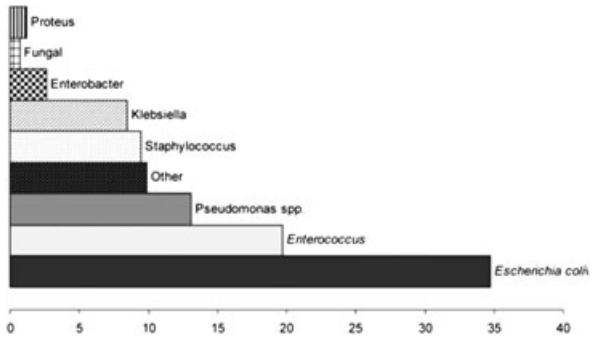

Figure 1: Microbiology of urinary tract infections in renal transplant recipients. Proportion of isolates in each category (N = 1519 isolates). Data taken from Chuang (15), Memikoglu (34), Pelle (6) and DiCocco (35).

more readily isolated on selective media, may be associated with obstructive uropathy and/or encrusted cystitis, and is not susceptible to most conventional oral antibiotics used for treatment of UTI (37). Unusual pathogens of the urinary tract include *M. tuberculosis*, *Salmonella* species, cytomegalovirus and adenovirus (the latter is associated with hemorrhagic cystitis; Refs.11,38). *Mycoplasma hominis* or *Ureaplasma urealyticum*, whose pathogenicity is often unclear when found in the genito-urinary tract, can rarely cause invasive infections (e.g. intrarenal or perinephric abscesses) after renal transplantation (39,40).

Candiduria

Candida species are the most common fungal cause of urinary tract infection in renal transplant recipients. While candiduria is frequent, occurring in 11% of renal transplant patients in one series, it is most often asymptomatic (29). As is the case with asymptomatic bacteriuria, there are no established diagnostic tests that reliably distinguish infection from colonization in patients with asymptomatic candiduria; no studies have unequivocally established the importance of pyuria or quantitative urine cultures for UTI due to candida. Candiduria can uncommonly have serious consequences and may cause ascending infection, candidemia, and/or obstructing fungal balls at the ureterovesical junction (41).

Antibiotic-resistant uropathogens

With widespread use of antibiotics for prevention and treatment in transplant recipients, the prevalence of resistance to antibiotics among uropathogenic bacteria is increasing. In patients receiving trimethoprim-sulfamethoxazole prophylaxis, 62% of urinary tract infections have been reported as caused by trimethoprim-sulfamethoxazole-resistant organisms (42). Use of fluroquinolones as prophylaxis for renal transplant recipients has been linked to surges in fluoroquinolone-resistant *Pseudomonas aeruginosa* (43). Frequent use of antibiotics for treatment of asymptomatic bacteriuria also has been associated with antimicrobial resistance. In a study of patients with asymptomatic *E. coli* or *E. faecalis* bacteriuria, treatment led to selection of resistant organisms in 78% of treated cases (44). Emergence of multidrug-resistant organisms, including ESBL-producing organisms or carbapenemase-producing organisms, have been observed in transplant units and may be associated with a poorer prognosis (45). In the RESITRA Spanish registry study, 26% of the 118 cases of symptomatic *E. coli* UTI were caused by ESBL-producing organisms (12). In a cohort of renal transplant recipients in Brazil, incidence of UTI caused by ESBL-producing organisms rose progressively from 13% among first episodes of infection to 45% of patients with a third episode of UTI (46). Outbreaks of organisms resistant to all commonly available antibiotics have occurred; treatment options may be restricted to nephrotoxic agents such as colistin (47). The prevalence of drug resistance varies considerably by region and country.

Pathophysiology

Virulence structures, like P fimbriae, are expressed on the surface of uropathogenic bacteria and facilitate adhesion to uroepithelial surface. *E. coli* that express P fimbriae account for more than 80% of the isolates from patients with pyelonephritis in the noncompromised host, and an even greater number of pyelonephritis isolates from immunosuppressed patients (20–22). In addition, a subset of O antigen serotype is present on the majority (80%) of *E. coli* isolates from patients with UTI (15,23).

In transplant pyelonephritis, acute elevations in creatinine are commonly observed, though may improve with treatment (48). Single center studies demonstrate that acute transplant pyelonephritis, especially in the first 3 months posttransplantation represents a risk factor for long-term kidney graft dysfunction but does not affect graft survival at five years (6,31). More controversial is the effect of asymptomatic bacteriuria in renal transplantation. The concern remains that at least in a subset of patients, asymptomatic bacteriuria may represent a pathologic state: (1) While not well documented, asymptomatic bacteriuria early after renal transplantion may be a risk factor for development of symptomatic urinary tract infection, especially if bacteriuria is found repeatedly in association with pyuria (49). (2) Furthermore, some studies have suggested that bacteriuria may be associated with injury to the graft. In one small study, renal transplant patients with asymptomatic bacteriuria had higher median urinary IL-8 cytokine levels than transplant patients without bacteriuria (50). A review of 225 patients with chronic rejection identified asymptomatic bacteriuria/symptomatic infection as a risk factor in a regular urine culture screening and antimicrobial treatment protocol (51). Data, however, on the clinical significance of asymptomatic bacteriuria are conflicting; even less clear is the impact of antimicrobials on asymptomatic bacteriuria.

Diagnosis of UTI: specimen collection

Since urine culture is the key to the diagnosis of a UTI, specimen collection technique is important. After use of antiseptic wipes to clean the perineum/glans, a midstream urine sample is collected in a sterile container. Straight catheterization to obtain a urine specimen can be considered as an alternative. For patients with indwelling catheters (especially those in place >2 weeks) and a suspected urinary tract infection, the Infectious Diseases Society of America recommends removal of the catheter and collecting either a midstream urine or via a newly placed urinary catheter (19).

Screening programs

It is common practice for transplant centers to regularly screen asymptomatic renal transplant recipients for bacteriuria to initiate antimicrobial therapy. However, the benefits of these screening/treatment programs have not been demonstrated. Recent guidelines from the Infectious Diseases Society of America acknowledge that at present, "no recommendations can be made for screening for or treatment of asymptomatic bacteriuria in renal or other SOT recipients" (20). If a screening strategy for asymptomatic bacteriuria is chosen, we recommend limiting routine collection of urine cultures (with an accompanying urinalysis with microscopy) to the first 1–3 months after renal transplantation; thereafter, these screening tests could be performed only if symptoms or signs of infection develop or if elevations in creatinine are observed. For women found to have bacteriuria, a second sample (minimizing risk of contamination) may be appropriate to document continued presence of bacteriuria. Not only have screening for asymptomatic bacteriuria not shown to be effective, a potential adverse effect is overexposure to antibiotics and selection for resistant microorganisms (see "Treatment" section on asymptomatic bacteriuria and "Microbiology" section on antibiotic-resistant uropathogens).

Diagnosis

Typically, diagnosis of a symptomatic UTI includes a quantitative count of bacteria ($\geq 10^5$) in an appropriately collected urine specimen in the presence of symptoms or signs of urinary infection (22). Not all organisms found in urine cultures are pathogens. For example, *Staphylococcus epidermidis* (except in the presence of ureteral stents), lactobacillus, and *Gardnerella vaginalis* are unlikely to be pathogens. Urine cultures containing multiple organisms (i.e. "mixed flora") indicate that contamination has likely occurred. Other true pathogens may not grow well on routine culture media (e.g. unusual pathogens such as *Corynebacterium urealyticum* or *M. tuberculosis*) and specific culture media may need to be requested. The usefulness of leukocyte esterase and nitrite screening by dipstick has not been demonstrated in renal transplant recipients. While pyuria (>10 WBC/HPF) does not necessarily confirm that the urinary tract is infected, absence of pyuria should lead the clinician to question the diagnosis of a urinary tract infection (19). Imaging with renal ultrasound or noncontrast CT scan should be considered to assess for complications such as obstruction and abscess, particularly in patients not fully responding to initial therapy or in patients with signs of severe infection.

Treatment of UTI

The overall treatment strategy depends on the severity of illness (Table 4; Ref.52). Selection of antimicrobial agents should be based on local epidemiological data and the patient's history of resistant organisms. It is important to be aware of the possibility of lack of correlation between signs and symptoms of UTI and the microbial load in immunocompromised patients. In clinical situations with severe infection with sepsis, the option of reduction/discontinuation of immunosuppression should also be considered.

Asymptomatic bacteriuria

There is no consensus whether asymptomatic bacteriuria should be treated in renal transplant recipients and if so, at what time periods posttransplantation (20). A small, prospective randomized controlled trial of 88 patients suggested that treatment of asymptomatic bacteriuria beyond 1 year post-KT does not prevent symptomatic UTI (53). In a study of 334 cases of asymptomatic *E. coli* or *E. faecalis* bacteriuria identified beyond 1 month after renal transplantation, evolution to symptomatic UTI was similar between treated and untreated groups (0/101 vs. 4/233; Ref.44). In this study, treatment led to selection of resistant pathogens in 78% of cases. While treatment of asymptomatic bacteriuria is a common practice in renal transplant centers, the few available studies indicate that antimicrobials in this setting are often unsuccessful in sustaining sterilization of urine and have not been demonstrated to prevent subsequent UTI or improve graft function (44,52–55). However, given that (1) symptomatic UTI are most common early after transplant (12) and pyelonephritis may be associated with at least short-term graft dysfunction (6,31) (2) since bacteriuria may theoretically be a precursor for symptomatic UTI, it may be reasonable to screen for and treat asymptomatic bacteriuria (particularly if associated with pyuria) for a limited period in the early posttransplant period, e.g. only 1–3 months posttransplant (4,49). A treatment duration of 5–7 days could be considered. However, these screening and treatment strategies may be too aggressive and lead to over-treatment and selection of resistant microorganisms. More data are needed to guide these strategies. Guidelines of the Infectious Diseases Society of America recommend treatment of asymptomatic bacteriuria in pregnancy and immediately prior to transurethral resection of the prostate or other urologic procedures in which

Table 4: Treatment of UTI in transplant recipients (52)

Clinical presentation	Suggested management
Asymptomatic bacteriuria	No consensus on management. Repeat culture with appropriate technique (consider straight catheterization) to rule-out contamination. In the first 1–3 months posttransplant, consider treatment for 5–7 days; beyond 3 months posttransplant, avoid treatment unless associated rise in creatinine. No need for empiric treatment—await culture susceptibility and select the most narrow-spectrum antibiotic available.
Symptomatic urinary tract infection—mild	Empiric oral therapy: ciprofloxacin +/- amoxicillin. Treatment duration 5–7 days.
Symptomatic urinary tract infection—moderately severe	Ciprofloxacin OR ceftriaxone OR ampicillin-sulbactam. Once culture susceptibility results available, complete 14 days of therapy with the most narrow-spectrum antibiotic available.
Symptomatic urinary tract infection—severe	Empiric piperacillin-tazobactam OR cefepime. Consider potential for multi-drug resistant organisms which may require a carbapenem or therapy for Vancomycin resistant enterococci. Once culture susceptibility results available, complete 14–21 days of therapy with the most narrow-spectrum antibiotic available.
Recurrent symptomatic urinary tract infection	Consider imaging to rule out structural causes or persistent foci of infection. Extend treatment to 6 weeks. (Review Fig. 2.)
Candiduria	Remove urinary catheters, stents. Avoid treatment of asymptomatic candiduria unless the patient is undergoing a urologic procedure or is neutropenic. If symptomatic or persistent candiduria, consider imaging of kidneys and collecting system to assess for fungal masses and request susceptibility testing; if fluconazole-susceptible, treat with fluconazole for 7–14 days. Note that voriconazole, posaconazole, caspofungin, and lipid-formulations of amphotericin attain only limited concentrations in urine but may achieve sufficient concentration in kidney tissue.

mucosal bleeding is anticipated in all persons, including nontransplant patients (20).

Symptomatic UTI

In patients with symptomatic UTI, removal (preferred) or replacement of urinary tract instruments such as urethral catheters and urologic stents is recommended. An oral fluoroquinolone, amoxicillin-clavulanate, or an oral third-generation cephalosporin (e.g. cefixime) is frequently selected for empiric treatment. For patients that require initial parenteral therapy because of severe illness or nausea and vomiting, beta-lactams such as cefepime, piperacillin-tazobactam or a fluoroquinolone may be used. It should be noted that drug resistant organisms such as ESBL-producing or carbapenemase-producing organisms are on the rise in many centers. In patients with signs of severe infection, choice of empiric antibiotic should take into account the patient's history of previous resistant organisms (e.g. previously isolated ESBL-producing organisms) as well as local epidemiologic data. Especially if resistant organisms are found, expanded antimicrobial testing should be requested from the microbiology lab to identify treatment options for completion of therapy (e.g. for Enterobacteriaceae—fosfomycin, an oral agent with limited supporting clinical data but potentially broad *in vitro* activity). Once susceptibility data are available, the most narrow-spectrum antibiotic should be used to complete course of therapy. Some authors recommend treatment of mild (e.g. cystitis) symptomatic UTI in renal transplant patients for 5–7 days. Others recommend that if the UTI occurs early posttransplant (e.g. in the first 6 months), even mild cases should be treated for 7–10 days (36,56). Transplant pyelonephritis or urosepsis warrants longer treatment, e.g. 14–21 days (56,57). Progression of upper urinary tract disease to a renal or perinephric abscess or emphysematous pyelonephritis may occur and usually requires a multidisciplinary approach to treatment, including urologic and/or interventional radiology consultation for percutaneous or surgical drainage of abscesses. While awaiting culture data, broad spectrum anti-infective therapy should be initiated with cefepime, an extended-spectrum penicillin (e.g. piperacillin-tazobactam), or a carbapenem. Duration of treatment should be for at least 2 weeks, and should be extended until adequate drainage of abscesses and clinical resolution of infection has been achieved.

Candiduria

Further research is necessary to determine whether asymptomatic candiduria warrants treatment in renal transplant patients as data on treatment of candiduria in renal transplantation are scant. In one observational case control study of 192 renal transplant recipients with candiduria, only 50% were treated with antifungal therapy; treatment of candiduria was not associated with improved clinical outcomes (29). Many asymptomatic patients with candiduria are treated because of the perceived risk to the allograft and the potential for involvement of the upper urinary tract (by obstruction-causing fungal balls). However, treatment of asymptomatic candiduria is generally discouraged unless the patient is undergoing a urologic procedure or is neutropenic (58).

In patients with symptomatic fluconazole-susceptible candiduria, the preferred agent is fluconazole, 200–400 mg orally per day for 14 days (58). Adjustments to calcineurin inhibitor dosages may be required with concurrent use of azole agents. Intravenous amphotericin B, 0.3–1 mg/kg/day for 1–7 days should only be used with extreme caution

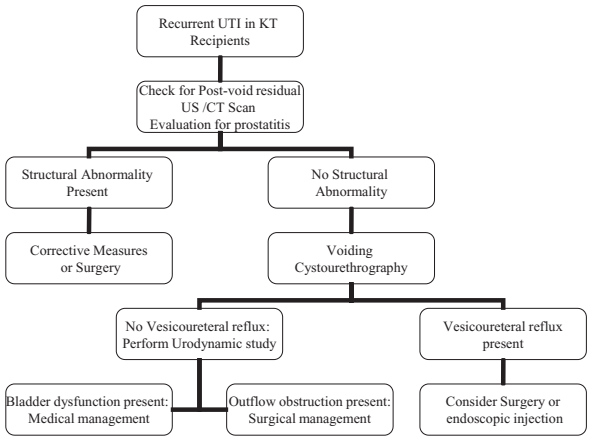

Figure 2: Suggested schema of evaluation for recurrent UTI (57). Figure modified from Ref. 57. KT = kidney transplantation; UTI = urinary tract infection.

given its nephrotoxicity. Short courses of flucytosine (25 mg/kg every 6 h for 7–10 days) can be considered; however, resistance can develop rapidly, dose adjustments are required for renal dysfunction, and patients must be monitored for cytopenias, rash, gastrointestinal symptoms and hepatoxicity (41). Continuous bladder irrigation with amphotericin B (50 mg diluted in 1 L of sterile water for 5–7 days) may be less nephrotoxic but is of limited value given high relapse rates (59). Voriconazole and echinocandins achieve low concentrations in the urinary collecting system, which reduces their usefulness for treatment of fungal urinary tract infection. However, these agents may be able to penetrate kidney tissue and thus may have a role in transplant pyelonephritis; clinical experience is limited. Lipid formulations of amphotericin should not be used to treat UTI because of poor levels in urine (41).

Recurrent UTI

Anatomic and functional abnormalities should be identified and corrected in renal transplant patients with recurrent symptomatic UTI (Fig. 2; Ref.57). Patients should be reminded of basic infection prevention measures, such as hydration, frequent voiding, and for females to void after sexual intercourse and wiping from front to back with toilet tissue. Prostatitis should also be considered in the differential diagnosis. Initial testing should include a quantification of postvoid residual volume and imaging (ultrasound and/or noncontrast computerized tomography (CT scan). It should be remembered that posttransplant UTI may originate in the transplant or native kidneys, and imaging should include ureters and bladder to identify obstruction, renal calculi, retained foreign bodies, and complex cysts (57). Voiding cystourethrograms, urodynamic studies, and cystoscopy should also be considered. However, given how common reflux is in renal transplant recipients even in the absence of symptomatic UTI or asymptomatic bacteriuria, not all findings of reflux warrant surgical or endoscopic correction (60). A schema of evaluation to identify a correctable abnormality is shown in Figure 2. In patients with polycystic kidney disease, gallium scanning (11) or CT-PET scans (57) may be helpful in identifying foci of infection. In KT recipients with relapsing asymptomatic bacteriuria, it remains unclear whether imaging and urologic work-up is necessary.

Some authors recommend treatment of relapsed, symptomatic UTI with 4–6 weeks of antibiotic therapy (11). If this fails, other strategies to prevent recurrent symptomatic UTI include topical vaginal estrogen in postmenopausal women. Trials have yielded mixed results regarding efficacy of concentrated cranberry tablets for prevention of recurrent symptomatic UTI in nonrenal transplant recipients (61). The potential benefits of extended antibiotic prophylaxis (e.g. 3 months) (36) should be carefully weighed against the risks of promoting bacterial resistance, *Clostridium difficile* infection, and other adverse events associated with antibiotics. If a long-term prophylaxis strategy is pursued, periodic (e.g. in 3–6 months) trials of stopping prophylaxis should be considered.

Prevention and Prophylaxis

General principles

Prevention of UTI should receive highest priority with a particular attention given to treatment of existing infections, and correction of structural abnormalities of the urinary tract when present in the potential recipients prior to transplantation. Neurogenic bladder, and in children, voiding dysfunction should be considered and addressed (62). In the immediate posttransplant period, vigilance for donor-transmitted infection is important. Transmission of infection via implantation of a contaminated organ is a potentially serious complication of SOT. Although, the optimal management of positive organ preservation fluid cultures is uncertain, increasing evidence suggests that organisms found in these cultures can subsequently cause infection in the recipient, and thus treatment can lead to improved outcomes (63).

Prevention of both asymptomatic bacteriuria and UTI after kidney transplantation has improved with the introduction of routine perioperative antibiotic prophylaxis, minimization of use of indwelling urethral catheters and ureteral stents, and long-term use of antimicrobial prophylaxis to prevent *Pneumocystis* pneumonia (64,65). A systematic review and meta-analysis of antibiotic prophylaxis for UTI in KT recipients showed prophylaxis to reduce the risk for developing sepsis with bacteremia by 87% (RR 0.13, 95% CI 0.02–0.7) and the risk for developing bacteriuria (symptomatic and asymptomatic) by 60% (RR 0.41, 95% CI 0.31–0.56); however, all cause mortality and graft outcome was not different in this meta-analysis. In addition TMP-SMX 160 mg daily seemed superior to 80 mg (42).

As uropathogenic bacteria have become more TMP-SMX resistant (66,67), prophylaxis with TMP-SMX may be less effective for the prevention of UTI in KT recipients. Most transplant centers utilize trimethoprim-sulfamethoxazole for prevention of *Pneumocystis* pneumonia; this may have an additional benefit of prevention of UTI in renal transplant recipients (68). For renal transplant recipients unable to receive trimethoprim-sulfamethoxazole, it remains unclear whether other antibiotics directed at prevention of urinary tract pathogens should be routinely used. Ciprofloxacin appears effective in the prevention of UTI in KT recipients (69). However, for patients who are allergic to trimethoprim–sulfamethoxazole, the recommended alternative agent would be nitrofurantoin in an effort to limit the emergence of fluoroquinolone resistance (70).

Specific Recommendations

(1) Presence of any obstructive and/or reflux uropathy needs to be corrected; remove or treat potential focus of infection in diseased end stage kidney(s) prior to transplantation (III) (25).
(2) Consider donor origin of UTI in the immediate postoperative period; recognition may require culture of preservative fluid for KTs (II 2) (25,63).
(3) In KT recipients, limit the duration of instrumentation, catheters and stents (64,71). Removal of ureteral stents within 4 weeks of renal transplantation is suggested (III) (33).
(4) Trimethoprim-sulphamethoxazole (TMP-SMX, cotrimoxazole 160 mg) antibiotic prophylaxis for 3–6 months significantly decreases asymptomatic bacteriuria and symptomatic UTI, and bacteremia in renal transplant recipients (I) (65,69,72). Antibiotic prophylaxis specifically for UTI is not recommended for nonkidney SOT recipients.
(5) Urine culture collection technique is important. After use of antiseptic wipes to clean the perineum/glans, a midstream urine sample is collected in a sterile container. In patients unable to perform these steps, straight catheterization to obtain a urine specimen can be considered. For patients with indwelling catheters (especially those in place >2 weeks) and a suspected urinary tract infection, the catheter should be removed and a specimen collected either via a midstream urine or newly placed urinary catheter (III) (19).
(6) Given the lack of evidence of efficacy and potential treatment of asymptomatic bacteriuria to select for antibiotic-resistant microorganisms, we recommend limiting routine screening for asymptomatic bacteriuria (collection of urine cultures with accompanying urinalyses with microscopy) to the first 1–3 months after renal transplantion; beyond this time period, screening for asymptomatic bacteriuria can be considered for patients with elevations in creatinine (III) (4,49).
(7) The duration of treatment for symptomatic UTI is 5–14 days, depending on the severity of illness. The efficacy of short course antibiotic therapy (single dose or 3 days) is unproven and not recommended in transplant recipients (III) (36,56,57).

Areas for Future Research

(1) Assess the true impact of UTI on patient and graft outcome using a standardized definition and classification both in the early and late post transplant period.
(2) Monitor outcomes of asymptomatic bacteriuria (with and without treatment) during first 3 months after transplantation in kidney and other SOT recipients.
(3) Evaluate the changing microbiological profile of UTI in transplant recipients with newer immunosuppressive agents.
(4) Determine the minimum necessary duration of indwelling urinary catheter and ureteral stent in renal transplant recipients.
(5) Re-assess the efficacy of antibiotic prophylaxis for prevention of UTI in renal transplant recipients in the era of rising antibiotic-resistance.
(6) Conduct randomized controlled trials (RCT) of treatment of asymptomatic bacteriuria occurring in the first 3 months after renal transplantation; conduct RCT of treatment of asymptomatic bacteriuria occurring more than 3 months after renal transplantation.
(7) Conduct studies to determine the optimal duration of treatment for symptomatic UTI, including whether there is a role for shortened courses of antimicrobials (e.g. 3-day regimens for mild cases) in SOT recipients.
(8) Investigate the pathophysiology of recurrent and relapsing UTI in renal transplant recipients and advance treatment strategies.

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Special Article

Travel Medicine and Transplant Tourism in Solid Organ Transplantation

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Key words: Solid organ transplant, travel medicine, vaccinations, vaccines

Abbreviations: BCG, Bacille Calmette-Guerin; DEET, N,N-diethyl-3-methylbenzamide; HAV, hepatitis A; HBV, hepatitis B; HIV, human immune deficiency virus; IPV/Salk, injected inactivated poliovirus vaccine; JE, Japanese encephalitis; MMR, measles, mumps and rubella; OPV/Sabin, orally administered, live, attenuated virus polio vaccine; PPD, purified protein derivative; SOT, solid organ transplant.

Introduction

The number of solid organ transplant (SOT) recipients continues to grow and as their overall health improves, they are increasingly traveling to areas with endemic and tropical infections. SOT recipients remain at increased risk of developing opportunistic and nonopportunistic infections, and are less likely to develop a robust response to vaccines against routine and travel related pathogens. We review the approach and evidence for prevention and treatment of travel related infections for adult and pediatric SOT recipients. We acknowledge that the quality of the evidence supporting the recommendations in SOT recipients is limited and mostly based on expert opinion (level III). We also acknowledge the increasing importance of the pretransplant evaluation during which future travel plans should be discussed.

Pretravel Evaluation, Timing and Travel Destination

SOT recipients who wish to travel should be seen by a travel medicine specialist familiar with their immunocompromised state and medications. Three recent surveys of transplant centers found significant rates of illness in transplant recipients during foreign travel, and insufficient rates of pretravel counseling and interventions. In one Canadian survey of 267 SOT recipients, 95 (36%) had recently traveled outside Canada and the United States, and while two-thirds of them sought pretravel advice from their transplant physician, many recommended preventative measures were overlooked. For example, 63% had traveled to areas where hepatitis A is endemic, but only 5% had received hepatitis A immunization; 50% traveled to dengue- and malaria-endemic areas, but only 25% adhered to mosquito prevention measures; and 10% reported behaviors that exposed them to blood or body fluids (1). A review at the Mayo Clinic, Rochester, Minnesota found that 303 (27%) of 1130 SOT recipients had traveled outside of the United States or Canada after their transplant; 16% to destinations at increased risk for infectious diseases. Travelers to these destinations were more likely to be men or born outside the United Stated or Canada (2). Liver recipients were more likely to travel than other SOT recipients and 96% of SOT travelers did not seek pretravel healthcare before their trip. Illness requiring medical attention occurred in 24 (8%) of the travelers and illness was significantly more likely among travelers to high-infection risk (18%) than low-risk (6%) destinations. In a Dutch study of 290 Dutch kidney transplant recipients, 34% had traveled outside Western Europe and Northern America; 22% of these travelers did not seek pretravel health advice and 29% were ill during their most recent journey, with 24% of ill travelers needing hospitalization for their illness (3). The Dutch SOT recipients tended to consult their transplant physician for pretravel advice (53%).

Given the well-recognized increased risk of infection during periods of greater immunosuppression (usually the first year after transplantation or during potent treatment of rejection), patients should be discouraged from traveling to higher infection risk areas (4,5). In addition, SOT recipients should carefully weigh the risks of travel to yellow fever endemic regions, locations with active outbreaks of disease and regions with limited health care, in the event they become ill and need medical attention. Cruise ships may provide lower infectious risk, although multiple cruises have been affected by large outbreaks of viral gastroenteritis. Those visiting friends and relatives may perceive such travel to be lower risk, although they should be aware of the augmented risk of food/water-borne illness (including typhoid fever), malaria, hepatitis A and others.

The pretravel visit should include information on vaccination and reducing the risk of nonvaccine preventable illness

Table 1: Recommended algorithm for travel visit, including travel planning, educational topics for travel medicine in immunocompromised hosts, and information on care abroad and evacuation insurance

Travel Planning: Defining and Moderating Risk
Timing after Transplant (>1 year for destinations with infection risk) (5)
Net State of Immunosuppression
Delay travel after treatment of rejection or increased immunosuppression
Location of travel
Specific infection(s); i.e. avoid yellow fever zones
Lack of adequate healthcare, medications, or experience in treating SOT patients
Type and length of travel
Backpacking vs. luxury travel vs. staying with relatives
Long stay trips
Educational topics to cover
Food and water precautions
Mosquito precautions
Blood/sex-borne infection precautions
Sun and altitude precautions
Traveler's diarrhea
Every patient travels with antibiotics and knows when to use them
Respiratory, skin, other infections (and give Rx's, renally adjusted, consider interactions)
Plan if sick in foreign country and medical evacuation insurance
Evacuation Insurance
U.S. Dept. of State Travel Health Evacuation http://travel.state.gov/travel/cis_pa_tw/cis/cis_1470.html
International SOS www.internationalsos.com
MEDEX www.medexassist.com
American Association of Retired Persons www.aarp.org
Resources to obtain overseas medical care
International Society of Travel Medicine www.istm.org
Intern. Assoc. for Med. Assistance to Travelers www.iamat.org
Joint Commission International www.jointcomissioninterantional.org
Travel Health Online www.tripprep.com
Review of routine and travel vaccine status (records, serologies)
Determine which vaccines should be administered
Malaria prophylaxis
Give Rx's (renally adjusted, consider interactions)

(Table 1). SOT recipients should bring a summary of their medical history, immunizations, and a written list of their current medications both to the travel clinic and on their travels. They should bring pills in the original containers and bring copies of prescriptions. Travelers should be aware of the risk of poor quality and counterfeit medications, and should try to bring full supplies with them. If needed, they should obtain medications from reputable sources, as much as possible. First aid kits can be assembled for the trip (Table 2). They should contact their health insurance plan to check coverage in their travel destination(s) and consider purchasing medical evacuation insurance (Table 1). Whenever possible, transplant recipients should obtain medical care at larger medical facilities with knowledge of transplant medicine and obtain a list of the closest transplant centers, specialists, dialysis centers, pharmacies, etc., before they travel (Table 1). Websites such as www.cdc.gov/travel and www.mdtravelhealth.com provide country-specific medical information.

Vaccine Preventable Illnesses

Vaccination for travel should be started several months before the trip, to allow time to reduce immunosuppression (if possible) and to immunize during periods of lowest levels of exogenous immunosuppression. Passive immunization with immune globulin should be considered for emergency travel situations. Since live attenuated vaccines continue to be contraindicated after SOT, it is important for these vaccines to be administered pretransplant whenever possible.

In certain circumstances, there may be advantages to evaluating serologic response to immunization and administering additional or booster doses of vaccines, should the response be inadequate, but the effectiveness of doing so in SOT recipients has not been studied, and this is rarely done in clinical practice. While a fourfold increase in titer is often considered evidence of seroconversion in normal hosts, SOT recipients are less likely to achieve this level of response, although they may still be partially protected. Immune responses to vaccination may wane more rapidly in SOT recipients, resulting in a need for more frequent booster vaccine administration.

Routine Vaccines for Adult SOT Recipients

All routine vaccines should be updated as needed before travel. Normal hosts often miss standard vaccines (6) and immunocompromised hosts are no exception (7). The recommendations for routine immunization of immunocompromised individuals are covered in "Guidelines for vaccination of SOT candidates and recipients" and are also available through the Centers for Disease Control and Prevention website (8), the Yellow Book (5) and other publications (9–12). Table 3 includes information on both routine and travel-related vaccinations for SOT recipients.

Tetanus, diphtheria and pertussis

Although tetanus is rare among travelers, all adults including SOT recipients should have a tetanus booster if they are not up to date before traveling. Diphtheria is common in resource-poor regions with 5–10% mortality among normal hosts, despite therapy. Patients vaccinated more than 10 years before travel should be revaccinated before entering an area in which diphtheria is endemic or resurgent. The incidence of pertussis has been increasing in the United States and worldwide over the last 20 years; as 90% of pertussis still occurs in developing countries, it is important to ensure that all travelers including SOT recipients are protected (8). A newer a cellular adult vaccine for pertussis is available, in combination with tetanus and diphtheria

Table 2: Considerations for First Aid Kit for Travelling SOT Recipients (adapted from wwwnc.cdc.gov/travel/yellowbook/2012/chapter-2-the-pretravel-consultation/travel-health-kits.htm)

Medications

- Destination-related, if applicable:
  - Antimalarial medications
  - Medication to prevent high-altitude illness
- Pain or fever (one or more of the following, or an alternative):
  - Acetaminophen
  - Aspirin
  - Ibuprofen
- Stomach upset or diarrhea:
  - Over-the-counter antidiarrheal medication (such as loperamide [Imodium])
  - Antibiotics for self-treatment of moderate to severe diarrhea - obtain before leaving home
  - Packets of oral rehydration salts for dehydration
  - Mild laxative
  - Antacid
- Throat and respiratory discomfort:
  - Antihistamine
  - Decongestant, alone or in combination with antihistamine
  - Cough suppressant or expectorant
  - Throat lozenges
- Anti-motion sickness medication
- Epinephrine auto-injector (such as an EpiPen), especially if history of severe allergic reaction; smaller-dose packages are available for children
- Any medications, prescription or over the counter, taken on a regular basis at home - recommend taking to cover more days than planned trip in case of delays

Basic First Aid

- Disposable gloves (≥2 pairs)
- Adhesive bandages, multiple sizes
- Gauze
- Adhesive tape
- Elastic bandage wrap for sprains and strains
- Antiseptic
- Cotton swabs
- Tweezers
- Scissors
- Antifungal and antibacterial ointments or creams
- 1% hydrocortisone cream
- Anti-itch gel or cream for insect bites and stings
- Aloe gel for sunburns
- Moleskin or molefoam for blisters - diabetic patients should be especially careful of foot injuries and should check their feet for early signs or irritation
- Digital thermometer
- Saline eye drops
- First aid quick reference card

Other Important Items

- Insect repellent (see the Protection against Mosquitoes, Ticks, and Other Insects and Arthropods <http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-2-the-pretravel-consultation/protection-against-mosquitoes-ticks-and-other-insects-and-arthropods.htm> section earlier in this chapter for recommended types)
- Sunscreen (≥15 SPF) given the risk of cancer in these patients - would recommend higher SPF than 15
- Antibacterial hand wipes or an alcohol-based hand cleaner, containing at least 60% alcohol
- Useful items in certain circumstances:
  - Extra pair of contact lenses, prescription glasses, or both, for people who wear corrective lenses
  - Mild sedative (such as zolpidem [Ambien]), other sleep aid, or antianxiety medication
  - Latex condoms
  - Water purification tablets
  - Commercial suture or syringe kits to be used by a local clinician. (These items will require a letter from the prescribing physician on letterhead stationery.)

(Tdap). Although administration of Tdap has not specifically been studied in SOT recipients, given the risk and consequences of developing pertussis in travelers, a single dose of Tdap should be given to adult travelers who have not recently received Tdap.

Influenza and streptococcus pneumoniae

SOT recipients should receive parenteral vaccination against influenza annually (unless they have a rare contraindication to vaccine administration; Refs.8,9). Influenza occurs year-round in the tropics; influenza immunization should be given to all SOT recipients who were not vaccinated within the past year, before such travel. Pneumococcal vaccine should be given to SOT recipients if it was not administered within the past 5 years.

Measles

Measles remains a risk for travelers, as there are millions of cases globally, with approximately 140 000 deaths annually. Measles vaccination (usually administered as the measles, mumps and rubella [MMR] live attenuated vaccine) is contraindicated in SOT recipients (5,13–15), because of the risk of major complications including encephalitis. Before travel to endemic areas, evidence of immunity against measles should be evaluated in all SOT recipients; those born before 1957, have evidence of two vaccinations, or a good history of clinical disease are likely protected. Others (especially those born in the late 1950s to 1970s) may be at risk for measles, and clinicians may wish to check serology (IgG). Immune globulin may be administered for short-term protection of nonimmune SOT recipients (5).

Varicella

Varicella is less common in childhood in the tropics, especially in rural areas. As with other live attenuated vaccines, varicella vaccine should not be administered to SOT recipients. Pretransplant immunization against varicella in seronegative individuals remains a priority. Options for SOT recipients who did not have evidence of immunity against varicella pretransplant with travel involving healthcare or other higher risk activity for varicella exposure include acyclovir and varicella immunoglobulin. Intramuscular pooled

Table 3: Recommendations for vaccination for solid organ transplant travelers (8,9,12)

Vaccine	Recommendations for adults	Recommendations for children
Routine		
Influenza-parenteral	Yearly	Yearly
Influenza-intranasal[1]	Contraindicated	Contraindicated
Pneumococcal polysaccharide and conjugated	Recommended; booster after five years	Administration at age 2 years
Tetanus/diphtheria	Recommended; booster every 10 years	Recommended per CDC guidelines
Pertussis	Recommended in combination with Tetanus and Diphtheria once	Recommended per CDC guidelines
Human papilloma virus	Recommended when indicated	Recommended per CDC guidelines
MMR[1]	Contraindicated	Contraindicated
Varicella[1]	Contraindicated	Contraindicated
Varicella zoster[1]	Contraindicated	Not applicable
Travel-related		
Hepatitis A	Recommended when indicated	Recommended per CDC guidelines, minimum age for first dose age 9 months
Hepatitis B	Recommended when indicated	Recommended per CDC guidelines, at birth
Meningococcal conjugate	Recommended when indicated	Recommended per CDC guidelines, minimum age for first dose 9 months
Inactivated polio (IPV)	Recommended when indicated	Recommended when indicated, minimum age 6 weeks
Rabies	Recommended when indicated	Recommended when indicated, any age
Japanese encephalitis	Recommended when indicated	Recommended when indicated; some vaccines not approved for pediatric use and pediatric vaccine not available in United States outside of clinical trials
Cholera vaccine	Recommended when indicated; not available in USA, available in Canada and elsewhere	Recommended when indicated; not available in USA, available in Canada and elsewhere; approved for use in Canada in 2003 for children 2 years of age and older
Typhim Vi	Recommended when indicated	Recommended when indicated for >6 years old
S. typhi Ty21a[1]	Contraindicated	Contraindicated
Oral polio (OPV)[1]	Contraindicated in patients/family members	Contraindicated in patients/family members
Bacille Calmette Guerin[1]	Contraindicated	Contraindicated
Yellow fever[1]	Contraindicated	Contraindicated

[1]Live, attenuated.
For quality of Evidence, please see "Guidelines for Vaccination of Solid Organ Transplant Candidates and Recipients."
Adapted from the Centers for Disease Control "Recommended Adult Immunization Schedule — United States, 2012" (8), "Advising Travelers with Specific Needs: The Immunocompromised Traveler" in Centers for Disease Control's "Health Information for International Travel" (5), and "Guidelines for vaccination of solid organ transplant candidates and recipients" (9).

immune globulin may convey some protection but has not been studied.

Hepatitis B

For those SOT recipients who were not vaccinated before transplant, immunization against hepatitis B before travel is indicated for travelers who will be living in endemic areas for extended periods, who are likely to need transfusions or medical procedures while traveling, or anticipate having new sexual partners. Immune response to the standard three doses of the hepatitis B vaccine post transplant is poor; some transplant clinicians use the high-dose vaccination scheme for dialysis patients, i.e. immunization with a vaccine containing 40 mcg of hepatitis B surface antigen (i.e. two 1 mL Engerix-B® vaccines, each containing 20 mcg, or a special formulation of Recombivax-HB®) given at one site, in a three- or four-dose schedule (8,16). Anti-HBs titers can be measured to assess vaccine efficacy. The need for booster doses is controversial in SOT recipients, although should be considered for high-risk travelers. Hepatitis B vaccination is usually given over a 6-month span; travel sometimes necessitates an accelerated series with variable efficacy, emphasizing the need for early pretravel evaluation.

Travel Vaccines for Adult SOT Travelers

Hepatitis A

The risk of hepatitis A in nonimmune travelers in resource-poor regions is estimated to be 1 in 1000 per week for those on a usual tourist route, and 1 in 200 for more remote travel (17). Hepatitis A can be a devastating illness in immunocompromised hosts.

Hepatitis A vaccine efficacy is suboptimal in SOT recipients. In a study of 37 hepatitis A seronegative liver transplant recipients given hepatitis A vaccine 6 months apart, only 8% had seroconverted 1 month following vaccination, and only 26% at 7 months (1 month after the second vaccination; Ref.18). In another study, zero of eight liver

transplant recipients responded to the two doses of vaccine given 2 months apart (19). In a third study, liver and renal transplant recipients (39 in each group) received two doses of hepatitis A vaccine 6 months apart (20); response after the primary dose occurred in 41% of the liver transplant patients and 24% of the renal transplant patients, while after the second dose, the respective conversion rates were 97% and 72%. Discrepancies between studies are likely due to differences in patient selection, severity of liver disease, immunosuppressive medications, and type of vaccine used. SOT recipients have a more rapid antibody decline than normal hosts: 2 years after vaccination, only 59% of liver transplant and 26% of renal transplant recipients who seroconverted retained protective titers (21), while mathematical models of vaccination in normal hosts predict antibodies to persist for 20–25 years (22). Use of higher or more doses of hepatitis A vaccine have not been studied in SOT recipients. Twinrix (combination HAV and HBV vaccine) can be considered for those needing both HAV and HBV vaccination. If there is enough time before travel, SOT recipient travelers should receive two doses of hepatitis A vaccine 6–12 months apart; titers can be checked to document seroconversion, although this is not common clinical practice. SOT recipients who do not have adequate time before travel or who are unlikely to respond to immunization (i.e. due to higher levels of immunosuppression) should be given intramuscular pooled immune globulin before travel as they provide 85–90% protection against hepatitis A infection (16). For up to 3 months of protection, a dose of 0.02 mL/kg is recommended; for more than 3 months of protection, a dose of 0.06 mL/kg is recommended, with the latter dose being repeated every 4–6 months for long-term travel. SOT recipients with hypogammaglobulinemia receiving routine immunoglobulin repletion with intravenous immunoglobulin do not need additional protection against hepatitis A (or measles or varicella).

Salmonella enterica serovar Typhi

An estimated 16–33 million cases of typhoid fever and 500 000–600 000 related deaths occur worldwide each year (23). SOT recipients are at significant risk of severe complications following infection with *Salmonella enterica* serovar Typhi. For this reason, immunization is indicated before travel to endemic areas. There are currently two vaccines commonly available: TyphimVi® (Aventis Pasteur SA), an injectable polysaccharide vaccine, and Vivotif® (Ty21a, Berna) an oral live, attenuated vaccine. The live oral typhoid vaccine is contraindicated in SOT recipients. SOT recipients should receive TyphimVi®. Travelers should be counseled that the immune response to this vaccine may be suboptimal. Travelers should be also counseled about food and water precautions.

Polio

Poliomyelitis caused by wild-type poliovirus has been eradicated from the Western hemisphere; wild type virus exists in regions of sub-Saharan Africa and South Asia. Outbreaks of vaccine-associated poliomyelitis occasionally occur, due to neurovirulent reversion of live attenuated poliovirus from the oral polio vaccine. Vaccine-associated outbreaks of poliomyelitis have recently occurred in Haiti and the Dominican Republic, the Philippines, Madagascar, and Cape Verde. Worldwide, two forms of the polio vaccine are available: the orally administered, live, attenuated virus (OPV/Sabin), and the injected inactivated poliovirus vaccine (IPV/Salk). Since attenuated vaccine strain polioviruses may spread through fecal-oral contact, immunocompromised hosts and household contacts of immunocompromised individuals should not receive OPV. OPV is no longer available in the United States and Canada. Adult SOT travelers should have received a primary series of polio vaccine during childhood, and should be given a parenteral booster when traveling to endemic areas. For subsequent travel, administration of additional booster doses is controversial because durability of antibody responses in SOT recipients has not been extensively studied, but booster immunization may be prudent for individuals traveling to locations with circulating wild-type polioviruses or polio outbreaks.

Meningococcus

Meningococcal disease has high case-fatality rates (5–15%). The meningococcal conjugate vaccine is recommended for travelers to endemic areas, such as the meningitis belt of sub-Saharan Africa (especially during the dry winter months of December–June), and for those traveling to Saudi Arabia for the Muslim pilgrimages of *hajj* or *umra*, where proof of vaccination is required (16). Periodic revaccination in SOT recipients may be indicated as immunity wanes.

Yellow fever

Yellow fever, a mosquito-borne viral hemorrhagic fever with a high case-fatality rate, occurs in tropical regions of South America and sub-Saharan Africa (http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/yellow-fever.htm) and kills an estimated 30 000 people every year. Case fatality may surpass 20%; no specific treatment exists. Whenever possible, SOT recipients should avoid travel to endemic regions entirely, or at least avoid peak season (i.e. January-March in Brazil, and July-October in rural West Africa; Ref.16).

The yellow fever vaccine contains a live attenuated viral strain and is distributed only through Department of Public Health-certified vaccination centers, including travel clinics and some county health departments. A listing of approved centers is available from local Departments of Public Health and the U.S. CDC (wwwnc.cdc.gov/travel/yellow-fever-vaccination-clinics/search.htm). As a live virus vaccine, it should not be given to SOT recipients (5,12,14,24–27). When vaccination is not given, a yellow fever vaccine waiver letter stating the contraindication to

vaccination is acceptable to most governments; such letters should bear the stamp of an official, approved yellow fever immunization center. While some less immunosuppressed travelers have tolerated the vaccine (including individuals with HIV infection (28–30), with rheumatologic disease (31) or on infliximab (32), a distant history of hematological malignancy not currently being treated with immunosuppressive agents) (33,34), including a small cohort of SOT recipients living in an endemic region (Brazil) (35), complications including death have been reported in immunosuppressed individuals who received the vaccine (36) and recommendations avoid its use in immunocompromised travelers (5). Further country-specific information is available from the Centers for Disease Control Yellow Book (16). Family members of immunosuppressed persons may receive yellow fever vaccine.

Rabies

Many travelers are at an increased risk of exposure to rabid animals while traveling. Only long-term travelers, individuals expecting intense animal exposure, and individuals who plan to be far from medical care should be considered candidates for preexposure vaccination against rabies. All travelers with a potential rabies exposure should receive postexposure prophylaxis, starting with immediate cleansing of the wound with soap and water. Those who have not previously been immunized receive multiple doses of intramuscular vaccine, plus rabies immune globulin (HRIG) (20 units/kg), half at the site and half intramuscular. Those who have received preexposure prophylaxis receive two more doses on days 0 and 3 and no HRIG. Since SOT recipients may not mount adequate antibody responses to the rabies vaccine (titers >0.5 IU/mL are considered adequate), however, some recommend administration of HRIG after all at-risk exposures (37).

Japanese encephalitis

Japanese encephalitis (JE), an arboviral infection transmitted by mosquitoes, may cause up to 10 000 deaths annually in Asia. Immunization against JE should be considered for individuals with intense rural travel in areas of Asia endemic for JE, especially during periods of increased transmission (varies by location) (16,38). Although JE is rare, the risk of serious neurologic sequelae is high. JE vaccine (Ixiaro®), approved in March 2009, is a killed viral vaccine; efficacy of the JE vaccine is unknown in SOT recipients, although its use would be recommended when indicated by travel plans. It is administered as 2 doses on days 0 and 28. This new vaccine is generally better tolerated and causes less clinical side effects than the prior vaccines, which are no longer available in the United States. If the primary series of Ixiaro® was administered ≥ 1 year previously, a booster dose should be given before potential re-exposure or if there is a continued risk for JEV infection (16). Travelers should be aware that some JE vaccines in Asia contain live virus; they should not receive such vaccines.

Bacille Calmette-Guerin and tuberculosis

Bacille Calmette-Guerin (BCG) is one of the most commonly administered vaccines in the world. It is a live, attenuated strain of *M. bovis*, and is used to prevent tuberculosis, especially in infants and children. BCG is rarely given in the travel medicine setting, and is contraindicated in SOT recipients because they can develop disseminated BCG. There are no specific approaches to prophylaxis other than wearing appropriate masks in health care settings in endemic regions. Pre- and post-travel tuberculosis skin tests with the purified protein derivative (PPD) or interferon-gamma release assays (T-SPOT.TB, Quantiferon Gold TB) may be helpful to assess whether a traveler has been exposed to tuberculosis, but SOT recipients are at risk of false negative tests.

Travel Vaccines for Pediatric SOT Travelers

For pediatric SOT recipients who are traveling, it is critical that their routine immunizations are up to date. Table 3 shows the minimum age for pediatric immunizations for travel related vaccines. Parents/guardians should be counseled against traveling to high-risk areas for pediatric patients before their child being on minimal immunosuppression and able to complete appropriate immunization schedules. Pediatric SOT recipients may not be protected against measles and varicella, as discussed above; immune globulin may provide coverage for unprotected travelers (and acyclovir for varicella). Pediatric SOT travelers should avoid yellow fever zones, as per above, or travel with waiver letters.

Vaccination of Close Contacts of SOT Recipients

Close contacts of immunocompromised hosts could transmit live, attenuated vaccine strains to the immunocompromised host. Certain live viral vaccines (including oral polio and smallpox vaccines) should be avoided by close contacts of SOT recipients. Administration of other live vaccines such as measles, mumps, rubella, yellow fever, oral Salmonella, varicella (Varivax®; Ref.39) and zoster (Zostavax®) vaccines are less likely to be transmitted, and their use is generally recommended in household contacts of immunocompromised hosts (16,40). Nasal influenza vaccine can be given to household member when injectable vaccine is not available. SOT recipients should be aware of the risk of exposure to oral polio vaccine via fecal-oral contact when abroad; a parenteral polio booster vaccine may help prevent subsequent illness.

Nonvaccine Preventable Illnesses

For optimal protection, education may be even more important than vaccination (Table 1).

Diarrhea

Diarrhea is the most common illness of travelers, affecting 10–60% of travelers to low resource countries. Travelers' diarrhea may be life threatening to SOT recipient travelers because dehydration may compromise renal function, particularly for those on tacrolimus and especially with *Cryptosporidium* (41). Complications of diarrhea include bacteremia, metastatic seeding, altered intestinal absorption and metabolism of immunosuppressive medications.

Before international travel, SOT recipients should be instructed in appropriate food and water precautions (16). SOT recipients should be instructed to drink only boiled or bottled water and beverages not containing local water or ice. Similarly they should avoid unpasteurized dairy products, food sold by street vendors and raw or undercooked foods (except fruit and vegetables that they can peel). Travelers should be counseled on the importance of fluid replacement (ideally with clean water and oral rehydration solution that is widely available in pharmacies throughout the world) if they develop diarrhea. All travelers should carry appropriate antibiotics (e.g. ciprofloxacin or azithromycin depending on the local resistance patterns of *Campylobacter* and *Salmonella* spp.) for self-treatment of diarrhea (not for prophylaxis). The threshold to start antibiotic therapy is usually more than three unformed stools in 24 h, fever, blood, pus or mucus in the stool. If fever, vomiting, and/or bloody stools accompany diarrhea, the SOT recipient should seek medical attention, as soon as possible. Trimethoprim/sulfamethoxazole is usually ineffective against travelers' diarrhea due to antibiotic resistance, and prophylaxis with rifaximin could result in drug interactions. Antimotility agents should be used with caution in SOT recipients with diarrhea, because they may delay clearance of toxins from the gut. In the gastrointestinal tract, bismuth subsalicylate (i.e. Pepto-Bismol) is converted to salicylic acid and insoluble bismuth salts; SOT recipients with decreased renal function are at risk of developing salicylate toxicity, and this medication should be avoided. Drug doses and interactions are highlighted in Tables 4 and 5.

Respiratory infections

Respiratory infections are the second most common infection affecting travelers (17). Although many infections are viral, endemic fungal pulmonary infections, such as histoplasmosis and coccidioidomycosis in North America, and penicilliosis due to *Penicillium marneffei* infection in Southeast Asia, can be acquired during travel (42–44). SOT recipients are at higher risk for invasive fungal infection, and should avoid activities such as spelunking and excavating, activities that have been associated with exposure to *Cryptococcus neoformans* or endemic fungi. Masks may be helpful in preventing these infections.

Insert-born illnesses: dengue and malaria

Malaria and dengue fever are the most common arthropod-borne illnesses of travelers. Travelers to endemic areas should be counseled about minimizing insect bites by use of repellents containing DEET (N, N-diethyl-3-methylbenzamide) or picaridin, bed nets, well-screened rooms or air conditioning, protective clothing, and permethrin-impregnated clothing (16). Such protection will also reduce other insect borne illnesses (e.g. Chikungunya). Most cases of dengue fever are self-limited in the normal host, but a recent case series of eight renal transplant recipients with dengue infection included three who developed dengue hemorrhagic shock syndrome and died (45). Up-to-date, country-specific information on dengue can be found on the dengue map (www.healthmap.org/dengue/index.php). Areas of outbreak should be avoided.

There is no evidence that malaria is more common or severe in SOT recipients (46). For prophylaxis against malaria, consult the CDC Yellow Book, which provides country-specific guidelines (16). Table 5 shows antimalarial interactions with calcineurin inhibitors and/or trimethoprim-sulfamethoxazole. In *Leishmania* endemic regions, transmission to SOT recipients has occurred (47), and precautions should be taken against sand fly bites.

Skin cancer

Transplant recipients have an augmented risk of skin cancer due to the immunosuppression medications, which is increased by intense sun exposure. Use of hats, sunglasses, protective clothing (also useful for arthropod-borne infections), and sun protection lotions with ultraviolet A and B protection are recommended.

High altitude and water exposure

Travelers who rapidly ascend to high altitudes are at risk for altitude sickness. Travelers should be advised to avoid vigorous activities for the first few days at altitude. A recent study found that muscular power and anaerobic performances during alpine skiing among a selected group of SOT recipients were similar to those of the general untrained population (48), suggesting that transplant recipients may not be at higher risk for complications at altitude. While acetazolamide accelerates acclimatization and decreases the risk of altitude sickness (49), its use in SOT recipients is unstudied. Acetazolamide should be offered to those travelers ascending rapidly above 2500 m since there is at least a 15–25% risk of altitude sickness. Travelers to regions where *Schistosoma* species are endemic should avoid swimming or wading in fresh water. Those with liver disease should be aware of the risk of *Vibrio* exposure in salt waters (50).

Bloodborne and sexually transmitted pathogens

Travelers should avoid contact with nonsterile needles, syringes and other medical equipment, and before travel, they or the transplant center should investigate whether transfusions of blood products are likely to be safe. Travelers are more likely to be sexually active with new partners,

Table 4: Travel-related medications, adult dosages and duration

	Dose	Duration	Change for reduced GFR	Change for hepatic dysfunction
Diarrhea treatment				
Ciprofloxacin	500 mg bid	For 3–7 days	Yes	No
Levofloxacin	500 mg qD	For 3–7 days	Yes	No
Azithromycin	500 mg qD	For 3–7 days	Use caution if Crcl<10 mL/min	No
Malaria prophylaxis				
Atovaquone-proguanil	Atovaquone/ proguanil 250 mg/100 mg daily	Start 1–2 days before entering a malaria-endemic area, continue throughout the stay and for 7 days after leaving malarial area	Not for mild to moderate renal impairment; avoid if severe renal impairment (Crcl <30 mL/min)	No dosage adjustment required in mild to moderate hepatic impairment. No data for use in severe hepatic impairment
Mefloquine	250 mg weekly	Begin 2 weeks before, arrival in endemic area, continuing weekly during travel and for 4 weeks after leaving endemic area	No	Half-life may be prolonged and plasma levels may be higher
Doxycycline	100 mg daily	Start 1–2 days before entering a malaria-endemic area, continue throughout the stay and for 28 days after leaving malarial area	No	No
Chloroquine	500 mg/week (300 mg base) weekly	Begin 1–2 weeks before exposure; continue for 4 weeks after leaving endemic area	Administer 50% of dose if CrCl <10 mL/min	No dosage adjustment required
Altitude illness prophylaxis				
Acetazolamide	125–250 mg bid	Begin 2 days before ascending and continue until 2 days after completing ascent	Yes	No

Table 5: Interactions between transplant and travel-related medications

	Calcineurin Trimethoprim/inhibitors (CNI)	Sirolimus	Sulfamethoxazole
Azithromycin	May ↑CNI levels		
Atovaquone			May increase risk of proguanil of bone marrow toxicity
Ciprofloxacin (or levofloxacin)	May cause QT prolongation when used in combination		
Doxycycline	May ↑CNI levels	May ↑ sirolimus levels	
Chloroquine	May ↑CNI levels		May increase risk of cardiac toxicity, QT prolongation, torsades de pointes or cardiac arrest
Mefloquine	May ↑CNI levels	May ↑ sirolimus levels	May increase risk of cardiac toxicity, QT prolongation, torsades de pointes or cardiac arrest
Primaquine	May ↑CNI levels		
Sulfadoxine/pyrimethamine	May ↓CNI levels		May increase risk of bone marrow toxicity
Acetazolamide	May ↑CNI levels		

Significant interactions of travel medicines and azathioprine, mycophenolate mofetil, and corticosteroids have not been reported; significant interactions of transplant medicines and diphenoxylate hydrochloride and atropine sulfate tablets or loperamide have not been reported; minimal data available.
Adapted from MicroMedex® DrugReax® Interactive Drug Interactions and Lexi-Comp Online™ Interaction Analysis.

acquire tattoos, and participate in other risky behaviors while traveling, and should be counseled on these topics.

Posttravel Evaluation

SOT recipients should be counseled to contact their transplant center should fever, diarrhea, rash or any new symptoms develop during or following their return from an international trip. In general, travelers who are well on return do not need to be seen after foreign travel, unless they have been away for prolonged periods or may have had with potential exposure to blood-borne pathogens or other high-risk situations.

Transplant Tourism

"Transplant tourism" (involving travel of either the organ donor or recipient strictly for purposes of organ transplantation), conveys significant infectious disease risks. A review of U.S. national waiting list data identified 373 patients who had transplants overseas in recipients who had previously been listed in the United States; most (89%) received kidney transplants, and were more likely to be male, Asian, either permanent residents or non US residents and college educated. The transplants occurred in 35 countries, led by China, the Philippines and India (51). Numerous international transplant organizations, including The Transplantation Society and The International Society of Nephrology, have made major efforts to decrease the purchase and sale of organs on ethical grounds (52).

Given their commercial and sometimes illegal nature, many of these organ transplants are not recorded in databases, so the incidence of infection in donors or recipients is unknown. The extent and quality of the pretransplant evaluation of the donor and recipient is likely to be quite variable. Communication and documentation from the transplant center may be limited, and prophylaxis against infection may be less than standard (53). Foreign-born transplant recipients may return to their country of origin for organ transplants (51,54), and are at risk both for reactivation of latent infections as well as acquisition of new indigenous infections; knowledge of the specific infections existing in those regions, as outlined in a recent review (55), can direct further evaluation.

There is a similar theme in the studies and case reports of transplantation overseas. One review of recipients who had undergone commercial transplants in foreign countries found that 4–6% had new, transplant-related human immune deficiency virus (HIV) infection and 2–12% had new hepatitis B infections (53). One study compared 540 patients who had received commercial renal transplants in India between 1978 and 1993 with 75 recipients of living nonrelated donors of renal transplants performed at two participating institutions in the Middle East. Although graft survival was similar, the commercial transplant recipients had a higher incidence of HIV infection (5% vs. 0%), and hepatitis B virus infection rate (8% vs. 1%; Ref.56). A case report from England documents *de novo* hepatitis B infection after a renal transplant in India, with subsequent infection of four patients in England (57). A series from Turkey compared 115 patients who had undergone commercial transplants in various countries (India, Iraq and Iran) with those with a living related transplant performed at their center (58); the commercially transplanted recipients had infections caused by malaria (10 cases), invasive fungal infections and tuberculosis (five cases each) and pneumonia. A review of 10 patients who underwent evaluation for transplant in Minnesota and subsequently had transplants abroad found that complications were primarily infectious, with six potentially life-threatening infections in four patients, including severe wound infection, *Acinetobacter* bacteremia/sepsis, central nervous system *Aspergillus* infection, severe urosepsis in 2, and CMV infection (59). Almost all of these and other studies (60–62) report reduced graft and patient survival, higher infectious complications and higher rates of rejection.

When these transplant recipients return to transplant centers and other clinicians in their usual area of residence, it is prudent to re-screen them for blood-borne pathogens, including HIV, HBV and HCV (with molecular diagnostics rather than serology) even if they are asymptomatic. Recipients with illnesses should be evaluated for bacteremia, urinary tract infections and other endemic pathogens depending on the location where they received the transplant and their clinical course (malaria, tuberculosis, Chagas disease, etc.). Optimizing their post-transplant prophylaxis against infection and obtaining further information about their surgical procedure(s) and immunosuppression may also help optimize their care.

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Special Article

Ventricular Assist Device Related Infections and Solid Organ Transplantation

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Key words: Bacterial infection, fungal infection, infectious complications, ventricular assist devices

Abbreviations: BTT, bridge to transplantation; DT, destination therapy; MRSA, methicillin resistant *Staphylococcus aureus*; VAC, vacuum assisted closure; VAD, ventricular assist device.

Introduction

A ventricular assist device (VAD) is a mechanical pump that augments the heart's ability to provide appropriate blood flow. VAD therapy is well established for the management of patients with refractory heart failure. Compared to medical therapy alone it improves survival, functional status and quality of life (1–4). VADs may be used to support either the right ventricle (RVAD) or the left ventricle (LVAD), or both; the vast majority today is LVAD.

Initial VADs were pulsatile pumps, intended to mimic the natural function of the heart. Continuous flow pumps, introduced in 2004, have showed improved survival and are now used almost exclusively (2,5). One type of continuous flow VAD (Heartmate II, Thoratec) that is now commonly used is represented in Figure 1. Because the advent of continuous flow pumps, the average time that patients live on VAD support has increased from 126 to 317 days and survival is now >80% at 1 year and >70% at 2 years (1,2,5,6). Seventy-three percent of recipients are bridge to transplant (BTT) at the time of VAD implantation and 25% receive theirs as "destination" therapy. However, far fewer of those identified as BTT go on to receive a heart transplant (5).

Epidemiology and Risk Factors

Infection occurs in up to 60% of VAD recipients (7,8). VAD-specific infections may involve the percutaneous driveline, the pump pocket, and the pump and/or cannula, and coexistent infection at multiple sites is common. Bacteremia or fungemia may be seen in these patients, and may be the result of a VAD-specific infection. The percutaneous driveline is quite susceptible to infection at the exit site, particularly when the skin seal is lost. The entire device, but especially the driveline, is susceptible to biofilm formation from infecting organisms and, thus, infection is nearly impossible to completely eradicate without device removal (6,9,10). Organisms that are commonly involved in the various sites are listed in Table 1.

Infection rates and the distribution of types of infections have changed since 2004 when the continuous flow pumps were first made available for use (13). Although infection accounted for 25% of deaths in the early years of device support (mostly due to early nosocomial sepsis events and bloodstream infections), more recent data show infectious complications accounting for only 7.7% of deaths, indicating that infection can now be managed quite effectively (4,15–17). Sepsis events and non-VAD infections still account for the majority of infections occurring within 30 days of implantation. Non-VAD infections predominate from 31 to 90 days postimplantation. Driveline and pump pocket infections predominate in the period beyond 90 days and likely account for the rise in incidence of bacteremic events during this late period (Figure 2; Refs.13,17).

Although the changes in timing and type of infection were largely attributed to differences between the pumps themselves (continuous flow pumps are smaller and their drivelines are of smaller caliber), data indicate that clinical experience with patient selection and management strategies are more closely associated with reduced infection rates overall (13). Additional factors associated with overall infection risk include patient co-morbidities, such as older age, diabetes, renal failure and nutritional status (12).

Risk factors for specific infections vary widely. Associated risk for early severe sepsis after VAD implantation includes older patient age and heart failure risk scores (13). Risk for driveline infection and other VAD specific infections include driveline trauma and time on the device (6,18). Risk for fungal infection includes use of parenteral nutrition (14).

Cellular and humoral immune dysfunction has been described after VAD implantation (19–22). However, many of these observations were made in the pulsatile pump era and may require further study in current populations. Currently, there are no recommendations for specific

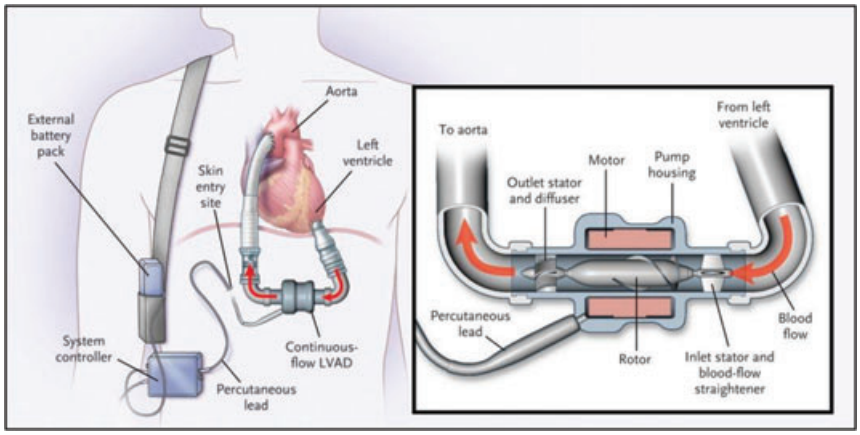

Figure 1: Components of the Heartmate II (Thoratec) continuous flow LVAD. Reproduced with permission from Ref. (1). Copyright 2007 Massachusetts Medical Society. All rights reserved.

Table 1: VAD-Specific Infections (Refs. 6,11–14)

Site of infection	Distribution of organisms
Driveline	*Staphylococcus aureus* 30–44%
	Pseudomonas aeruginosa 10–28%
	Enteric gram-negative bacteria 13–30%
	Coagulase negative staphylococci 7–20%
	Enterococcus spp 5–15%
	Corynebacterium spp 2–15%
	Candida spp 0–8%
Pocket	Coagulase negative staphylococci 15–40%
	S. aureus 20–30%
	Enterococcus spp 20–24%
	Enteric gram-negative bacteria 5–25%
	P. aeruginosa 5–19%
	Candida spp 10%
Pump/cannula	Coagulase negative staphylococci 20–40%
	S. aureus 20%
	P. aeruginosa 8–20%
	Corynebacterium spp 8–20%
	Enteric gram-negative bacteria 0–15%
	Enterococcus spp 0–30%

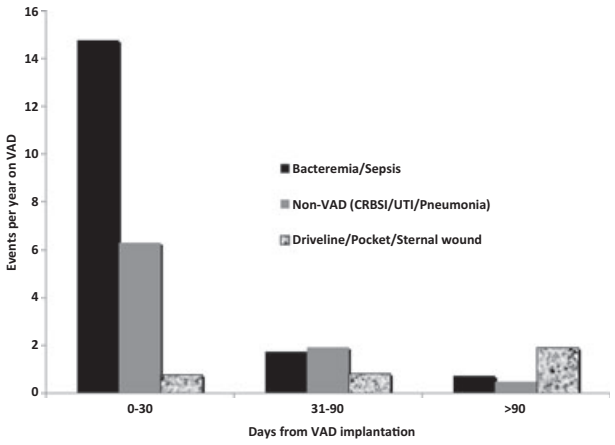

Figure 2: Timing and type of infection with continuous flow VAD. Adapted from Schaeffer et al. (13). CRBSI, catheter related blood stream infection; UTI, urinary tract infection.

monitoring tools for routine assessment of cellular and humoral immune status after VAD implantation.

Patients with controlled HIV infection have successfully survived on VAD as BTT and DT and there does not seem to be an increased risk for infection in this population, though reported case series are small (23).

There are no randomized controlled trials related to VAD infections, and thus recommendations are based on observational studies and expert opinion. It is generally recommended that composite risk for overall patient outcome be assessed on a case by case basis before VAD implantation with particular attention to recognized risk factors related to early severe sepsis, namely older patient age and heart failure risk score.

Diagnosis

Althoguh infection-related complications of VAD therapy have been described for many years, standardized definitions for VAD infections were developed only recently (24–26). Such definitions are intended to create consistency in language of reporting of infections and to guide diagnostic criteria in clinical practice. However, these definitions have not yet been validated in clinical studies.

In general, patients with VADs may not present with classic signs of infection. If infection is suspected, a diagnostic approach is recommended that captures VAD-specific, VAD-related and non-VAD infections (24). All patients should have a white blood cell count, basic chemistries, a chest X-ray, at least two sets of initial blood cultures, urinalysis and urine culture. If a central venous catheter or PICC line is present, a culture from that line should be obtained coincident with the peripheral blood culture.

Driveline infections

Driveline infections are most commonly superficial but may evolve into or present coincidently with deep infection that involves the fascial and muscle layers. Diagnosis

of both types of infection can be problematic. The driveline should be inspected visually and palpated along the driveline tract away from the cutaneous exit site. Although at times the driveline seems clearly infected, with purulent discharge and/or surrounding cellulitis, often infection can be more difficult to recognize, with just wound dehiscence or serous discharge. Conversely, surrounding skin erythema may be due to other factors, such as trauma from the driveline or reactions to topical agents, in the absence of infection. Even with infection, systemic signs, such as fever, leukocytosis or elevated inflammatory markers, may not be present (6).

If purulent drainage is present, a sterile aspirate from the exit site should be obtained for bacterial and fungal culture. If an aspirate is not feasible due to low quantity of material, a sterile swab may be used.

Imaging with ultrasound or computed tomography (CT) are recommended to assess for deep infection by identifying collections along the driveline tract, though artifact from the pump hardware may limit the utility of CT. Leukocyte SPECT/CT imaging may be more sensitive than CT at detecting anatomic location and extent of infection along the driveline cable and near the pump pocket (27). However, this testing may not be uniformly available.

Pocket infections

VAD pocket infections may arise from direct extension due to primary driveline infection or may develop due to inoculation at the time of surgery or thereafter, in a manner similar to that seen with other implanted devices such as pacemakers. Although infections may develop slowly, systemic signs often emerge. Coincident bloodstream infection may emerge as well and may indicate the involved pathogen.

Imaging with ultrasound or CT may be helpful in suggesting the diagnosis. Again, leukocyte SPECT/CT imaging may be more sensitive than CT at identifying a deeper infection (27). Ultrasound or CT guided aspiration of fluid for gram stain and culture is recommended particularly if an organism has not yet been identified.

Cannula/pump infections

Infection of the internal portions of the pump or cannula (VAD endocarditis) presents in a manner similar to prosthetic valve endocarditis, frequently with persistent bacteremia and fever. In addition, this may be associated with internal VAD thrombosis, obstruction, and dysfunction.

Blood cultures are imperative for diagnosis. At least two but preferably four or more cultures may be required before initiation of empiric antibiotics to properly identify infecting pathogenic bacteria. Echocardiography, particularly transesophageal echocardiography, is recommended to identify vegetations or turbulent flow through the device, abscess and/or cannula dehiscence. Other imaging modalities, specifically CT or SPECT/CT may define inflammatory changes around the cannula. Clinical identification of classic vascular and immunologic phenomena of endocarditis may enhance the diagnostic yield in certain settings.

Recommendations:

(1) Have a heightened suspicion for infection in patients with a VAD, as classic symptoms and signs of infection may be absent (II-3).
(2) Imaging, including ultrasound, CT, leukocyte SPECT/CT, or echocardiography, may be helpful in identifying infected areas (II-3).
(3) Culture any potentially infected material evident on exam or imaging to guide antimicrobial therapy (II-3).

Management of Infection

Management strategies are directed in part by the site and severity of infection. However there is often substantial overlap. Driveline infections may be associated with pump pocket infection, and bacteremic spread from these sites can result in cannula/pump infection. For patients who present with sepsis, broad-spectrum empiric therapy is advised, including activity against both gram-positive and gram-negative organisms pending further investigation. Depending on patient-specific risk, an antifungal agent might also be included. As with other device-associated infections, all infected VAD components should ideally be removed for cure of the infection, as would occur with transplantation (or with VAD explant in the setting of recovery of cardiac function). However, donor hearts are often not immediately available. And although surgical VAD exchange is an option, this carries a significant operative risk, and relapse of infection may still occur (28).

Several reports have indicated that active VAD infection is not a contraindication to transplant. However, the timing of transplantation is important. Septic shock would be an obvious contraindication to transplant. However, transplantation in the setting of reasonably controlled infection is often lifesaving. For further discussion of management strategies, these VAD-specific infections are listed separately.

Driveline infection

For driveline infections, after appropriate cultures are obtained empiric antibiotic therapy is typically initiated. A gram stain from the exit site may help guide initial antibiotic choice. The choice of agent(s) should be based on the local institution's pattern of infecting organisms and antimicrobial resistance, along with the patient's prior history of infections and antibiotic therapy. Treatment can typically be narrowed once the pathogen-specific antimicrobial susceptibilities are known.

If localized abscesses associated with driveline infection are found by exam or imaging, percutaneous or surgical drainage is recommended. Vacuum-assisted closure (VAC)

treatment of sizable resultant wounds may promote healing and reduce time to complete closure (10,29).

For superficial driveline exit site infections, a short course of antibiotics until the area has healed is reasonable. Once infected, the driveline is rarely (if ever) infection free and recurrent treatment courses are often required. Due to the possibility of progression of superficial infection to deep, some advocate continuous antibiotics until transplantation (10,11). However, an approach that weighs the risk of prolonged antibiotics with the risk for infection progression is warranted. This is particularly problematic in patients with VAD as DT (30).

Pump pocket infection

For suspected pump pocket infection intravenous antibiotics are warranted initially and drainage is often required. Occasionally this may necessitate surgical revision, with intraperitoneal relocation of the pump and use of an omental flap (28,31,32). Even with surgical revision, chronic suppressive antibiotic therapy is typically used, initially with intravenous followed by oral administration, if an oral option is available (28,31). Complete eradication of the infection is unlikely unless the VAD can be explanted in the setting of cardiac recovery or transplantation.

VAD cannula/pump infection

VAD cannula/pump infections (referred to by some as "VAD endocarditis") are the least common but amongst the most serious of VAD specific infections. Infection along the cannula or within the pump can lead to dehiscence of the pump anastamoses, pump failure due to obstruction of blood flow and septic embolic phenomena, including mycotic aneurysms. Control of infection, initially with intravenous antibiotics with possible transition to oral therapy for chronic suppression, until VAD exchange or transplantation is recommended. Specific antibiotics should be tailored to the organism involved, as one might for a prosthetic heart valve or other infected endovascular device. Specific additions like synergistic aminoglycosides or rifampin have not specifically been studied in this context, but have been reported as beneficial (in relapse of *S. aureus* bacteremia) and may be considered in the appropriate setting (11). Attention to drug interactions between rifampin and other drugs metabolized through CYP-3A4 and CYP-2C9 (particularly warfarin) is required.

Length of treatment

One report found that for a group with VAD-related infections (mixed local and bloodstream infections) use of continuous antibiotic therapy through the time of transplant was superior to limited courses of antibiotics, with fewer relapses and shorter time to transplant (11). In this report, infections with *S. aureus* were more likely to relapse with shorter courses of antibiotics. For more invasive infections, including pump pocket, VAD endocarditis, and VAD-related bacteremia, continuous antibiotics are recommended through the time of VAD removal. However, despite continuous antibiotic suppressive therapy, both local and disseminated breakthrough infections may occur, most likely due to inadequate source control.

Outcomes

In general, several studies have shown that VAD infection does not significantly affect survival to transplantation or survival after transplant (11,18,33–36). However, with certain subgroups, such as sepsis post-VAD implantation (typically occurring early after surgery), VAD endocarditis, or bloodstream infection (which may or may not be VAD-related) mortality is higher and overall survival is impacted (12,16,32). Although fungal VAD infection, typically due to *Candida* spp, is less common, mortality is quite high, though this has not been found universally (14,16,37). Non-*Candida* fungal infections, particularly with *Aspergillus* species have been reported and are often fatal (35,38,39). Infection with such organisms would be a strong but relative contraindication to transplantation, as it would in the setting of mold infections for any patient being assessed for transplant.

Pretransplant evaluation

For BTT VAD patients, routine and specific vaccinations should be updated (Vaccination chapter of Guidelines, 2013). Pretransplant exposure risks should be ascertained and appropriate screening performed (Screening chapter of Guidelines, 2013). Routine VAD care must continue. Perioperative antibiotic therapy around the time of transplant may be altered from a standard regimen based on known new infections or colonization (40,41).

Posttransplant management

Intra-operative cultures should be obtained from any suspected infected site, including the mediastinum and the interior and exterior surfaces of the VAD (24). Pus, along with tissue samples from suspicious tissue surrounding the VAD, driveline or anastomoses, should be sent for gram stain, fungal stain, bacterial and fungal cultures, and tissue samples should also be sent for histopathology (24). Antibiotic therapy may be modified based on culture results. For patients who have been maintained on suppressive antibiotics for VAD infection, antibiotic therapy should be continued posttransplant, with length of therapy dependent on severity of infection. Mild infections may only require 1 week or less of ongoing antibiotics. For patients with more severe infections, such as VAD-related bacteremia or endocarditis, antibiotic therapy should be continued for at least 2 weeks posttransplant, and often longer.

Recommendations:

(1) For VAD related bloodstream infections, especially VAD endocarditis, and pump pocket infection, antibiotic therapy should be continued through the time of transplantation or device removal (II-2). For those patients with

VAD as destination therapy, a treatment course with intravenous therapy followed by the best-tolerated suppressive antibiotic regimen given indefinitely is recommended (III).
(2) For superficial driveline infection, short courses of antibiotics may suffice (II-3). However, the specific infecting organism, degree of local inflammation and expected time to transplantation (if patient bridged to transplant) should affect the treatment duration (III).
(3) In general, VAD infection is not a contraindication to transplant, with certain exceptions such as septic shock or mold infection (II-2).
(4) Antimicrobials should be continued posttransplant, with the length of therapy dependent on the severity of infection (III).

Infection Prevention

Perioperative prophylaxis

Antibiotic prophylaxis for VAD-related infections is typically confined to the peri-operative setting. To date there have been no trials comparing peri-operative antibiotic regimens and VAD-related infection outcomes. Use of surgical infection prophylaxis is extrapolated in part from cardiothoracic surgery guidelines, which recommend peri-operative cefazolin beginning within 1 h of surgical incision and continuing no longer than 48 h postoperatively (40,41). Vancomycin substitution is recommended in selected environments where MRSA colonization is likely or documented. These would constitute minimum recommended guidelines for surgical infection prophylaxis for VAD. Due to the distribution of pathogens involved in VAD-related infections, particularly a greater frequency and broader array of both gram positives and gram negatives including *Pseudomonas* species, a wider spectrum of coverage has traditionally been used in most programs. Surveys indicate that antibiotic regimens differ between centers and range from vancomycin or cefazolin alone to four agents, typically vancomycin, an antipseudomonal beta-lactam or quinolone, rifampin and fluconazole (42,43). However, it is not clear that the gram-negative bacteria and yeast implicated in VAD-related infections are introduced at the time of VAD implantation, as they often emerge weeks to months after implantation. Thus, they may not be impacted by surgical infection prophylaxis. The most recent VAD manuals recommend antimicrobial prophylaxis based on the hospital microbial sensitivity profile with sufficient coverage for gram-positive organisms including *Staphylococcus aureus*, coagulase-negative staphylococcal species and enterococcus species. This has evolved from recommendations for broader coverage of gram negative and fungal pathogens.

Driveline care

Trauma to the driveline exit site, such as dropping the battery pack or pulling on the driveline, has been associated with onset of driveline infection (18). It is thought that loss of tissue in-growth and exposure of nonepithelialized skin provides a medium for organism growth and biofilm formation. Methods to restrain the driveline are highly recommended and various devices for this purpose are available.

Careful attention to topical care is critical for infection prevention, but the optimal method is not known. The standard dressing change protocol has been daily cleaning with soap and 2% chlorhexidine gluconate, with a gauze covering. A method using Tegaderm Ag mesh (3 M, St. Paul, MN), foam gauze, and clear dressing changes every 3 days did not increase infections and improved caregiver satisfaction (44). Dressing changes typically start within 24–72 h after surgery. Showering may be permitted within the first week, though this practice varies by center.

Recommendations:

(1) At the time of VAD implantation, peri-operative antibiotic prophylaxis is mandatory with coverage provided at a minimum against staphylococcal species (II-2), but the choice of antimicrobials has not been standardized.
(2) Strict attention to driveline care, including avoiding trauma to the exit site, use of driveline fixation devices and careful cleaning and dressing changes, is critical for infection prevention (III).

Pediatrics

The same principles for VAD management discussed above apply to pediatric populations. However, biventricular devices, often paracorporeal, pulsatile, and pneumatically driven, are used more often (20–30%) in pediatric patients due to the common involvement of the right ventricle in childhood cardiomyopathies and viral myocarditis (45–47). When reported according to time on device, the incidence of VAD-specific infections (at "percutaneous and pump pocket sites") and early sepsis events seem to be similar to that reported in adults (47,52). However, localized non-VAD related infections seem to be more frequent in children (52). As with reporting of infection with adults, attention to definitions of infection and risk according to pre-VAD illness severity will illuminate true differences in infection risk in this population. Further study related to features of infection particular to pediatric related devices is warranted.

Future Directions/Research

With the absence of controlled trials in this area, there are many opportunities to improve the evidence-based approach to VAD infection prophylaxis and management. Cooperative, multi-institutional studies are warranted to best define risk factors and prevention strategies for the less frequent, but more serious VAD-specific infections. Research on technological improvements is ongoing. Smaller continuous flow pumps are presently being used on an

investigational basis, though infections remain a significant issue (48,49). Analogous to attempts to prevent central line associated bloodstream infections, drivelines have been coated with antimicrobials (50). And as the driveline itself is the primary risk factor for the majority of VAD-associated infections, elimination of the percutaneous driveline with the use of transcutaneous energy transfer systems has been a longstanding goal (51).

Conclusions

Patients with VAD infection can, with rare exception, be managed with antibiotics and surgical interventions. Heart transplantation is not contraindicated in patients with VAD infection and, in fact, is curative as the VAD is explanted at the time of transplant. As a general rule, infection does not impact posttransplant survival, though VAD-related bacteremia or fungal infection may be associated with higher mortality. Careful attention to driveline care is critical for infection prevention.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Special Article

Human T Cell Lymphotrophic Virus 1/2 in Solid Organ Transplantation

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Key words: donor-to-host transmission, human T lymphotrophic virus, leukemia, screening

Abbreviations: ATL, adult T cell leukemia/lymphoma; EIA, enzyme linked immunosorbent assays; FDA, Food and Drug Administration; HAM/TSP, human T cell lymphotrophic virus associated myelopathy/tropical spastic paraparesis; HTLV, human T cell lymphotrophic virus; NHL, Non-Hodgkins lymphoma; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PTLD, posttransplant lymphoproliferative disease; SOT, solid organ transplantation; US, United States.

Epidemiology and Risk Factors

General epidemiology

Human T cell lymphotrophic virus 1 (HTLV)-1 is a delta retrovirus endemic in the Caribbean, parts of South America (highest rates reported in Brazil, Peru, Ecuador and Venezuela), West Africa, Asia (particularly Southwestern Japan) and Oceania. In areas of highest endemicity, 2–6% of adults are infected (1,2). Infection is much less common in North America. For example, in the United States (US) 0.035–0.046% of blood donors are infected with HTLV-1 or HTLV-2 (3). Among potential organ donors in France and the United States, similarly low rates of HTLV-1 (0.03–0.067%) have been reported (4,5). In endemic areas, breastfeeding is the predominant mode of transmission (2). HTLV-1 may also be transmitted via intravenous drug use, sexual intercourse (inefficiently), solid organ transplantation (SOT) and transfusion of cell-containing blood products (14.4–47.3% of recipients) (6,7). HTLV-2, in contrast, is primarily found in intravenous drug users and sexual contacts of infected persons and is endemic in some indigenous populations of North, Central and South America and in West and Central Africa.

HTLV-1 establishes latent infection in lymphocytes and infection persists for life. While most patients remain asymptomatic, following a prolonged period of latency 2–5% of infected patients develop adult T cell leukemia/lymphoma (ATL). In Southwestern Japan, 75% of non-Hodgkins lymphoma (NHL) is ATL (7). In addition to ATL, a small percentage of infected individuals develop severe neurological disease termed HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). Other inflammatory disorders and less severe neurological disease have also been associated with HTLV-1 and no reliably effective treatment is available. Unlike HTLV-1, the link between HTLV-2 and human disease is uncertain, although there have been occasional case reports of neurological disease, inflammatory disorders, and leukemia in infected patients (8). Thus at present, for the purpose of organ donation, HTLV-2 is not considered a human pathogen and organs from HTLV-2 positive donors are generally not considered to present an increased risk of donor-derived disease compared to organs from HTLV-2 negative donors. This guideline will focus on HTLV-1 infection.

Recipients positive pretransplantation

The effect of immunosuppression on the natural history of HTLV-1 is not well defined as very few cases have been described. This is an important issue in determining the safety of organ transplantation in HTLV-1 positive recipients. Case series from Japan describe 35 HTLV-1 positive kidney recipients with long-term follow up; no HTLV-1 disease occurred (9–11). In contrast, a series of patients with posttransplant lymphoproliferative disease (PTLD) describes the development of HTLV-1 associated ATL in five renal transplant recipients (unknown if all cases were infected pre-transplant) with death occurring in four or five patients (12). Among 26 HTLV-1 positive living donor liver recipients, four (15%) developed ATL with fatal outcomes in all cases (13). Overall survival, however, did not differ between HTLV-1 positive and negative recipients.

Donor derived HTLV-1 infection

Since 1999, 162 HTLV 1/2 screen positive organs have been transplanted in the United States with no HTLV-1 associated disease described in recipients (5,14,15). In virtually all cases, however, confirmatory tests were not performed on donors and analysis based on the performance of the HTLV-1/2 screening assay in a low seroprevalence population indicates that most of these donors had HTLV-2 or a false positive screening assay (5). Further, the OPTN

database tracks malignancy but not neurological outcome. Thus, the absence of reports of HTLV-1 associated disease in this population does not indicate that true positive HTLV-1 organs can be safely transplanted.

Only a few case reports describe proven HTLV-1 transmission from seropositive donor to seronegative recipient (16–19). The most convincing case of donor-derived disease occurred in Spain in which three seronegative recipients of an HTLV-1 seropositive donor developed myelopathy within 2 years of transplantation (17). A heart transplant who received HTLV-1 infected blood at the time of transplantation developed severe HAM 18 weeks after transplantation (20). A number of other reports describe donor to recipient HTLV-1 transmission without known development of disease (13,16,18,19).

Recommendation: While the impact of immunosuppression on the natural history of HTLV-1 is not fully understood, persons seropositive for HTLV-1 can be considered for transplantation. Given that these recipients may face a higher (but difficult to quantify) risk of serious disease (ATL and HAM/TSP), information regarding this risk should be provided to HTLV-1 positive potential recipients as part of the informed consent process (Category II-2).

Diagnosis

Laboratory diagnosis of HTLV-1

Enzyme linked immunosorbent assays (EIA) are currently used as screening tests for HTLV-1/2. These tests do not distinguish between HTLV-1 and HTLV-2. Further, diagnosis of HTLV-1 infection is a two-step process requiring a confirmatory assay. The most commonly used confirmatory assays include Western blot and line immunoassays. Depending on the assay design and the results in a particular patient, these confirmatory immunoassays may distinguish between HTLV-1 and HTLV-2. Polymerase chain reaction (PCR) tests may also be useful to confirm infection (particularly in the case of an indeterminate confirmatory test) and can distinguish between infection with HTLV-1 and HTLV-2. As plasma viremia is not prominent in HTLV-1 infection, PCR tests are best performed on peripheral blood mononuclear cells (PBMCs). In some studies, however, PCR is less sensitive than serological methods for the diagnosis of HTLV-1/2, and may be even lower for HTLV-2 (21). No confirmatory tests or nucleic acid based tests are currently approved by the FDA, and in most settings confirmatory results are not available in a time frame adequate to make decisions regarding deceased donor organ donation.

Screening EIA tests are highly sensitive but have poor positive predictive value when applied to a low seroprevalence population. For example, using the Abbott HTLV I/II EIA assay (now discontinued) 15 215 blood donors, 51 (0.35%) were repeatedly reactive; only 10 of these had positive confirmatory tests and only 4 had confirmed HTLV-1. Thus, only 4/51 (7.9%) of screen positive patients had confirmed HTLV-1 infection (22). In patients with medical conditions unrelated to HTLV-1/2, higher rates of positive screens are obtained (26/639), but only 3/26 were confirmed to have HTLV-1 infection (22). Likewise, among potential organ donors, HTLV-1 infection could not be confirmed in the majority of screen positive donors (5).

Recommendations: Whenever possible, HTLV-1/2 screen positive results should be confirmed with Western blot, line immunoassay, or polymerase chain reaction (Category II-1).

Treatment

Currently no proven medical treatment for asymptomatic carriers of HTLV-1 exists. Antiretrovirals effective in HIV infection have achieved mixed results at best in reducing HTLV proviral loads (the typically stable amount of virus present in infected cells) (23–27) and this is unsurprising as viral replication is sustained by cellular division rather than highly active viral production (24). Other proposed treatments for asymptomatic carriers or patients with HAM/TSP include corticosteroids, alpha interferon, anti-CD25 monoclonal antibody, cyclosporine and valproic acid which increases viral expression theoretically leading to enhanced immune surveillance (24). Overall, treatment is focused on management of sequelae of HTLV-1 infection—namely ATL and HAM/TSP—in carriers who develop HTLV-1 associated disease.

Recommendations: No specific proven treatment for asymptomatic HTLV-1 infection is currently available.

Prevention

Donor screening

As a result of both the impending discontinuation of the Abbott HTLV I/II assay in 2009 and concern regarding the high false positive rate of available assays, an analysis of universal HTLV-1 screening in deceased donors was undertaken. This suggested that 167–227 uninfected organs were discarded yearly due to false positive screening tests (5). A separate analysis estimated that in a low prevalence population the ratio of false positive to true positive HTLV-1 screening assays was 40:1 (28). Based on these considerations, the requirement for HTLV-1 screening of deceased donors was removed by OPTN/UNOS in 2009.

In general, OPTN/UNOS policy limits recommendations for laboratory testing to assays approved by the FDA for purpose of donor screening. Currently, three assays are FDA approved for screening in the United States (29). The characteristics of each assay are described in Table 1. A major limitation to donor screening is the inability of any licensed screening test to distinguish HTLV-1 from HTLV-2 and the

Table 1: FDA approved HTLV-1/2 screening assays

Assay	Comments
Abbott HTLV-I/II	Practical for OPO use No longer available in the United States Does not distinguish between HTLV-1 and HTLV-2
Abbott prism HTLV-I/II	Designed for large scale use (blood produce donor screening) Not practical in most OPO laboratories Requires significant investment in expensive equipment and reagents Does not distinguish between HTLV-1 and HTLV-2
Avioq HTLV-I/II microelisa system	Approved for HTLV screening Recently approved (March 2012) and practicality for OPO use unproven Does not distinguish between HTLV-1 and HTLV-2

HTLV = human T cell lymphotrophic virus; OPO = organ procurement organizations.

lack of a confirmatory test that can be completed prior to organ donation.

Follow up of recipients at risk for donor-derived HTLV-1

The optimal management and follow up of recipients receiving organs from donors proven or suspected to have HTLV-1 is unknown. In cases of screen positive donors, every effort should be made to perform confirmatory tests on stored donor samples to determine if the donor is actually HTLV-1 infected. Recipients of HTLV-2 positive donors or those with negative confirmatory assays do not require specific follow-up. If the donor is proven to have HTLV-1 or confirmatory tests cannot be done or are indeterminate, periodic testing for HTLV-1 using both serological (may have low sensitivity in immunosuppressed patients) and nucleic acid based testing on the recipient is indicated. Testing quarterly for 1 year and then biannually for 1 year would be a reasonable approach. While therapeutic options are uncertain, recipients would benefit from knowing their HTLV-1 status to prevent secondary (sexual or breastfeeding) transmission (see later). Further, HTLV-1 viral loads are higher in patients with neurological disease than in asymptomatic carriers (30,31) and patients with donor-derived infection might benefit from viral load guided modulation in their immunosuppression. While HTLV-1 viral loads appear to be maintained by cell division rather than production of new virus and tend to remain stable, this may not be true in immunosuppressed patients.

Standardized clinical monitoring for complications of HTLV-1 infection is not well established. ATL may present with any of a number of clinical features, including generalized adenopathy, cutaneous lesions, hypercalcemia, bony lesions and/or isolated peripheral blood abnormalities/leukemia. HAM/TSP is equally variable in clinical manifestation, and may present with stiff gait, spasticity and lower extremity weakness, back pain, urinary incontinence, impotence, paresthesias, decreased sensation (particularly for posterior column modalities such as vibratory sense) and upper motor neuron signs (32) .

Risk to Staff

HTLV-1 is spread by cell-associated virus, rather than by cell-free virus and body fluids, and is transmitted by blood products, sexual activity and breastfeeding. As with other blood borne pathogens, the greatest risk for healthcare workers caring for an HTLV-1 infected patient is accidental inoculation via contaminated sharps. While transmission of both HTLV-1 and HTLV-2 in the occupational setting have been reported (33,34), in another report no seroconversions occurred among 34 healthcare workers exposed by puncture wounds (35). No data exist on appropriate prophylaxis for individuals exposed to HTLV. While some have recommended the use of antiretroviral agents in settings of severe exposure (e.g. zidovudine/lamivudine/raltegravir; Refs. (27,36,37), the CDC and other US agencies do not recommend postexposure prophylaxis due to the lack of available data. As with other blood borne pathogens, universal, standard precautions and scrupulous sharps safety are considered sufficient for the prevention of HTLV acquisition.

Risk to others (secondary transmission)

In the nonoccupational setting, transmission may occur horizontally (usually through sexual activity, or through sharing of injection drug needles) or vertically (mother-to-child, almost entirely through breastfeeding). These can be issues for a recipient who received an HTLV-1 infected or possibly infected organ, or for an HTLV-uninfected SOT recipient who may be entering a sexual relationship with an HTLV-infected partner. The effects of immunosuppression on the risk of acquisition of HTLV are not understood, though at least one animal model suggests that cyclosporine at the time of HTLV-1 infection increased the viral set point and might result in increased risk of the development of HTLV-1 associated disease (38). For the HTLV-1 infected SOT recipient, a few general comments apply with respect to transmission. Sexual transmission of HTLV-1 can be prevented effectively with condom use and other safer sex practices (as recommended for prevention of HIV transmission). Transmission by (injection) needles can be minimized by employment of sterile needles with each use, and by avoidance of sharing of needles and other potentially contaminated equipment. Vertical transmission can be decreased by avoidance of breastfeeding (particularly in the United States and other resource-rich settings, where breast milk alternatives are available).

Recommendations: (Table 2)

(1) In low seroprevalence areas (like North America), only in extreme circumstances should confirmed HTLV-1

Table 2: Summary of recommendations

Recommendation		Level of evidence	Comment
Epidemiology	HTLV-1 seropositive individuals should not be excluded from transplantation, but informed consent should be obtained.	II-2	Reports demonstrate good outcomes without the development of HTLV-1 disease after transplantation. Immunosuppression may speed the development of HTLV-1 disease; HTLV-1 related deaths have been reported after organ transplantation.
Diagnosis	Whenever possible, screen positive results should be confirmed with Western blot, line immunoassay, or PCR.	III	Most screen positive donors or recipients will not have HTLV-1.
Treatment	No specific proven treatment of asymptomatic HTLV-1 infection is available.	III	Proposed treatments include corticosteroids, alpha interferon, anti-CD25 monoclonal antibody, cyclosporine and valproic acid.
Prevention	In low seroprevalence areas, confirmed HTLV-1 positive donors should only be used in extreme circumstances.	II-3	While routine screening is no longer required, in some circumstances (e.g. living donors) confirmed serostatus may be available.
Donor screening	Routine screening of all deceased donors for HTLV-1 is not recommended.	III	In low seroprevalence areas, most screen positive donors do not have HTLV-1 resulting in significant wastage of uninfected organs.
	Individual OPO's with higher prevalence populations (e.g. immigrants from high prevalence countries) could consider targeted screening.	III	While a positive screening test in a higher risk donor is more likely to represent a true positive, even in this circumstance if a timely confirmatory test cannot be performed most screen positive donors will likely not have HTLV-1.
	Living donors with epidemiological risk factors (e.g. previous residence in endemic area) should be screened as time frame allows for performance of confirmatory testing.	III	Reports of donor derived HTLV-1 disease justify testing in higher epidemiological risk donors when adequate time for confirmatory testing is available.
Recipient issues	Periodic testing (quarterly for 1 year and then biannually for 1 year) with both PCR and serology should be performed on recipients of proven or suspected HTLV-1 infected donors.	III	While no proven intervention is available, recipients with the potential for donor-derived HTLV-1 should be made aware of the risk of secondary transmission (sexual or breastfeeding) and investigational treatments/prophylaxis could be considered.
	Follow up of HTLV-1 positive SOT recipients should include regular clinical monitoring for complications of infection, including ATL and HAM/TSP.	III	Investigational (HAM/TSP) and standard (ATL) treatments could be considered.
	SOT recipients who are HTLV-1 infected (or received potentially infected organs) should be counseled about risks of transmission to others, including how to minimize those risks.	III	HTLV-1 can be transmitted through sexual contact, breastfeeding, or sharing injection needles. HTLV-1 cannot be transmitted through casual contact.
	SOT recipients who are at risk for acquiring HTLV-1 should be counseled on modes of transmission and how to minimize the risk of acquisition. In general, these recommendations follow those for other viruses such at HIV or hepatitis C (Category III).	III	This would primarily apply to transplant recipients who are sexual partners of HTLV-1 infected individuals.
Infection control	Standard, universal precautions should be employed when providing care to patients with HTLV infection.	III	In occupational settings, HTLV-1 transmission is similar to other blood borne viruses (HIV).
	There is insufficient evidence to recommend occupational postexposure prophylaxis for those who are exposed to HTLV-1.	III	The use of antiretrovirals immediately after exposure could theoretically prevent the establishment of infection, but there are only *in vitro* data to support this.

ATL = adult T cell leukemia; HAM/TSP = HTLV-associated myelopathy/tropical spastic paraparesis; HIV = human immunodeficiency virus; HTLV = human T cell lymphotrophic virus; OPO = organ procurement organizations; PCR = polymerase chain reaction; SOT = solid organ transplant.

seropositive donors be used. As routine HTLV-1 screening of deceased donors is no longer performed by most OPOs, the most likely scenario would be a living donor in whom confirmatory testing could be performed or a high risk deceased donor in whom screening and confirmatory testing is performed (Category II-3).

(2) Due to the low seroprevalence of HTLV-1 in the United States and the poor positive predictive value of screening HTLV-1/2 assays in this population, routine screening of all deceased donors is not recommended (Category II-3).

(3) Individual OPOs with higher prevalence populations (e.g. a high proportion of immigrants from endemic countries) could consider targeted or universal screening. However, even in these higher risk donors, most screen positive donors likely will not have HTLV-I (Category III).

(4) Living donors with epidemiological risk factors for HTLV-1 should be screened for HTLV-1 as in this situation adequate time to perform confirmatory testing is available (Category III).

(5) Recipients of confirmed or suspected HTLV-1 infected organs should undergo periodic monitoring using both serological and nucleic acid based testing (quarterly for 1 year then every 6 months for 1 year) (Category III).

(6) Follow up of HTLV-positive SOT recipients should include regular clinical monitoring for complications of infection, including ATL and HAM/TSP (focusing on the skin, lymph nodes, hematologic system and neurologic system) (Category III).

(7) SOT recipients who are HTLV-infected (or received potentially infected organs) should be counseled about risks of transmission to others, including how to minimize those risks (Category III).

(8) SOT recipients who are at risk for acquiring HTLV-1 should be counseled on modes of transmission and how to minimize the risk of acquisition. In general, these recommendations follow those for other viruses such at HIV or hepatitis C (Category III).

(9) Standard, universal precautions should be employed when providing care to patients with HTLV-1 infection (Category III).

(10) There is insufficient evidence to recommend occupational postexposure prophylaxis for those who are exposed to HTLV-1 (Category III).

Future Research

A number of important issues regarding HTLV-1/2 and SOT recipients remain undefined. Perhaps most importantly, given the recent elimination of the requirement for deceased donor HTLV-1/2 screening, the transplant community should monitor for cases of ATL or HAM in recipients that could represent donor derived infection. If significant numbers of cases are noted, targeted donor screening or universal screening using improved assays could be considered. We also need to better understand the effect of immunosuppression on the natural history of asymptomatic HTLV-1 infection, and additional case series from endemic regions are needed. Finally, further studies are needed to better define the role of antiretrovirals as postexposure prophylaxis.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Special Article

Arenavirus and West Nile Virus in Solid Organ Transplantation

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Key words: Arenavirus, donor infection, donor-to-host transmission, encephalitis, lymphocytic choriomeningitis virus, West Nile virus

Abbreviations: ARF, acute renal failure; CDC, Centers for Diseases Control; CSF, cerebrospinal fluid; EIND, emergency investigational new drug; ELISA, enzyme linked immunosorbent assay; FDA, Federal Drug Administration; ID, individual donor; IFA, immunofluorescence assay; IgG, immunoglobulin G; IgM, immunoglobulin M; IVIG, intravenous immunoglobulin; LCM, lymphocytic choriomeningitis; LCMV, lymphocytic choriomeningitis virus; MP, Minipool; MRI, magnetic resonance imaging; NAT, Nucleic Acid Amplification Test; NVD, neuroinvasive disease; PCR, polymerase chain reaction; PRNT, plaque reduction neutralization assay; RNA, ribonucleic acid; SLEV, St. Louis Encephalitis virus; SNHL, sensorineural hearing loss; TMA, transcription mediated amplification; WNV, West Nile virus.

Introduction

Over the past several years, emerging pathogens such as arenaviruses and West Nile virus (WNV) have been identified as sources of both donor-derived and posttransplant infections. Thus far, data on these infections has been published primarily in case reports. Herein, we present discussions of WNV and arenavirus infections in solid organ transplant recipients.

Arenaviruses

Epidemiology and risk factors

Arenaviruses are single-stranded enveloped RNA viruses associated with rodent-transmitted diseases of humans. Their family name is derived from the sandy appearance on electron microscopy (Latin arena, or "sand"). They are divided into two groups: The Old World complex (family *Muridae*, subfamily *Murinae*) which includes lymphocytic chorimeningitis virus (LCMV), Lassa virus and other closely related viruses and the New World complex (Family *Muridae*, subfamily *Sigmodontinae*) which includes Junin, Machupo, Guanarito, Sabia and other closely related New World viruses, commonly referred to as South American hemorrhagic fever viruses due to similar clinical presentation.

Arenaviruses are maintained in nature through chronic asymptomatic infection in rodents. LCMV differs from other arenaviruses as common house mice (*Mus domesticus* and *Mus musculus*), a rodent with global distribution as opposed to geographically restricted field mice are its natural reservoir (1). Pet hamsters and guinea pigs are not the natural reservoirs for LCMV but pet and laboratory rodents can become infected if they come in contact with house mice (e.g. in a breeding facility, pet store or home). Among rodents, transmission may occur vertically or horizontally or both depending on the specific virus. The virus exhibits high species specificity with a given rodent species providing reservoir to a specific virus. The geographic distribution of the respective rodent species in turn determines the regional distribution of the disease. In United States, the seroprevalence of LCMV in rodents is quite variable with reported rates of 9% in Baltimore, Maryland (2), to up to 95% in a case study from Michigan (3).

Humans are primarily infected by inhaling infectious aerosolized particles of rodent secretions (saliva, urine or droppings). In addition, contact with infectious rodent excreta, ingestion of contaminated food, rodent consumption and rodent bite have all been known to cause infection in humans. Lower socioeconomic status, substandard housing and agricultural activities have been associated with rodent infestation and a higher risk of infection (4). Transplant recipients may become infected with arenavirus if they are exposed to conditions conducive to contact with wild rodents or infected pet rodents. Isolated reported cases of LCMV infections have been reported in laboratory personnel after contact with infected hamsters or infected rodent cell lines (5,6). Person-to-person transmission via aerosol spread and contact with infected fluid can occur in Lassa fever and some other South American viral hemorrhagic fevers. Transmission by sex and breast feeding can also occur in Lassa fever and potentially other viral hemorrhagic

fevers even during recovery from acute illness. In case of LCMV, person-to-person transmission has occurred only through maternal–fetal transmission (7,8) and organ transplantation (9).

Anti-LCMV IgG antibody prevalence in healthy human populations ranging from 0.3% to 4.7% has been reported from different parts of the world (1,10–13). The seroprevalence of latent Lassa virus infection in West African population is reported anywhere from 12% to 50% (14,15). The seroprevalence of subclinical Junin virus infection in two rural populations in Argentina was found to be 1.9% and 4.4%, respectively (16). Table 1 describes the geographic distribution, incubation period, peak season and clinical features of arenavirus related diseases (8,17,18).

LCMV infection in immunocompetent patients is asymptomatic or mild in most patients. When symptomatic, illness is often subtle with self-limiting symptoms of fever, malaise, headache, photophobia, listlessness, myalgia, confusion, memory deficits and abdominal pain. Some cases may progress to meningitis, encephalitis and other central nervous system manifestations (19) but the overall case fatality is <1%. In immunosuppressed patients, such as organ transplant recipients, the disease is more systemic with a much higher case fatality (>90%). To date, five clusters of transmission of LCMV and an LCMV-like arenavirus via organ transplantation (9,20–22) have been described (Table 2). Fourteen of the 17 recipients died of multisystem organ failure, with LCMV-associated hepatitis as a prominent feature. A common donor was recognized in each cluster. In the 2005 cluster, the donor had contact in her home with a pet hamster infected with an LCMV strain identical to that detected in the organ recipients. It is unclear if LCMV infection can happen in transplant recipients due to reactivation of latent virus posttransplant. LCMV is often underrecognized and underdiagnosed because the clinical characteristics of LCMV meningitis are similar to those of other viral meningitis. In addition, there is lack of awareness of the virus among physicians, and the diagnostic assays are not commercially available.

Lassa fever is mild or asymptomatic in most of the infected individuals (15) but in a small proportion, it is severe and may progress to multisystem organ failure with shock, coma or death. The presentation of South American viral hemorrhagic fevers is similar to Lassa fever but with more frequent hemorrhagic and neurological complications. In contrast to South American hemorrhagic fevers, hepatitis is more frequent and severe in Lassa fever. The case fatality in Lassa fever and South American hemorrhagic fevers can be as high as 25–30% (23–25). There have no reported cases of Lassa fever and South American hemorrhagic fever in organ transplant recipients, but cases are likely missed and not reported.

Diagnosis

Diagnosis of LCMV should be strongly considered in organ transplant recipients presenting with aseptic meningitis and encephalitis, especially with unexplained fever, hepatitis or multisystem organ failure. Lassa fever and South American Hemorrhagic fever should be considered in travelers to the endemic areas with compatible clinical picture and potential exposure to rodents or a person with viral hemorrhagic fever. Possible alternate diagnosis including Yellow fever, dengue fever, malaria, Crimean-Congo hemorrhagic fever, Rift valley fever, Ebola and Marburg viral fevers, viral hepatitis and typhoid fever must also be considered.

The laboratory diagnosis can be made by detection of antivirus immunoglobulin M (IgM) and/or a fourfold rise in IgG in serum or CSF samples using enzyme-linked immunosorbent assay (ELISA) (preferred) and/or immunofluorescence assay (IFA; Refs.26,27). Reverse transcriptase polymerase chain reaction (PCR) can detect viral RNA rapidly and help identify strains but poses limitations due to natural genetic diversity of the virus and currently, remains as a research tool. Viral culture using cell lines can be confirmatory but is time consuming. Immunohistochemical staining of viral antigens in tissue specimens can be helpful in case of negative serological assays. Virus can be isolated from blood, CSF or throat swabs. "These tests are available at state and public health reference laboratories, such as the US Centers for Disease Control and Prevention (CDC), although they may also be available in few commercial laboratories."

Donor screening: There is currently no evidence of benefit of screening of potential organ donors for LCMV infection. Deceased donors may be asymptomatic at the time of death prior to donation. If the potential donor has died of aseptic meningitis or encephalitis of unknown cause, risks and benefits to potential transplant recipients in offering and accepting organs from such donors should be carefully considered.

Treatment

Supportive care with meticulous fluid balance and electrolyte management is the mainstay of therapy in arenavirus infection. One surviving transplant patient in the 2005 cluster of donor-transmitted cases was treated with ribavirin and reduction of immunosuppressive therapy (9). However, in 2011 cluster, 2 of the 4 infected recipients survived without receiving treatment with ribavirin (22). Although, ribavirin has in vitro activity against LCMV (7), it is not FDA approved for this indication. The surviving patient with LCMV infection in the 2005 cluster was treated with intravenous ribavirin (administered at a loading dose of 30-mg/kg, followed by 16-mg/kg every 6 h for 4 days; followed by 8-mg/kg every 8 h). After the patient's clinical condition stabilized, ribavirin was changed to oral route (400 mg every morning and 600 mg every evening). The

Table 1: Geographic distribution, incubation period, peak season and clinical features of arenavirus related diseases

Arena virus	Disease	Geographic distribution	Incubation period	Peak season	Clinical features
LCMV	LCM	Worldwide including Americas, Europe and Asia	1–3 weeks	July–August	– Prodrome: Fever, malaise, myalgia, headache, photophobia, listlessness, confusion, memory deficits and abdominal pain. – Meningitis and encephalitis – Complications: Hepatitis, ARF, coagulopathy, thrombocytopenia orchitis and parotitis. – Infection during pregnancy may cause congenital abnormalities or abortion (8)
Lassa	Lassa fever	West Africa	3–21 days	January to April	– Prodrome of fever, myalgia, malaise, headache, vomiting, diarrhea, abdominal and retrosternal chest pain. – Purulent pharyngitis, mouth ulcers, conjunctivitis, facial edema, lymphadenopathy, upper body rash – Hypovolemic shock – Hemorrhage and neurologic complications – Sudden SNHL during convalescence (18) – Abortion during pregnancy (17)
South American HF viruses			7–14 days		– Fever, malaise, myalgia – Hemorrhagic shock-Leukopenia, thrombocytopenia – Neurological signs-tremors, seizures, encephalopathy.
Junin	Argentine HF	North-central Argentina		March to June	
Machupo	Bolivian HF	Northeast Bolivia		April to July	
Guanarito	Venezuelan HF	Central Venezuela		November to January	
Sabia	Brazilian, HF	Sao Paulo, Brazil		Unknown	

HF = hemorrhagic fever; LCMV = lymphocytic chorimeningitis virus; LCM = lymphocytic choriomeningitis; ARF = acute renal failure; SNHL = sensorineural hearing loss.

Table 2: Donor-derived cases of LCMV and LCMV-like arenavirus in solid organ transplant recipients

Year/ location	Donor risk factors	Cause of donor death	Organs donated	Number of recipients	Number of recipients who died	Time of recipient death after transplantation
Dec 2003/Wisconsin	None identified	Head trauma/ subdural hematoma	2 Kidneys 1 Liver 1 Lung	4	4	1–19 weeks
April 2005/Rhode Island	Infected pet hamster	Right MCA stroke/IC hemorrhage	2 Kidneys 1 Liver 1 Lung	4	3	1–19 weeks
2007/Australia	None identified	IC hemorrhage	2 Kidneys 1 Liver	3	3	4–6 weeks
April 2008/Massachusetts	None identified	Aseptic meningitis	2 Kidneys	2	2	4–10 weeks
Feb 2011/Arkansas	Possible rodent exposure	DKA/Possible meningitis	2 Kidneys 1 Liver 1 Lung	4	2	3–4 weeks

DKA = diabetic ketoacidosis; IC = intracerebral; MCA = middle cerebral artery.

drug was discontinued on day 37 when the virus became undetectable. Intravenous ribavirin is not available in United States for routine use, but it may be available through an Emergency Investigational New Drug (EIND) application as an investigational agent for patients with serious viral infections. IV ribavirin has also been used successfully to treat other arenavirus hemorrhagic fevers including Lassa fever (28), Argentine hemorrhagic fever (29,30) and Bolivian hemorrhagic fever (31). It has been found to reduce the risk of developing oliguria in patients with confirmed

hemorrhagic fever with renal syndrome (32). Convalescent plasma has been reported to reduce case fatality in Argentinine hemorrhagic fever when given within first 8 days of illness (33).

Prevention/Prophylaxis

Special care must be taken to prevent person-to-person spread of Lassa fever and potentially South American viral hemorrhagic fever with both airborne and contact isolation of patients (34,35). Household members should avoid close physical contact with infected person and their body fluids. Nursing mothers with viral hemorrhagic fever should avoid breast feeding even 2–3 months into recovery. Oral ribavirin may be considered for postexposure prophylaxis of Lassa fever in health care workers and close contacts that have been exposed to blood or body fluids of an infected person or animal. One of the recommended dose is: 35 mg/kg loading dose, maximum 2.5 g followed by 15 mg/kg, maximum 1 g three times a day for 10 days (36). If the exposed person develops manifestations of hemorrhagic fever, they should be immediately converted to intravenous ribavirin (36). The drug is however poorly tolerated (37), is teratogenic and cause hemolytic anemia. The vaccine against Lassa virus remains in the development phase (38), but is critically needed. A live attenuated vaccine against Junin virus (Argentine viral hemorrhagic fever) was found effective in a prospective, randomized, double-blind, placebo-controlled trial (39) and has drastically reduced the incidence of disease in Argentina (40). The vaccine has also been found effective against Machupo virus (Bolivian hemorrhagic fever) (41). The vaccine is however not approved or available in the United States. Since live viral vaccines are not generally recommended posttransplant, solid organ transplant candidates in high risk areas may be vaccinated before transplant. However, its efficacy is unknown in transplant recipients.

In experimental mice models, an immunosuppressive drug, sirolimus has been shown to enhance virus-specific CD8 T cells following acute LCMV infection as well as after immunization (42). The strategy can be potentially utilized to help in the future development of LCMV-specific vaccine.

Rodent avoidance, control and elimination, safe disposal of rodent nests and droppings and rodent contaminated foods, proper hand washing and cleaning of rodent infested areas are important interventions for preventing spread of arenaviral infections.

Future research directions

Future research should focus on making the molecular and serological assays for diagnosis of arenavirus infections commercially available, development of better and safer drugs and development of effective vaccines against LCMV and Lassa fever virus.

Recommendations for the prevention, diagnosis and treatment of arenavirus infections in transplant recipients: Transplant recipients should avoid contact with house mice, and wild and pet rodents by taking adequate measures as outlined by CDC (http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lcmv.htm). In general, patients should be advised to keep away from wild rodents, avoid close contact with pet rodents, observe proper hand hygiene after handling pet rodents, to ask another family member or friend to clean the cage and care for the pet, and maintaining adequate environmental cleaning. Although the risk of LCMV infection from pet rodents (i.e. mice, hamsters or guinea pigs) is generally low, proper precautions should be observed (Grade III).

LCMV infection should be strongly considered in transplant recipients presenting with unexplained fever, hepatitis, encephalitis or multisystem organ failure with prompt reporting to CDC and initiation of testing (Grade III). Serum, CSF and tissue samples should be obtained for viral culture, serology and immunohistochemical staining.

Universal organ donor screening for arenavirus is not recommended due to lack of evidence for its utility and lack of readily available diagnostic tests (Grade III).

Donors with unexplained meningoencephalitis should be assessed for risk factors for arenaviruses, and organs from donors with suspected or proven arenavirus infection should not be used (Grade III).

Intravenous ribavirin is the drug of choice for Lassa fever (Grade I), and should be considered for the treatment of Argentine and Bolivian hemorrhagic fever (Grade II-3). There is insufficient evidence for its efficacy and use in LCMV and other South American hemorrhagic fevers.

West Nile Virus

Epidemiology and risk factors

WNV is a mosquito borne single-stranded RNA arbovirus that belongs to the Flaviviridae family, which also includes St. Louis Encephalitis (SLEV), Japanese B encephalitis virus, Dengue, Yellow Fever, Murray Valley encephalitis and Kunjin viruses. In 1937, the first human case of WNV was reported in Uganda (43). Since then, WNV outbreaks have occurred in Africa, Asia, Europe and the Middle East where the virus is endemic. In 1999, the first outbreak of WNV in the Western hemisphere occurred in New York City (43). Over the ensuing years, WNV has spread westward over the continental United States, northward to Canada and southward to the Caribbean islands and Latin America (44). WNV and SLEV are the only flaviviridae endemic in the United States (45) with WNV now the most common, and reported in 48 states.

Infected mosquitoes, most commonly of the *Culex* genera, acquire WNV by feeding on infected birds who serve as the primary amplifying hosts of WNV (46). As the seasons progress from summer to fall, a bird-mosquito enzootic cycle develops with increasing viral amplification and infectivity of "bridge vector" mosquitoes (47,48). The net result is the successful transmission of WNV to incidental hosts, including humans. The incidence and geographic location of WNV varies yearly depending on environmental conditions such as the presence of *Culex* spp. mosquitoes and their ability to grow in number and have access to bird vectors (80).

The incubation period for WNV is between three and 14 days (average of 6 days). 80% of immunocompetent individuals remain asymptomatic (46) while 20% commonly have mild symptoms such as fever, myalgias, malaise, nausea, vomiting, diarrhea and transient rash. Only one of 140 symptomatic patients develops neuroinvasive disease including meningitis, encephalitis or meningoencephalitis (46), a poliomyelitis-like flaccid paralysis, Parkinsonian cogwheel rigidity and profound cognitive impairment. Following acute infection, 50% of patients have residual difficulties with memory loss, fatigue, ambulation and depression (49). Groups at high risk for the development of WNV associated neuroinvasive disease are immunosuppressed individuals such as solid organ transplant recipients (51,52) and recipients of chemotherapy (52) such as rituximab and B cell depleting agents (54–56), inferring the importance of humoral mediated immunity in controlling WNV infection.

In 2002 and 2003, WNV epidemics in the United States and Canada identified nonmosquito borne transmission of WNV through solid organ donation, blood transfusions (46), breast milk and placental transmission during pregnancy (49). Between 2002 and 2009, a total of five cases of solid organ donor transmission of WNV have been identified (Table 3). In these cases, the mean duration of incubation period was 13.5 days (range 7–17 days; Ref.50).

While donor transmission of WNV has been of major concern, a majority of reports of WNV infection in transplant recipients are related to infected mosquito bites (51,52). A seroprevalence study suggested that while less than 1% of immunocompetent individuals infected with WNV develop neuroinvasive disease, the incidence may be as high as 40% in solid organ recipients (57). However, this was not confirmed in another seroprevalence study (58).

Diagnosis

The diagnosis of WNV depends on a high index of suspicion and laboratory testing (46). The clinician should consider WNV in the differential diagnosis of a patient presenting with fevers, altered mental status, lower extremity paralysis, Parkinsonian cogwheel rigidity or other neurologic symptoms during the "typical WNV season", defined as May 1 to November 30 (59). To assist the clinician, local and state health departments and the CDC via Arbonet reporting (www.cdc.gov/ncidod/dvbid/westnile/index.htm) websites report cases of WNV infections in mosquito, bird and/or humans in specific locales.

Laboratory studies for diagnosis include serum and CSF WNV IgM and IgG antibodies and viral PCR testing. Interpretation of the results is facilitated by review of the timeline of WNV infection (Figure 1). In most cases, WNV infected mosquito bites are followed by an average incubation period of 6 days. After the incubation period, asymptomatic viremia lasting for 5–14 days can be identified by serum and CSF WNV PCR testing. Longer periods of viremia may occur, especially in immunocompromised patients (59). Patients with defective humoral immunity, such as those treated with rituximab, may be unable to produce WNV IgM or IgG antibodies (54–56) and have a persistent WNV viremia. Therefore, serum and CSF PCR testing may be the primary means of diagnosing WNV infection in this population (54).

Commonly, decline in WNV viremia is followed by the production of IgM antibodies. IgM is produced within 8 days after the initial WNV exposure (58) and an average of 3.9 days after the onset of viremia. Serum WNV IgG is then produced within the following 3.4 days in one study (61). Serum IgM may persist for over 500 days and therefore may not be indicative of acute infection (60). Serum IgG antibody confers lifelong protection against reinfection. Given the prolonged positive serologies, acute and convalescent serologies for IgM and IgG may be helpful in confirming the diagnosis of acute WNV infection.

For the diagnosis of WNV neuroinvasive disease, CSF should be obtained for cell count with differential, protein, glucose, WNV IgM/IgG and PCR. Studies of solid organ transplant recipients with naturally occurring WNV disease showed CSF pleocytosis ranging from 5 to 540 cells with half of cases showing a lymphocytic pleiocytosis and the other half demonstrating a neutrophilic predominance. CSF protein levels have ranged between 41 and 142 with primarily normal glucose levels (51,52). The presence of WNV IgM in the CSF is pathognomonic for central nervous system disease, as the IgM antibody does not cross the blood–brain barrier.

A complicating factor in the interpretation of WNV serologies is the cross reactivity with other flaviviridae, such as St. Louis and Japanese Encephalitis and Dengue viruses. Furthermore, the Yellow Fever vaccine may also result in false positive serologies for WNV. To assist in differentiation, the CDC utilizes IgM-ELISA microsphere assays that are specific to the different flaviviridae. For specific confirmation, plaque reduction neutralization testing (PRNT) may be obtained through the CDC (76), although results are not available prior to organ harvesting.

Table 3: Donor derived cases of WNV in solid organ transplant recipients

Year/ location	Donor risk factors and serologies/NAT	Organ donated	Onset of symptoms post transplant	Recipient serum serologies/NAT	CSF	Treatment	Symptoms/ Outcome
2009 (76) Italy	Mosquito bite Serum WNV NAT+	Liver	No symptoms	Serum WNV IgM+ and NAT	Not examined	Prophylaxis with daily local WNV IgG+ IVIG followed by Omr-IgG-am^{TM2} for total 33 days, both 0.4 g/kg	Remained without symptoms
2009 (47) California	Possible mosquito bite Serum WNV PCR+, IgM neg	Liver	Day 15	+serum WNV IgM positive, NAT and IgG neg 3 weeks later – WNV IgG+	Day 19: WNV IgM+	IVIG 0.4 g/kg on days 15 and 19	Resolved with no neurologic deficits
2008 (41) Louisiana	Blood transfusion	Heart	Day 8	Day 22: WNV IgM+	Day 31: WNV IgM+	Supportive care	NVD[3]
2005 (40,82,86) New York	Probable mosquito bite Serum WNV IgM and IgG+, RNA by mpNAT[1]	Liver	Day 13	WNV IgM+	WNV IgM+ WNV RNA +	Omr-IgG-am^{TM2}(WNV specific IVIG)	NVD, coma
		Lung	Day 17	Day 19: WNV IgM - Day 23: WNV IgM and IgG +	Day 24: WNV IgM and RNA neg Day 27: WNV IgM and IgG+	Omr-IgG-am^{TM}	NVD, coma
		Kidney #1	Asymptomatic	Day 22: WNV IgM- WNV IgG+ WNV RNA +	Not obtained	Prophylactic Omr-IgG-am	Asymptomatic
		Kidney #2	Asymptomatic	Day 16: WNV IgM, IgG and RNA negative	Not obtained	Prophylactic Omr-IgG-am	Asymptomatic
2002 (80) Georgia	Blood transfusion	Kidney	Day 17	Day 22: WNV IgM and IgG border line Day 42: WNV IgM1:5120	Day 21 and 28: WNV IgM+ CSF WBC 675 (92% PMNS[4]), Protein 87, Glucose 67	None	NVD, transferred to rehabilitation center
		Kidney	Day 19	Day 23: WNV IgM and IgG-	Day 23: WNV IgM and IgG – CSF WBC10	None	Death (Brain tissue + for WNV PCR)
		Heart	Day 13	Day 14 and 23: WNV PCR+	Day 23: WNV IgM	None	Neuroinvasive disease, resolved
		Liver	Day 9	Day 28: WNV IgM+	CSF not obtained	None	Mild cognitive impairment, resolved

[1]minipool nucleic acid-amplification test.
[2]Omrix Biopharmaceuticals, Tel Aviv, Israel.
[3]Neuroinvasive disease.
[4]Polymorphonuclear leukocytes.

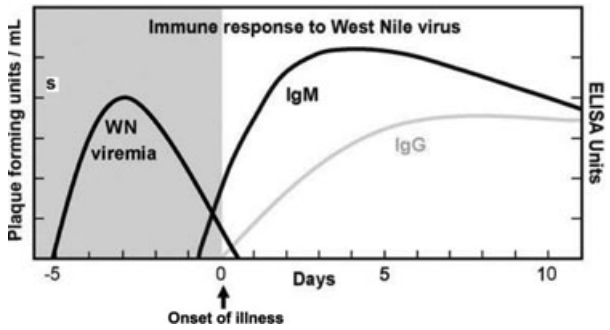

Figure 1: The basic phases of WNV viremia, the onset of illness, and the immune response to a WNV infection (77). (Zhang et al., copied with permission). There may be variability in the timeline in transplant recipients.

CT imaging has been reportedly normal with WNV meningoencephalitis (46). In contrast, diffusion and T2 weighted MRI imaging are commonly helpful by showing enhancement, which is similar to other forms of acute or chronic demyelinating processes. Both symmetric and asymmetric enhancement have been reported in the leptomeninges (61), brainstem, basal ganglia, thalami (51), pons, parietal and frontal lobes (50). T2 weighted enhancement of the spinal cord has been reported with acute flaccid paralysis (60). These findings have been observed in over 70% of transplant recipients (50,53) as compared to only one third of immunocompetent patients (60). If the initial MRI of the brain is unremarkable but the index of suspicion for WNV is high, a repeat MRI of the brain may be considered after 24–48 h to look for progression of disease. Additional electromyelographic studies may show findings of anterior horn cell disease suggestive of WNV disease (51).

- Recommendation for laboratory diagnosis of WNV: Serum and cerebrospinal fluid WNV IgM, IgG and PCR (Grade III).

Treatment

The primary treatment of WNV is supportive care such as hydration, hospitalization and use of ventilatory support, if needed. Temporary reduction in immunosuppression should be considered in order to allow for restoration of natural immunity to WNV. There are no solid data to support use of specific antivirals for treatment, but several agents have shown encouraging results (70).

Intravenous immunoglobulins: Intravenous immunoglobulin (IVIG) containing WNV specific antibodies has shown promise in the treatment of acute infection (61–64). WNV appears to have greater susceptibility to humoral mediated, as opposed to cell mediated immunity (55). Passive transfer of monoclonal or polyclonal virus-specific antibodies has been shown to play a key role in both prophylaxis and treatment (65,66). This was shown in a mouse model where WNV infection was lessened or completely aborted in a dose dependent manner with transfer of passive antibodies (67).

The presence of adequate WNV antibodies in the IVIG product initially required use of high titer WNV-specific immunoglobulin (Omr-IgG-am®, Omrix Biopharmaceutical Ltd, Kiryat-Ono, Israel) from the Middle East, where there are areas of high endemicity for WNV (62,67) and was granted orphan drug status by the FDA in 2007 (68). However, the seroprevalence of WNV in the United States has increased, resulting in the presence of high titer WNV antibodies in US plasma derived products (69) although the concentrations may vary from region to region depending on WNV endemicity. Successful use of U.S. derived IVIG for the treatment of acute WNV infection has been reported, with two doses of 0.4 g/kg administered four days apart in one report (62) and 1000 mg/kg followed by 500 mg/kg in a second report (63). Early administration of IVIG at the time of viremia may improve the outcome of WNV infection (62,65,66). A delay in dosing has been shown to decrease survival benefit (70), so that empiric early administration may be prudent if there is a high level of suspicion for the presence of WNV infection, prior to obtaining results of studies.

A small randomized controlled trial of Omr-IgG-am® versus standard IVIG failed to show a clinical benefit in adults with symptomatic disease (76). Further studies with higher doses or prophylaxis of donor infections will need to be performed utilizing either the United States derived IVIG or the Omr-IgG-am product.

Interferons-α 2b: Interferons restrict viral replication by activation of cytotoxic T cell responses (71) with animal studies suggesting improved survival with WNV infection (72). Reports of successful treatments with interferon-α 2b 3 million units daily × 14 days have been published (70,72,73). However, due to concern that interferon may be associated with organ rejection, its use in transplant recipients has not been studied.

Ribavirin: Ribavirin has demonstrated *in vitro* activity against WNV infection, but has not shown clinical efficacy (70).

- Recommendations for treatment of WNV infections: Supportive care, reduction in immunosuppression, consider intravenous immunoglobulin (Grade III).

Prevention/Prophylaxis

In an effort to avoid donor-derived WNV infections, recommendations for pretransplant screening of potentially infected SOT donors have focused on symptomatology as outlined by the Health Resources and Services Administration (HRSA) and Food and Drug Administration (FDA; Ref.53; Table 4). Actively viremic individuals (i.e. serum PCR positive) are most likely to transmit WNV by blood and

Table 4: Recommendations for identification of potentially infected WNV SOT donors (54,77)

(Grade III – Opinions of respected authorities, descriptive epidemiology)
1. Defer donors with meningitis, encephalitis or flaccid paralysis of unknown etiology from regions with reported WNV activity
2. Live donor screening:
a. Consider live donor screening with WNV NAT as close to time of donation as possible
b. Live donors with positive WNV NAT should be deferred for 120 days
c. Live donors who report a postdonation febrile illness with headache, eye pain, body aches, generalized weakness, new skin rash or swollen lymph nodes within two weeks of donation during WNV season should be tested with WNV IgM and NAT
3. Obtain WNV IgM and NAT for SOT recipients with febrile illnesses if WNV is suspected
4. Deceased donors who are WNV NAT positive prior to organ harvesting:
a. Consider transplantation only in emergent, life threatening situations.
b. Counsel patient and family with regard to risks of transplantation of potentially infected organ

organ donation (73). In addition to symptoms, predonation laboratory screening has been based on techniques implemented in 2003 to identify viremic blood products (75). Identification of virus is performed by nucleic acid amplification testing (NAT) utilizing either:

(1) Procleix System (semi-automated) or TIGRIS (automated) transcription mediated amplification (TMA) WNV test (Novartis Diagnostics) with results available in 5–6 h. This test has a sensitivity of 100 copies for live donor tissue samples, or
(2) Cobas Taq-Screen MPX test (automated) utilizing real-time PCR (Roche Molecular Diagnostics Products) with a sensitivity of 117–365 copies and results available in several hours.

Testing is initially performed on 6–16 pooled samples (minipooled or MP-NAT) with 75% sensitivity. If the MP-NAT identifies the presence of WNV in a region, the local blood bank triggers a change to an individual donation testing (ID-NAT) which can detect very low levels of virus (75,77). However, there are several main challenges in the use of these techniques in the screening of potential organ donors:

(1) Testing requires specialized laboratory facilities that are not logistically available for all organ procurement organizations (OPOs) so that NAT results may not be available prior to transplant.
(2) WNV NAT testing is performed on large platforms with as many as 500 samples for blood donor screening and is not conducive to single donor sampling. Efforts are being made to provide smaller donor sampling testing capabilities by billing only for portions used (RTI Biologics Inc.; Ref.76).
(3) NAT negative donors may transmit WNV. WNV infection was unexpectedly transmitted in 2005 to three of four solid organ recipients from a donor who was seropositive for WNV IgM and IgG but had a negative WNV NAT (85). In 2008, a donor who was WNV IgM, IgG and NAT negative received an IgM positive/NAT negative blood donation and transmitted WNV to the heart transplant recipient (Ref.44); Table 3). These two episodes suggest that tissue concentrations of virus may remain after the viremia clears (76) or that the RNA copy number may have been below the level of detection of the NAT assay.
(4) WNV NAT false positive rate of upwards of 80% in blood donor screening has been observed (personal communication, Dr. Michael Bauer, Labs, Inc), resulting in potential organ loss. FDA approved confirmatory testing is available for blood donor screening for the Procleix TIGRIS platform but not for organ donation.

Due to the significant false positive rate and potential organ loss, WNV NAT screening for organ donor screening is not routinely performed unless specifically requested. In comparison, WNV NAT screening is performed routinely for blood donation with serologic confirmation of positive results. Patients with positive results are asked to hold on further donations for 120 days.

In the posttransplant population, prevention of WNV infection focuses on avoidance of mosquito bites, specifically with the use of long sleeves and long pants, and application of topical insecticides on exposed skin, such as DEET, picardin, oil of lemon eucalyptus or IR3535 in concentrations between 10% and 50%. As mosquitoes are most active in the evenings, they should be advised to avoid outdoor activities from dusk to dawn whenever possible. A brochure specifically designed for transplant patients can be downloaded through the CDC website (82).

Infection Control Issues

Human to human transmission of WNV does not occur through contact, respiratory or droplet exposure. Patients with WNV who are hospitalized require standard universal infection control precautions.

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