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Comprehensive Review

Infection in Organ Transplantation

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The prevention, diagnosis, and management of infectious disease in transplantation are major contributors to improved outcomes in organ transplantation. The risk of serious infections in organ recipients is determined by interactions between the patient's epidemiological exposures and net state of immune suppression. In organ recipients, there is a significant incidence of drug toxicity and a propensity for drug interactions with immunosuppressive agents used to maintain graft function. Thus, every effort must be made to establish specific microbiologic diagnoses to optimize therapy. A timeline can be created to develop a differential diagnosis of infection in transplantation based on common patterns of infectious exposures, immunosuppressive management, and antimicrobial prophylaxis. Application of quantitative molecular microbial assays and advanced antimicrobial therapies have advanced care. Pathogen-specific immunity, genetic polymorphisms in immune responses, and dynamic interactions between the microbiome and the risk of infection are beginning to be explored. The role of infection in the stimulation of alloimmune responses awaits further definition. Major hurdles include the shifting worldwide epidemiology of infections, increasing antimicrobial resistance, suboptimal assays for the microbiologic screening of organ donors, and virus-associated malignancies. Transplant infectious disease remains a key to the clinical and scientific investigation of organ transplantation.

Abbreviations: CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; EBV, Epstein-Barr virus; GM, galactomannan; HAART, highly active antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HSV, herpes simplex virus; MDRO, multidrug-resistant organism; NAT, nucleic acid testing; PCP, *Pneumocystis jiroveci* pneumonia; PML, progressive multifocal leukoencephalopathy; PTLD, posttransplant lymphoproliferative disorder; PyVAN, polyomavirus-associated nephropathy; QNAT, quantitative molecular assays; TMP-SMZ, trimethoprim-sulfamethoxazole; VZV, varicella zoster virus; WNV, West Nile virus

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Introduction

The prevention, diagnosis, and management of infectious diseases are major contributors to clinical organ transplantation. The emergence of Transplant Infectious Disease as a specialty has paralleled the expansion of organ transplantation with prolonged allograft and patient survivals and increasingly effective immunosuppressive agents. Recent advances include the availability of international standards for quantitative molecular assays for common viruses, demonstration of links between genetic polymorphisms in immune responses with the risk for specific infections, and newer antimicrobial therapies including those for hepatitis C virus (HCV) as well as the development of some detailed practice guidelines (1–4). Challenges include the paucity of assays to assess risk for specific infections or graft rejection, increasing antimicrobial resistance, suboptimal screening paradigms for microbiologic evaluation of organ donors, virus-associated malignancies, and shifts in global patterns of infection (e.g. Zika and West Nile [WNV] viruses). Bedside clinical skills remain paramount in immunocompromised hosts who manifest few clinical signs of infection. This review will describe an approach to the management of infection in transplantation.

General concepts

A wide spectrum of potential pathogens infects immunocompromised hosts; many are infrequent pathogens in normal individuals. Fever and physical signs of infection (e.g. erythema) are diminished; infection may be signaled by more subtle laboratory (e.g. liver function tests) or radiographic abnormalities. Antimetabolites (azathioprine and mycophenolate mofetil) are associated with lower leukocyte counts and lower maximum temperatures. Significant infections such as peritonitis may lack fever or localizing signs. Up to 40% of infections cause no fever, notably in fungal infections, and up to 22% of fevers are noninfectious in origin (5,6).

Given hepatic metabolism and renal toxicity of calcineurin inhibitors, drug interactions and renal injuries are common. Thus, *every effort must be made to establish*

specific microbiologic diagnoses to optimize the therapy for infection while minimizing antimicrobial resistance and associated toxicities. This may necessitate invasive procedures to obtain samples for histopathology, cell counts, and cultures. Reduction in immunosuppression may be a useful component of antimicrobial therapy but risks graft rejection and increased inflammation in the form of immune reconstitution syndromes (7). Pathogen-specific immune assays suggest the relative risk of certain infections; however, in the face of intensive immunosuppression, protective immunity, while useful, tends to dissipate.

Risk of Infection and the Timeline of Infection

The risk of infection for the recipient at any point in time after transplantation is a function of two factors:

1 The *epidemiologic exposures* of the patient and the organ donor including recent, nosocomial, and remote exposures (Table 1) (8).
2 The patient's "*net state of immunosuppression*" including all factors contributing to the risk of infection (Table 2).

Table 1: Epidemiologic exposures relevant to transplantation[1]

- Virus
  - Herpes group (CMV, EBV, HHV6, 7, 8, HSV, VZV)
  - Hepatitis viruses (HAV, HBV, HCV, HEV)
  - Retroviruses (HIV, HTLV-1 and 2)
  - Others: West Nile (WNV), Chikungunya, Zika, Dengue, lymphocytic choriomeningitis virus, rabies
- Bacteria
  - Gram-positive and gram-negative bacteria (*Staphylococcus* spp., *Pseudomonas* spp., Enterobacteriaceae, antimicrobial-resistant organisms), *Legionella* spp.
  - Mycobacteria (Tuberculosis and nontuberculous)
  - *Nocardia* spp.
- Fungus
  - *Candida* spp.
  - *Aspergillus* spp.
  - *Cryptococcus* spp.
  - Geographic fungi (*Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides* species)
  - Opportunistic molds (*Scedosporium*, Agents of Mucormycosis, Phaeohyphomycoses)
- Parasites
  - *Toxoplasma gondii*
  - *Trypanosoma cruzi*
  - *Strongyloides stercoralis*
  - *Leishmania* spp.
  - *Balamuthia* spp.
- Nosocomial exposures
  - Methicillin-resistant staphylococci
  - Antimicrobial-resistant enterococci (vancomycin, linezolid, daptomycin, quinupristin-dalfopristin)
  - Multidrug-resistant gram-negative bacilli
  - *Clostridium difficile*
  - *Aspergillus* spp.
  - *Candida* nonalbicans strains
- Community exposures
  - Food- and water-borne (*Listeria monocytogenes*, *Salmonella* spp., *Cryptosporidium* spp., hepatitis A, *Campylobacter* spp.)
  - Respiratory viruses (RSV, influenza, parainfluenza, adenovirus, metapneumovirus)
  - Common viruses, often with exposure to children (Coxsackie, Parvovirus)
  - Polyomavirus, papillomavirus
  - Atypical respiratory pathogens (*Legionella* spp., *Mycoplasma* spp., Chlamydia)
  - Geographic fungi and *Cryptococcus*, *Pneumocystis jiroveci*
- Parasites (often distant)
  - *Strongyloides stercoralis*
  - *Leishmania* spp.
  - *Toxoplasma gondii*
  - *Trypanosoma cruzi*
  - *Naegleria* spp.

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpes virus; HSV, herpes simplex virus; VZV, varicella zoster virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; HTLV, human T cell lymphotrophic virus; RSV, respiratory syncytial virus.
[1]Both known and unrecognized infections from organ and in recipient.

Table 2: Factors contributing to the "net state of immunosuppression"

- Immunosuppressive Therapy: Type, Temporal Sequence, and Intensity
- Prior therapies (Chemotherapy or Antimicrobials)
- Mucocutaneous Barrier Integrity (catheters, lines, drains)
- Neutropenia, Lymphopenia, Hypogammaglobulinemia (often drug-induced)
- Technical complications (graft injury, fluid collections, wounds)
- Underlying immune defects (e.g. Genetic polymorphisms, autoimmune disease)
- Metabolic conditions: uremia, malnutrition, diabetes, alcoholism/cirrhosis, advanced age
- Viral infection (e.g., herpesviruses, hepatitis B and C, HIV, RSV, influenza)

HIV, human immunodeficiency virus; RSV, respiratory syncytial virus.

Increased degrees of immune dysfunction predispose to infection at lower microbial inoculums or with less virulent organisms or to infections of greater than expected severity. With lower levels of immunosuppression, the incidence of infection is less and drug side-effects less frequent, but graft rejection is more common. Immunosuppression for transplantation impacts all limbs of innate and adaptive immunity; specific immune defects tend to favor specific types of infection, e.g. T-lymphocyte depletion predisposes to viral infections, B cell depletion to encapsulated bacteria, and corticosteroids to *Pneumocystis* and other fungi (Table 3). Antimicrobial prophylaxis should reflect the risk of specific infections for each immunosuppressive regimen over time. Transplantation requires adjustment of preventative strategies considering local immunosuppressive regimens, technical approaches, and understanding of local patterns of infection, factors that can be adapted to the management of individual recipients.

Epidemiologic exposures and the microbiome

The microbiome: Microorganisms in tissues and on barrier surfaces are collectively termed the "microbiome" including both commensal flora and acute exposures (infection). The microbiome of the transplant recipient is derived from multiple sources: prior colonization of mucosal surfaces, latent (viral, parasitic, fungal) infections, infection from the organ donor, and new community-derived or nosocomial exposures. Recent data suggest that these microbes have a dynamic and well-regulated interaction with the normal immune system but may contribute to immune dysregulation and graft rejection in the transplant recipient (9). The impact of the microbiome on immune function is under investigation but depends on the context in which microbial shifts occur: during immune development; with acute infections; or during homeostatic repopulation after

Table 3: Common associations of immunosuppression and infectious syndromes

Antilymphocyte globulins (lytic depletion)
T-lymphocytes: Activation of latent viruses, fever, cytokines
B-lymphocytes: encapsulated bacteria
Plasmapheresis: Encapsulated bacteria, line infections
Co-stimulatory blockade: Unknown; possible increased risk for EBV/PTLD
Corticosteroids: Bacteria, fungi (PCP), hepatitis B, wound healing
Azathioprine: Neutropenia, possibly papillomavirus
Mycophenolate mofetil: Early bacterial infection, B-cells, late CMV
Calcineurin inhibitors: enhanced herpesviral replication, gingival infection, intracellular pathogens
mTOR inhibitors: Poor wound healing, excess infections in combination with other agents, idiosyncratic interstitial pneumonitis

EBV, Epstein-Barr virus; PTLD, posttransplant lymphoproliferative disorder; PCP, *Pneumocystis jiroveci* pneumonia; CMV, cytomegalovirus.

lymphocyte depletion. In transplant recipients, microbial networks are disrupted by immunosuppression, infectious exposures, antimicrobial therapies, metabolic disarray, and surgery (Figure 1). Changes in microbial diversity (types, distribution, and concentrations of organisms) and new exposures alter local and systemic immunity and may affect graft outcomes (9). Viruses (termed the "virome") have diverse effects on immune function including allograft injury (e.g. inflammation, priming of adaptive responses, altered antigen expression) and predisposition to opportunistic infections—loosely termed "indirect effects" (10). The resiliency of the "normal" host microbiome ("microbiome homeostasis") may promote more tolerant immune responses. Infectious exposures may promote rejection, block tolerance, or stimulate cross-reactive cellular alloimmunity. Memory T cell responses to previously encountered pathogens that cross-react with alloantigens constitute "heterologous immunity," the clinical significance of which requires clarification (11,12). Further study is needed of the interactions between the microbiome and immunity in allograft recipients.

Donor- and recipient-derived infections: Donor and recipient microbiologic screening provide essential data for development of posttransplant preventative strategies (Tables 4 and 5) (13–15). These strategies are individualized to include interventions including treatment of recipients with isoniazid for latent tuberculosis or ivermectin for *Strongyloides stercoralis*, vaccination of seronegative recipients, or empiric antifungal therapy in lung recipients. Antiviral strategies for the herpesviruses are based on an assessment of risk of infection based on donor and recipient pathogen-specific serologies and, increasingly, cellular immune assays (16,17).

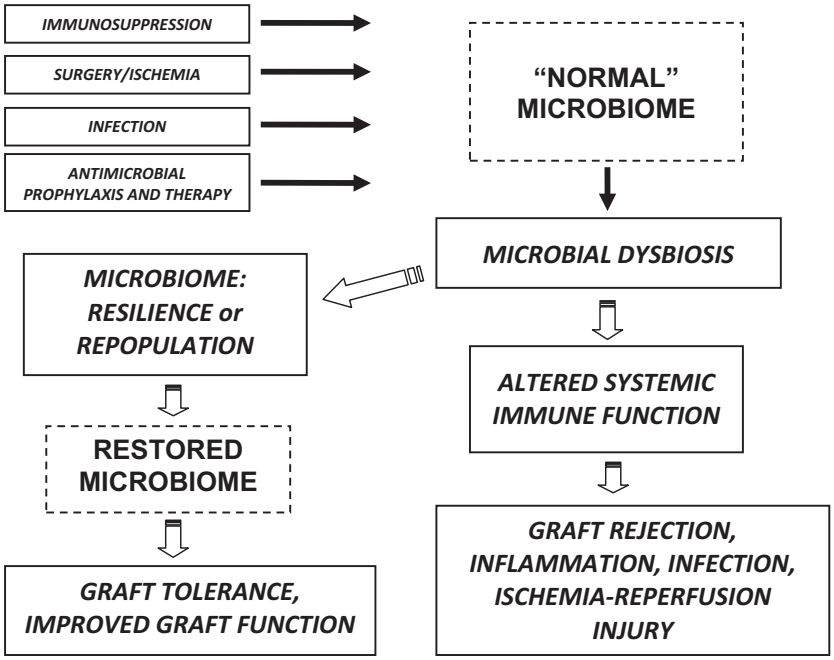

Figure 1: Transplantation and the microbiome. Transplantation disrupts the composition of commensal microbial flora through a variety of mechanisms, including surgery, diet, immunosuppression, antimicrobial prophylaxis and therapies, infection, and vaccination. Microbial disruption of the host's baseline microbiome ("Normal") or "dysbiosis" has been associated with the development of chronic rejection, injury from ischemia–reperfusion injury, and infection (9). Conversely, the resilience of the microbiome or restoration of the diversity of the pretransplant flora ("Restored Microbiome") has been associated with improved allograft outcomes. The role of microbial manipulation (e.g. by fecal transplantation) as a therapeutic measure to improve allograft outcomes is unclear.

Some donor-derived infections (e.g. *Strongyloides stercoralis*, tuberculosis, or coccidioidomycosis) may emerge decades after initial exposure. Donor colonization (e.g. *Aspergillus* in donor lungs) may also increase susceptibility to graft injury, rejection, vascular or tracheal anastomotic defects, or drug (e.g. m-TOR inhibitor) toxicity. Epidemiologic history and microbiologic assays are applied to screening of organ donors for common pathogens. Active infections are best treated prior to transplantation. Perioperative prophylaxis may be adapted for unusual colonizing organisms including multidrug-resistant organisms (MDRO) or molds; this may be continued postoperatively in select situations (*Aspergillus* colonization in lung recipients) (24). Immunosuppressed patients with incompletely treated infections often relapse. Prolonged prophylaxis risks emergence of antimicrobial resistance.

Universal prophylaxis involves giving preventative therapy to all "at-risk" patients posttransplant for a defined time period; e.g. trimethoprim-sulfamethoxazole (TMP-SMZ) for *Pneumocystis* and urinary prophylaxis. *Pre-emptive therapy* employs monitoring of patients at predefined intervals using a sensitive assay (e.g. nucleic acid assays for cytomegalovirus [CMV] or BK polyomavirus) to detect early, active infection. Positive assays result in therapy initiation. Pre-emptive therapy incurs extra costs for monitoring and coordination of outpatient care while reducing the cost of drugs and the inherent toxicities of drug exposure (25–28).

Unexpected donor-derived infections occur in less than 1% of grafts and may manifest as a cluster of infections among recipients of organs from a common donor (15,29–31). Uncommon pathogens (e.g. rabies or lymphocytic choriomeningitis virus) may not be considered or testing may be restricted to specialty laboratories; such assays are generally of low yield, too costly, and too slow for routine use (30,32). Certain pathogens such as WNV, Dengue, or *Trypanosoma cruzi* vary with geography or season; donor screening must be adapted to local requirements. Testing using highly sensitive assays in low-yield situations risks false-positive assays and unnecessary organ donor exclusion. Recent guidelines for donor screening have focused on identification of high-risk behaviors for common viruses (human immunodeficiency virus [HIV], HCV, hepatitis B virus [HBV]) using a combination of serologic and nucleic acid testing (NAT) (33). NAT screening reduces, but does not eliminate ("residual risk") the window period following infection in which an infected individual has a negative screening result using serologic assays (Table 5) (19–22). In early infection, donors may have viral loads below the limits of detection and lack seroconversion; unexpected viral transmission to recipients from deceased or living donors

Table 4: Microbiologic screening assays for potential organ recipients

Medical and family history (countries of origin, habitation, travel, endemic exposures, tuberculosis, dietary habits, water source)
Occupational exposures (health care worker, animal contacts, environmental)
Hobbies (caves, pigeons, garden, travel), pets, drugs (inhaled or injected)
Sexual history
Review microbiological data, vaccinations (BCG vaccine or therapy).
Assays to assess recipient risk
• Cytomegalovirus antibody
• Epstein-Barr virus (EBV) antibody panel (EBV viral capsid antigen, +/− early antigen and nuclear antigen antibody levels)
• Measles, mumps, rubella serologies
• Syphilis: Nontreponemal and treponemal testing
• Human immunodeficiency virus serology (ELISA) or fourth-generation ELISA
• Hepatitis B (HBV) serologies including HBV surface antigen (or HepB NAT), core antibody, surface antibody, QNAT if +
• Hepatitis C antibody, QNAT if +
• Toxoplasma antibody (notably in cardiac recipients)
• Tuberculosis by skin test or interferon-γ release assay
May add:
• Herpes simplex virus antibody
• Varicella-zoster virus antibody
• Strongyloides stercoralis (HTLV 1 and 2), *Trypanosoma cruzi*, *Schistosoma mansoni* (travel or exposure)
• Endemic fungi (In USA: Histoplasma, Coccidioides)
Sputum cultures for lung recipients
Consider: Rectal swab for resistant bacteria (Based on local epidemiology: vancomycin-resistant enterococcus, multidrug resistant organisms)
Vaccines if nonimmune (18)
• Hepatitis B
• Hepatitis A (livers, travel)
• Influenza
• Pneumovax/PCV13
• Tetanus (Tdap)
• MMR (review serologies)
• Varicella zoster virus (>50 years)
• Meningococcal (including type B), *Haemophilus influenzae* (desensitization protocols, splenectomy)
• Human papillomavirus

BCG, bacille Calmette-Guérin; ELISA, enzyme-linked immunoassay; NAT, nucleic acid testing; QNAT, quantitative molecular assays; HTLV, human T-cell lymphotrophic virus; PCV, pneumococcal vaccine; MMR, measles, mumps, rubella.

Table 5: The estimated "window period" in microbiologic screening of potential organ donors

Time to positive assay after infectious exposure	Serology (days)	Nucleic acid testing (days)	Approximate reduction in window period (days)
HIV	22 (to 180)[1]	5.6–10.2	12
Hepatitis C	38–94	6.1–8.7	30
Hepatitis B	38.3–49.7	20.4–25.7	12

The development of an antibody response measured using serologic assays may occur weeks to months after an initial infectious exposure. Nucleic acid testing (NAT) measures viral nucleic acids, often using signal amplification techniques. Depending on the performance characteristics of the assay and the amount of virus present in the clinical specimen, NAT tends to detect infection earlier and with greater sensitivity than the corresponding serologic test. The time between the infectious exposure and the development of a positive assay result is called the window period (19–22). False-positive assays have been reported with all tests but are generally more common in NAT testing. HIV, human immunodeficiency virus.

[1]Combination HIV Ab/Ag fourth-generation assays detect HIV p24 antigen and may have a shorter window period compared with antibody testing alone; this test is not as sensitive as HIV RNA testing (23).

has occurred. Conversely, such testing has allowed the use of "U.S. Public Health Service (PHS) increased risk for transmission of infection" donors such as those dying of drug overdoses who represent up to 40% of the potential donor pool in some regions. The use of HIV+ organs for HIV+ recipients (kidney and liver) is increasing (under research protocols in the United States) and requires expertise in antiviral management (34). With effective antiviral therapies, HCV+ donor organs are increasingly used for both HCV+ and HCV-negative recipients.

Community exposures: Travel, hobbies, young children, and work environments provide exposures to contaminated food and water (*Listeria, Cryptosporidium*), soil (*Aspergillus* or *Nocardia*), birds (*Cryptococcus*), and geographically restricted mycoses (*Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides* species, and *Histoplasma capsulatum*) in addition to outbreaks of respiratory viruses and arthropod-borne diseases.

Nosocomial exposures: Colonization with antimicrobial-resistant organisms may result from prolonged hospitalizations of organ donors and transplant candidates. The mortality associated with MDRO infections in transplant recipients is increased, likely as a result of delayed recognition and therapy (24,35,36). Common postsurgical infections include vancomycin-resistant enterococci, methicillin-resistant staphylococci, *Clostridium difficile* colitis, and fluconazole-resistant *Candida* species. MDROs include carbapenem-resistant enteric gram-negative bacteria, often *Klebsiella* species. Respiratory viral infections may be acquired from medical staff. Increasingly, candidates for transplantation have multiple comorbidities that may require advanced cardiopulmonary supports (ventricular assist devices, extracorporeal circulation membrane oxygenation, hemodialysis), prolonged intubation, antimicrobials or immunosuppressive therapies, and are at increased risk for colonization by MDRO.

Net State of Immunosuppression

The net state of immunosuppression is a conceptual measure of all factors contributing to the patient's risk of infection (Table 2) (8). Among these are the following:

- The specific immunosuppressive therapy, including dose, duration, and temporal sequence of agents—Intensive treatment of graft rejection poses greater acute risk than chronic immunosuppression.
- Technical problems in the transplant procedure, resulting in leaks (blood, lymph, urine) and fluid collections, devitalized tissue, and poor wound healing.
- Prolonged airway intubation.
- Use of broad-spectrum antimicrobial agents.
- Posttransplant renal, hepatic, pulmonary, or cardiac dysfunction, malnutrition or diabetes, advanced age.
- Prolonged use of urinary, vascular access, or dialysis catheters, surgical drains, or other breaks in skin or mucosal defenses.
- Infection with immunomodulating viruses including CMV, Epstein-Barr virus (EBV), HBV, HCV, or HIV. Most viruses, including community-acquired respiratory viruses, have local innate or adaptive immune effects predisposing to superinfection.
- Neutropenia or lymphocytopenia.
- Genetic polymorphisms in immune response pathways.

The synergies of combinations of immunosuppressive agents or defects in immune function are not quantifiable (3). The assessment of pathogen-specific (i.e. cellular) immune function and its relation to the intensity of immunosuppression correlates roughly with the risk of infection in an individual (3,37). Few commercialized assays exist other than for CMV and tuberculosis but include interferon-γ-release assays, ELISpot, MHC-tetramer staining, or intracellular cytokine staining. Serologic assays determine past exposures to various pathogens but are poorly predictive of the efficacy of immune response to specific pathogens in the immunosuppressed host and are often not useful for acute diagnosis. Low serum antibody levels correlate with an overall risk of infection but specific cutoff values and indications for replacement therapy are lacking (38). Other measures of "global" immune function lack the desired predictive values for infectious risk (39). Individual drugs are associated with increased risk of certain infections (Table 3) (40); combinations of agents may enhance risk or cause toxicity. Few data exist on functional immune reconstitution after T- or B-lymphocyte depletion or with costimulatory blockade. The contribution of organ dysfunction (e.g. cirrhosis, renal dysfunction) to immune function resists quantification. Superimposed surgery (i.e. returns to the operating room) or invasive radiologic and endoscopic procedures to address complications such as bleeding, vascular thromboses, and urinary or biliary leaks or strictures risk contamination or dissemination of organisms. These effects are amplified by drug-induced leukopenia or dysfunction. Recent data support the importance of genetic polymorphisms among transplant recipients in risk of microbial colonization and infection (1,2,41). Such predispositions are often unrecognized until additional alterations in immune function are introduced.

Timetable of infection: With standardized immunosuppressive regimens, most common infections occur in a relatively predictable pattern depending on the time elapsed since transplantation (Figure 2). This is a reflection of changing risk factors over time: surgery/hospitalization, immunosuppression, emergence of latent infections, and community exposures (8). The pattern of infection changes with alterations in the immunosuppressive regimen, including side effects, viral infections, graft dysfunction, or significant epidemiologic exposures (e.g., travel or food). Risk also depends on exposures via donor organs. Prophylactic antimicrobial agents will delay, but not eliminate, the "normal" appearance of infections. Any reduction in the net state of immunosuppression will reduce risk of infection when prophylaxis is discontinued (43–46). The time line represents three overlapping periods of risk: (1) the perioperative period to approximately 30 days after transplantation; (2) the period from 1 to 6–12 months after transplantation (reflecting immunosuppression including "induction" therapies and prophylaxis); and (3) beyond 6–12 months posttransplantation.

The time line is used to (1) establish a differential diagnosis for the transplant patient suspected of having infection, (2) identify excess environmental hazards or over-immunosuppression, and (3) design preventative antimicrobial strategies. Infections occurring at the "wrong" time suggest an excessive epidemiologic hazard or excessive immunosuppression (8). The prevention of infection is linked to the expected risk of infection (47) (Tables 6 and 7).

Phase I: 1 month posttransplantation: During the first month after transplantation, infections result from surgical complications, donor-derived infections, preexisting recipient infections, and nosocomial infections including aspiration or *C. difficile* colitis. Early infections often reflect technical issues (bleeding, strictures, leaks, graft injury) or hospital environmental exposures (e.g. *Aspergillus* pneumonia with hospital construction). Fevers may be associated with antibody for cellular depletion, transfusions, drug reactions, or graft rejection. Drainage of fluid collections and early removal of lines and drains, limiting antimicrobial agents, and meticulous wound care are essential. Early opportunistic infections are uncommon as sustained administration of immunosuppressive agents is generally required to allow organisms of low native virulence to cause invasive disease. Thus, early *Pneumocystis* pneumonia is rare without pretransplant immunosuppression.

Figure 2: The timeline of infections following organ transplantation. The pattern of common infections following organ transplantation varies with the net state of immunosuppression and the epidemiology of infectious exposures. Development of disease is delayed, but not eliminated by prophylaxis including vaccinations and antimicrobial agents. Individual risk is modified by events including treatment for graft rejection or malignancy. Thickness of line indicates relative risk. Bold type indicates infections potentially preventable by prophylaxis. PML, progressive multifocal leukoencephalopathy, PTLD, posttransplant lymphoproliferative disorder. (Reprinted by permission from Fishman (42), John Wiley and Sons, NY.)

Phase II: 1 to 6–12 months posttransplant: Multiple causes of infectious syndromes exist in the transplant recipient 1 to 6–12 months posttransplantation. Anti-CMV strategies and TMP-SMZ prophylaxis have altered patterns of posttransplant infections (Figure 2, Tables 6 and 7). TMP-SMZ virtually eliminates *Pneumocystis jiroveci* pneumonia (PCP) and given daily, prevents *Toxoplasma gondii*, and reduces urinary tract infections, *Listeria monocytogenes* meningitis, and many *Nocardia* species infections (8,48). Notably, other agents substituted for TMP-SMZ in sulfa-allergic patients offer less protection. Effective anti-CMV prophylaxis should prevent most CMV infections (and herpes zoster, herpes simplex virus [HSV], human herpesvirus [HHV] 6 and 7, and primary EBV) for the duration of therapy (Table 6). In the patient not receiving anti-CMV prophylaxis, protection against varicella zoster virus (VZV) and HSV remain useful (49–51). The differential diagnosis of infectious syndromes in this period includes the following:

- Graft rejection, particularly in regimens without induction, corticosteroids, or calcineurin inhibitors
- Lingering infection from the perisurgical period including *C. difficile* colitis, residual pneumonia, or technical issues (e.g. anastomotic leaks, empyema, cholangitis, infected hematoma).

Table 6: Prophylaxis for herpes group viruses[1]

CMV universal antiviral prophylaxis (47)		
CMV serologic status +/− T cell depletion in induction therapy	Possible regimen[2]	Monitoring (viral load NAT)
D+/R− with induction using T cell depletion (Highest risk)	Kidney: Valganciclovir 900 mg po × QD (or iv ganciclovir 5 mg/kg iv until taking po) (corrected for renal function) for 6 months Heart/Liver/Intestine/Pancreas/VCA: 3–6 months prophylaxis Lung: ≥12 months prophylaxis	Monthly for 6 months after discontinuation of therapy[3]
D+/R− without T cell depletion (costimulatory blockade) (High risk)	Valganciclovir 900 mg po × QD (or iv ganciclovir 5 mg/kg iv until taking po) (corrected for renal function) for 3–6 months Heart: 3–6 months prophylaxis Lung: ≥12 months prophylaxis	Monthly for 6 months after discontinuation of therapy[3]
R+ without T cell depletion (costimulatory blockade) (Intermediate risk)	Kidney/Liver/Heart/Pancreas: Oral valganciclovir (900 mg/d corrected for renal function) × *3 months or pre-emptive therapy* Lung: 6–12 months prophylaxis	For symptoms (May monitor monthly 3–6 months after therapy)
R+ with T cell depletion or desensitization, (D− at Intermediate risk) (D+ at Higher risk)	Kidney/Liver/Heart/Pancreas/VCA: Oral valganciclovir (900 mg/d corrected for renal function) × 3–6 *months or pre-emptive therapy* Lung: 6–12 months prophylaxis	For symptoms (May monitor monthly 3–6 months after therapy)
D−/R− (Lowest risk) Target HSV/VZV	Oral famciclovir 500 mg po qd × 3–4 months (or valacyclovir 500 bid or acyclovir 400 tid) Use of CMV-negative or leukocyte-filtered blood	Symptoms, fever/neutropenia

Neutropenia: The doses of antiviral therapies are not reduced for neutropenia. Formal creatinine clearance measurement may be useful in dose adjustment. Alternatives to valganciclovir: High-dose valacyclovir (≥ 8 g/day)—compliance is difficult and efficacy is not well studied; po ganciclovir (3 g/day)—lower bioavailability. CMV, cytomegalovirus; D, donor serology; R, recipient serology; VCA, vascularized composite allograft; NAT, nucleic acid test normalized to World Health Organization international standard; HSV, herpes simplex virus; VZV, varicella zoster virus.

[1]Pre-emptive therapy: Pre-emptive therapy requires a carefully organized monitoring program and patient compliance. Either a molecular CMV viral load test or a pp65 antigenemia assay may be used for monitoring. Monitoring should be performed once weekly after transplantation for 12–24 weeks. Infections indicated by positive assays are treated with either oral valganciclovir or intravenous ganciclovir. Therapy is continued at least until viremia is undetectable.

[2]Longer prophylaxis for heart and lung recipients is commonly employed although data are limited.

[3]Hybrid prophylaxis: Many centers prefer universal prophylaxis for highest risk kidney recipients (D+/R− or R+ with lymphocyte depletion) and pre-emptive therapy for lower-risk groups.

Table 7: Prophylaxis for *Pneumocystis jiroveci* pneumonia (PCP)

Regimen: One single-strength trimethoprim-sulfamethoxazole tablet (TMP-SMZ, containing 80 mg trimethoprim, 400 mg sulfamethoxazole) or one double-strength tablet po daily for 3–6 months posttransplant. Patients infected with CMV, with chronic rejection, recurrent infections, and most lung, liver, and heart recipients may benefit from prolonged prophylaxis.
Alternative regimen: TMP-SMZ three times weekly (does not prevent bacterial infections)

Allergy: For patients *proven* not to tolerate TMP-SMZ, regimens include (1) a combination of atovaquone 1500 mg po with meals once daily plus levofloxacin (or equivalent fluoroquinolone without anti-anaerobic spectrum) 250 mg once daily; (2) pentamidine (300 mg iv or inhaled q 3–4 weeks, effective only after 2–3 doses); (3) dapsone (100 mg po qd to biw, test for G6PD-deficiency) +/− pyrimethamine.
None of these alternative programs offer the same broad protection of TMP-SMZ.

CMV, cytomegalovirus.

- Viral infections including CMV, HSV, herpes zoster (VZV), EBV, HHV 6 or 7, BK polyomavirus, relapsed hepatitis (HBV, HCV), and the community-acquired respiratory viruses (adenovirus, influenza, parainfluenza, respiratory syncytial virus, and metapneumovirus) (49–51).

Table 8: Common radiographic appearance of pulmonary infiltrates in immunocompromised hosts

	Common etiologies by rate of disease progression[1]	
Radiographic abnormality	Rapid (<24–48 h)	Subacute-chronic
Consolidation (lobar pneumonia)	Any organism (usually bacterial) Aspiration Pulmonary hemorrhage	Superinfection of viral or other diffuse injury Molds Mycobacteria *Nocardia* spp. *Actinomycetes* spp. Bronchiolitis obliterans
Bronchopneumonia and peribronchiolar opacity	Community acquired respiratory viruses Nontuberculous mycobacteria *Mycoplasma*, *Chlamydia*, *Neisseria*, *Haemophilus* spp. Aspiration	Mycobacteria Bronchiolitis obliterans Sarcoidosis Pneumoconiosis Alveolar cell carcinoma
Diffuse interstitial infiltrates ("ground glass," septal widening, multifocal)	*Pneumocystis jiroveci* (PCP) Community-acquired respiratory viruses Cytomegalovirus, Epstein-Barr virus, herpes simplex virus Pulmonary edema Pulmonary hemorrhage Acute respiratory distress syndrome (ARDS)	Drug toxicity (m-TOR inhibitors) Radiation toxicity Mycobacteria Metastatic cancer Alveolar proteinosis
Nodular infiltrates[2]	Bacteria (incl. Legionnaire's) Fungi (esp. Aspergillus spp.) CMV (uncommon) PCP (uncommon)	*Nocardia* spp. Mycobacteria Fungi (Cryptococcus, Histoplasma, Coccidioides) Kaposi sarcoma Castleman's disease Other tumors (lung cancer)
Adenopathy	Tuberculosis *Cryptococcus neoformans* PTLD	PTLD/Lymphoma Kaposi sarcoma Castleman disease Lung cancer
Pleural effusion	Bacteria (parapneumonic) Postoperative Empyema Tuberculosis	
Pneumothorax	Pneumocystis	

CMV, cytomegalovirus; PCP, *Pneumocystis jiroveci* pneumonia; PTLD, posttransplant lymphoproliferative disorder.
[1]An acute illness develops and requires medical attention in a matter of relatively few hours. A subacute-chronic process develops over several days to weeks. Note that unusual causes of a process are in parentheses. Immune reconstitution may accelerate the radiographic appearance of subacute to chronic processes.
[2]A nodular infiltrate is defined as one or more large (>1 cm^2 on chest radiography) focal defects with well-defined, more or less rounded edges, surrounded by aerated lung. Multiple tiny nodules of smaller size, as sometimes caused by such an agent as CMV or varicella-zoster virus, are not included here.

- Opportunistic infection due to *Pneumocystis jiroveci*, *L. monocytogenes*, *T. gondii*, *Nocardia* species, *Aspergillus* species, endemic fungi, often following immunomodulating viral infection (Table 8).

Viral infections have a central role, notably in immunosuppressed and immunologically naïve seronegative recipients of seropositive donor organs. Each virus produces a set of clinical syndromes or "direct effects" (e.g. fever, pneumonitis, hepatitis, leukopenia) as well as a variety of "indirect" or cellular effects including (1) local or systemic immunosuppression predisposing to subsequent opportunistic infections; (2) stimulation of innate immune responses that may augment alloreactivity; and (3) cellular proliferation including malignancies (posttransplant lymphoproliferative disorder [PTLD], anogenital cancers) and organ-specific injuries including accelerated atherogenesis (hearts) or chronic lung allograft dysfunction (CLAD) with bronchiolitis obliterans syndrome (lungs) (52,53).

Phase III: more than 6–12 months posttransplant: Later posttransplant, recipients with satisfactory allograft function will tolerate reduced maintenance immunosuppression with lowered risk of infection. Healthy recipients suffer community-based epidemiological exposures including "viruses," foodborne gastroenteritis, or molds from work or

gardening. Most clinicians have patients who decide to clean their attic (*Aspergillus, Cryptococcus*), muck out the barn (*Rhodococcus equi*, hantavirus), or explore the world (malaria, *Salmonella* sp., dengue). Occasionally, such patients will develop primary CMV infection (socially acquired) or infections related to underlying conditions (e.g. skin infections in diabetics). Some recipients will develop relapsing viral infection. In the past, and in regions without access to antiviral therapies, this was driven by CMV, HBV, HCV, and HIV. At present, major challenges include late CMV (occasionally with antiviral resistance), EBV (as PTLD), BK polyomavirus infection, and papillomavirus (anogenital cancers and warts).

Most attention is required by individuals with tenuous graft function with higher levels of maintenance suppression. These patients suffer recurrent infections (pancreatitis, cholangitis, abscesses, urinary tract infections, pneumonia) necessitating hospitalization and antimicrobial therapy. Attempts to reduce the intensity of immunosuppression provoke humoral and cellular graft rejection. They develop progressive colonization by antimicrobial-resistant flora including fungi, complications of therapy (e.g. *C. difficile* colitis, bleeding from graft biopsies), renal dysfunction (calcineurin inhibitor toxicity, sepsis, radiographic contrast exposure), and increased immunosuppression to "save" the graft. This subgroup of transplant recipients has been termed the "chronic ne'er-do-wells," and risk common opportunistic pathogens (e.g. *P. jiroveci*, *L. monocytogenes*, *N. asteroides*, *Aspergillus* species, or *Cryptococcus neoformans*) (48) and more unusual infections (e.g. *Listeria, Rhodococcus, Cryptosporidium, Microsporidium*), molds (*Scedosporium*, agents of mucormycosis, Phaeohyphomycoses), and common diseases (herpes zoster, HSV) of unusual severity. *Minimal clinical signs or symptoms merit careful evaluation in this group of "high-risk" individuals.* This group may benefit from lifelong TMP-SMZ or prolonged antifungal prophylaxis.

Approaches to common infectious syndromes in transplantation

General approaches

As was noted, the benefits of early, specific antimicrobial therapy and avoidance of therapeutic toxicities emphasize the need for *rapid and specific diagnosis in transplant recipients with infectious syndromes. Appropriate cultures must be obtained in advance of antimicrobial therapy to avoid prolonged courses of unnecessary agents.* Similarly, technical problems merit early and definitive therapy—drainage of fluid collections, opening of blocked vessels or ducts—before the impact on graft and patient become irreversible or patients become too ill for such procedures. Invasive approaches are often subject to interspecialty negotiations—as in any sick, complex patient. Advanced imaging with interventional radiology may assist in selection of the best approach (Table 8). Reduction in immunosuppression, while conceptually sound, risks graft rejection or immune reconstitution syndromes (e.g. in cryptococcal meningitis) (7,54). Acute allograft rejection may be less common during some acute viral infections (e.g. CMV, EBV), risking subsequent graft rejection with antiviral therapy or reduction in immunosuppression. This effect is inconsistent and viral infection and rejection may be observed together (e.g. with BK polyomavirus nephropathy, HCV). Reduction in specific immunosuppressives should be roughly linked to the host responses desired for the pathogens encountered—e.g. steroids for bacteria and fungi, calcineurin inhibitors for viruses, cell cycle agents in neutropenia, m-TOR inhibitors for wound dehiscence and pulmonary processes—recognizing that each agent has multiple effects on the immune system. Some immune deficits (neutropenia, hypogammaglobulinemia) may respond to adjunctive therapies (colony-stimulating factors or antibody repletion) (38).

Cytomegalovirus (CMV)

CMV remains an important pathogen in transplant recipients despite generally effective antiviral therapies (46,55,56). The nature of CMV infection varies with the specific viral isolate, antiviral susceptibility, the site of infection, and the quality of the host's immune response (52). The reservoir for latent CMV infection is largely monocytes, affecting innate immune responses to organisms such as *Pneumocystis* and *Aspergillus*. CMV replicates in all transplanted organs (nephritis, hepatitis, carditis, pneumonitis, pancreatitis) and vessels in fibroblasts, epithelial, endothelial, and other parenchymal cells. Infections caused by multiple viral strains are commonly associated with higher viral loads with delayed clearance and higher rates of recurrence (41,57).

Epidemiology: Without prophylaxis, infection is most common 1 to 6 months after transplantation depending on the organ, the immunosuppressive regimen, and the host's immune status. The greatest risk in transplant recipients is primary infection in immunologically naïve, seronegative recipients (R−) of seropositive organs (D+) with up to a 91.9% incidence of viremia and 50–65% rate of symptomatic infection by 90 days posttransplant without prophylaxis (47,55). In D+R− transplant recipients, virus-specific, cytotoxic T cell immunity may be impaired in the graft due to MHC mismatch between donor and recipient. Measurement of CMV-specific T cell function is a useful guide to the need for prophylaxis; serology is a surrogate marker (16,58). Approximately 40–60% of seropositive recipients (R+) develop viremia without prophylaxis (55). Lung recipients (D+ or R+) are at high risk for viral activation and for adverse effects of CMV infection. Primary CMV infection in seronegative individuals after transfusion or sexual contact is often severe; transmission may occur (≈4%) even with use of leukoreduced or seronegative blood products. Viremia and

symptomatic infections are rare during effective antiviral prophylaxis but may occur after the cessation of prophylaxis with 25–40% developing symptomatic disease (46). Risk is amplified by other inflammatory processes (graft rejection, fever) with tumor necrosis factor-α release, coinfection with other herpesviruses (HHV-6 and -7), and intensification of immunosuppression with bolus corticosteroids or T cell depletion. Risk appears to be reduced by regimens including m-TOR inhibitors (sirolimus, everolimus) (59).

Clinical features: Many viremic infections are asymptomatic. "Viral syndrome" presents with fever, leukopenia, and myalgias, often with mild hepatitis. Invasive disease is often focused in the allograft. Common presentations include pancolitis with ulceration and bleeding as well as inflammation of multiple organs. Retinitis, encephalitis, and central nervous system (CNS) vasculitis are uncommon. Immune effects amplify the risk for opportunistic infections including *P. jiroveci*, *Listeria*, *Candida*, and *Aspergillus* species, EBV (PTLD), and HHV6 and 7 (60).

Diagnosis and management: International standards exist for quantitative molecular assays (QNAT) used in pre-emptive therapy, diagnosis, and management of CMV infections (58,61–64). QNAT has largely supplanted antigenemia assays. Serologic tests are useful prior to transplantation to predict risk but neither IgG nor IgM serum levels are useful for the diagnosis of acute infection; in transplantation, seroconversion is generally delayed. CMV cell cultures are slow and insensitive for diagnosis and were used in antiviral resistance studies. Notably, CMV secretion in sputum or urine is of little diagnostic utility. The presence of CMV inclusions or immunostaining by histology in the appropriate clinical setting is the "gold standard" for the diagnosis of tissue-invasive CMV disease. QNAT testing of blood detects most systemic disease but viral loads during infection of the gastrointestinal tract and CNS are generally lower than for other sites. In transplant recipients with ulcerative colonic or gastric lesions or diffuse erythema or bleeding, CMV must be considered even with low or undetectable viremia (65).

In practice, the use of QNAT in the management of CMV infections varies between clinical centers and by patient populations (66). In CMV treatment, assays are used to demonstrate virologic responses and endpoints (negative assay) for therapy (26). Virologic responses to therapy generally require 7 or more days; thus, repeated samples at less than weekly intervals carry no advantage. Monitoring schedules for prevention are linked to the patient's status. In the high-risk patient (D+/R− or R+ with antithymocyte globulins) most centers use prophylaxis for 3–6 months posttransplant, and may use biweekly or monthly screening to assure the absence of infection for an additional 3–6 months (66). In pre-emptive monitoring for R+ or D−/R− patients, weekly or biweekly monitoring is employed in the first months posttransplant with therapy initiated for positive assays. Monitoring or prophylaxis is required following intensified immunosuppression for graft rejection. No specific viral load cutoffs are available to initiate antiviral therapy. However, persistent low-level viremia (<2500 IU) suggests excess immunosuppression or stimulation by other infections or processes (e.g. rejection).

Cellular and immunological effects: Control of CMV infection is largely via MHC-restricted, virus-specific, cytotoxic T lymphocyte response (CD8+ cells) (16,26,67). CD4+ lymphocytes are important in the maintenance of the cellular immune response. Neutralizing antibody responses appear to be important clinically; seroconversion correlates roughly with the presence but not the quality of cellular immune responses. Human CMV (HCMV) glycoprotein B is involved in cellular attachment and penetration by CMV and is a target of neutralizing antibodies and a major component of recent HCMV vaccines (68). HCMV interacts with Toll-like receptors (TLR9 and TLR3) to activate inflammatory cytokine pathways and activate costimulatory pathways (55). Macrophages, dendritic cells, and natural killer (NK) cells participate in the innate immune response to CMV. The precise role of γδT-cells remains to be defined (52).

The cellular and immunological effects of CMV ("indirect effects") may be as important in transplantation as invasive viral infections (8,52,53). The mechanisms for these effects are complex and relate to viral evasion strategies to antiviral responses. The bidirectional linkage between CMV infection and graft injury has been observed in multiple clinical trials of cardiac, lung, liver, and kidney transplantation (60,69–72). CMV contributes to graft fibrosis, possibly via profibrotic and vasculopathic growth factors such as TGF-β, platelet-derived growth factor, connective tissue growth factor, vascular endothelial growth factor, and adhesion molecules including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (52). Intensive prophylaxis targeting both symptomatic disease and asymptomatic infection protects against chronic graft injury including cardiac vasculopathy and CLAD (60,69–71).

Prevention: Prevention of CMV infection varies by the organ transplanted, the immunosuppressive regimen, and patient risk (27,28,47,55,73). Prophylaxis using ganciclovir, valganciclovir, high-dose acyclovir, or related agents has the possible advantage of preventing both CMV as well as many HSV, VZV, HHV6, and HHV7 infections. Breakthrough VZV and HSV may reflect neurologic latency or reduced susceptibility. Furthermore, in the highest risk cohorts (D+R−), the indirect effects of

CMV (i.e. graft rejection, opportunistic infections, and mortality) are reduced by universal prophylaxis compared with pre-emptive therapy; breakthrough disease during prophylaxis with valganciclovir is uncommon. In practice neither strategy is perfect. Replication after completion of prophylaxis reaches 17–37% in D+/R− recipients, emphasizing benefits of reduced immunosuppression and postprophylaxis monitoring (45,74,75). CMV seropositivity is a useful guide to risk for viremia and invasive disease; up to a third of D+/R− recipients have delayed seroconversion. CMV-specific T cell immune response assays are useful for this purpose (16,17).

In renal recipients, prophylaxis for 200 days or a hybrid scheme (3 months of prophylaxis and 3 months of pre-emptive monitoring) in D+/R− renal recipients is common (45,66). In seropositive (R+) recipients, 3 months of prophylaxis or pre-emptive therapy is commonly employed in the absence of T-cell depletion for induction. With T cell depletion, 3–6 months of prophylaxis may be preferred. Valganciclovir dosing should not be reduced for neutropenia during prophylaxis to avoid selection of ganciclovir-resistant strains. Instead, consider reduction in mycophenolate mofetil dosing or use of granulocyte colony-stimulating factor to support the neutrophil count with formal measurements of creatinine clearance to guide dose adjustments. Prophylactic TMP-SMZ is uncommonly the source of clinically significant neutropenia. If the correct dose of ganciclovir cannot be tolerated, prophylaxis can be replaced with pre-emptive monitoring.

Heart, liver, pancreas, intestine, and vascularized composite allograft recipients at risk for primary infection (CMV D+/R−) generally receive prophylaxis for 3–6 months after transplantation (Table 6) (46,76). Many centers utilize 6 months of prophylaxis in CMV D+/R− or R+ patients receiving lymphocyte-depleting induction (45,66). Lung transplant recipients may benefit from prolonged (1 year) prophylaxis (43,77). Other groups are candidates for preemptive therapy *if* an appropriate monitoring system is in place and patient compliance is adequate (66). The added benefit of monthly CMV hyperimmune globulin for 3–6 months in cardiac and lung recipients is not well studied with current antiviral agents. Hypogammaglobulinemia requires intravenous immune globulin therapy (46,78,79).

Treatment: The standard of care for treating CMV disease is at least 2–3 weeks of therapy with intravenous ganciclovir (5 mg/kg twice daily, with dosage adjustments for renal dysfunction) or valganciclovir (900 mg po twice daily corrected for renal function). As clearance of viremia within 21 days is approximately 50%, most centers treat for two negative weekly QNAT assays followed by monitoring or prophylaxis for up to 3 months. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of patients with mild-to-moderately severe CMV disease with modest viral loads (80). Oral therapy may fail in patients with high viral loads and invasive gastrointestinal disease. Seronegative patients with poor clinical or virologic responses to therapy are at heightened risk for relapse and antiviral resistance; prolonged therapy, or secondary prophylaxis for 2–4 months of oral valganciclovir (900 mg daily based on creatinine clearance) is reasonable (81). The addition of 3 months of CMV hyperimmune globulin (150 mg/kg/dose iv monthly) does not appear to be justified in the absence of hypogammaglobulinemia. Relapses appear to be more frequent in gastrointestinal disease initially treated with oral therapy and in individuals not treated to a negative quantitative assay. In practice, therapy may be initiated with intravenous or oral therapy with monitoring weekly to demonstrate a response.

While spontaneous CMV ganciclovir antiviral resistance occurs, this complication is most common in the higher-risk groups with inadequate dosing of antiviral agents, often following dose reduction for neutropenia. Ganciclovir resistance is most common in lung transplant recipients, in D+/R− recipients, with high viral loads, inadequate dosing of prophylactic or therapeutic ganciclovir, more intensive immunosuppression including antilymphocyte antibody induction, and with prolonged antiviral prophylaxis (82). Clinically, the viral load or clinical syndrome fails to respond to appropriate therapy. Genetic resistance testing is useful in managing resistant CMV infection; mutations in the viral UL97 (thymidine kinase) or UL54 (DNA polymerase) genes can confer ganciclovir resistance (83,84). Some common UL97 mutations respond to higher doses of intravenous ganciclovir while combined mutations (UL97 and UL54) may manifest high-level resistance to ganciclovir and cidofovir. Alternative antiviral therapies are available in intravenous form only. These include foscarnet and cidofovir. Foscarnet is active against many ganciclovir-resistant strains of CMV, although associated with marked magnesium and potassium wasting, seizures (notably with calcineurin inhibitor therapy), and some renal toxicity. Cidofovir risks significant nephrotoxicity and ocular toxicity. Combination therapy (ganciclovir and foscarnet) is not widely used (85). Specific UL54 mutations may cause resistance to foscarnet and cidofovir (83,84). Some centers reduce immunosuppression during therapy. Addition of hyperimmune globulins may reduce viral load but clinical benefit is undocumented. Adoptive CMV-specific or polyfunctional T cell therapies are increasingly available for treatment of resistant infections (86,87). Multiple courses of antiviral therapy may be needed to cure resistant CMV infection. Investigational drugs include: Maribavir, a UL97 protein kinase inhibitor, with *in vitro* activity against ganciclovir- or cidofovir-resistant CMV; Letermovir (AIC-246), a CMV-specific terminase inhibitor, available orally or intravenously with activity against drug-resistant CMV *in vitro*; and Brincidofovir (CMX-001), an oral agent with broad activity against herpesviruses, adenoviruses,

polyomaviruses, papillomaviruses, and variola virus with some gastrointestinal toxicities. Leflunomide has some activity against CMV and BK virus but is often ineffective for therapy; possible hepatotoxicity requires drug-level monitoring. Artesunate has *in vitro* activity against herpes viruses but clinical trials are lacking.

Posttransplant lymphoproliferative disorder and Epstein-Barr virus (EBV)

Epidemiology: EBV is a gamma herpesvirus with seroprevalence rates of approximately 50% by age 5 in developed countries and over 90% in adults worldwide. In immunocompetent individuals, infection presents as a childhood febrile respiratory illness or as infectious mononucleosis of young adults with fever, lymphadenopathy, hepatosplenomegaly, and hepatitis. After transplantation, EBV seronegative individuals are at risk for primary infection, which is associated with greatly increased risk of PTLD (88,89).

Epidemiology: PTLD occurs in up to 20% of pediatric organ recipients but less than 1% of adults. Most PTLD reflects EBV infection of B-lymphocytes, but T cell, NK cell, null cell, and EBV-negative forms occur. Risk factors for PTLD include primary EBV infection (10- to 76-fold increased risk), CMV donor–recipient serostatus mismatch (D+R−), T cell depletion, younger age in children, older age in adults, and intensity of immunosuppression (88,90). Viral burden in transplanted lymphoid tissues may contribute to increased rates of disease in intestinal transplantation in children (up to 32%) while kidney (1–2%), heart, lung, liver, and pancreas are lower (3–12%) (91,92). PTLD may also occur in the absence of EBV infection or in seropositive recipients (93,94). Specific calcineurin inhibitors do not appear to increase risk, while m-TOR inhibitors may increase risk (95). Belatacept as maintenance immunotherapy has a higher associated rate of PTLD in EBV D+R− recipients, notably involving the CNS. (96,97). HLA polymorphisms may modulate the risk for PTLD (98).

Clinical presentation: Primary EBV infection tends to present in the first year posttransplant. In immunosuppressed transplant recipients, primary EBV infection may be asymptomatic or cause a febrile mononucleosis syndrome with B cell lymphocytosis with or without lymphadenopathy, atypical lymphocytosis, exudative pharyngitis, meningitis, hepatitis, or pancreatitis. Remitting–relapsing EBV infection is common in children and may suggest relative overimmune suppression. Persistent low-level EBV viremia may suggest increased risk for PTLD; the management of such patients remains unclear.

The clinical presentation of EBV-associated disease includes the following:

- Asymptomatic
- Unexplained fever or weight loss.
- Mononucleosis-like syndromes or tonsillar swelling.
- Gastrointestinal bleeding, obstruction, perforation, or abdominal mass lesions
- Infiltrative disease of the allograft (often donor-derived; confused with rejection)
- Focal CNS dysfunction or meningitis
- Pulmonary or other organ infiltration

PTLD presents at any time posttransplantation with a bimodal pattern of onset in the first year and then beyond 5–7 years. The spectrum of PTLD includes polymorphic (polyclonal) and monomorphic hyperplasias to B cell neoplasms, T cell neoplasms, and Hodgkin lymphomas. Less commonly, other forms of lymphoid neoplasms and smooth muscle tumors occur. Compared with the general population, PTLD has increased extranodal involvement, poor response to conventional therapies, and poor outcomes. Late PTLD includes more monomorphic B cell tumors, EBV-negative proliferations (up to ≈20%), or NK cell or T cell tumors (99,100). T cell, NK cell, and null cell tumors are common in some adult series and are more often monomorphic with worse prognosis. Other negative prognostic indicators include CNS disease, disease in multiple body sites, EBV-negative PTLD, disease of recipient origin, and the presence of mutations in proto-oncogenes or tumor-suppressor genes. Atypical presentations include hemolytic anemia, hemophagocytosis, and thrombocytopenia.

Prevention: All donors and recipients should undergo EBV serologic testing prior to transplantation. There are insufficient data to support routine use of antiviral prophylaxis in the D+R− population (101); reduction in the intensity of immunosuppression risks rejection. A preemptive strategy is most often applied based on posttransplant QNAT screening of higher-risk patients (EBV serology D+R−) (93,94,102). Viremic patients are candidates for reduction in immunosuppression; failure to clear viremia should raise concerns for PTLD (103,104). Insufficient data exist to support routine use of antivirals, anti-B cell therapies, or adoptive immunotherapy in such individuals.

Diagnosis and therapy: The diverse presentations of EBV-associated disease complicate diagnosis. Graft dysfunction in the presence of EBV viremia should suggest infiltrating PTLD as well as rejection. Radiologic imaging for perforation, obstruction, or gastrointestinal bleeding will often be the first clue to the presence of mass lesions; EBV and CMV QNAT assays should be obtained. Specific viral load cutoffs are not available with serial assays most useful in individuals (88,94,102,105–107).

In normal hosts, primary infection is assessed using IgG and IgM antibodies to viral capsid antigen, antibodies to

early antigen, and nuclear antigen (EBNA). These responses are often delayed with immunosuppression; anti-EBNA titers may fall in seropositive hosts with onset of PTLD (108). Transfusion of blood products confuses analysis. Tissue histopathology is required for diagnosis and staging with the spectrum of PTLD defined by World Health Organization standards (109,110). EBV-specific nucleic acids can be detected in tissue by *in situ* hybridization for EBV-encoded small nuclear RNAs (EBER) and EBV lytic or latent antigens (EBNA-1, EBNA-2, LMP-1, BZLF1) by immunostaining. Immunophenotyping (e.g. CD20) for cell lineage determination of tumors is essential. Molecular genetic markers are used to assess tumor clonality and HLA staining to assess donor versus recipient origins. Immune assays (ELISPOT or EBV-specific tetramer assays) may be useful in strategizing; commercial ATP release assays require further study. In EBV-negative PTLD, standard hematologic diagnostic criteria must be applied.

Therapy must be individualized (111). In general, EBV+ polyclonal disease in seropositive individuals has some response to reduction of immunosuppression. Prospective trials of antiviral therapy for PTLD are lacking; current data do not support a role for antivirals in PTLD (104). Treatment of CMV coinfection is warranted. Alternate therapies may be required for extranodal and monoclonal malignant PTLDs. Combinations of anti-B cell therapy (anti-CD20), chemotherapy (CHOP), and/or adoptive immunotherapy with stimulated T cells have been utilized (88,112,113). CNS disease may require addition of irradiation or chemotherapy. The duration of response is often disappointing. Relapses may be accompanied by EBV viremia. Graft rejection may complicate withdrawal of immunosuppression during treatment of malignancy.

Polyomaviruses

Epidemiology: Polyomaviruses are a growing family of common, small, nonenveloped, double-stranded DNA viruses that infect multiple species including humans (114). SV40 was identified from African Green Monkey cells used to produce polio and adenovirus vaccines. Polyomaviruses have been identified in transplant recipients in association with tubulointerstitial nephritis and nephropathy (polyomavirus-associated nephropathy [PyVAN] with BK virus [BKV], and JC virus [JCV]) and ureteric stenosis (BKV) demyelinating disease of the brain (JCV in progressive multifocal encephalopathy, PML), in Trichodysplasia spinulosa, in some malignancies (Merkel cell carcinoma) and condylomata as well as in polyomavirus hemorrhagic cystitis (PvVHC) and occasionally nephritis in stem cell transplant recipients. Tissue receptors for the human viruses are ubiquitous. Disease is generally restricted to immunocompromised hosts.

Adult seroprevalence is 40–100% for all of the polyomaviruses; transmission is thought to be oral or respiratory and is generally asymptomatic. BKV infection (type 1 in 70–80%, type 4 in 10–20%) occurs in 1–10% of renal recipients. BKV achieves latency in renal tubular epithelial cells with asymptomatic urinary shedding in up to half of renal recipients. BKV infection generally originates from the kidney allograft. Up to 30% of renal recipients with viruria develop viremia and will generally progress to nephropathy without intervention. Outside of renal transplantation, viruria is rarely associated with viremia (115). The risk for PyVAN is increased with greater MHC mismatch, deceased donor organs, BKV serologic mismatch (D+/R−), high-titer donor antibodies to BKV (possibly due to recent infection), older age recipients, lower-titer donor antibodies to BKV, greater intensity of immunosuppression including T cell depletion and bolus steroids, acute graft rejection, ureteric stenting, and low cellular immune responses to BKV. Retransplantation for PyVAN is also a risk factor for reinfection. JCV has been isolated from renal tissues and may cause nephropathy but has major tropism for neural tissues. Reactivation occurs with immunodeficiency and tissue injury (e.g. ischemia–reperfusion). In the absence of effective antiviral therapies, screening and management of infection in renal recipients is recommended.

Clinical presentation: *BK polyomavirus (BKPyV) infection:* In renal transplant recipients, BKPyV is associated with viruria and viremia, ureteric ulceration and stenosis, and polyomavirus-associated nephropathy (PyVAN or PVAN) (115). PVAN is rarely recognized in recipients of nonrenal organs, suggesting the need for renal injury (e.g. ischemia–reperfusion or drugs) to cause nephropathy. The role of BKPyV in other syndromes (pneumonitis, hemophagocytic syndrome, encephalitis, or PML) is unclear. In the absence of screening, patients often present with diminished renal allograft function or, less often, with obstruction due to ureteric smooth muscle proliferation.

In the face of renal dysfunction, a presumptive diagnosis of PVAN may be made based on elevated plasma BK viral load (discussed below) without obstruction by renal ultrasound. Biopsy with immunohistopathology is generally needed to distinguish PVAN from graft rejection and/or drug toxicity. Patients with BK nephropathy treated with bolus corticosteroids have a high rate of graft loss, while reducing immunosuppression risks rejection (115).

Screening and diagnosis: Screening of renal recipients for BKV both for renal dysfunction and routinely (e.g. months 1, 3, 6, 9, 12, and possibly annually years 2–5) allows reduction in immunosuppression for immunological viral clearance before significant renal injury has occurred. Screening generally utilizes plasma (or whole blood) viral load (VL) molecular assays. Urine studies (cytology for "decoy cells," BKV DNA or VP-1 mRNA) are less specific. Decoy cells are shed infected tubular and ureteric epithelial cells with an enlarged

nucleus with a large basophilic intranuclear inclusion by urine cytology. Cytology cannot distinguish BKV from adenovirus and false negative tests also occur. A urinary test for BKV (cytology for decoy cells or urine BKV loads over 7 log geq/mL) is adequate for screening; if negative, the risk for PVAN is low. Quantitative cutoffs for presumptive diagnosis of BKV nephropathy include plasma DNA VL $>10^4$ copies/mL (whole blood polymerase chain reaction [PCR] VL >1500–3500 copies/mL), urine VP-1 mRNA load $>6.5 \times 10^5$ copies/ng total RNA, or urine DNA load $>10^7$ copies/mL; higher viral loads are increasingly predictive of PyVAN. Interlaboratory variability in the performance of BKV QNAT should be reduced using World Health Organization standards. Biopsy is suggested for confirmation if creatinine is elevated. Renal histopathology provides definitive diagnosis of PyVAN, although the lesions are often focal and may be missed on biopsy. The histopathology of PyVAN includes tubulointerstitial nephritis with cytopathic changes and positive immunohistochemistry using antibodies generally targeting cross-reacting SV40 large T antigen or BKV antigens or by *in situ* hybridization for BKV nucleic acids. Fibrosis is often prominent with occasional calcification. The histologic appearance may mimic or coexist with cellular rejection. Recognition of tubulitis in areas distinct from those containing viral cytopathic changes suggests that acute rejection is present. SV40 staining does not distinguish BKV from JCV. Tissue (intranuclear viral inclusions) and urine electron microscopy may detect BKV particles and tubular injury. The histologic staging of PyVAN is reviewed elsewhere (115). JCV-mediated PyVAN should be considered in those with histologic evidence of disease in the absence of BKV by NAT and urine assays.

Treatment

There is no accepted treatment for PVAN other than reduction in the intensity of immune suppression for those with sustained viral loads. It is reasonable, and controversial, to reduce dosing of both calcineurin inhibitors and antimetabolites in a stepwise fashion (25–50% per step) to allow anti-BK T cell activity while monitoring BKV plasma loads. Regardless of approach, renal function (1–2 times per week), drug levels, and viral loads (alternate weeks) must be monitored during reductions (115). Rebiopsy may be needed for poor response.

Adjunctive antiviral therapies remain controversial. Some centers use cidofovir for BK nephropathy in low doses (0.25–1 mg/kg every 2 weeks). Significant renal toxicity is common with cidofovir; lipid-conjugated cidofovir is under study for this indication. Leflunomide, an agent with some antiviral activity for BKV (with monitoring drug levels) and intravenous immunoglobulin lack clinical trials data and US Food and Drug Administration approval for this indication. Fluoroquinolones have not proven useful for therapy (116).

Immunology: Retransplantation has been successful in PVAN patients with failed allografts, possibly due to developing immunity. Retransplantation is best delayed until immunosuppression has been completely discontinued for some period (6 months) and BKV is undetectable in blood and low in urine. Measurements of viral loads, BKV-specific cellular immunity, and humoral alloimmunity off immunosuppression may guide timing for retransplantation (117). Surgical removal of the allograft does not protect against future BK infection or PVAN but may be needed if immunosuppression cannot be reduced (double transplants, allosensitization) or elevated viral loads persist. Graft rejection may occur after reduction in immunosuppression and treatment may provoke relapse. A brisk inflammatory response may occur with immune reconstitution as immunosuppression is reduced.

JC polyomavirus (JCV): PML results from CNS infection by JCV and is uncommon in solid organ recipients (118). This demyelinating disorder presents with focal neurologic deficits or seizures as well as with more slowly progressive neurologic lesions and may progress to death. PML may be confused with calcineurin neurotoxicity; both may respond to a reduction in drug levels. No proven therapies exist for PML, although reduction of immunosuppression is commonly employed. Diagnosis is suggested by a compatible neurologic syndrome, radiologic evidence of demyelination, and evidence of JCV infection of cerebrospinal fluid (CSF). Confirmation requires brain biopsy with immunohistologic staining. JCV has been implicated in some cases of BKV-negative PyVAN.

Human papillomavirus (HPV)

HPV is a family of double-stranded DNA viruses, members of which have tropism for basal epithelial cells of the transformation zone of the cervix, mucous membranes, and keratinized skin. Spread is by person-to-person, generally sexual contact. HPV infection is associated with anogenital and skin precancers, cancers, and warts associated with human HPV infections. The existence of latent infection by HPV is unproven. Immunosuppression, notably of cell-mediated responses, increases the rate of HPV-associated premalignancy and relapse of prior HPV infections (119–123). Anogenital warts tend to be caused by lower cancer risk HPV types (e.g. 6 and 11) but should be seen as a marker for possible carriage of higher-risk types for cervical and anal cancers including 16 and 18. Association with head and neck cancers and respiratory infections require further study. Routine gynecological and skin screening is mandatory for transplant recipients. Pretransplant vaccination appears to protect against vaccine strains in uninfected individuals.

HIV

Successful organ transplantation in HIV-infected individuals is a reflection of advances in highly active

antiretroviral therapy (HAART) and immune monitoring in transplantation (124–127). This success should be enhanced by HCV antiviral therapy in coinfected individuals. Successful management of HIV transplants includes a stable pretransplant ART regimen with adequate CD4+ cell counts and full transplant immunosuppression including T cell depletion therapy if indicated. Failure to provide adequate immunosuppression has been associated with increased rates of acute graft rejection by 30% among kidney and twofold among liver recipients with HIV (127,128). Meticulous tracking of immunosuppressive drug levels and toxicities, avoidance of protease inhibitors in ART selection if possible, and knowledge of HIV susceptibility patterns are required (129,130). HCV-HIV coinfection carries an important negative impact on liver transplant outcomes (128,131,132); the effect of directly active anti-HCV therapies is under investigation. Prevention of opportunistic infections in HIV+ recipients is based on reconstitution with HAART. Individuals with exposures to potential pathogens prior to transplantation and/or prior to receiving HAART (e.g. Toxoplasma gondii, thrush, HPV, or Pneumocystis, mycobacteria, Coccidioides, and Histoplasma species) may require monitoring and prophylaxis posttransplantation beyond the standard of care. Donors with HIV infection or HCV infection are now being used at some centers with expertise in antiviral management in transplantation.

Fungal infections

Reduced corticosteroid use and newer antifungal agents have improved outcomes for fungal infections when coupled with therapeutic drug monitoring and fungal susceptibility testing. In transplantation, the most common fungal pathogens include *Candida* and *Aspergillus* species, *Cryptococcus neoformans*, and the endemic mycoses (133–135) (Figure 2). Highly aggressive species with increased resistance (agents of mucormycosis, *Scedosporium, Fusarium, Penicillium* species) are becoming more common. Common risk factors include prior colonization, neutropenia, intensity of immunosuppression, T cell depletion, viral coinfections (CMV, HHV6, and community-acquired respiratory infections), diabetes, broad-spectrum antimicrobial agents, renal and hepatic dysfunction, leukopenia, and critical illness. The azole antifungal agents have important interactions and toxicities in association with immunosuppressive regimens, notably increasing blood levels of the calcineurin and m-TOR inhibitors (136). The echinocandins may alter calcineurin and m-TOR inhibitor levels (137–140).

Candida *species*

Candidemia is most often an early posttransplant nosocomial infection associated with vascular access catheters, surgical drains, antimicrobial use, peripheral hyperalimentation, and diabetes (133). Candidemia may be observed in liver recipients with cholangitis, bile leaks, or hematomas. The risk of *Candida* infection is increased in liver recipients with choledochojejunostomy over duct-to-duct anastomoses and in pancreas transplantation, notably with enteric drainage (141). Other risk factors include kidney and liver allograft dysfunction, intensive care unit stays, vascular access catheters, large volume blood transfusions, re-exploration surgery after abdominal transplantation, graft pancreatitis, parenteral hyperalimentation, colonization, and broad-spectrum antimicrobial therapy (142). Targeted prophylaxis has been applied to reduce *Candida* infections in liver, small bowel, and pancreas recipients.

All cases of candidemia merit antifungal therapy in addition to removal of vascular access catheters; delayed therapy carries significant mortality (143,144). Candida retinal lesions may be due to endocarditis or endophthalmitis. *Candida* isolates from sterile sites should have susceptibility testing for fluconazole; fluconazole-resistant species are generally echinocandin susceptible. Echinocandin resistance is increasingly identified (145). Thrush is common; *Candida* may superinfect esophageal lesions due to HSV, CMV, or cancer. Other than at tracheal anastomoses in lung recipients, pulmonary candidiasis is rare without tissue infarction. Vascular anastomotic infections may lead to the development of mycotic aneurysms with risk of rupture (Figure 3A). Mycotic aneurysms may reflect donor-derived infection, contamination during procurement or via preservation fluid (Figure 3A) (146). Candiduria must be evaluated in the absence of urinary catheters and with good urine flow; fungal pyelonephritis is uncommon and fungus balls must be excluded.

Aspergillus *species*

Epidemiology: Invasive *Aspergillus* infection generally occurs in more debilitated or immunosuppressed organ recipients with mortality estimates with invasive disease of 20 to >50% (133,147–149). The risk of *Aspergillus* infection is increased by factors including organ retransplantation and reexploration, posttransplant renal or hepatic failure with renal replacement therapy, CMV infection, and hepatitis C coinfection (150–154). The rates of *Aspergillus* infections vary by organ and center including livers (1–9.2%), hearts (1–14%) (155,156), kidney (0.7–4%), and pancreas (135,157). *Aspergillus* isolation is most common in lung recipients with colonization rates >25%, and invasive infection approaching 6% including tracheobronchitis, bronchial anastomotic infections, and invasive pulmonary (32%) and disseminated (22%) infections; these rates are greater in cystic fibrosis patients and in the native lungs of single lung recipients (148). Airway ischemia and CLAD are risk factors in this population. *A. fumigatus* is the most common species isolated, while other species are increasingly noted including *A. terreus*, *A. flavus*, and *A. niger*. All transplant recipients are at risk for sinopulmonary

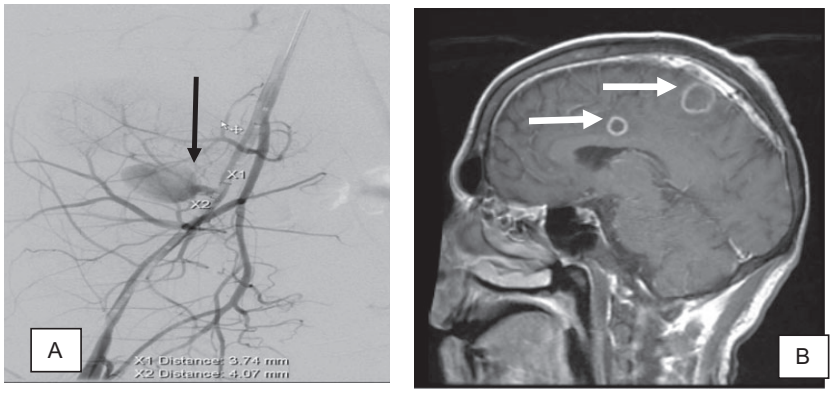

Figure 3: (A) Donor-derived infection of vascular anastomosis due to *Candida glabrata*. The patient presented day 25 after pediatric *en bloc* renal transplantation with abdominal pain and fever. Pseudoaneurysm (arrow) had ruptured at the cephalad aortic margin anastomosis. The graft was resected and aorta repaired. Recipients of liver and heart developed infections due to the same organism. (B) Brain abscesses (arrows) in neutropenic liver transplant recipient with *Aspergillus fumigatus* pneumonia. Microbiologic diagnosis was made on samples from needle aspiration of an abscess cavity. Pulmonary infection may spread to the central nervous system in some common infections including those due to *Aspergillus*, *Scedosporium*, *Rhodococcus*, and *Norcardia* species, in mycobacterial infections and with other lung abscesses.

aspergillosis. Invasive *Aspergillus* infection may extend to the CNS (Figure 3B), although intracerebral invasion is increasingly attributed to other fungal species including the agents of mucormycosis and *Scedosporium* species (158).

Clinical presentation and evaluation: Pulmonary aspergillosis generally presents with fever, cough with or without hemoptysis, and occasionally pleurisy; CNS symptoms may suggest metastatic infection (Figure 3B). Invasive aspergillosis is a medical emergency. Early specific diagnosis is essential. Patients with suspicion of infection require imaging, generally by computed tomography (CT) scan, and sampling by bronchoscopy (bronchoalveolar lavage [BAL]) or biopsy for cultures and antifungal susceptibility testing. CT scans may reveal consolidation, nodules with or without halos, or cavitary lesions. Blood cultures are generally negative for *Aspergillus*; if available, serum PCR assays are useful when positive. In general, the serum galactomannan (GM) antigenemia assay does not perform well in solid organ recipients and BAL GM samples are preferred. Interpretation of GM in the lungs of patients colonized by *Aspergillus* requires radiologic or histopathologic confirmation. If available, BAL PCR complements GM when positive.

Recommendations for prophylaxis, diagnosis, and treatment are reviewed in current Infectious Disease Society of America and International Society for Heart and Lung Transplantation guidelines (148,159). Prospective trials data are lacking to define general requirements for anti-*Aspergillus* prophylaxis other than for lung recipients. Practitioners should develop strategies based on local epidemiology of infection and individual risk factors.

CNS infection and* Cryptococcus *species

CNS infection in the transplant recipient is a medical emergency. The spectrum of causative organisms is broad. Classic signs (headache, meningismus, fever, Kernig and Brudzinski signs, or papilledema) are often absent. Subtle cranial nerve abnormalities may be useful in diagnosis. Neurologic signs of infection may be obscured by hepatic encephalopathy, uremia, hypoxemia, drug effects (calcineurin inhibitors, fluoroquinolones, TMP-SMZ), systemic infection, or alcohol withdrawal and depression.

Many CNS infections spread from the lungs or sinuses (Figure 3B). Thus, "metastatic" evaluations are needed, notably for infections due to *Aspergillus,* agents of mucormycosis, *Scedosporium, Cryptococcus*, *Nocardia species*, or *Strongyloides stercoralis*. Important viral infections include HSV meningoencephalitis, cytomegalovirus, JC virus (PML), West Nile virus, and varicella zoster virus. Common bacterial infections include *Listeria monocytogenes*, mycobacteria, *Nocardia* species, and occasionally *Salmonella* species. Parasites include *Toxoplasma gondii*, Microsporidia, and *Strongyloides*.

Specific diagnosis is essential. Empiric therapy must "cover" *Listeria* (ampicillin), *Cryptococcus* (fluconazole or amphotericin), and herpes simplex virus (acyclovir or ganciclovir), common bacterial pathogens (vancomycin, ceftriaxone), and known colonizing organisms while awaiting data from lumbar puncture, blood cultures, and radiographic studies. Included in the differential diagnosis are noninfectious etiologies including calcineurin inhibitor toxicity, posterior reversible encephalopathy, PML, lymphoma (PTLD), and other malignancies. Unique epidemiologic exposures (e.g. Chagas disease, Lyme) must be considered.

Cryptococcus *species:* Infections due to *Cryptococcus neoformans* generally occur later posttransplant (160–162). Meningitis presents with unexplained headaches and fever or altered state of consciousness. Pneumonia, cellulitis, or nodular or papular skin lesions or asymptomatic pulmonary nodules may occur. Unique risk factors include exposures to bird and bat excreta and T cell depletion. Liver recipients are at the greatest risk among transplant recipients (Figure 4).

Diagnosis is by serum and/or CSF cryptococcal antigen assays; all patients require lumbar puncture for cell counts and cultures and CSF pressure measurements. Initial treatment is best with lipid amphotericin and 5-flucytosine until cultures are negative and clinical improvement is noted; high-dose fluconazole is used until the cryptococcal antigen clears in the CSF and suppression is maintained for life. *Cryptococcus* is intrinsically resistant to the echinocandins. Cryptococcal antigenemia may persist in serum despite clinical and apparent microbiologic resolution. Immune reconstitution syndrome or scarring may cause ventricular obstruction with increased CSF pressure and hydrocephalus (161,164).

Non-*neoformans* species including *C. gattii*, *C. albidus*, and *C. laurentii* have been recognized as pathogens in transplant recipients. These isolates may have elevated minimum inhibitory concentrations to fluconazole; *C. gattii* may be hypervirulent and present with disseminated disease with low CSF cryptococcal antigen titers and with a high attributable mortality. (165,166).

Strongyloides stercoralis*:* *S. stercoralis* infection may activate decades after exposure with immunosuppressive therapy. Reactivation presents with fever and diarrheal illness or with parasite migration and hyperinfestation syndrome (characterized by hemorrhagic enterocolitis and/or hemorrhagic pneumonia) or disseminated infection with accompanying polymicrobial bacteremia or meningitis (167). Eosinophilia is common but is decreased by corticosteroids. Patients from endemic regions including the southeastern United States should be screened with *Strongyloides* IgG serology prior to transplantation, and can be treated with ivermectin preemptively if seropositive.

Pneumonitis and Pneumocystis *infection*

Epidemiology: Transplant recipients carry synergistic gaps in pulmonary defenses created by immunosuppression, neutropenia, and immunosuppressive effects of viral infections on clearance mechanisms. These effects are amplified in lung recipients by tracheal anastomotic narrowing and tissue ischemia, impaired cough reflexes, CLAD, decreased pulmonary T cell and macrophage functions with MHC mismatch, hypogammaglobulinemia, and disrupted lymphatic drainage. The timeline of infection is useful in evaluating pneumonitis. In the first month after transplantation, pneumonia is caused by reactivation of infection present prior to transplantation (donor or recipient derived) or nosocomially acquired gram-negative bacilli (aspiration) and fungal species. The risk of antimicrobial-resistant infection increases with the duration of pretransplant hospitalizations and posttransplant intubation. Superinfection of dysfunctional lung grafts is common, with special concern with bronchial anastomotic infection by *Aspergillus*. Opportunistic infections are uncommon early without pretransplant immunosuppression.

In the period 1 to 12 months after transplant, opportunistic infections emerge including PCP, toxoplasmosis,

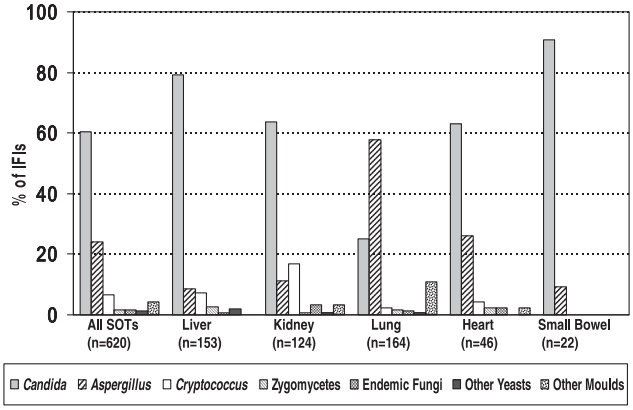

Figure 4: The incidence of specific invasive fungal infections varies with the organ transplanted. Infection due to *Candida* species is most common in all groups. Cryptococcal infection is more common in liver recipients and *Aspergillus* infection in lung recipients. Recipients of single organ transplants (SOT) are included for each group while multiorgan transplants are included in the total SOT category. Data were collected from 19 U.S. Medical Centers participating in the Prospective Antifungal Therapy (PATH) Alliance registry database. IFIs, invasive fungal infections. (From Fishman et al (163). Reprinted with permission. Copyright ©2008 Massachusetts Medical Society).

endemic fungi (e.g. histoplasmosis), and intracellular pathogens including mycobacteria. In this period, viral infections, particularly CMV and community-acquired respiratory viruses (including metapneumovirus or picornavirus) cause pneumonia and impair ciliary and macrophage functions to predispose to subsequent infections, including *Pneumocystis jirovecii, Aspergillus* and endemic fungi, and *Nocardia* species (168). Increasingly, *Nocardia* and bacterial species resistant to TMP-SMZ are observed. Greater degrees of immunosuppression and alternative prophylaxis regimens risk *Pneumocystis*, *Nocardia*, and more unusual pathogens including *Rhodococcus, Toxoplasma, Scedosporium*, and *Penicillium* species. PTLD, mycobacterial infections, and endemic fungal infections may not be distinguishable on clinical grounds. Specific microbiological diagnosis is essential.

Radiology in the diagnosis of pneumonia: The presentation and evolution of the chest radiograph provide clues to the differential diagnosis of pulmonary infection and the diagnostic workup (Table 8). Attention must be paid to comparison with prior studies and the rates of progression of roentgenographic abnormalities in relation to clinical symptoms. Any radiologic abnormalities must be considered in light of immunosuppression. Thoracic CT scans have greater sensitivity than routine chest radiographs and allow definition of the extent of disease processes, selection of sites for invasive diagnostic procedures, and response to therapy. Focal or multifocal consolidations of acute onset are most often bacterial. Multifocal nodular lesions with subacute to chronic progression are more often fungal, tuberculous, PTLD, or nocardial infections. Subacute disease with diffuse peribronchovascular or miliary abnormalities may be seen with viruses, nontuberculous mycobacteria, PCP, or graft rejection in lung allografts. Cavitation suggests angioinvasive disease caused by fungi, *Nocardia,* gram-negative bacilli, and embolic infection. Sirolimus toxicity may cause diffuse interstitial disease often with superimposed infectious pneumonitis.

Pneumocystis jiroveci *pneumonia*

Epidemiology: The risk of infection with PCP is greatest in the first 6 months after transplantation (≈10% of unprophylaxed recipients) and during periods of increased immunosuppression (169). Lung transplant recipients retain lifelong risk of PCP. PCP should be suspected with significant hypoxemia out of proportion to subtle findings on chest examination and by radiography. The natural reservoir of infection remains unknown. Aerosol transmission of infection has been demonstrated in animal models and clusters of infections have been described among immunocompromised hosts. Bolus corticosteroids, rapamycin lung syndrome, CMV, or community-acquired respiratory viral infection may precede PCP.

Diagnosis: PCP is generally acute to subacute in development. PCP presents with a broad alveolar-arterial PO_2 gradient, elevated serum lactic dehydrogenase (>300 IU/mL), and often elevated beta-1,3,glucan levels. No specific radiographic pattern exists for PCP. The chest radiograph may be normal or develop a pattern of perihilar and interstitial "ground glass" infiltrates with or without microabscesses, nodules, small effusions, or lymphadenopathy. Atypical *Pneumocystis* infection (radiographically or clinically) is seen in patients with coexisting pulmonary infections or who develop disease while receiving prophylaxis with second-choice agents (e.g. pentamidine or atovaquone). The manifestations of PCP are virtually identical to those of CMV pneumonia and these may coexist. Extrapulmonary disease is uncommon in the transplant recipient.

Identification of organisms in PCP should lead to successful treatment. Sputum induction with immunostaining should reveal organisms; otherwise, invasive techniques should be used and alternative diagnoses considered. The burden of organisms in acquired immunodeficiency syndrome patients with PCP is generally greater than in transplantation. Antibody staining reveals both cysts and trophozoites. The cyst wall can be displayed by a variety of staining techniques; the Gomori methenamine-silver nitrate method (which stains organisms brown or black) is most consistent. Sporozoites and trophozoites are stained by polychrome stains including Giemsa stains, but organisms are harder to visualize. Molecular assays are a useful adjunct to diagnosis (170,171).

Therapy: Early therapy, ideally with TMP-SMZ, is preferred; few transplant patients tolerate full-dose TMP-SMZ for prolonged periods of time. Recommended treatment dosing (15–20 mg/kg per day of the trimethoprim component) is often excessive. Creatinine may rise due to competition with trimethoprim for renal excretion and renal toxicity of sulfa. Hydration is essential. Alternate therapies are less desirable including atovaquone, clindamycin with primaquine or pyrimethamine, or intravenous pentamidine. The use of short courses of adjunctive steroids with a gradual taper and treatment of concomitant CMV infections are useful in transplant recipients (169).

Prophylaxis using low-dose TMP-SMZ is well tolerated, and allergies and toxicities are overestimated in the absence of history of true allergy or interstitial nephritis (Table 7). Alternative prophylactic strategies including dapsone, atovaquone, and inhaled or intravenous pentamidine are less effective than TMP-SMZ. The advantages of TMP-SMZ include increased efficacy, lower cost, multiple oral preparations, and possible protection against other organisms including *Toxoplasma*, *Isospora*, *Cyclospora*, and *Nocardia* species, and common urinary, respiratory, and gastrointestinal bacterial pathogens (169).

Summary

Infection must be considered in the differential for changes in clinical status of transplant recipients even in the absence of common signs or symptoms of infection. Specific microbiological diagnosis is essential for appropriate therapy and to avoid drug toxicities. Management of transplant recipients is increasingly dependent on assays for pathogen-specific immune function and molecular microbiological assays deployed in organ donors and recipients. These tools and the complexity of transplant management provide a basis for practice of Transplant Infectious Disease. Despite advances, emerging infections, increasing antimicrobial resistance, new immunosuppressive regimens, and newer technologies including extracorporeal organ resuscitation will add new challenges to clinical management. Investigative approaches including pathogen-specific immunotherapies and risk stratification based on genetic polymorphisms of immunoregulatory pathways will allow the individualization of immunosuppression and prophylaxis.

Disclosure

The author of this manuscript has no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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