American Journal of Transplantation 2013; 13: 272–279
Wiley Periodicals Inc.

doi: 10.1111/ajt.12119

Special Article

Pneumocystis Pneumonia in Solid Organ Transplantation

S. I. Martin[a,*], J. A. Fishman[b] and the AST Infectious Diseases Community of Practice

[a] *Division of Infectious Diseases and the Comprehensive Transplant Center at The Ohio State University, Wexner Medical Center, Columbus, OH*
[b] *Division of Infectious Diseases and Transplant Center at Massachusetts General Hospital, Boston, MA*
* *Corresponding author: Stanley Martin, stanley.martin@osumc.edu*

Key words: opportunistic infection, *Pneumocystis*, pneumonia, posttransplant infection, trimethoprim sulfamethoxazole

Abbreviation: PCP, *Pneumocystis* pneumonia.

Epidemiology and Risk Factors

Pneumocystis jiroveci, previously *P. carinii*, is the quintessential opportunistic infection among immunocompromised patients (1). Despite the availability of effective prophylaxis, *P. jiroveci* remains an important pathogen among solid organ transplant recipients. *Pneumocystis* spp. are thought to be ubiquitous in nature with serologic studies suggesting exposure occurs commonly in childhood (2). The existence and degree of respiratory tract colonization by *Pneumocystis* is a topic of great interest (3,4). Symptomatic *Pneumocystis* pneumonia (PCP) is generally limited, however, to individuals with immune deficits. Animal models suggest that *de novo* infection via airborne transmission and reactivation of previously established infection can occur (5). Clusters of infection have been described in medical facilities among solid organ transplant recipients as well, suggesting the possibility of direct or indirect person-to-person transmission (6–11).

Based on studies prior to routine implementation of prophylaxis, the overall incidence of infection among solid organ transplant recipients varied in the range of 5–15% depending on organ type, transplant center and immunosuppressive regimens. The attack rate appeared highest in lung and combined heart–lung transplant recipients with an overall incidence ranging from less than 10% to just over 40% (12). Infection has decreased with reduction in the routine use of corticosteroids in organ transplantation and the adoption of effective prophylactic measures. As with most infections, the overall net state of immunosuppression is the main contributor to risk rather than any specific immunosuppressive agent. Inconsistent use of prophylaxis is linked to a number of the published outbreaks in recent years (9). Risk factors for disease are outlined in Table 1.

Diagnosis

Symptomatic progression of PCP in HIV-negative patients is variable but classically more acute than in HIV-infected individuals. In the setting of transplantation, symptoms often develop over the course of a few days, though evolution over 1–2 weeks may also occur. The signs and symptoms of infection are outlined in Table 2 and are based on studies from the 1980s when the AIDS epidemic in the United States was just underway (27). As PCP may present as part of concomitant viral infection, symptoms may be masked by coinfection or by other causes of respiratory distress (e.g. congestive heart failure). In general, patients present with marked hypoxemia out of proportion to physical findings. Some common signs (e.g. fever) may be absent while others (e.g. dyspnea) should be expected.

Chest radiography may be normal or reveal diffuse bilateral interstitial pulmonary infiltrates. Computed tomographic (CT) scans are more sensitive than routine chest radiography. No specific radiological diagnostic pattern exists, however (28). Direct demonstration of the organism in the respiratory tract or secretions is the diagnostic method of choice. Diagnosis can be accomplished using noninvasive or invasive methods. The diagnosis of PCP has been markedly improved by the use of immunofluorescent monoclonal antibody stains against the organism (29,30). Direct staining of samples from respiratory tract secretions, or from transbronchial or open lung biopsies, bind to both the cyst and trophozoite forms of *Pneumocystis*, increasing the sensitivity of detection of the organism. Without antibody staining, routine stains such as Gomori methenamine-silver (GMS) can stain the cyst form only while Giemsa and Wright's stains also can stain trophozoites, the most common form of the organism in the alveolus. Diff-Quick staining (a modified Wright stain) may be the least sensitive method in identifying organisms from respiratory samples when used alone, and Calcoflour white and GMS staining may have the best overall predictive values for routine clinical use when monoclonal antibody staining is not available (31). For successfully treated patients, the organism may persist in sputa; this should not be considered a failure of therapy as relapse is uncommon with completion of therapy (32). Use of molecular techniques such as PCR has been increasingly

Table 1: Risk factors for the development of *Pneumocystis* pneumonia expected or observed in solid organ transplant recipients

Risk factors	Comments
Immunosuppressive therapies	
Corticosteroids	• Retrospective case series in non-HIV patients identified corticosteroids in up to 90% • Median dose and duration of therapy in one series of non-HIV patients with PCP was 30 mg/day of prednisone for 12 weeks (13)
Antilymphocyte therapy	• Antilymphocyte antibodies are linked to the highest risk for PCP in the 1–6 month posttransplant period (14) • Alemtuzumab, a monoclonal antibody with activity against B-, T-, and NK cells may confer the highest risk (15)
Mycophenolate mofetil	• The anti-*Pneumocystis* effects of mycophenolate mofetil *in vitro* and in animal models have not been confirmed in prospective clinical trials (16)
Calcineurin inhibitors	• At a single institution where cyclosporine A replaced azathioprine in renal transplantation, the incidence of PCP increased from 3% to 9% (17) • One retrospective study suggested a higher incidence of PCP among renal transplant recipients on tacrolimus-based regimens compared to cyclosporine A (18)
Other clinical factors	
CMV disease	• CMV may be an independent risk factor for PCP (19) • Coinfection with CMV and PCP may be observed in solid organ transplantation (20–22)
Allograft rejection	• PCP has been related to the intensity of immunosuppression in transplant recipients (18) • PCP has been linked to treatment and number of episodes of acute rejection (21)
Low CD4+ T cell counts	• In HIV infection, the risk for PCP is linked to CD4+T cell counts <200 cells/mL, or <20% of the total circulating lymphocytes (23) • PCP has been linked to decreased CD4+T cell counts in HSCT recipients (24), solid tumor patients receiving chemotherapy (25), autoimmune disease and hematological malignancy patients (26) • Transplant patients with CD4+T cell lymphopenia are expected to be at risk for PCP, though clinical data to support this are lacking (19)
Neutropenia	• Prolonged neutropenia is a potential risk factor for PCP in transplant recipients (19)
Exposure	• In solid organ transplant recipients not taking effective prophylaxis, being in close proximity to other transplant recipients with PCP may increase the risk for developing infection (6–11)

CMV = cytomegalovirus; GVHD = graft vs. host disease; HIV = human immunodeficiency virus; HSCT = hematopoietic stem cell transplant; PCP = *Pneumocystis* pneumonia.

Table 2: Signs and symptoms of *Pneumocystis* pneumonia as originally described in HIV-infected patients

Sign or symptom of PCP	Incidence
Fever	81–87%
Dyspnea	66–68%
Cough	71–81%
Chest pain	23–24%
Abnormal lung auscultation on exam	30–34%
Abnormal chest radiography	92–96%
Hypoxemia	78–91%

studied as a diagnostic tool for PCP (33–37). Concerns about lack of specificity linger, though quantification-based assays may increase the specificity of the approach. Application in bronchoalveolar lavage (BAL) fluid may have an increased sensitivity in detecting *P. jiroveci* compared to routine staining and antigen detection (38).

Coinfection with CMV is common and other respiratory viral infections may precede or coincide with PCP (1). Infection with *Pneumocystis* has also been observed in concert with abnormal lung changes due to sirolimus. Diagnostic tests are outlined in Table 3.

Practice recommendations for the diagnosis of PCP in transplant recipients include:

(1) Patients should undergo initial screening via multiple induced sputum samples (Grade II-2). All respiratory secretions should be stained using antibodies for PCP (immunoflourescent, immunoperoxidase, or similar) as well as routine stains for *Pneumocystis* and other organisms (Giemsa, Silver, and others) (Grade II-1). Use of PCR-based diagnostics on respiratory secretions can be considered (Grade III). Samples should also be assayed for routine bacterial, fungal, mycobacterial, and other organisms to rule out concomitant infections (Grade II-2). Evaluation for CMV or other respiratory viral coinfection, in particular, should be considered (Grade II-2).
(2) Clinicians should have a low threshold for bronchoscopy with BAL to obtain diagnostic samples (Grade II-2). This may have the dual advantage of increasing the yield and helping expedite the diagnosis of other and/or concomitant infections.
(3) Patients undergoing bronchoscopy should be considered for transbronchial biopsies. Increased yield is likely obtained by multiple samples (Grade II).
(4) Measurement of plasma (1→3) β-D-glucan levels can be considered and may suggest the diagnosis (Grade II-2). This assay lacks specificity for *Pneumocystis*, however, and can be positive in the setting of other invasive fungal infections.

Table 3: Diagnostic approaches to *Pneumocystis* pneumonia in transplantation

Test	Estimated yield	Comments
Routine sputum smears	Generally poor	• Organ transplant patients with PCP may have smaller burden of infecting organisms than AIDS patients (39) • Use of fluorescent monoclonal antibody staining may increase the sensitivity of finding the organism over other stains
Induced sputum smears	Improved over routine sputum exam when coupled with antibody staining; yield ≥50% (29)	• Yield from induced sputum in transplant patients may not reflect that found in HIV-infected patients • Sensitivity and specificity in transplant patients unknown • Repeat testing may improve yield (30)
Bronchoalveolar	Generally ≥70% in non-AIDS immunocompromised hosts when coupled with antibody staining	• Older data involving immunosuppressed patients with PCP suggested a yield close to 80% (40)
Transbronchial biopsy	Increases yield of routine BAL (1)	• Multiple biopsies preferred to increase sensitivity with some increased procedural risk
Open lung biopsy	Often considered to be a gold standard, but early patchy disease may decrease yield	• Case reports highlight PCP infections missed on BAL that were subsequently identified from open lung biopsies (41,42) • Cases of missed infection in open lung biopsy also reported (30)
PCR testing of samples	Sensitivity and specificity vary depending on manner of sampling (sputum vs. BAL) and assay employed	• Multiple assays are not standardized. Generally target genes for conserved surface glycoproteins or rRNAs • Specificity unknown
Plasma (1→3) β-D-glucan	Some reports in transplant and HIV patients (43–46). Meta-analysis suggests a sensitivity of almost 95%, but with a specificity in the mid-80% (47)	• (1→3) β-D-glucan is produced in the cyst cell wall and detection in the serum has been associated with underlying infection (also positive in other invasive fungal infections) (48) • Clinical trials data lacking

AIDS = acquired immunodeficiency syndrome; BAL = bronchoalveolar lavage; HIV = human immunodeficiency virus; PCP = *Pneumocystis* pneumonia; PCR = polymerase chain reaction; rRNA = ribosomal ribonucleic acid.

(5) Open lung biopsies can be obtained when other diagnostic approaches have been unrevealing or where other concomitant diseases may be a concern (Grade II). Video-assisted thoracoscopic (VATS) biopsies may be appropriate for some patients in this regard.

Treatment

For the established or presumed diagnosis of PCP, therapeutic options are outlined in Table 4.

Practice recommendations regarding the treatment of PCP in transplant recipients include:

(1) Trimethoprim-sulfamethoxazole (TMP-SMX) is the first-line agent and drug of choice (Grade I). No agent has been shown to have outcomes superior to TMP-SMX.
(2) In severe infections, intravenous pentamidine probably remains the second-line agent after TMP-SMX (Grade II-1). Although pentamidine is effective, use can be complicated by numerous toxicities. Most experts recommend alternative therapies in pancreas or islet transplant recipients due to the potential for islet cell necrosis (Grade III).
(3) In patients with hypoxemia ($pAO_2 < 70$ mmHg on room air), adjunctive corticosteroids should be administered with antimicrobial therapy, ideally within 72 hours of initiating antimicrobial therapy for maximum benefit (Grade II-1). Though the optimal dose of corticosteroids has not been well-established, recommendations of 40–60 mg of prednisone (or equivalent) given twice daily for 5–7 days before being tapered over a period of at least 7–14 days is often recommended (Grade III).
(4) Duration of antimicrobial therapy should be extended for at least 14 days, although clinicians treat for 21 days total in severe infection (Grade III).

Prophylaxis

Routine anti-*Pneumocystis* prophylaxis is recommended for most centers with an incidence of PCP of at least 3–5% among transplant recipients (19). With widespread use of prophylaxis and diverse immunosuppressive regimens, the true incidence of posttransplant PCP is unknown. For those patients who have risk factors such as the need

Table 4: Therapeutic options for treating *Pneumocystis* pneumonia

Agents	Dosing	Comments
Trimethoprim-sulfamethoxazole (TMP-SMX)	15–20 mg/kg/day of the TMP component given IV in divided doses every 6–8 hours often in combination with corticosteroids (see below); for milder disease, two double-strength tablets can be given po bid-tid	• TMP-SMX is the **drug of choice** and is considered to be the most effective systemic therapy for PCP. Hydration should be maintained • Patients on high-dose TMP-SMX should have regular monitoring of cell counts, creatinine and potassium
Pentamidine isesthionate	4 mg/kg/day IV initially over 1–2 hours; dose reduction to 2–3 mg/kg/day if needed	• Pentamidine side effects include pancreatitis, hypoglycemia, hyperglycemia, bone marrow suppression, renal failure and electrolyte disturbances • Pancreatic dysfunction may suggest the need for avoidance in pancreas transplantation
Atovaquone	750 mg po bid (optimal dose uncertain; 1500 bid used anecdotally)	• Atovaquone is available in an oral suspension only • Atovaquone has variable oral absorption (best with fatty foods) • Atovaquone is approved only for mild and moderate PCP
Primaquine and clindamycin	Primaquine 15–30 mg po qd in combination with clindamycin 600–900 mg IV or po q6–8 hours	• This combination has been studied in mild to moderate PCP in AIDS • Long-term use of clindamycin can predispose to infection with *Clostridium difficile* • Primaquine should be avoided in G6PD deficiency
Dapsone and trimethoprim	Dapsone 100 mg po qd used in combination with trimethoprim 15 mg/kg/day po divided tid	• This combination has been used with sulfa allergy, though dapsone may elicit sulfa allergies as well
Trimetrexate with folinic acid	Trimetrexate 45 mg/m^2/day IV (or 1.5 mg/kg/day IV in patients <50 kg) with folinic acid 20 mg/m^2 po or IV every 6 hours (80 mg/m^2 total daily); Folinic acid therapy extends $\geq$ 3 days beyond trimetrexate therapy	• Trimetrexate causes bone marrow suppression and must be used with folinic acid, 10 mg po qd • Outcomes are inferior to TMP-SMX in AIDS • Trimetrexate is no longer commercially available in the United States
Pyrimethamine and sulfadiazine	Pyrimethamine load of 100–200 mg po, followed by 50–100 mg po qd in combination with sulfadiazine 4 g po qd in divided doses	• Limited data available on this regimen • Usually with folinic acid 10mg po qd to reduce bone marrow toxicity
Macrolide and SMX	Macrolides such as clarithromycin or azithromycin in combination with sulfamethoxazole may be synergistic *in vivo* (49)	• Few clinical data to support the use of this combination. No recommendations available for dosing or duration of therapy
Caspofungin and TMP-SMX	70 mg IV loading dose of caspofungin on day one, followed by 50 mg IV daily after in combination with TMP-SMX (dose reduced in the setting of moderate to severe hepatic dysfunction)	• Echinocandins have activity against *Pneumocystis* in animal models (50,51) • Case reports exist of caspofungin use in combination with TMP-SMX and other drugs for PCP (52–55) • Clinical efficacy compared to TMP-SMX alone remains unknown
Adjunctive agents		
Corticosteroids	40 mg–60 mg of prednisone (or equivalent) po bid with taper after 5–7 days over a period of 1–2 weeks	• Corticosteroids are best administered within 72 hours in the setting of hypoxia ($pAO_2 < 70$ mmHg) • Commonly used but not well studied in transplantation • May require prolonged taper to avoid immune reconstitution pneumonitis
Colony-stimulating factors	Ideal dosing unknown	• Use of GM-CSF as an adjuvant has been studied in animal models (56) • No clinical data in humans

AIDS = acquired immunodeficiency syndrome; G6PD = glucose-6-phosphate dehydrogenase; GM-CSF = granulocyte/macrophage colony stimulating factor; PCP = *Pneumocystis* pneumonia; TMP-SMX = trimethoprim-sulfamethoxazole.

Table 5: Specific prophylactic agents for prevention of *Pneumocystis* listed by preference

Agents	Dosing	Comments
Trimethoprim-sulfamethoxazole (TMP-SMX, cotrimoxazole)	Can be given at 80 mg TMP/400 mg SMX or 160 mg TMP/800 mg SMX po (single or double strength) daily or three times weekly	• TMP-SMX remains the **drug of choice** for PCP prophylaxis (59) • Daily regimens may be required to have efficacy for other forms of posttransplant infections
Dapsone(4,4′-diaminodiphenylsulfone)	50–100 mg po qd	• Dapsone is considered a second-line agent for the prophylaxis of PCP (60) • Side effects may be more common among solid organ transplant recipients (61) • Avoid in G6PD deficiency, methemoglobin reductase deficiency • Uncommon allergy to sulfone or sulfa-containing agents • Generally not recommended in with history of severe sulfa reactions (desquamation, neutropenia, interstitial nephritis or hepatitis).
Atovaquone	1500 mg po qd (as single dose)	• Clinical trial data in HIV patients who could not tolerate TMP-SMX showed atovaquone to be equivalent to dapsone in preventing PCP (62) • Data in solid organ transplant recipients show it to be well-tolerated (19,63) • Failures of atovaquone have been reported at doses of 1000 mg or less daily (19,64)
Pentamidine	300 mg administered through aerosolized nebulizer q 3–4 weeks	• Pentamidine requires administration by experienced personnel with a nebulizer producing droplets of 1–3μ • Pentamidine is well-tolerated with minimal side effects other than cough and bronchospasm • There is a higher incidence of breakthrough infection compared to TMP-SMX or dapsone • Reports of disseminated infection involving the thyroid in HIV cases receiving inhaled pentamidine as prophylaxis (65)
Clindamycin and pyrimethamine	Up to 300 mg of clindamycin po qd with 15 mg of pyrimethamine po qd (some clinicians have administered this regimen 3 times weekly instead of daily)	• Somewhat efficacious in AIDS, though less effective than TMP-SMX or dapsone (66) • Failure rate higher than for aerosolized pentamidine • Gastrointestinal intolerance may be limiting

AIDS = acquired immunodeficiency syndrome; HIV = human immunodeficiency virus; PCP = *Pneumocystis* pneumonia; TMP-SMX = trimethoprim-sulfamethoxazole.

for increasing immunosuppression in the face of graft rejection, recurrent or chronic active infection with CMV, prolonged courses of corticosteroid therapy (e.g. >20 mg daily of prednisone for at least 2 weeks), prolonged neutropenia, or flares of autoimmune disease, prophylaxis is generally indicated. Lung transplant recipients are always considered at high risk for PCP (57). In any transplant population, the risk has always been considered highest within the first 6 months posttransplant, though features outlined above may prolong that risk. A more recent single center study found that most PCP cases occurring among transplant recipients now occur several years after the procedure and involve patients no longer taking effective prophylaxis (58).

In general, anti-*Pneumocystis* prophylaxis is recommended for all solid organ transplant recipients for at least 6–12 months posttransplant, though longer durations should be considered (Grade III). For lung and small bowel transplant recipients, as well as any transplant patient with a history of prior PCP infection or chronic CMV disease, lifelong prophylaxis may be indicated (Grade III). Agents used for prophylaxis are outlined in Table 5.

Practice recommendations regarding prophylaxis include TMP-SMX as the drug of choice for prophylaxis of PCP (Grade I). All other prophylactic agents should be considered second-line agents due to breadth of coverage, drug intolerances, cost, and efficacy issues that are not favorable compared to TMP-SMX.

The side effects of TMP-SMX dosing in prophylaxis are less common than with therapy, and rarely necessitate cessation of treatment. Bone marrow suppression may be potentiated by concomitant administration of other myelosuppressive agents. Rash may occur, spanning the gamut of benign reactions to Stevens-Johnson syndrome. Other

potential adverse effects include hepatitis, interstitial nephritis, aseptic meninigits, and pancreatitis. Trimethoprim has the capacity to inhibit potassium and creatinine secretion in the renal tubules, resulting in hyperkalemia and an elevation of serum creatinine that does not necessarily reflect true renal function. Patients on TMP-SMX may need laboratory monitoring of renal function and electrolytes including potassium levels. Other lab testing may be indicated in select cases.

Dapsone is often used as a second-line agent for PCP prophylaxis. Some reports of daily dapsone use have included it in combination with pyrimethamine at 25–50 mg once weekly. Although it may be tolerated in transplant patients who cannot receive TMP-SMX, it is generally not recommended in those who suffer severe TMP-SMX or sulfa reactions such as desquamation, neutropenia, interstitial nephritis, or hepatitis. It is also generally contraindicated in those patients with documented glucose-6-phosphate dehydrogenase (G6PD) deficiencies. The most commonly associated side effects of dapsone include hemolytic anemia and methemoglobinemia. Classically these symptoms are associated with G6PD enzyme deficiency, though G6PD deficiency is not a prerequisite (61).

Atovaquone is well-studied in the HIV population and has also been studied in small prospective trials of stem cell and solid organ transplant recipients (19). Available only in a suspension, atovaquone acts by inhibiting mitochondrial electron transport in susceptible *Pneumocystis*. Absorption is enhanced by fatty foods and decreased in the setting of diarrhea. Rash and gastrointestinal complaints are the most common side effects. Increased hepatic transaminases are rarely noted. Although ideal dosing may be unclear, breakthrough infections have been documented in patients taking 1000 mg or less daily (19,64).

Inhaled pentamidine should be considered a third-line agent. It is less effective overall compared to TMP-SMX, dapsone or atovaquone. Use of inhaled pentamidinehas been associated with breakthrough infections, notably in the upper lung zones. There is also some concern that inhaled pentamidine may negatively affect the sensitivity of diagnostic assays using respiratory secretions in patients with PCP (67).

Infection control issues

Pneumocystis jiroveci has traditionally not been thought of as a healthcare associated infection. Outbreaks among susceptible transplant recipients have been documented (6–11,68). A possible explanation for clustered infections could be person-to-person transmission—a hypothesis supported by some molecular typing studies of *Pneumocystis* from infected cases (6,7,9) and animal studies. Older studies have also shown that *Pneumocystis* can be detected in air samples from hospital patient care rooms using PCR techniques (69,70). The debate for a role of person-to-person transmission versus an unidentified environmental common source exposure is unresolved in these outbreaks. Some authors recommend strict hospital segregation of immunocompromised patients with PCP and the use of facemask filtering to prevent transmission among infected individuals (7). However, prophylaxis in susceptible patients is effective at preventing infection. Without definitive data, formal recommendations regarding infection control in the hospital or healthcare clinic cannot yet be made.

Acknowledgment

This manuscript was modified from a previous guideline written by Stanley I. Martin and Jay A. Fishman published in the *American Journal of Transplantation* 2009; 9(Suppl 4): S227–S233, and endorsed by the American Society of Transplantation/Canadian Society of Transplantation.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation.* Dr. Fishman is a consultant for Primera Dx.

References

1. Rodriguez M, Fishman JA. Prevention of infection due to *Pneumocystis* spp. in human immunodeficiency virus-negative immunocompromised patients. Clin Microbiol Rev 2004; 17: 770–782, table of contents.
2. Pifer LL, Hughes WT, Stagno S, Woods D. *Pneumocystis carinii* infection: Evidence for high prevalence in normal and immunosuppressed children. Pediatrics 1978; 61: 35–41.
3. Morris A, Wei K, Afshar K, Huang L. Epidemiology and clinical significance of pneumocystis colonization. J Infect Dis 2008; 197: 10–17.
4. Barlett MS, Fishman JA, Durkin MM, Queener SF, Smith JW. An improved rat model to study efficacy of drugs for treatment or prophylaxis of *Pneumocystis carinii* pneumonia. J Protozool 1989; 36: 77S–78S.
5. Kovacs JA, Gill VJ, Meshnick S, Masur H. New insights into transmission, diagnosis, and drug treatment of *Pneumocystis carinii* pneumonia. JAMA 2001; 286: 2450–2460.
6. de Boer MG, Bruijnesteijn van Coppenraet LE, Gaasbeek A, et al. An outbreak of *Pneumocystis jiroveci* pneumonia with 1 predominant genotype among renal transplant recipients: Interhuman transmission or a common environmental source? Clin Infect Dis 2007; 44: 1143–1149.
7. Schmoldt S, Schuhegger R, Wendler T, et al. Molecular evidence of nosocomial *Pneumocystis jirovecii* transmission among 16 patients after kidney transplantation. J Clin Microbiol 2008; 46: 966–971.
8. Hocker B, Wendt C, Nahimana A, Tonshoff B, Hauser PM. Molecular evidence of *Pneumocystis* transmission in pediatric transplant unit. Emerg Infect Dis 2005; 11: 330–332.
9. Sassi M, Ripamonti C, Mueller NJ, et al. Outbreaks of *Pneumocystis* pneumonia in 2 renal transplant centers linked to a single strain

of *Pneumocystis*: Implications for transmission and virulence. Clin Infect Dis 2012; 54: 1437–1444.
10. Arichi N, Kishikawa H, Mitsui Y, et al. Cluster outbreak of *Pneumocystis* pneumonia among kidney transplant patients within a single center. Transplant Proc 2009; 41: 170–172.
11. Yazaki H, Goto N, Uchida K, Kobayashi T, Gatanaga H, Oka S. Outbreak of *Pneumocystis jiroveci* pneumonia in renal transplant recipients: *P. jiroveci* is contagious to the susceptible host. Transplantation 2009; 88: 380–385.
12. Fishman JA. Prevention of infection due to *Pneumocystis carinii*. Antimicrob Agents Chemother 1998; 42: 995–1004.
13. Yale SH, Limper AH. *Pneumocystis carinii* pneumonia in patients without acquired immunodeficiency syndrome: Associated illness and prior corticosteroid therapy. Mayo Clin Proc 1996; 71: 5–13.
14. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med 2007; 357: 2601–2614.
15. Martin SI, Marty FM, Fiumara K, Treon SP, Gribben JG, Baden LR. Infectious complications associated with alemtuzumab use for lymphoproliferative disorders. Clin Infect Dis 2006; 43: 16–24.
16. Oz HS, Hughes WT. Novel anti-*Pneumocystis carinii* effects of the immunosuppressant mycophenolate mofetil in contrast to provocative effects of tacrolimus, sirolimus, and dexamethasone. J Infect Dis 1997; 175: 901–904.
17. Hardy AM, Wajszczuk CP, Suffredini AF, Hakala TR, Ho M. *Pneumocystis carinii* pneumonia in renal-transplant recipients treated with cyclosporine and steroids. J Infect Dis 1984; 149: 143–147.
18. Lufft V, Kliem V, Behrend M, Pichlmayr R, Koch KM, Brunkhorst R. Incidence of *Pneumocystis carinii* pneumonia after renal transplantation. Impact of immunosuppression. Transplantation 1996; 62: 421–423.
19. Fishman JA. Prevention of infection caused by *Pneumocystis carinii* in transplant recipients. Clin Infect Dis 2001; 33: 1397–1405.
20. Munoz P, Munoz RM, Palomo J, Rodriguez-Creixems M, Munoz R, Bouza E. *Pneumocystis carinii* infection in heart transplant recipients. Efficacy of a weekend prophylaxis schedule. Medicine (Baltimore) 1997; 76: 415–422.
21. Arend SM, Westendorp RG, Kroon FP, et al. Rejection treatment and cytomegalovirus infection as risk factors for *Pneumocystis carinii* pneumonia in renal transplant recipients. Clin Infect Dis 1996; 22: 920–925.
22. Pliquett RU, Asbe-Vollkopf A, Hauser PM, et al. A *Pneumocystis jirovecii* pneumonia outbreak in a single kidney-transplant center: Role of cytomegalovirus co-infection. Eur J Clin Microbiol Infect Dis 2012; 31: 2429–2437.
23. Masur H, Ognibene FP, Yarchoan R, et al. CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection. Ann Intern Med 1989; 111: 223–231.
24. Castagnola E, Dini G, Lanino E, et al. Low CD4 lymphocyte count in a patient with *P. carinii* pneumonia after autologous bone marrow transplantation. Bone Marrow Transplant 1995; 15: 977–978.
25. Sepkowitz KA, Brown AE, Telzak EE, Gottlieb S, Armstrong D. *Pneumocystis carinii* pneumonia among patients without AIDS at a cancer hospital. JAMA 1992; 267: 832–837.
26. Mansharamani NG, Balachandran D, Vernovsky I, Garland R, Koziel H. Peripheral blood CD4+ T-lymphocyte counts during *Pneumocystis carinii* pneumonia in immunocompromised patients without HIV infection. Chest 2000; 118: 712–720.
27. Kovacs JA, Hiemenz JW, Macher AM, et al. *Pneumocystis carinii* pneumonia: A comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med 1984; 100: 663–671.
28. Fujii T, Nakamura T, Iwamoto A. Pneumocystis pneumonia in patients with HIV infection: Clinical manifestations, laboratory findings, and radiological features. J Infect Chemother 2007; 13: 1–7.
29. Kovacs JA, Ng VL, Masur H, et al. Diagnosis of *Pneumocystis carinii* pneumonia: Improved detection in sputum with use of monoclonal antibodies. N Engl J Med 1988; 318: 589–593.
30. LaRocque RC, Katz JT, Perruzzi P, Baden LR. The utility of sputum induction for diagnosis of *Pneumocystis* pneumonia in immunocompromised patients without human immunodeficiency virus. Clin Infect Dis 2003; 37: 1380–1383.
31. Procop GW, Haddad S, Quinn J, et al. Detection of *Pneumocystis jiroveci* in respiratory specimens by four staining methods. J Clin Microbiol 2004; 42: 3333–3335.
32. O'Donnell WJ, Pieciak W, Chertow GM, Sanabria J, Lahive KC. Clearance of *Pneumocystis carinii* cysts in acute *P. carinii* pneumonia: Assessment by serial sputum induction. Chest 1998; 114: 1264–1268.
33. Alanio A, Desoubeaux G, Sarfati C, et al. Real-time PCR assay-based strategy for differentiation between active *Pneumocystis jirovecii* pneumonia and colonization in immunocompromised patients. Clin Microbiol Infect 2011; 17: 1531–1537.
34. Arcenas RC, Uhl JR, Buckwalter SP, et al. A real-time polymerase chain reaction assay for detection of *Pneumocystis* from bronchoalveolar lavage fluid. Diagn Microbiol Infect Dis 2006; 54: 169–175.
35. Brancart F, Rodriguez-Villalobos H, Fonteyne PA, Peres-Bota D, Liesnard C. Quantitative TaqMan PCR for detection of *Pneumocystis jiroveci*. J Microbiol Methods 2005; 61: 381–387.
36. Linssen CF, Jacobs JA, Beckers P, et al. Inter-laboratory comparison of three different real-time PCR assays for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage fluid samples. J Med Microbiol 2006; 55(Pt 9): 1229–1235.
37. Larsen HH, Kovacs JA, Stock F, et al. Development of a rapid real-time PCR assay for quantitation of *Pneumocystis carinii* f. sp. carinii. J Clin Microbiol 2002; 40: 2989–2993.
38. Botterel F, Cabaret O, Foulet F, Cordonnier C, Costa JM, Bretagne S. Clinical significance of quantifying *Pneumocystis jirovecii* DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. J Clin Microbiol 2012; 50: 227–231.
39. Thomas CF, Jr., Limper AH. *Pneumocystis* pneumonia: Clinical presentation and diagnosis in patients with and without acquired immune deficiency syndrome. Semin Respir Infect 1998; 13: 289–295.
40. Stover DE, Zaman MB, Hajdu SI, Lange M, Gold J, Armstrong D. Bronchoalveolar lavage in the diagnosis of diffuse pulmonary infiltrates in the immunosuppressed host. Ann Intern Med 1984; 101: 1–7.
41. Bondoc AY, White DA. Granulomatous *Pneumocystis carinii* pneumonia in patients with malignancy. Thorax 2002; 57: 435–437.
42. Studemeister A, Dass S. A patient with dyspnea, cough, and fever. Clin Infect Dis 2006; 43: 1461–1462, 1490–1461.
43. Akamatsu N, Sugawara Y, Kaneko J, Tamura S, Makuuchi M. Preemptive treatment of fungal infection based on plasma (1 –> 3)beta-D-glucan levels after liver transplantation. Infection 2007; 35: 346–351.
44. Kawagishi N, Miyagi S, Satoh K, Akamatsu Y, Sekiguchi S, Satomi S. Usefulness of beta-D glucan in diagnosing *Pneumocystis carinii* pneumonia and monitoring its treatment in a living-donor liver-transplant recipient. J Hepatobiliary Pancreat Surg 2007; 14: 308–311.
45. Costa JM, Botterel F, Cabaret O. Association between circulating DNA, serum (1->3)-beta-D-glucan, and pulmonary fungal burden in *Pneumocystis* pneumonia. Clin Infect Dis 2012; 55: e5–e8.

46. Sax PE, Komarow L, Finkelman MA, et al. Blood (1->3)-beta-D-glucan as a diagnostic test for HIV-related *Pneumocystis jirovecii* pneumonia. Clin Infect Dis 2011; 53: 197–202.
47. Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME. Accuracy of β-D-glucan for the diagnosis of *Pneumocystis jirovecii* pneumonia: A meta-analysis. Clin Microbiol Infect 2013; 19: 39–49.
48. Yasuoka A, Tachikawa N, Shimada K, Kimura S, Oka S. (1–>3) beta-D-glucan as a quantitative serological marker for *Pneumocystis carinii* pneumonia. Clin Diagn Lab Immunol 1996; 3: 197–199.
49. Fishman JA. Treatment of infection due to *Pneumocystis carinii*. Antimicrob Agents Chemother 1998; 42: 1309–1314.
50. Schmatz DM, Romancheck MA, Pittarelli LA, et al. Treatment of *Pneumocystis carinii* pneumonia with 1,3-beta-glucan synthesis inhibitors. Proc Natl Acad Sci USA 1990; 87: 5950–5954.
51. Powles MA, Liberator P, Anderson J, et al. Efficacy of MK-991 (L-743,872), a semisynthetic pneumocandin, in murine models of *Pneumocystis carinii*. Antimicrob Agents Chemother 1998; 42: 1985–1989.
52. Annaloro C, Della Volpe A, Usardi P, Lambertenghi Deliliers G. Caspofungin treatment of *Pneumocystis* pneumonia during conditioning for bone marrow transplantation. Eur J Clin Microbiol Infect Dis 2006; 25: 52–54.
53. Utili R, Durante-Mangoni E, Basilico C. Efficacy of caspofungin addition to trimethoprim-sulfamethoxazole treatment for severe pneumocystis pneumonia in solid organ transplant recipients. Transplantation 2007; 84: 685–688.
54. Armstrong-James D, Stebbing J, John L, et al. A trial of caspofungin salvage treatment in PCP pneumonia. Thorax 2011; 66: 537–538.
55. Ceballos ME, Ortega M, Andresen M. Successful treatment with echinocandin in an HIV-infected individual failing first-line therapy for *Pneumocystis jirovecii* pneumonia. AIDS 2011; 25: 2192–2193.
56. Mandujano JF, D'Souza NB, Nelson S. Granulocyte-macrophage colony stimulating factor and *Pneumocystis carinii* pneumonia in mice. Am J Respir Crit Care Med 1995; 151: 1233–1238.
57. Gordon SM, LaRosa SP, Kalmadi S. Should prophylaxis for *Pneumocystis carinii* pneumonia in solid organ transplant recipients ever be discontinued? Clin Infect Dis 1999; 28: 240–246.
58. McKinnell JA, Cannella AP, Kunz DF, et al. *Pneumocystis* pneumonia in hospitalized patients: A detailed examination of symptoms, management, and outcomes in human immunodeficiency virus (HIV)-infected and HIV-uninfected persons. Transpl Infect Dis 2012; 14: 510–518.
59. Ioannidis JP, Cappelleri JC, Skolnik PR, Lau J, Sacks HS. A meta-analysis of the relative efficacy and toxicity of *Pneumocystis carinii* prophylactic regimens. Arch Intern Med 1996; 156: 177–188.
60. Hughes WT. Use of dapsone in the prevention and treatment of *Pneumocystis carinii* pneumonia: A review. Clin Infect Dis 1998; 27: 191–204.
61. Lee I, Barton TD, Goral S, et al. Complications related to dapsone use for *Pneumocystis jirovecii* pneumonia prophylaxis in solid organ transplant recipients. Am J Transplant 2005; 5: 2791–2795.
62. El-Sadr WM, Murphy RL, Yurik TM, et al. Atovaquone compared with dapsone for the prevention of *Pneumocystis carinii* pneumonia in patients with HIV infection who cannot tolerate trimethoprim, sulfonamides, or both. Community Program for Clinical Research on AIDS and the AIDS Clinical Trials Group. N Engl J Med 1998; 339: 1889–1895.
63. Gabardi S, Millen P, Hurwitz S, Martin S, Roberts K, Chandraker A. Atovaquone versus trimethoprim-sulfamethoxazole as *Pneumocystis jirovecii* pneumonia prophylaxis following renal transplantation. Clin Transplant 2012; 26: E184–E190.
64. Rodriguez M, Sifri CD, Fishman JA. Failure of low-dose atovaquone prophylaxis against *Pneumocystis jiroveci* infection in transplant recipients. Clin Infect Dis 2004; 38: e76–e78.
65. Zavascki AP, Maia AL, Goldani LZ. *Pneumocystis jiroveci* thyroiditis: Report of 15 cases in the literature. Mycoses 2007; 50: 443–446.
66. Barber BA, Pegram PS, High KP. Clindamycin/primaquine as prophylaxis for *Pneumocystis carinii* pneumonia. Clin Infect Dis 1996; 23: 718–722.
67. Levine SJ, Masur H, Gill VJ, et al. Effect of aerosolized pentamidine prophylaxis on the diagnosis of *Pneumocystis carinii* pneumonia by induced sputum examination in patients infected with the human immunodeficiency virus. Am Rev Respir Dis 1991; 144: 760–764.
68. Thomas CF, Jr., Limper AH. Current insights into the biology and pathogenesis of *Pneumocystis* pneumonia. Nat Rev Microbiol 2007; 5: 298–308.
69. Bartlett MS, Vermund SH, Jacobs R, et al. Detection of *Pneumocystis carinii* DNA in air samples: Likely environmental risk to susceptible persons. J Clin Microbiol 1997; 35: 2511–2513.
70. Olsson M, Lidman C, Latouche S, et al. Identification of *Pneumocystis carinii* f. sp. *hominis* gene sequences in filtered air in hospital environments. J Clin Microbiol 1998; 36: 1737–1740.