Curr Fungal Infect Rep (2015) 9:285–291
DOI 10.1007/s12281-015-0244-z

Pneumocystis Pneumonia in Solid Organ Transplant Recipients

Ricardo M. La Hoz[1] · **John W. Baddley**[2,3]

Published online: 30 September 2015

Abstract *Pneumocystis* pneumonia (PCP) remains an important opportunistic infection among solid organ transplant (SOT) recipients. The diagnosis of PCP should be considered in patients with new onset of fever, pulmonary symptoms, and hypoxemia. The introduction of PCP prophylaxis for SOT recipients has dramatically modified the epidemiological landscape of this infection; we are currently experiencing the era of "late PCP," where the majority of cases occur more than 12 months after transplantation in patients with PCP risk factors in whom prophylaxis has been discontinued. Despite remarkable advancement in our understanding of the biology, mode of transmission, epidemiology, and clinical manifestations of PCP, there remains a paucity of data regarding the performance of contemporary diagnostic tools for PCP in transplant recipients. Although there is a low incidence of PCP in this population, associated morbidity and mortality may be high, necessitating additional studies aimed at diagnosis.

This article is part of the Topical Collection on *Epidemiology of Fungal Infections*

✉ Ricardo M. La Hoz
Ricardo.LaHoz@UTSouthwestern.edu

[1] Division of Infectious Diseases, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9113, USA

[2] Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA

[3] Birmingham Veterans Affairs Medical Center, Birmingham, AL, USA

Keywords Transplantation · Opportunistic infection · Pneumocystosis · *Pneumocystis jiroveci* · Fungal infection · Epidemiology · Solid organ transplant · *Pneumocystis* pneumonia · PCP · PCP prophylaxis · Diagnostic tools

Introduction

Pneumocystis jiroveci, a fungus, is the causative agent of *Pneumocystis* pneumonia (PCP). The diagnosis of PCP should be considered in solid organ transplant (SOT) recipients with new onset of fever, pulmonary symptoms, and hypoxemia. The contemporary incidence of this infection among SOT recipients is on average <1 %; however, the average incidence of PCP in SOT recipients prior to the implementation of prophylaxis was 10–12 % but varied depending on the on the organ type and immunosuppressive regimen [1, 2]. The introduction of PCP prophylaxis for SOT recipients has dramatically modified the epidemiological landscape of this infection. Currently, we are experiencing the era of "late PCP," where the majority of infections are diagnosed greater than 12-months post-transplant in patients who are no longer receiving prophylaxis.

Because there is no available culture system for *Pneumocystis jiroveci*, the mainstay of the diagnosis of PCP has been microscopic examination of lower respiratory tract samples. Traditionally, the diagnostic yield of lower respiratory tract microscopy with staining in non-HIV patients has historically been considered lower when compared to those who are HIV-infected [2]. Newer diagnostic methods are being explored and will hopefully increase the validity of diagnosis in SOT patients.

Herein, we review the epidemiology of PCP in solid organ transplant recipients, with a focus on changing epidemiology, transmission, and diagnosis.

Epidemiology

The average incidence of PCP in SOT recipients prior to the implementation of prophylaxis was 10–12 % but varied depending on the on the organ type and immunosuppressive regimen [1, 2]. During this era, the risk of PCP in kidney transplant recipients ranged from 0.6 to 14 %; similar frequency of infection was described in liver transplant recipients (3–11 %) [3–5]. The likelihood of developing disease in heart transplant recipients was in the order of 2–41 %; whereas lung and combined heart-lung recipients had the highest risk ranging from 6.5 to 43 % [6–9]. Timing of PCP infection was highest between the second- and sixth-month post-transplantation [2]. Proposed risks factors for early-onset PCP included immunosuppressive therapies (anti-thymocyte globulin, alemtuzumab, calcineurin inhibitors), CMV infection, and allograft rejection [2, 8, 10–15].

The American Society of Transplantation (AST) Infectious Diseases Community of Practice recommends PCP prophylaxis for all solid organ transplant recipients for at least 6–12 months [16]. At completion of initial prophylaxis, the need for additional prophylaxis should be evaluated. Transplant recipients who require increased immunosuppression in the face of graft rejection may have a longer at-risk period. In addition, the need to re-initiate PCP prophylaxis should be considered in those patients who develop CMV infection or acquire other risk factors for PCP. Many transplant professionals advocate for life-long PCP prophylaxis in higher-risk organ groups such as heart, lung, and heart-lung transplant recipients [13, 16, 17].

Trimethoprim-sulfamethoxazole (TMP-SMX) is the prophylactic agent of choice for PCP, while dapsone, atovaquone, clindamycin plus pyrimethamine, and pentamidine are considered alternative or second-line agents [13, 16]. The advantages of TMP-SMX over other prophylactic agents include higher efficacy, lower cost, and breadth of coverage for other opportunistic infections. Multiple studies have demonstrated the efficacy of TMP-SMX as a prophylactic agent for PCP in SOT recipients [18–20]; breakthrough infections are rare [21]. Breakthrough infections are more common in patients taking a non-TMP-SMX agent; these breakthrough episodes tend to have atypical presentations, and microscopy on respiratory samples is often negative [13]. Due to the benefits of TMP-SMX over other agents, it is worthwhile to consider re-introduction of TMP-SMX in those SOT recipients on other prophylactic regimens in the following scenarios: no clear contraindication to TMP-SMX; an alternative explanation for the adverse effect initially attributed to TMP-SMX is identified; or other drugs that could have lead to additive toxicity have been discontinued or dose reduced.

The introduction of prophylaxis has modified the landscape of the epidemiology of PCP in SOT recipients. In order to understand recent changes, it is necessary to evaluate studies describing the epidemiology of this disease among patients transplanted after the year 2000. The Transplant Associated Infection Surveillance Network (TRANSNET), consisting of 23 US transplant centers, performed prospective surveillance for fungal infections from 2001 to 2006 [22]. The surveillance cohort, defined as prevalent and incident transplant patients from the centers who developed infection, revealed that pneumocystosis represents 0–3 % of all invasive fungal infections in SOT recipients. The incidence cohort estimated a cumulative incidence of PCP at 12 months of <0.1 %; 75, 50, and 20 % of the cases occurred after 6, 12, and 36 months, respectively [22]. A single center retrospective study from Slovenia also conducted between 2001 and 2006 described 13 (2.2 %) cases among 601 renal transplant recipients [23]. All patients received induction with basiliximab, and the protocol for PCP prophylaxis consisted of TMP-SMX (80/400 mg daily) for 12 months. The median time from transplantation to diagnosis was 17 months (range 3–148 months). Ten of the 13 cases occurred more than 12 months after transplantation; none of the 3 patients who developed PCP within the first 12 months was receiving prophylaxis. Six of the 13 cases had recent or concomitant CMV infection. The mortality among patients with PCP was 23 %, owing to bacterial, viral, or fungal super-infection.

A single center retrospective study from Korea performed between 2008 and 2009 described a 12-month incidence of PCP of 0.9 % among liver transplant recipients [24]. The protocol for PCP prophylaxis at this high-volume center (performing more than 300 liver transplants per year) was TMP/SMX 160/800 mg every other day for 6 months. The median time from transplantation to PCP was 9.5 months (range 1–67 months); half of the cases experienced a recent episode of rejection. The crude mortality in this study for patients who developed PCP was 50 %. Two other studies from Canada and Spain have also described similar findings [25, 26]. Collectively, these data suggests that the incidence of PCP among SOT recipients receiving prophylaxis is low. Most cases of PCP currently occur more than 12 months after transplant and are associated with treatment for rejection and CMV infection. These findings emphasize the need to consider re-introduction of prophylaxis for PCP among those at risk.

Transmission

During the last two decades, there has been a dramatic increase in the number of studies reporting outbreaks and clusters of infections in transplant recipients [10, 18, 27–36]. To understand the results and implications of these studies, an overview of the pathogenesis and mode of transmission of this infection is warranted. When symptomatic PCP infection occurs, more than 50 % of patients have more than one strain of the organism [37–39]. Animal and human studies favor an airborne transmission route [40]. With regards to the mode of

acquisition, multiple facts point toward human-to-human transmission in cases of *Pneumocystis* pneumonia: (1) *Pneumocystis* organisms are host specific; rat, mouse, ferret, or horse species infect only their specific host and are not sources of human infection; and (2) there is no identifiable reservoir for *P. jiroveci* other than individuals infected with this organism. Serological data from infants indicate that most humans are infected within the first 2 to 4 years of life [41]. Given the ubiquitous nature of this early exposure, it is unlikely that the organism is transmitted only by humans with symptomatic infection. In the past, it was thought that most cases of PCP were due to reactivation. Recent data support the notion that disease can be the result of a recently acquired infection.

A systematic review of outbreaks and clusters of PCP in renal transplant recipients from 1980 to 2010 was recently published [33]. This study included a total of 16 outbreaks in renal transplant recipients, described in 15 articles. Interestingly, none of the studies reported a simultaneous increase in the incidence of pneumocystosis in immunocompromised hosts, including non-renal transplant recipients. The median number of cases was 12 per outbreak. Most outbreaks were marked by cases occurring in patients who were not receiving PCP prophylaxis. A period of low incidence of PCP (<2 %) preceding the outbreak was described in 75 % of the outbreaks and quoted as the rationale for the lack of chemoprophylaxis for this organism. Of note, 11 of the 15 studies describe a temporal association between the cessation of the outbreak and the introduction of prophylaxis with TMP-SMX. Treatment for rejection and CMV infection were risk factors for developing PCP during the outbreaks. A transmission map was frequently a part of the analysis and suggested either a common environmental source or patient-to-patient transmission. The lack of evidence for an environmental source and molecular analysis points toward patient-to-patient transmission. The use of more advanced molecular techniques, namely restriction fragment length polymorphisms (RFLP), reveals that no two cases of sporadic PCP are caused by the same stain [42]. Using RFLP, investigators were able to demonstrate that a single strain was responsible for outbreaks in two geographically distinct regions of Europe [36]. In an additional study [34], colonized individuals were purported as potential infectious sources for PCP cases. In summary, human-to-human transmission is likely to be more common than reactivation of prior infection. Although controversial, these findings provide support to the recommendations made by some experts to isolate patients with PCP from other susceptible hosts [16, 43, 44]. Without definitive data, formal recommendations regarding infection control cannot be made.

Clinical Presentation of PCP

The diagnosis of PCP should be considered in SOT recipients with new onset of fever, pulmonary symptoms, and hypoxemia. Groups at increased risk include those not receiving prophylaxis, those prescribed prophylactic regimes other than trimethoprim-sulfamethoxazole, or in those whose compliance and/or tolerance to prophylaxis has been poor [45, 46].

It is important to recognize that the clinical presentation of SOT recipients with PCP differs from that of HIV-infected patients with PCP [47–51]. Studies from the early HIV epidemic suggested a shorter duration of symptoms in HIV-negative patients with PCP when compared with those who had AIDS [47]. More contemporary studies that included and compared transplant recipients confirm these findings [48–50]. A retrospective single center study from the USA conducted from 1996 to 2008 included a total of 97 cases of confirmed PCP: 65 in HIV-positive and 32 in HIV-negative patients (19 SOT recipients) [49]. HIV-negative patients reported a shorter duration of symptoms, fewer days of dyspnea (10±2 days vs. 17±2 days; $P=0.02$) and of fever (8±2 days vs. 15±4 days; $P=0.02$). Otherwise, there were no statistical differences in the frequency of tachycardia, tachypnea, hypoxemia, and laboratory abnormalities including lactate dehydrogenase (354.9±29.2 vs. 496±50.5 U/L; $P=0.10$). A prospective multicenter study from France included a total of 544 cases of confirmed PCP from 2007 to 2010, 223 HIV-positive and 321 HIV-negative [50]. The HIV-negative sub-group included 99 SOT recipients (80 kidney, 8 liver, 8 heart, 3 lung). The median time from onset of respiratory symptoms to diagnosis was significantly shorter for non-HIV patients (5 vs. 21; $P<0.0001$). However, hypoxemia, intensive care admission, need for invasive or noninvasive mechanical ventilation, and shock were more common among non-HIV patients. Viral, bacterial, and fungal infections were common in the HIV-negative group, with 30.5 % having ≥1 microbial co-infection and 6 % having ≥2 microbial co-infections. These data emphasize the importance of ruling out concomitant infections in SOT recipients with PCP.

Radiography

There is a paucity of contemporary data regarding radiographic findings in SOT recipients with PCP. The most common findings on chest radiograph are diffuse bilateral alveolar or interstitial pulmonary infiltrates [49, 50]. Normal roentgenograms, consolidation, solitary, or multiple nodules with or without cavitations have also been described [52, 53]. High resolution computed tomography (HRCT) is considered more sensitive in the early phases of the infection [54]. Patchy or diffuse ground glass opacities sparing the lung periphery are the characteristic findings in HRCT; consolidations, nodules, cysts, pneumoceles, pneumothoraces, and other patterns have also been described [53]. Use of aerosolized pentamidine as prophylaxis has been associated with upper lobe infiltrates [55, 56]. In a study from France, all patients diagnosed with

PCP had a chest radiograph while computed tomography scans were performed when deemed necessary by the clinicians. For the 231 non-HIV patients, the percentage of chest radiographic findings was typical (bilateral interstitial or alveolointerstitial opacities) in 77 %; atypical findings (focal consolidation, pleural effusion, subpleural nodules, and cavitations) in 15 %; pneumothorax in 2.2 %; and a normal chest radiograph in 8 %. Diffuse ground glass opacities were considered typical findings in CT scans; septal lines and centrilobular nodules were also interpreted as supportive of a PCP diagnosis.

Diagnosis

Because there is no available culture system for this organism, the mainstay of the diagnosis of PCP has been microscopic examination of lower respiratory tract samples (induced sputum, bronchoalveolar lavage, transbronchial or open lung tissue biopsies). In general, more invasive procedures produce a higher diagnostic yield [2]. Noninvasive testing of a lower respiratory sample microscopic examination should be attempted as an initial step to confirm the diagnosis. Although *Pneumocystis* is rarely identified in expectorated sputum, the organism can be frequently detected in sputum induced by inhalation of aerosolized hypertonic saline [16, 57]. If PCP is not identified using this diagnostic modality, then a bronchoscopy with bronchoalveolar lavage (BAL) should be performed with consideration of a transbronchial biopsy. Video-assisted thoracoscopic biopsies are often reserved for those patients in whom other diagnostic approaches have been unrevealing [58, 59].

Numerous staining methods have been utilized for microscopy of lower respiratory tract samples [2]. Direct fluorescent antibody staining using a fluorescein-conjugated monoclonal antibody has the highest sensitivity as it can identify both trophic forms and cysts and has become a common technique used [60]. The diagnostic yield of lower respiratory tract microscopy with staining in non-HIV patients has historically been considered lower when compared to those who are HIV-infected [2, 45]. However, contemporary studies challenge this notion [49, 50]. A single center retrospective study from the USA suggested similar diagnostic yields in these two groups, while the study from France showed a higher diagnostic yield when using bronchoscopy in non-HIV patients (87 vs. 97 % P=0.0003). Despite the advances in diagnostic techniques, the exact performance in SOT recipients remains unknown.

The notion that the sensitivity of microscopy is lower in non-HIV patients, perhaps as a result of lower organism burden, has fueled the interest in other diagnostic modalities: polymerase chain reaction (PCR) and serology with serum ß-D-glucan. The studies with these diagnostic tools have the following limitations: scarcity of data specific to SOT recipients and lack specificity [16]. Using different techniques and target genes, PCR assays have been developed for the detection of PCP in induced sputum, BAL, and oral-wash samples [61–67]. A single center prospective study conducted from 2002 to 2005 enrolled 448 HIV-negative patients (21 SOT recipients) [65]. Using conventional PCR targeting the large subunit of mitochondrial RNA, the sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) were 87.2, 92.2, 51.5, and 98.7 %, respectively. The lack of specificity and low PPV result from inability of the assay to distinguish disease from colonization. A more recent study published by the same group suggests a better performance using real-time PCR; unfortunately, only 6 % (14/238) of the patients included in the study were SOT recipients [66]. Data assessing the performance of the ß-D-glucan test has similar limitations [68–71]. A study in HIV-infected patients, using a dichotomized value of 80 pg/ml showed a sensitivity, specificity, PPV, and NPV of 92, 65, 85, and 80 %, respectively (27). A retrospective study from Japan that included 295 consecutive patients (HIV-positive and HIV-negative) from 1998 to 2005 revealed a PPV and NPV of 61 and 89 % when using a cut-off level of 31.1 pg/ml [68]. A meta-analysis that included both HIV-positive and HIV-negative patients disclosed a sensitivity and specificity of 94.8 and 86.3 %, respectively; unfortunately, only 5 of the 14 studies included enrolled SOT recipients [71]. In clinical practice, the results of these two assays should be interpreted in the context of the clinical characteristics and pre-test probability. To optimize the performance of these diagnostic tests, clinicians should avoid using them indiscriminately and reserve them for patients with a compatible clinical presentation when other diagnostic tests are either not feasible or available.

Table 1 summarizes the AST recommendations for the diagnosis of PCP in SOT recipients [16]. Clinicians should consider PCP in their differential diagnosis in patients at risk as prompt diagnosis and initiation of therapy is essential to optimize outcomes [49, 50].

Conclusions

Since the first description of *Pneumocystis* pneumonia more than 100 years ago, there has been remarkable advancement in our understanding of the epidemiology, clinical manifestations, and diagnostic armamentarium. PCP remains an important opportunistic infection among transplant recipients, and the introduction of prophylaxis has dramatically modified the epidemiological landscape of this infection; we are currently experiencing the era of late PCP. Current studies emphasize the need to consider prolonging the prophylaxis course and reintroducing prophylaxis among patients who remain at risk for PCP. Although molecular diagnostic methods have improved our understanding of the epidemiology of PCP, there is a dearth of diagnostic testing data in SOT recipients;

Table 1 Recommendations for the diagnosis of PCP in SOT recipients [16]

1. Patients should undergo initial screening via multiple induced sputum samples All respiratory secretions should be stained using antibodies for PCP (immunofluorescent, immunoperoxidase, or similar) as well as routine stains for *Pneumocystis* and other organisms (Giemsa, Silver, and others). Use of PCR-based diagnostics on respiratory secretions can be considered. Samples should also be assayed for routine bacterial, fungal, mycobacterial, and other organisms to rule out concomitant infections. Evaluation for CMV or other respiratory viral co-infection, in particular, should be considered.
2. Clinicians should have a low threshold for bronchoscopy with BAL to obtain diagnostic samples. 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.
4. Measurement of plasma (1→3) ß-D-glucan levels can be considered and may suggest the diagnosis. This assay lacks specificity for *Pneumocystis*, however, and can be positive in the setting of other invasive fungal infections.
5. Open lung biopsies can be obtained when other diagnostic approaches have been unrevealing or where other concomitant diseases may be a concern. Video-assisted thoracoscopic (VATS) biopsies may be appropriate for some patients in this regard.

traditionally, sensitivity of testing is lower when compared to HIV-infected patients. Although there is a low incidence of PCP in the SOT population, associated morbidity and mortality may be high, necessitating additional studies aimed at diagnosis.

Compliance with Ethical Standards

Conflict of Interest Ricardo M. La Hoz declares that he has no conflict of interest. John W. Baddley has served as a consultant for Merck, Pfizer, and Astellas.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Fishman JA, Rubin RH. Infection in organ-transplant recipients. N Engl J Med. 1998;338(24):1741–51.
2. Rodriguez M, Fishman JA. Prevention of infection due to Pneumocystis spp. in human immunodeficiency virus-negative immunocompromised patients. Clin Microbiol Rev. 2004;17(4):770–82.
3. Colombo JL, Sammut PH, Langnas AN, et al. The spectrum of Pneumocystis carinii infection after liver transplantation in children. Transplantation. 1992;54(4):621–4.
4. Hayes MJ, Torzillo PJ, Sheil AG, et al. Pneumocystis carinii pneumonia after liver transplantation in adults. Clin Transplant. 1994;8(6):499–503.
5. Paya CV, Hermans PE, Washington 2nd JA, et al. Incidence, distribution, and outcome of episodes of infection in 100 orthotopic liver transplantations. Mayo Clin Proc. 1989;64(5):555–64.
6. Gryzan S, Paradis IL, Zeevi A, et al. Unexpectedly high incidence of Pneumocystis carinii infection after lung-heart transplantation. Implications for lung defense and allograft survival. Am Rev Respir Dis. 1988;137(6):1268–74.
7. Janner D, Bork J, Baum M, et al. Pneumocystis carinii pneumonia in infants after heart transplantation. J Heart Lung Transplant. 1996;15(8):758–63.
8. Muñoz P, Muñoz RM, Palomo J. Pneumocystis carinii infection in heart transplant recipients. efficacy of a weekend prophylaxis schedule. Medicine (Baltimore). 1997;76(6):415–22.
9. Dummer JS, Montero CG, Griffith BP, et al. Infections in heart-lung transplant recipients. Transplantation. 1986;41(6):725–9.
10. Lufft V, Kliem V, Behrend M, et al. Incidence of Pneumocystis carinii pneumonia after renal transplantation. Impact Immunosuppress Transplant. 1996;62(3):421–3.
11. 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(6):920–5.
12. Hardy AM, Wajszczuk CP, Suffredini AF, et al. Pneumocystis carinii pneumonia in renal-transplant recipients treated with cyclosporine and steroids. J Infect Dis. 1984;149(2):143–7.
13. Fishman JA. Prevention of infection caused by Pneumocystis carinii in transplant recipients. Clin Infect Dis. 2001;33(8):1397–405.
14. Ballardie FW, Winearls CG, Williams G. Cyclosporin and steroids in renal transplantation: risk of Pneumocystis carinii pneumonia [letter]? Lancet. 1984;2:638–9.
15. Franson TR, Kauffman Jr HM, Adams MB. Cyclosporine therapy and refractory Pneumocystis carinii pneumonia: a potential association. Arch Surg. 1987;122:1034–5.
16. Martin SI, Fishman JA, AST Infectious Diseases Community of Practice. Pneumocystis pneumonia in solid organ transplantation. Am J Transplant. 2013;13 Suppl 4:272–9.
17. Gordon SM, LaRosa SP, Kalmadi S, et al. Should prophylaxis for Pneumocystis carinii pneumonia in solid organ transplant recipients ever be discontinued? Clin Infect Dis. 1999;28(2):240–6.
18. Branten AJ, Beckers PJ, Tiggeler RG, et al. Pneumocystis carinii pneumonia in renal transplant recipients. Nephrol Dial Transplant. 1995;10(7):1194–7.
19. Olsen SL, Renlund DG, O'Connell JB, et al. Prevention of Pneumocystis carinii pneumonia in cardiac transplant recipients by trimethoprim sulfamethoxazole. Transplantation. 1993;56(2):359–62.
20. Torre-Cisneros J, de la Mata M, Lopez-Cillero P, et al. Effectiveness of daily low-dose cotrimoxazole prophylaxis for Pneumocystis carinii pneumonia in liver transplantation—an open clinical trial. Transplantation. 1996;62(10):1519–21.
21. Saah AJ, Hoover DR, Peng Y, et al. Predictors for failure of Pneumocystis carinii pneumonia prophylaxis. Multicenter AIDS Cohort Study. JAMA. 1995;273(15):1197–202.
22. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–11.
23. Borstnar S, Lindic J, Tomazic J, et al. Pneumocystis jirovecii pneumonia in renal transplant recipients: a national center experience. Transplant Proc. 2013;45(4):1614–7.
24. Choi YI, Hwang S, Park GC, et al. Clinical outcomes of Pneumocystis carinii pneumonia in adult liver transplant recipients. Transplant Proc. 2013;45(8):3057–60.

25. Wang EH, Partovi N, Levy RD, et al. Pneumocystis pneumonia in solid organ transplant recipients: not yet an infection of the past. Transpl Infect Dis. 2012;14(5):519–25.
26. Perez-Ordoño L, Hoyo I, Sanclemente G, et al. Late-onset Pneumocystis jirovecii pneumonia in solid organ transplant recipients. Transpl Infect Dis. 2014;16(2):324–8.
27. Hennequin C, Page B, Roux P, et al. Outbreak of Pneumocystis carinii pneumonia in a renal transplant unit. Eur J Clin Microbiol Infect Dis. 1995;14(2):122–6.
28. Rabodonirina M, Vanhems P, Couray-Targe S, et al. Molecular evidence of interhuman transmission of Pneumocystis pneumonia among renal transplant recipients hospitalized with HIV-infected patients. Emerg Infect Dis. 2004;10(10):1766–73.
29. de Boer MG, Bruijnesteijn van Coppenraet LE. 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(9): 1143–9.
30. 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(3):966–71.
31. Yazaki H, Goto N, Uchida K, et al. Outbreak of Pneumocystis jiroveci pneumonia in renal transplant recipients: P. jiroveci is contagious to the susceptible host. Transplantation. 2009;88(3):380–5.
32. 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(1):170–2.
33. de Boer MG, de Fijter JW, Kroon FP. Outbreaks and clustering of Pneumocystis pneumonia in kidney transplant recipients: a systematic review. Med Mycol. 2011;49(7):673–80.
34. Le Gal S, Damiani C, Rouillé A, et al. A cluster of Pneumocystis infections among renal transplant recipients: molecular evidence of colonized patients as potential infectious sources of Pneumocystis jirovecii. Clin Infect Dis. 2012;54(7):e62–71.
35. 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(10):1437–44.
36. Rostved AA, Sassi M, Kurtzhals JA, et al. Outbreak of pneumocystis pneumonia in renal and liver transplant patients caused by genotypically distinct strains of Pneumocystis jirovecii. Transplantation. 2013;96(9):834–42.
37. Nahimana A, Blanc DS, Francioli P, Bille J, Hauser PM. Typing of Pneumocystis carinii f. sp. hominis by PCR-SSCP to indicate a high frequency of co-infections. J Med Microbiol. 2000;49(8):753–8.
38. Hauser PM, Blanc DS, Sudre P, et al. Genetic diversity of Pneumocystis carinii in HIV-positive and -negative patients as revealed by PCR-SSCP typing. AIDS. 2001;15(4):461–6.
39. Ma L, Kutty G, Jia Q, et al. Analysis of variation in tandem repeats in the intron of the major surface glycoprotein expression site of the human form of Pneumocystis carinii. J Infect Dis. 2002;186(11): 1647–54.
40. Kovacs JA, Gill VJ, Meshnick S, et al. New insights into transmission, diagnosis and drug treatment of Pneymocystis carinii pneumonia. JAMA. 2001;286:2450–60.
41. Meuwissen JH, Tauber I, Leeuwenberg AD, et al. Parasitologic and serologic observations of infection with Pneumocystis in humans. J Infect Dis. 1977;136(1):43–9.
42. Ripamonti C, Orenstein A, Kutty G, et al. Restriction fragment length polymorphism typing demonstrates substantial diversity among Pneumocystis jirovecii isolates. J Infect Dis. 2009;200(10):1616–22.
43. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Health Care Infection Control Practices Advisory Committee. 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Am J Infect Control. 2007;35(10 Suppl 2):S65–164.
44. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238.
45. Hiemenz JW, Groll AH, Walsh TJ. Infections caused by uncommon fungi in patients undergoing hematopoietic stem cell or solid organ transplantation. In: Bowden RA, Ljungman P, Snydman DR, editors. Transplant infections. 3rd ed. Phildelphia: Lippincott Williams & Wilkins; 2010.
46. Kovacs JA, Masur H. Evolving health effect of Pneumocystis: one hundred years of progress in diagnosis and treatment. JAMA. 2009;301(24):2578–85.
47. Kovacs HJW, Macher AM, et al. Pneumocystic carinii Pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med. 1984;100(5):666–71.
48. Nüesch R, Bellini C, Zimmerli W. Pneumocystis carinii pneumonia in human immunodeficiency virus (HIV)-positive and HIV-negative immunocompromised patients. Clin Infect Dis. 1999;29(6):1519–23.
49. 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(5):510–8.
50. Roux A, Canet E, Valade S, Gangneux-Robert F, et al. Pneumocystis jirovecii pneumonia in patients with or without AIDS. France Emerg Infect Dis. 2014;20(9):1490–7.
51. Ng VL, Yajko DM, Hadley WK. Extrapulmonary Pneumocystosis. Clin Microbiol Rev. 1997;10(3):401–18.
52. Opravil M, Marincek B, Fuchs WA, et al. Shortcomings of chest radiography in detecting Pneumocystis carinii pneumonia. J Acquir Immune Defic Syndr. 1994;7(1):39–45.
53. 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):1–7.
54. Gruden JF, Huang L, Turner J, et al. High-resolution CT in the evaluation of clinically suspected Pneumocystis carinii pneumonia in AIDS patients with normal, equivocal, or nonspecific radiographic findings. AJR Am J Roentgenol. 1997;169(4):967–75.
55. Jules-Elysee KM, Stover DE, Zaman MB, et al. Aerosolized pentamidine: effect on diagnosis and presentation of Pneumocystis carinii pneumonia. Ann Intern Med. 1990;112(10):750–7.
56. Ewig S, Schäfer H, Rockstroh JK, et al. Effect of long-term primary aerosolized pentamidine prophylaxis on breakthrough Pneumocystis carinii pneumonia. Eur Respir J. 1996;9(5):1006–12.
57. 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(10):589–93.
58. Bondoc AY, White DA. Granulomatous Pneumocystis carinii pneumonia in patients with malignancy. Thorax. 2002;57:435–7.
59. Studemeister A, Dass S. A patient with dyspnea, cough, and fever. Clin Infect Dis. 2006;43:1461–2. 1490–1461.
60. Procop GW, Haddad S, Quinn J, et al. Detection of Pneumocystis jiroveci in respiratory specimens by four staining methods. J Clin Microbiol. 2004;42(7):3333–5.
61. Caliendo AM, Hewitt PL, Allega. Performance of a PCR assay for detection of Pneumocystis carinii from respiratory specimens. J Clin Microbiol. 1998;36(4):979–82.
62. Larsen HH, Huang L, Kovacs JA, et al. A prospective, blinded study of quantitative touch-down polymerase chain reaction using oral-wash samples for diagnosis of Pneumocystis pneumonia in HIV-infected patients. J Infect Dis. 2004;189(9):1679–83.

63. 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):1229–35.
64. 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(3):169–75.
65. Azoulay E, Bergeron A, Chevret S, et al. Polymerase chain reaction for diagnosing pneumocystis pneumonia in non-HIV immunocompromised patients with pulmonary infiltrates. Chest. 2009;135(3):655–61.
66. 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(10):1531–7.
67. Botterel F, Cabaret O, Foulet F, et al. Clinical significance of quantifying Pneumocystis jirovecii DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. J Clin Microbiol. 2012;50(2):227–31.
68. Tasaka S, Hasegawa N, Kobayashi S, et al. Serum indicators for the diagnosis of pneumocystis pneumonia. Chest. 2007;131(4):1173–80.
69. 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(2):197–202.
70. Morris AM, Masur H. A serologic test to diagnose pneumocystis pneumonia: are we there yet? Clin Infect Dis. 2011;53(2):203–4.
71. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, et al. β-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin Infect Dis. 2011;52(6):750–70.