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Review

Pneumocystis as a Co-Factor in Pulmonary Diseases

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Abstract

Pneumocystis causes life-threatening pneumonia in immunocompromised populations. More recently it has been implicated as a co-factor in a number of chronic lung diseases including chronic obstructive pulmonary disease (COPD), severe asthma, and cystic fibrosis (CF). In this review, we will examine the current literature regarding *Pneumocystis* and lung diseases in the HIV-infected patients and non-HIV immunocompromised populations, and the barriers to prophylaxis and treatment in these patients. Trimethoprim sulfamethoxazole (TMP-SMX) is an effective therapeutic against *Pneumocystis* but this approach remains problematic due to drug interactions, treatment-limiting adverse events, and break-through *Pneumocystis* pneumonia (PCP) despite prophylaxis. This review summarizes the shortcomings of current prophylaxis and treatment strategies, and the advances that have been made toward the development of novel diagnostics and therapeutics, with a focus on vaccine development.

Keywords

Pneumocystis; lung disease; HIV; COPD; severe asthma; cystic fibrosis; vaccine; immunosuppression; kexin

1. Lung Disease in the HIV-Infected Population

1.1 HIV Infection and PCP

Pneumocystis jirovecii is an opportunistic extracellular fungal pathogen that causes life-threatening pneumonia in immunocompromised individuals. Persistent *Pneumocystis* colonization and acute pneumonia are associated with permanent obstructive lung damage [1]. Recent reports suggest that this organism may also play a role in the development of severe asthma [2, 3] and cystic fibrosis [4] and potentially other lung diseases. The clinical relevance of *Pneumocystis* emerged in the 1980s as a result of the AIDS epidemic [5]. For many years, *Pneumocystis* pneumonia (PCP) was the leading cause of morbidity and mortality in HIV-infected patients. Despite improvements in trimethoprim sulfamethoxazole (TMP-SMX) prophylaxis and antiretroviral therapy (ART), PCP remains the most common serious opportunistic infection among those with AIDS [6, 7]. The overall rate of PCP in the United States is 4.7 cases per 100 person-years and may be significantly higher in those who are either not using or not responding to ART or are undiagnosed [8]. Even where treatment is initiated, mortality in this population remains high at 10-40%, but may be as high as 60-80% in patients who have progressive lung damage leading to respiratory failure [9, 10]. Due to the high rate of mortality with the current standard of care, PCP remains a significant co-morbidity among HIV-infected patients.

1.2 HIV Infection and COPD

In addition to causing PCP, several laboratories have reported an association between *Pneumocystis* colonization and chronic obstructive pulmonary disease (COPD) in both HIV-infected and non-HIV infected populations [1, 10-23]. The pathogenesis of HIV-related COPD is poorly understood, but it is hypothesized that persistent colonization with *Pneumocystis* or other microbes may amplify inflammatory responses and tissue damage [11, 14, 19, 24, 25]. Increasing evidence suggests that *Pneumocystis* colonization is associated with the development and exacerbation of COPD in both HIV-infected and non-HIV infected populations, independent of smoking [1, 10-23]. In HIV-infected persons, *Pneumocystis* colonization is frequent even among individuals receiving anti-*Pneumocystis* prophylaxis and those with high CD4 cell counts who are receiving ART [19, 20]. COPD-like changes have been found in HIV-infected patients following PCP [10, 26, 27], and we have demonstrated that even low levels of *Pneumocystis* correlate with COPD [13]. *Pneumocystis* colonization in HIV-infected patients is associated with worse pulmonary airway obstruction and emphysema [1], as well as *Pneumocystis*-related pulmonary inflammation that may contribute to the pathogenesis of COPD [16, 21]. Our laboratory was the first to demonstrate that in a non-human primate (NHP) model of HIV infection, persistent *Pneumocystis* colonization leads to the development of airway obstruction and emphysema [15]. We found that the decline in pulmonary function occurs early after *Pneumocystis* colonization and these *Pneumocystis*-induced obstructive changes are not reversible following treatment with TMP-SMX or albuterol [28]. Our results, along with mounting clinical evidence, support the concept that *Pneumocystis* colonization contributes to the development of COPD in HIV-infected individuals.

2. Pneumocystis-Related Lung Disease in Non-HIV-Infected Populations

2.1 PCP in Non-HIV-Infected Patients

In contrast to the HIV-infected population, PCP is of increasing concern in non-HIV infected persons receiving immunosuppressive therapies. These at-risk populations include cancer patients, transplant recipients, individuals treated for inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease, and persons experiencing natural immunosuppression to due aging, congenital or acquired immunosuppressive states [29]. In the non-HIV immunosuppressed population, approximately 15-20% are at risk for PCP, with mortality rates as high as 60% [24, 30, 31]. Systemic immunosuppressive steroid therapy is the treatment most commonly associated with the development of PCP, and combination cytotoxic therapies (e.g. cyclophosphamide, methotrexate, CHOP regimen (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone)) that have a synergistic T cell suppressive effect are associated with increased risk. Patients treated with monotherapies, even at low doses, as well corticosteroid-sparing regimens, are also at risk of PCP [32]. As immunologic control of *Pneumocystis* infection is dependent upon T and B cells, there is an increased incidence of PCP among patients treated with immune-therapeutics (*e.g.* B cell therapeutics, TNF inhibitors). As the number of cancer cases worldwide is expected to increase to approximately 21 million by 2030, along with increases in patients receiving organ transplants and immunosuppressive therapies, over two million patients are estimated to be at risk of development of PCP. Of increasing significance is a widespread lack of PCP prophylaxis guidelines in many of the non-HIV immunocompromised patient populations and there is a growing list of immunosuppressive agents associated with the development of PCP [32]

2.2 COPD in Non-HIV-Infected Patients

Several groups, including our own, have identified an association between *Pneumocystis* colonization and chronic obstructive pulmonary disease (COPD) in the non-HIV population [13, 33-36]. Smoking is still considered to be the primary cause of COPD, but there is increasing evidence that *Pneumocystis* colonization is a co-factor that maintains or increases an inflammatory response, accelerating the development of severe COPD [21, 37, 38]. Although non-immunosuppressed patients rarely develop PCP [39], persistent low levels of *Pneumocystis* DNA have been documented in patients with chronic bronchial disease [40]. Our study found that *Pneumocystis* colonization is increased in smokers with COPD (non-HIV-infected) compared to smokers without COPD and correlates with disease severity [13]. These data were further supported by later clinical studies which found that *Pneumocystis*-colonized patients have higher levels of systemic pro-inflammatory cytokines than non-colonized patients [16, 21], suggesting that *Pneumocystis* may play a role in disease progression.

Clinical studies profiling the cytokine environment in non-HIV-infected COPD patients have revealed that *Pneumocystis* colonization is associated with the expression of TNF-α, IL-6 and IL-18 [21], and Th1-associated genes such as IFN-γ and chemokine ligands CXCL9, CXCL10, and CXCL11 [16]. Likewise, fungal β-1,3-glucans (BG) found in *Pneumocystis* can act as potent inducers of alveolar macrophage activation that initiate inflammatory responses by lung parenchymal cells, characterized by the secretion of TNF-α, IL-6, IL-8, MIP-2, eicosanoid metabolites, and reactive oxidant species [41, 42]. Thus, inflammatory responses that occur in response to chronic

Pneumocystis colonization resemble the inflammatory environment observed in the lungs non-HIV-infected COPD patients [16, 21], supporting the hypothesis that *Pneumocystis* exacerbates COPD.

2.3 Severe Asthma

Patients with severe asthma exhibit sustained symptoms such as coughing, wheezing, and shortness of breath despite the use of high levels of inhaled corticosteroids or oral corticosteroids [3]. Clinical studies have documented the presence of *Pneumocystis* in individuals with severe asthma patients [43]; however, there has been a lack of data to discern whether *Pneumocystis* drives pathogenesis in this disease or if *Pneumocystis* colonization is due to high corticosteroid use within this population. Supporting this hypothesis, a murine model of *Pneumocystis* infection has been shown to promote several pathologic features associated with asthma, including increased Th2 inflammation, increased mucus production, airway remodeling, and eosinophil recruitment [2]. Two recent studies support the correlation between *Pneumocystis* colonization and the development of severe asthma [2, 44]. Eddens et al., reported that higher levels of sera IgG and IgE titers against whole *Pneumocystis* lysate (PC-hi) were associated with worse symptoms and lung functions in patients with severe asthma when compared to patients with lower levels of *Pneumocystis* antibodies (PC-low), despite similar levels of inhaled corticosteroids or oral steroids [2]. Moreover, when comparing the lung microbiomes of cohorts with pediatric lung disease, children with severe asthma had significantly higher levels of several fungal species including *Pneumocystis* in bronchoalveolar lavage fluid than children with cystic fibrosis [44], demonstrating a connection between *Pneumocystis* and severe asthma independent of pulmonary disease. Together these studies support the hypothesis that *Pneumocystis* may be a co-factor in the development of severe asthma.

2.4 Cystic Fibrosis

Cystic fibrosis (CF) is an inherited chronic lung disease caused by a defect in the CFTR gene that controls fluid and electrolyte homeostasis of epithelial cells [45, 46]. This deficiency results in unusually thick and viscous mucus that accumulates in small airways, leading to airway obstruction, frequent bacterial infections, and inflammatory lung disease. Although specific fungal agents such as *Aspergillus* have well documented roles in disease exacerbation in mouse models of CF [47-49], *Pneumocystis* has been thought to be an unrelated side effect of pre-existing lung disease. Of note, recent evidence suggests that *Pneumocystis* may play a more an active role in CF than previously thought [50-56]. Several studies have reported *Pneumocystis* colonization in CF patients that support this hypothesis. *Pneumocystis* colonization in this population is highly variable but is estimated to occur in 7.4-32.8% of CF patients [4, 57, 58], significantly higher than in healthy populations, and may be dependent upon geographical location [59, 60]. Despite the paucity of *Pneumocystis* colonization within CF patients, a prospective study found that the rate of colonization was five times higher in CF patients undergoing acute respiratory attacks (9.2%) than in stable CF patients (2%), implicating colonization incidence with the severity of disease [61]. Moreover, it has been suggested that these patients may serve as a reservoir for human transmission that put other susceptible populations at risk during outpatient treatment. More

research is necessary to investigate the pathogenic relationship between CF and *Pneumocystis* to determine if *Pneumocystis* intervention ameliorates disease severity.

3. Critical Barriers and Challenges to Prevention and Treatment of PCP

The preferred therapeutic for the prevention and treatment of *Pneumocystis* is trimethoprim-sulfamethoxaxone (TMP-SMX, Bactrim). Prophylactic use of TMP-SMX is effective in reducing the incidence of PCP in immunocompromised individuals but this approach remains problematic due to drug interactions, treatment-limiting adverse events, and break-through PCP despite prophylaxis [62-69]. Even when alternative antibiotics are used, such as clindamycin-primaquine, pentamide [70] or atovaquone [71], there are increasing concerns for antibiotic resistance and break-through PCP. PCP infection rates have increased in incidence from 2002 to 2010 [72] with approximately 15,000 hospitalization per year in the US. Despite low frequency of PCP in the non-HIV immunosuppressed population (5-15%), there is a high mortality rate with the current standard of care (10-40%) [24, 30, 31].

In addition to the challenges of TMP-SMX use, many barriers to treatment and prevention of *Pneumocystis*-related sequalae may stem from an inability to identify highly susceptible individuals within the immunosuppressed and control hospital-acquired infections during outpatient visits. It has been well documented that *Pneumocystis*-colonized patients have served as a source of infection during outbreaks [73, 74]. There have been numerous reports which have attempted to identify risk factors for PCP among the non-HIV immunosuppressed by correlating traits such as immunologic disorders, graft rejection in transplantation recipients, cytomegalovirus, and corticosteroid use [75, 76]. Despite these studies, there remains no reliable diagnostic method to identify the highest risk patients within the growing number of patients receiving immunotherapy. As a result, transplantation centers have been pressed to adopt TMP-SMX prophylaxis from 3-12 months for many transplant recipients, especially in renal transplant patients [77, 78]; however, the length and uniformity of prophylaxis has been debated.

Lapses in prophylaxis can lead to the development of PCP, even in patients with a successful history of long-term graft control and chronic disease maintenance. One case report even identified a renal transplant patient that succumbed to PCP following withdrawal of 24 years of TMP-SMX prophylaxis [79]. The difficulty in adopting uniform PCP prophylaxis guidelines is further exemplified by a recent report of serial outbreaks of PCP over a 10-year period in a renal transplant unit, despite *Pneumocystis* prophylaxis in some individuals [74]. In this study, intermittent use of TMP-SMX prophylaxis failed to prevent serial outbreaks among patients that were spread via human-to-human transmission during visits to the outpatient clinic [74]. These authors conclude that life-long PCP prophylaxis may be necessary in renal transplant recipients [74, 79]. With extensive use of TMP-SMX for both prophylaxis and treatment, it is not surprising that the rising prevalence of drug resistance increasingly complicates treatment options. Finally, as with all antibiotics, treatment of PCP with TMP-SMX does not prevent subsequent infections. These challenges to PCP treatment and prophylaxis, along with the inability to identify highly susceptible patients, emphasize the need for alternative strategies for disease prevention and treatment, such as vaccines and neutralizing antibodies, to supplement the current standard of care.

4. Development of Pneumocystis Prevention and Treatment Strategies

The development of a vaccine for the prevention of PCP has long been a goal of the field [80]. Several antigenic components have been evaluated in murine models and the roles of CD4 T cells and antibodies have been clearly established [18, 81-91]. These vaccine candidates include the major surface glycoprotein (MSG) [89], SPD1 [88], and kexin [90, 91]. Our laboratory has evaluated host responses to the *Pneumocystis* protein kexin and we have shown that humoral response against components of kexin (KEX1) may provide promising approaches toward the development of preventive and therapeutic vaccines, novel antibody-based therapeutics and improved diagnostic methods. KEX1 is a subtilisin-like serine protease that is highly expressed in *Pneumocystis* and related fungal species [92-94]. From animal and clinical studies, we know that *Pneumocystis* antibodies are important for protection against *Pneumocystis*-related pulmonary sequalae [18, 81-87], but KEX1-specific antibodies in particular play major role in abrogating *Pneumocystis*-related disease [46-52,57].

Most individual are seropositive against *Pneumocystis* antigens by early childhood [95-97] and have pre-existing humoral immunity against KEX1; however, the levels of *Pneumocystis* KEX1-specific immunity can vary between individuals. Based on these clinical studies, our laboratory has explored the utility of the humoral response to KEX1 as a biomarker for predicting susceptibility to PCP and *Pneumocystis*-related sequalae. Indeed, we have shown that low *Pneumocystis* KEX1-specific natural plasma antibodies are associated with increased risk of *Pneumocystis* colonization levels and are an independent predictor of susceptibility to PCP in HIV-infected patients [18] and in an experimental NHP model of HIV-associated PCP [23]. In a prospective study of newly diagnosed HIV-infected individuals, we demonstrated that high antibodies against KEX1, but not the *Pneumocystis* major surface glycoprotein (MSG), correlated with reduced incidence of PCP [18]. The association of low *Pneumocystis* KEX1 antibody levels as a predictor of subsequent PCP development was also demonstrated in a NHP model of HIV and *Pneumocystis* co-infection [23]. SHIV-infected macaques with high baseline KEX1 titers, were able to generate KEX1-specific antibodies in response to natural exposure to *Pneumocystis* and correlated with protection from colonization and preserved lung function[6].

A negative correlation between KEX1 antibody levels and lung disease was also found in HIV-negative smokers and COPD patients. In these populations, low anti-*Pneumocystis* KEX1 antibody titers were independently associated with more severe airway obstruction, suggesting that KEX1 antibodies may contribute to protection from *Pneumocystis* colonization and progressive COPD [12, 98]. These results provide further evidence that low KEX1 IgG titers may be a novel biomarker to predict PCP risk and may be a useful parameter to refine PCP prophylactic protocols, particularly in non-HIV immunocompromised populations where long-term prophylaxis may not be well tolerated by all individuals or necessary for prevention of PCP [32].

Currently, there are no clinically approved vaccines for the prevention of fungal infection. The observation that most individuals have been primed to KEX1, and that higher KEX1 plasma titers inversely correlate with susceptibility to PCP in HIV-infected individuals, suggest that “boosting” immune responses to KEX1 may induce protection against PCP. Using a non-human primate model of HIV and PCP co-infection, we demonstrated that vaccination of healthy macaques with KEX1 prior to SHIV protects against PCP, despite SHIV-associated immunosuppression [90]. These studies in pre-clinical primate models of HIV-associated PCP support the exploration of KEX1 as a

vaccine for the prevention of PCP in the HIV-infected and non-HIV-infected populations. In addition, combined vaccination with other protective *Pneumocystis* antigens such as SPD1 [88] could provide enhanced protection.

In summary, as a ubiquitous organism among human populations, *Pneumocystis* exposure in healthy and immunocompromised individuals elicits a spectrum of immunologic responses that can be protective or alternatively can contribute to immune-mediated lung pathology. Insights into the host responses against *Pneumocystis*-protective antigens, such as KEX1, provide a path toward the anti-*Pneumocystis* vaccine development, improved diagnostic, and the development of novel immune-therapeutics for the treatment of acute PCP.

Author Contributions

All authors made equal contributions to this work.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Morris A, Alexander T, Radhi S, Lucht L, Sciurba FC, Kolls JK, et al. Airway obstruction is increased in pneumocystis-colonized human immunodeficiency virus-infected outpatients. J Clin Microbiol. 2009; 47: 3773-3776.
2. Eddens T, Campfield BT, Serody K, Manni ML, Horne W, Elsegeiny W, et al. A novel CD4+ T cell-dependent murine model of Pneumocystis-driven asthma-like pathology. Am J Respir Crit Care Med. 2016; 194: 807-820.
3. Guilbert TW, Bacharier LB, Fitzpatrick AM. Severe Asthma in Children. J Allergy Clin Immunol Pract. 2014; 2: 489-500.
4. Sing A, Geiger AM, Hogardt M, Heesemann J. Pneumocystis carinii carriage among cystic fibrosis patients, as detected by nested PCR. J Clin Microbiol. 2001; 39: 2717-2718.
5. Masur H, Michelis MA, Greene JB, Onorato I, Stouwe RA, Holzman RS, et al. An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. N Engl J Med. 1981; 305: 1431-1438.
6. HIV/AIDS Surveillance Report. Centers for Disease Control and Prevention, 1997 Contract No.: 2.
7. Coyle PV, McCaughey C, Nager A, McKenna J, O'Neill H, Feeney SA, et al. Rising incidence of Pneumocystis jirovecii pneumonia suggests iatrogenic exposure of immune-compromised patients may be becoming a significant problem. J Med Microbiol. 2012; 61: 1009-1015.
8. Kaplan JE, Hanson D, Dworkin MS, Frederick T, Bertolli J, Lindegren ML, et al. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. Clin Infect Dis. 2000; 30 Suppl 1: S5-14.
9. De Palo VA, Millstein BH, Mayo PH, Salzman SH, Rosen MJ. Outcome of intensive care in patients with HIV infection. Chest. 1995; 107: 506-510.
10. Morris AM, Huang L, Bacchetti P, Turner J, Hopewell PC, Wallace JM, et al. Permanent declines in pulmonary function following pneumonia in human immunodeficiency virus-

infected persons. The Pulmonary Complications of HIV Infection Study Group. Am J Respir Crit Care Med. 2000; 162: 612-616.

11. Fernandes EF, Patil SP, Morris A, Sciurba FC, Rogers RM, Norris KA. Colonization with Pneumocystis in a simian model of AIDS results in chronic inflammation and airflow obstruction. Am J Resp Crit Care Med. 2005; 2: A867.
12. Morris A, Netravali M, Kling HM, Shipley T, Ross T, Sciurba FC, et al. Relationship of pneumocystis antibody response to severity of chronic obstructive pulmonary disease. Clin Infect Dis. 2008; 47: e64-68.
13. Morris A, Sciurba FC, Lebedeva IP, Githaiga A, Elliott WM, Hogg JC, et al. Association of chronic obstructive pulmonary disease severity and Pneumocystis colonization. Am J Respir Crit Care Med. 2004; 170: 408-413.
14. Norris KA, Morris A, Patil S, Fernandes E. Pneumocystis colonization, airway inflammation, and pulmonary function decline in acquired immunodeficiency syndrome. Immunol Res. 2006; 36: 175-187.
15. Shipley TW, Kling HM, Morris A, Patil S, Kristoff J, Guyach SE, et al. Persistent pneumocystis colonization leads to the development of chronic obstructive pulmonary disease in a nonhuman primate model of AIDS. J Infect Dis. 2010; 202: 302-312.
16. Fitzpatrick ME, Tedrow JR, Hillenbrand ME, Lucht L, Richards T, Norris KA, et al. Pneumocystis jirovecii colonization is associated with enhanced Th1 inflammatory gene expression in lungs of humans with chronic obstructive pulmonary disease. Microbiol Immunol. 2014; 58: 202-211.
17. Sivam S, Sciurba FC, Lucht LA, Zhang Y, Duncan SR, Norris KA, et al. Distribution of Pneumocystis jirovecii in lungs from colonized COPD patients. Diagn Microbiol Infect Dis. 2011; 71: 24-28.
18. Gingo MR, Lucht L, Daly KR, Djawe K, Palella FJ, Abraham AG, et al. Serologic responses to pneumocystis proteins in HIV patients with and without Pneumocystis jirovecii pneumonia. J Acquir Immune Defic Syndr. 2011; 57: 190-196.
19. Morris A, Wei K, Afshar K, Huang L. Epidemiology and clinical significance of pneumocystis colonization. J Infect Dis. 2008; 197: 10-17.
20. Morris A, Kingsley LA, Groner G, Lebedeva IP, Beard CB, Norris KA. Prevalence and clinical predictors of Pneumocystis colonization among HIV-infected men. AIDS. 2004; 18: 793-798.
21. Calderon EJ, Rivero L, Respaldiza N, Morilla R, Montes-Cano MA, Friaza V, et al. Systemic inflammation in patients with chronic obstructive pulmonary disease who are colonized with Pneumocystis jiroveci. Clin Infect Dis. 2007; 45: e17-19.
22. Cardenal R, Medrano FJ, Varela JM, Ordonez A, Regordan C, Rincon M, et al. Pneumocystis carinii pneumonia in heart transplant recipients. Eur J Cardiothorac Surg. 2001; 20: 799-802.
23. Kling HM, Shipley TW, Patil SP, Kristoff J, Bryan M, Montelaro RC, et al. Relationship of Pneumocystis jiroveci humoral immunity to prevention of colonization and chronic obstructive pulmonary disease in a primate model of HIV infection. Infect Immun. 2010; 78: 4320-4330.
24. Morris A, Norris KA. Colonization by Pneumocystis jirovecii and its role in disease. Clin Microbiol Rev. 2012; 25: 297-317.
25. Norris KA, Morris A. Pneumocystis infection and the pathogenesis of chronic obstructive pulmonary disease. Immunol Res. 2011; 50: 175-180.

26. Mitchell DM, Fleming J, Pinching AJ, Harris JR, Moss FM, Veale D, et al. Pulmonary function in human immunodeficiency virus infection. A prospective 18-month study of serial lung function in 474 patients. Am Rev Respir Dis. 1992; 146: 745-751.
27. Shaw RJ, Roussak C, Forster SM, Harris JR, Pinching AJ, Mitchell DM. Lung function abnormalities in patients infected with the human immunodeficiency virus with and without overt pneumonitis. Thorax. 1988; 43: 436-440.
28. Kling HM, Shipley TW, Guyach S, Tarantelli R, Morris A, Norris KA. Trimethoprim-sulfamethoxazole treatment does not reverse obstructive pulmonary changes in pneumocystis-colonized nonhuman primates with SHIV infection. J Acquir Immune Defic Syndr. 2014; 65: 381-389.
29. Roux A, Gonzalez F, Roux M, Mehrad M, Menotti J, Zahar JR, et al. Update on pulmonary Pneumocystis jirovecii infection in non-HIV patients. Med Mal Infect. 2014; 44: 185-198.
30. Sepkowitz KA. Opportunistic infections in patients with and patients without Acquired Immunodeficiency Syndrome. Clin Infect Dis. 2002; 34: 1098-1107.
31. Pareja JG, Garland R, Koziel H. Use of adjunctive corticosteroids in severe adult non-HIV Pneumocystis carinii pneumonia. Chest. 1998; 113: 1215-1224.
32. Carmona EM, Limper AH. Update on the diagnosis and treatment of Pneumocystis pneumonia. Ther Adv Respir Dis. 2011; 5: 41-59.
33. Calderon EJ, Regordan C, Medrano FJ, Ollero M, Varela JM. Pneumocystis carinii infection in patients with chronic bronchial disease. Lancet. 1996; 347: 977.
34. Varela JM, Respaldiza N, Sanchez B, de la Horra C, Montes-Cano M, Rincon M, et al. Lymphocyte response in subjects with chronic pulmonary disease colonized by Pneumocystis jirovecii. J Eukaryot Microbiol. 2003; 50 Suppl: 672-673.
35. Calderon E, de la Horra C, Medrano FJ, Lopez-Suarez A, Montes-Cano MA, Respaldiza N, et al. Pneumocystis jiroveci isolates with dihydropteroate synthase mutations in patients with chronic bronchitis. Eur J Clin Microbiol Infect Dis. 2004; 23: 545-549.
36. Nevez G, Magois E, Duwat H, Gouilleux V, Jounieaux V, Totet A. Apparent absence of pneumocystis jirovecii in healthy subjects. Clin Infect Dis. 2006; 42: e99-101.
37. Vidal S, de la Horra C, Martin J, Montes-Cano MA, Rodriguez E, Respaldiza N, et al. Pneumocystis jirovecii colonisation in patients with interstitial lung disease. Clin Microbiol Infect. 2006; 12: 231-235.
38. Morris A, Sciurba FC, Norris KA. Pneumocystis: a novel pathogen in chronic obstructive pulmonary disease? Copd. 2008; 5: 43-51.
39. Cano S, Capote F, Pereira A, Calderon E, Castillo J. Pneumocystis carinii pneumonia in patients without predisposing illnesses. Acute episode and follow-up of five cases. Chest. 1993; 104: 376-381.
40. Wissmann G, Morilla R, Friaza V, Calderon E, Varela JM. [Human reservoirs of Pneumocystis]. Enferm Infecc Microbiol Clin. 2010; 28: 38-43.
41. Lebron F, Vassallo R, Puri V, Limper AH. Pneumocystis carinii cell wall beta-glucans initiate macrophage inflammatory responses through NF-kappaB activation. J Biol Chem. 2003; 278: 25001-25008.
42. Limper AH, Lebron F, Evans SE, Hahn RY. Pneumocystis carinii: cell wall beta-glucan-mediated pulmonary inflammation. J Eukaryot Microbiol. 2003; 50 Suppl: 646.

43. Sy ML, Chin TW, Nussbaum E. Pneumocystis carinii pneumonia associated with inhaled corticosteroids in an immunocompetent child with asthma. J Pediatr. 1995; 127: 1000-1002.
44. Goldman DL, Chen Z, Shankar V, Tyberg M, Vicencio A, Burk R. Lower airway microbiota and mycobiota in children with severe asthma. J Allergy Clin Immunol. 2018; 141: 808-811.e807.
45. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med. 2005; 352: 1992-2001.
46. Mogayzel PJ, Jr., Flume PA. Update in cystic fibrosis 2009. Am J Respir Crit Care Med. 2010; 181: 539-544.
47. Chaudhary N, Datta K, Askin FB, Staab JF, Marr KA. Cystic fibrosis transmembrane conductance regulator regulates epithelial cell response to Aspergillus and resultant pulmonary inflammation. Am J Respir Crit Care Med. 2012; 185: 301-310.
48. Moretti S, Bozza S, Oikonomou V, Renga G, Casagrande A, Iannitti RG, et al. IL-37 inhibits inflammasome activation and disease severity in murine aspergillosis. Plos Pathog. 2014; 10: e1004462.
49. Iannitti RG, Napolioni V, Oikonomou V, De Luca A, Galosi C, Pariano M, et al. IL-1 receptor antagonist ameliorates inflammasome-dependent inflammation in murine and human cystic fibrosis. Nat Commun. 2016; 7: 10791.
50. Ramsey KA, Ranganathan S, Park J, Skoric B, Adams AM, Simpson SJ, et al. Early respiratory infection is associated with reduced spirometry in children with cystic fibrosis. Am J Respir Crit Care Med. 2014; 190: 1111-1116.
51. Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with Aspergillus fumigatus on lung function and hospitalization in patients with cystic fibrosis. Chest. 2010; 137: 171-176.
52. Noni M, Katelari A, Dimopoulos G, Doudounakis SE, Tzoumaka-Bakoula C, Spoulou V. Aspergillus fumigatus chronic colonization and lung function decline in cystic fibrosis may have a two-way relationship. Eur J Clin Microbiol Infect Dis. 2015; 34: 2235-2241.
53. Chotirmall SH, O'Donoghue E, Bennett K, Gunaratnam C, O'Neill SJ, McElvaney NG. Sputum Candida albicans presages FEV(1) decline and hospital-treated exacerbations in cystic fibrosis. Chest. 2010; 138: 1186-1195.
54. Gileles-Hillel A, Shoseyov D, Polacheck I, Korem M, Kerem E, Cohen-Cymberknoh M. Association of chronic Candida albicans respiratory infection with a more severe lung disease in patients with cystic fibrosis. Pediatr Pulmonol. 2015; 50: 1082-1089.
55. de Boer K, Vandemheen KL, Tullis E, Doucette S, Fergusson D, Freitag A, et al. Exacerbation frequency and clinical outcomes in adult patients with cystic fibrosis. Thorax. 2011; 66: 680-685.
56. Hector A, Kirn T, Ralhan A, Graepler-Mainka U, Berenbrinker S, Riethmueller J, et al. Microbial colonization and lung function in adolescents with cystic fibrosis. J Cyst Fibros. 2016; 15: 340-349.
57. Respaldiza N, Montes-Cano MA, Dapena FJ, de la Horra C, Mateos I, Medrano FJ, et al. Prevalence of colonisation and genotypic characterisation of Pneumocystis jirovecii among cystic fibrosis patients in Spain. Clin Microbiol Infect. 2005; 11: 1012-1015.
58. Hernandez-Hernandez F, Frealle E, Caneiro P, Salleron J, Durand-Joly I, Accoceberry I, et al. Prospective multicenter study of Pneumocystis jirovecii colonization among cystic fibrosis patients in France. J Clin Microbiol. 2012; 50: 4107-4110.

59. Gal SL, Héry-Arnaud G, Ramel S, Virmaux M, Damiani C, Totet A, et al. Pneumocystis jirovecii and cystic fibrosis in France. Scand J Infect Dis. 2010; 42: 225-227.
60. Nevez G, Robert-Gangneux F, Pougnet L, Virmaux M, Belleguic C, Deneuville E, et al. Pneumocystis jirovecii and Cystic Fibrosis in Brittany, France. Mycopathologia. 2018; 183: 81-87.
61. Green HD, Bright-Thomas RJ, Mutton KJ, Guiver M, Jones AM. Increased prevalence of Pneumocystis jirovecii colonisation in acute pulmonary exacerbations of cystic fibrosis. J Infect. 2016; 73: 1-7.
62. Arend SM, van't Wout JW. Editorial response: Prophylaxis for Pneumocystis carinii pneumonia in solid organ transplant recipients--as long as the pros outweigh the cons [editorial; comment]. Clin Infect Dis. 1999; 28: 247-249.
63. Faul JL, Akindipe OA, Berry GJ, Doyle RL, Theodore J. Recurrent Pneumocystis carinii colonization in a heart-lung transplant recipient on long-term trimethoprim-sulfamethoxazole prophylaxis. J Heart Lung Transplant. 1999; 18: 384-387.
64. Fisk DT, Meshnick S, Kazanjian PH. Pneumocystis carinii pneumonia in patients in the developing world who have acquired immunodeficiency syndrome. Clin Infect Dis. 2003; 36: 70-78.
65. Gordon SM, LaRosa SP, Kalmadi S, Arroliga AC, Avery RK, Truesdell-LaRosa L, et al. Should prophylaxis for Pneumocystis carinii pneumonia in solid organ transplant recipients ever be discontinued? Clin Infect Dis. 1999; 28: 240-246.
66. Hugle B, Solomon M, Harvey E, James A, Wadhwa A, Amin R, et al. Pneumocystis jiroveci pneumonia following rituximab treatment in Wegener's granulomatosis. Arthritis Care Res (Hoboken). 2010; 62: 1661-1664.
67. Kurokawa T, Kaya H, Yoshida T. Two cases of Pneumocystis jiroveci pneumonia with non-Hodgkin's lymphoma after CHOP-based chemotherapy containing rituximab. J Clin Exp Hematop. 2010; 50: 159-162.
68. Lemaire CM, Browning JC, Hsu S. Medical Pearl: Pneumocystis pneumonia prophylaxis for patients on chronic systemic corticosteroids. J Am Acad Dermatol. 2006; 55: 124-125.
69. 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.
70. Barber BA, Pegram PS, High KP. Clindamycin/primaquine as prophylaxis for Pneumocystis carinii pneumonia. Clin Infect Dis. 1996; 23: 718-722.
71. Argy N, Le Gal S, Coppée R, Song Z, Vindrios W, Massias L, et al. Pneumocystis cytochrome b mutants associated with atovaquone prophylaxis failure as the cause of Pneumocystis infection outbreak among heart transplant recipients. Clin Infect Dis. 2018; 67: 913-919.
72. de Armas Rodriguez Y, Wissmann G, Muller AL, Pederiva MA, Brum MC, Brackmann RL, et al. Pneumocystis jirovecii pneumonia in developing countries. Parasite. 2011; 18: 219-228.
73. Le Gal S, Damiani C, Rouillé A, Grall A, Tréguer L, Virmaux M, 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: e62-e71.
74. Goto N, Takahashi-Nakazato A, Futamura K, Okada M, Yamamoto T, Tsujita M, et al. Lifelong prophylaxis with trimethoprim-sulfamethoxazole for prevention of outbreak of Pneumocystis jirovecii Pneumonia in kidney transplant recipients. Transplant Direct. 2017; 3: e151.

75. Lee SH, Huh KH, Joo DJ, Kim MS, Kim SI, Lee J, et al. Risk factors for Pneumocystis jirovecii pneumonia (PJP) in kidney transplantation recipients. Sci Rep. 2017; 7: 1571.
76. Liu Y, Su L, Jiang SJ, Qu H. Risk factors for mortality from pneumocystis carinii pneumonia (PCP) in non-HIV patients: a meta-analysis. Oncotarget. 2017; 8: 59729-59739.
77. Kasiske BL, Zeier MG, Chapman JR, Craig JC, Ekberg H, Garvey CA, et al. KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary. Kidney Int. 2010; 77: 299-311.
78. Martin SI, Fishman JA. Pneumocystis pneumonia in solid organ transplantation. Am J Transplant. 2013; 13: 272-279.
79. Kono M, Kojima K, Wakai S, Shirakawa H. A case of a Pneumocystis pneumonia twenty-four years after living kidney transplantation due to withdrawal of sulfamethoxazole/trimethoprim prophylaxis. Transplant Direct. 2018; 4: e359.
80. Garvy BA. Overcoming hurdles to development of a vaccine against Pneumocystis jirovecii. Infect Immun. 2017; 85.
81. Pascale JM, Shaw MM, Durant PJ, Amador AA, Bartlett MS, Smith JW, et al. Intranasal immunization confers protection against murine Pneumocystis carinii lung infection. Infect Immun. 1999; 67: 805-809.
82. Empey KM, Hollifield M, Schuer K, Gigliotti F, Garvy BA. Passive immunization of neonatal mice against Pneumocystis carinii f. sp. muris enhances control of infection without stimulating inflammation. Infect Immun. 2004; 72: 6211-6220.
83. Garvy BA, Wiley JA, Gigliotti F, Harmsen AG. Protection against Pneumocystis carinii pneumonia by antibodies generated from either T helper 1 or T helper 2 responses. Infect Immun. 1997; 65: 5052-5056.
84. Gigliotti F, Garvy BA, Haidaris CG, Harmsen AG. Recognition of Pneumocystis carinii antigens by local antibody-secreting cells following resolution of P. carinii pneumonia in mice. J Infect Dis. 1998; 178: 235-242.
85. Gigliotti F, Haidaris CG, Wright TW, Harmsen AG. Passive intranasal monoclonal antibody prophylaxis against murine Pneumocystis carinii pneumonia. Infect Immun. 2002; 70: 1069-1074.
86. Gigliotti F, Hughes WT. Passive immunoprophylaxis with specific monoclonal antibody confers partial protection against Pneumocystis carinii pneumonitis in animal models. J Clin Invest. 1988; 81: 1666-1668.
87. Wells J, Haidaris CG, Wright TW, Gigliotti F. Active immunization against Pneumocystis carinii with a recombinant P. carinii antigen. Infect Immun. 2006; 74: 2446-2448.
88. Ruan S, Cai Y, Ramsay AJ, Welsh DA, Norris K, Shellito JE. B cell and antibody responses in mice induced by a putative cell surface peptidase of Pneumocystis murina protect against experimental infection. Vaccine. 2017; 35: 672-679.
89. Theus SA, Smulian AG, Steele P, Linke MJ, Walzer PD. Immunization with the major surface glycoprotein of Pneumocystis carinii elicits a protective response. Vaccine. 1998; 16: 1149-1157.
90. Kling H, Norris KA. Vaccine-induced Immunogenicity and Protection against Pneumocystis pneumonia in Non-human Primate Model of HIV-Pneumocystis Co-Infection. J Infect Dis. 2016; doi: 10.1093/infdis/jiw032.

91. Zheng M, Shellito JE, Marrero L, Zhong Q, Julian S, Ye P, et al. CD4+ T cell-independent vaccination against Pneumocystis carinii in mice. J Clin Invest. 2001; 108: 1469-1474.
92. Fuller RS, Brake A, Thorner J. Yeast prohormone processing enzyme (KEX2 gene product) is a Ca2+-dependent serine protease. Proc Natl Acad Sci U S A. 1989; 86: 1434-1438.
93. Van de Ven WJ, Creemers JW, Roebroek AJ. Furin: the prototype mammalian subtilisin-like proprotein-processing enzyme. Endoproteolytic cleavage at paired basic residues of proproteins of the eukaryotic secretory pathway. Enzyme. 1991; 45: 257-270.
94. van den Ouweland AM, van Duijnhoven HL, Keizer GD, Dorssers LC, Van de Ven WJ. Structural homology between the human fur gene product and the subtilisin-like protease encoded by yeast KEX2. Nucleic Acids Res. 1990; 18: 664.
95. Peglow SL, Smulian AG, Linke MJ, Pogue CL, Nurre S, Crisler J, et al. Serologic responses to Pneumocystis carinii antigens in health and disease. J Infect Dis. 1990; 161: 296-306.
96. Respaldiza N, Medrano FJ, Medrano AC, Varela JM, de la Horra C, Montes-Cano M, et al. High seroprevalence of Pneumocystis infection in Spanish children. Clin Microbiol Infect. 2004; 10: 1029-1031.
97. Vargas SL, Hughes WT, Santolaya ME, Ulloa AV, Ponce CA, Cabrera CE, et al. Search for primary infection by Pneumocystis carinii in a cohort of normal, healthy infants. Clin Infect Dis. 2001; 32: 855-861.
98. Kling HM, Shipley TW, Patil S, Morris A, Norris KA. Pneumocystis colonization in immunocompetent and simian immunodeficiency virus-infected cynomolgus macaques. J Infect Dis. 2009; 199: 89-96.