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Update on the diagnosis and treatment of *Pneumocystis* pneumonia

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Abstract

Pneumocystis is an opportunistic fungal pathogen that causes an often-lethal pneumonia in immunocompromised hosts. Although the organism was discovered in the early 1900s, the first cases of *Pneumocystis* pneumonia in humans were initially recognized in Central Europe after the Second World War in premature and malnourished infants. This unusual lung infection was known as plasma cellular interstitial pneumonitis of the newborn, and was characterized by severe respiratory distress and cyanosis with little or no fever and no pathognomic physical signs. At that time, only anecdotal cases were reported in adults and usually these patients had a baseline malignancy that led to a malnourished state. In the 1960–1970s additional cases were described in adults and children with hematological malignancies, but *Pneumocystis* pneumonia was still considered a rare disease. However, in the 1980s, with the onset of the HIV epidemic, *Pneumocystis* prevalence increased dramatically and became widely recognized as an opportunistic infection that caused potentially life-threatening pneumonia in patients with impaired immunity. During this time period, prophylaxis against this organism was more generally instituted in high-risk patients. In the 1990s, with widespread use of prophylaxis and the initiation of highly active antiretroviral therapy (HAART) in the treatment of HIV-infected patients, the number of cases in this specific population decreased. However, *Pneumocystis* pneumonia still remains an important cause of severe pneumonia in patients with HIV infection and is still considered a principal AIDS-defining illness. Despite the decreased number of cases among HIV-infected patients over the past decade, *Pneumocystis* pneumonia continues to be a serious problem in immunodeficient patients with other immunosuppressive conditions. This is mostly due to increased use of immunosuppressive medications to treat patients with autoimmune diseases, following bone marrow and solid organ transplantation, and in patients with hematological and solid malignancies. Patients with hematologic disorders and solid organ and hematopoietic stem cell transplantation are currently the most vulnerable groups at risk for developing this infection.

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Conflict of interest statement

AH Limper holds a US patent on a *Pneumocystis* diagnostic method using real time PCR recognizing the *PcCdc2* target gene.

However, any patient with an impaired immunity, such as those receiving moderate doses of oral steroids for greater than 4 weeks or those receiving other immunosuppressive medications are at also at significant risk.

Keywords

Pneumocystis; pneumonia; AIDS; immune suppression; prophylaxis; therapy

Introduction

Pneumocystis encompasses a genus of opportunistic fungal pathogens that cause potentially lethal pneumonia in immunocompromised hosts. Although the organism was discovered in the early 1900s, the first cases of *Pneumocystis* pneumonia in humans were initially recognized in Central Europe after the Second World War, in premature and malnourished infants. This unusual lung infection was known as plasma cellular interstitial pneumonitis of the newborn, and was characterized by severe respiratory distress and cyanosis with little or no fever and no pathognomic physical signs [Baar, 1955]. At that time, only anecdotal cases were reported in adults. Usually these patients had an underlying malignancy that led to a malnourished state. In the 1960–1970s, additional cases were described in adults and children with hematological malignancies, but *Pneumocystis* pneumonia was still considered a rare disease. However, in the 1980s, with the onset of the HIV epidemic, the prevalence of *Pneumocystis* increased dramatically and became widely recognized as an opportunistic infection that caused often-lethal pneumonia in patients with impaired immunity. During this time period, prophylaxis against this organism was more generally instituted in high-risk patients [Fischl *et al.* 1988]. In the 1990s, with widespread use of prophylaxis and the initiation of highly active antiretroviral therapy (HAART) in the treatment of HIV-infected patients, the number of cases in this specific population decreased. However, *Pneumocystis* pneumonia still remains an important cause of severe pneumonia in patients with HIV infection and is still considered a principal AIDS-defining illness [Kaplan *et al.* 2000; Jones *et al.* 1999].

Despite the decreased number of cases among HIV-infected patients over the past decade, *Pneumocystis* pneumonia continues to be a serious problem among other immunosuppressed patients. This is mostly due to the increased use of immunosuppressive medications to treat patients with hematological and solid malignancies, bone marrow and solid organ transplantation, and patients with autoimmune diseases [Dungarwalla *et al.* 2007; Harigai *et al.* 2007; Kalyoncu *et al.* 2007; Kaur and Mahl, 2007; Kolstad *et al.* 2007; Lahiff *et al.* 2007; Yale and Limper, 1996]. This article summarizes the current knowledge about the biology, pathogenesis, and diagnosis of *Pneumocystis* infection as well as prophylaxis and treatment recommendations, as used by our group and as supported by the existing guidelines.

Historical considerations and taxonomy

Current concepts propose that *Pneumocystis* species have likely co-evolved and co-existed with their respective mammalian hosts for thousands of years (as reviewed by Aliouat-Denis *et al.* [2008]). However, it was not until 1909 that Carlos Chagas first described members of

this genus when working in a guinea-pig model of trypanosome infection. Chagas initially believed that he had found a new type of trypanosomal organism or life form. Antonio Carini came to the same conclusion 1 year later when he identified similar organisms in the lungs of his rat colony. It was not until 1912 that the Delanoës working at the Pasteur Institute in Paris recognized that *Pneumocystis* in rat represented a unique species and christened this organism *Pneumocystis carinii* in honor of Antonio Carini. Despite the knowledge of its existence in animals, the first human cases were not described until 1942 by two Dutch investigators, Van der Meer and Brug [Van der Meer and Brug, 1942]. In 1952, Vanek and Jírovec reported that *Pneumocystis* “*carinii*”, later renamed *Pneumocystis jiroveci*, was the cause of interstitial pneumonia in neonates [Vanek and Jírovec, 1952]. The persisting belief of that era was that *Pneumocystis* was a protozoan and was classified as such for many years. This was likely due to the existence of two life forms that are morphologically parallel to other protozoan organisms, as well as its response to the antiprotozoan medication pentamidine. Despite this prevailing theory, Giese is first credited with proposing that *Pneumocystis* may be a yeast and that the foamy material described in the airway of these patients were spores [Giese, 1953]. However, it was not until 1988 when Edman and colleagues sequenced the small ribosomal RNA subunit that *Pneumocystis* species were correctly classified as ascomycetous fungi [Edman *et al.* 1988].

The genus *Pneumocystis* encompasses a variety of ubiquitous fungi that can colonize and infect almost every species of mammalian hosts. In addition, nucleic acids from *Pneumocystis carinii*, one of the species that infects rats, have been found in ambient air and pond water [Wakefield *et al.* 1998]. However, a true environmental reservoir of viable *Pneumocystis* organisms has not been established. *Pneumocystis* species are also quite different in their unique specificity for a particular mammalian host. For example, the human-specific species *Pneumocystis jiroveci* is only capable of infecting humans and is unable to infect other animals and vice versa. The host specificity of *Pneumocystis* across respective host species was further investigated by Dr Wakefield using a polymerase chain reaction (PCR) approach. She developed a set of primers from the mitochondrial large subunit rRNA of all known species of *Pneumocystis* and showed that when the nonhuman primers were used in human samples no amplification was detected and conversely human primers failed to amplify *Pneumocystis* from nonhuman samples (as reviewed by Stringer [2002]). Interestingly, human *Pneumocystis jiroveci* was found to be closest in homology to primate-derived *Pneumocystis* organisms. However, infection of a *Pneumocystis* species into its nonnative mammalian host has never been documented conclusively.

As a result of this absolute host species specificity, *Pneumocystis* organisms have been speciated, in part, according to the mammalian host that it infects. For instance, rats are infected with *P. carinii* and *P. wakefieldiae*, mice are infected by *P. murina*, and humans are susceptible to *P. jiroveci*, in honor of Dr Jírovec, who first associated this organism with human disease. For the purpose of this review we use the term *Pneumocystis* as the general genus name, unless otherwise specified.

Life cycle and pathogenesis

The study of the *Pneumocystis* life cycle has been challenging for many years due to both the fact that an environmental niche is still unknown, and that a long-term *in vitro* culture system has remained elusive. Transient proliferation has been achieved on lung epithelial and other feeder cells [Limper *et al.* 1997b; Cushion *et al.* 1985]. However, despite isolated reports of short-term *in vitro* culture in the absence of feeder cells [Merali *et al.* 1999], sustained cultures have not been consistently observed. Thus, the *Pneumocystis* scientific community remains without a reproducible and valid culture system to study this organism. Despite these challenges, considerable advances have been made over the years using molecular techniques. Immune-suppressed animal models of *Pneumocystis* pneumonia continue to serve as a reliable source of organisms for study. More recently, the analysis of the *P. carinii* genome project, begun in 1997 by Cushion and Arnold, has been of considerable benefit to the *Pneumocystis* research community. It is important to note that all of the currently available agents used to treat *P. jiroveci* pneumonia in humans, were initially derived from study of *P. carinii* and other species in rodents.

Ultrastructural studies of animal and human derived organisms have provide some insight into the life cycle of *Pneumocystis* and have established that there are at least two different life forms, the trophic form and the cyst. The trophic form generally measures about 2 μm , possesses a single nucleus, and is surrounded by a plasma membrane. In contrast, the cyst is significantly larger, approximately 8–10 μm in size, contains up to eight intracystic bodies and is surrounded by a characteristic thick cyst wall. Upon rupture of the wall, known as excystment, the intracystic bodies are released to become new trophic forms of the organism. The *Pneumocystis* cyst wall is formed largely of β -glucans, a complex branching polysaccharide, but other components such as mannoproteins, chitins, and other proteins are also present. The main function of this wall is to confer rigidity and support for the organism, and putatively, to protect the organism from harsh environmental conditions outside the host. The maintenance of the cyst wall is a dynamic process of formation and degradation. While the cyst wall is essential to provide rigidity and viability of the organism, degradation of this wall is also necessary during excystment for the organism to complete its life cycle. To facilitate this dynamic process, β -glucan synthetases facilitate formation of the β -1,3-glucan homopolymers that comprise the cysts wall, and β -glucanases and other proteins drive the active process of degradation and restructuring of the wall. Our group first described the *Pneumocystis* *PcGsc1* gene responsible for glucan synthesis, and recently characterized the *PcEng2* β -1,3-endoglucanase that participates in cell wall remodeling [Villegas *et al.* 2009; Kottom and Limper, 2000]. Such enzymes responsible for cell wall integrity are essential to the *Pneumocystis* life cycle, and may represent attractive targets for future therapeutic strategies. This is noteworthy, since emerging resistance to current therapeutic agents has been described recently (see the treatment section for further details).

Other important activities of *Pneumocystis* cell wall β -glucans have recently been described by a number of investigators. Multiple lines of evidence indicate that *Pneumocystis* β -glucans have the capacity to induce multiple inflammatory pathways in the host. Thus, it has been proposed that cell wall β -glucans contribute to the exaggerated inflammatory responses elicited by alveolar macrophages and respiratory epithelial cells, promoting respiratory

failure in patients with severe *Pneumocystis* pneumonia [Carmona *et al.* 2006; Lebron *et al.* 2003; Vassallo *et al.* 1999a, 1999b; Limper *et al.* 1989]. In addition, *Pneumocystis* β -glucans have also been shown to activate dendritic cells and modulate T-cell responses [Carmona *et al.* 2006], crucial for *Pneumocystis* clearance. Moreover, β -glucans have been demonstrated to prime dendritic cells in a manner that they polarized T cells toward Th17 differentiation, which further promotes host antifungal immune resistance [Zelante *et al.* 2007]. Several excellent reviews of host defense and immunity during *Pneumocystis* pneumonia have been published recently [Goodridge *et al.* 2009; Thomas and Limper, 2007; Gigliotti and Wright, 2005].

Unlike most other fungi, the route of *Pneumocystis* infection is not completely understood. The most commonly accepted theory is that infectious organisms are transmitted as aerosolized particles from host to host. However, the exact mechanisms of infection continue to be debated. Some investigators propose a model of initial infection in infancy or early childhood, with reactivation later in life during periods of immune suppression. In contrast, other investigators propose that while initial infection is nearly universal in early childhood, the infection is actually re-acquired again, later in life, if the host lacks T-cell-based immune responses necessary for effective defense. Observations that support the first theory are from the fact that it is not unusual to detect the presence of *Pneumocystis* in otherwise healthy animals [Demanche *et al.* 2005] implying that otherwise normal healthy hosts may serve as a reservoir for infection. Additional data in both animal models, and humans, indicate that exposure and colonization can occur very early in life, and may further support that reactivation later in life is possible [Icenhour *et al.* 2002].

In humans, serologic evidence of anti-*Pneumocystis* antibodies or positive PCR in healthy children supports early exposure to this organism. Additional data indicate that adults with chronic obstructive pulmonary disease (COPD) may be colonized with subsequent reactivation during periods of immune suppression. In addition, a recent study by Vargas and colleagues demonstrated that over 60% of individuals who underwent a legal autopsy in Santiago, Chile, were colonized with *Pneumocystis*. The cause of death in these cases was unrelated to active *Pneumocystis* infection, and was either a violent cause or other indications that required legal autopsy. Unfortunately, prior medical histories of the autopsied cases were not available [Ponce *et al.* 2010]. Nonetheless, these data further support the notion of colonization of otherwise healthy individuals. In contrast, however, other studies have failed to detect *Pneumocystis* organisms in postmortem evaluation of immuno-competent adults [Peters *et al.* 1992b; Peglow *et al.* 1990; Wakefield *et al.* 1990a, 1990b; Pifer *et al.* 1978]. This contradictory data may be explained by a state of transient colonization of otherwise healthy individuals or by different sensitivities of the methodologies used in these studies. However, the fact that some individual are at least transiently colonized supports the concept of possible reservoirs of *Pneumocystis* within immune-competent humans. Whether *Pneumocystis* pneumonia itself is due to reactivation of latent colonization or newly acquired infection or a combination of the two ultimately remains a matter of debate at the present time.

Once inhaled, *Pneumocystis* has considerable tropism for the lung and rarely disseminates to other organs, although occasional cases of extrapulmonary dissemination have been

reported, specifically in patients with advanced HIV infection [Panos *et al.* 2007; Hagmann *et al.* 2001; Bartlett and Hulette, 1997; Ruggli *et al.* 1997; Guttler *et al.* 1993; Dieterich *et al.* 1992]. When the infective form is inhaled it reaches the alveoli. Trophic forms bind tightly to alveolar epithelial cells and extracellular matrix proteins [Walzer, 1986]. Several studies have shown that interactions with the extracellular matrix proteins and in particular with fibronectin facilitate binding of *Pneumocystis* to lung epithelial cells [Limper *et al.* 1993]. More recently, our laboratory has identified a novel integrin-like extracellular matrix adhesion receptor, PcInt1, expressed only on the trophic form, which mediates attachment of the organism to fibronectin [Kottom *et al.* 2008]. Adhesion of *Pneumocystis* to the epithelium triggers several signaling pathways, which mediate mating, morphological changes and proliferation. It is hypothesized that a sexual conjugation-mating phase takes place followed by meiosis and mitosis resulting in generation of the mature cyst [Burgess *et al.* 2008]. Attachment to the alveolar epithelium also has several consequences for host such as suppression of the lung epithelial cell growth and repair and initiation of multiple inflammatory pathways.

Risk factors

It is important to emphasize that while the underlying condition that may have lead to the infection has clinical and prognostic implications to the host, the degree of the impaired innate and acquire cellular immunity are critical predicting factors for lung function and survival during *Pneumocystis* pneumonia. For instance, CD4 lymphocytes are essential for the clearance of the organisms and patients with a CD4 count of less than 200 cells per cubic meter have been shown to be at higher risk for infection [Phair *et al.* 1990; Shellito *et al.* 1990]. The *Pneumocystis*-infected SCID mice model have provided evidence that *Pneumocystis* clearance is impaired due to a lack of CD4 and CD8 cells [Wright *et al.* 1999]. Interestingly, these animals do not present gas exchange abnormalities until the very late phase of the infection suggesting that T cells may also play a role in oxygenation. Immune reconstitution of these mice with normal spleen cells results in significant deterioration of their lung function as a result of an intense CD8 T-cell-mediated inflammatory response [Wright *et al.* 1999; Beck *et al.* 1991]. Similar findings have been observed in HIV-infected patients during immune reconstitution while on HARRT treatment or bone marrow transplant patients during engraftment [Wislez *et al.* 2001]. Other findings such as prolonged neutropenia, impaired macrophage activity, inhibition or neutralization of tumor necrosis factor (TNF)- α responses and other factors are also considered important [Lasbury *et al.* 2006; Thomas and Limper, 2004; Fishman, 2001; Koziel *et al.* 1998; Kolls *et al.* 1997; Limper *et al.* 1997a; Hoffman *et al.* 1993; Chen *et al.* 1992]. These studies indicate that a balance immune response is necessary to successfully defend against *Pneumocystis* infection.

As mentioned earlier in this review, the classical groups affected by *Pneumocystis* pneumonia have been patients with HIV infection and hematological malignancies. However, the incidence of *Pneumocystis* pneumonia is increasing in other groups such as posttransplant patients (including bone marrow transplant) and those with autoimmune and other inflammatory conditions; especially if they are receiving prolonged corticosteroid therapy as demonstrated in a retrospective case series from the Mayo Clinic. In that study,

116 consecutive non-HIV patients with a new diagnosis of *Pneumocystis* pneumonia were investigated. Interestingly, more than half of the patients (57%) in the study did not have an underlying malignant process. Some of these individuals were transplantation patients (25%), but the majority had an inflammatory or autoimmune condition. Most of the patients received systemic corticosteroids within a month of diagnosis with an equivalent dose of prednisone that averaged 30 mg/day. However, for 25% of the individuals, the averaged prednisone dose was as little as 16 mg a day. The mean duration of corticosteroid therapy was 12 weeks, but one quarter of the patients developed *Pneumocystis* pneumonia after 8 weeks or less [Yale and Limper, 1996]. Overall, these data suggested that not only patients on high-dose steroids are at risk, but, in fact, doses of prednisone between 16 and 20 mg/day for at least 4–6 weeks are great enough to increase the risk of *Pneumocystis* pneumonia in these patients. Interestingly, the study also indicated that up to 10% of the patients affected with *Pneumocystis* pneumonia were not on systemic steroids. Unfortunately, no concomitant or past use of other immunosuppressant agents was reported in the study [Yale and Limper, 1996]. Although, it is possible that these patients had significant immune impairment due to their baseline condition, it is likely that they were receiving other immunosuppressant agents or even had prolonged impaired immunity from past immunosuppressive therapy.

Classically, steroids and other cytotoxic agents such as methotrexate, cyclosporine and cyclophosphamide have also been associated with the development of *Pneumocystis* pneumonia [Hardy *et al.* 1984; Perruquet *et al.* 1983]. However, new immunomodulating agents such as TNF- α inhibitors and other monoclonal antibodies have also more recently been implicated [Segal *et al.* 2008; Lahiff *et al.* 2007]. A list of the most frequent drugs associated with the development of *Pneumocystis* pneumonia is given in Table 1. It is important to be aware that new immunosuppressant agents are continuously being developed that may enhance the potential risk of developing *Pneumocystis* pneumonia. Therefore, a high index of suspicion should be maintained in patients who are receiving or who have taken immunosuppressants in the past. This is particularly true if the underlying medical condition itself may also affect their overall immune competency.

Another important observation is the widespread lack of chemoprophylaxis in many immune-compromised patients without HIV infection. The authors of the Mayo Clinic study commented that none of the cases that developed *Pneumocystis* pneumonia in their series had received adequate chemoprophylaxis specifically directed against *Pneumocystis*. In addition, a recent study surveyed physician members of the American College of Rheumatology revealing that one-third of the physicians never used *Pneumocystis* prophylaxis and of those that did employ prophylaxis, only a small percentage would prescribe it for patients on monotherapy with steroids. In addition, only 17% of patients receiving prednisone 20 mg or more a day for at least 4 weeks were found to have received prophylaxis [Cettomai *et al.* 2010]. Preliminary observations from an ongoing studies of patients diagnosed with their first episode of *Pneumocystis* pneumonia at our institution further reveals a lack of prophylaxis in patients with underlying immunosuppressive conditions treated with steroids or other immunosuppressive agents (unpublished data). In our opinion, these data highlight that lack of prophylaxis in individuals at risk is one of the principal risk factors for *Pneumocystis* pneumonia. However, one of the main challenges for physician that care for immune-suppressed patients is to accurately define which patients are

‘at risk’ of developing *Pneumocystis* pneumonia. Unfortunately a variety of immune-altering conditions place individuals at risk, as are a wide variety of specific medications, dosages and treatment duration. Prophylaxis guidelines that highlight situations at risk have been developed for certain groups such as HIV and cancer patients [Segal *et al.* 2008; Kaplan *et al.* 2002]. Unfortunately, a consensus opinion is lacking for other groups including patients with rheumatologic and other anti-inflammatory processes. A synopsis of these recommendations as well as additional evidence for indication of prophylaxis in these other groups is given in Table 2 [Moosig *et al.* 2009; Mukhtyar *et al.* 2009; Lapraik *et al.* 2007; Godeau *et al.* 1995; Ognibene *et al.* 1995].

Clinical manifestations and radiological findings

Clinical presentation of patients affected with *Pneumocystis* pneumonia differs among HIV and non-HIV immunosuppressed patients. For instance, HIV patients tend to present with sub-acute onset of progressive dyspnea, nonproductive or minimally productive cough, low-grade fever and malaise. However, up to 7% of these patients can be asymptomatic. In contrast, patients that are immunocompromised but HIV-negative usually present more acutely, with substantial dyspnea, fever, chills and some may initially present in urgent need of mechanical ventilation. Respiratory failure in this group carries an overall mortality of up to 40% [Festic *et al.* 2005]. Interestingly, HIV patients usually have significantly more organisms in the bronchoalveolar lavage (BAL) than non-HIV patients, but this does not seem to correlate with worse oxygenation. Indeed, the opposite has been demonstrated, in that patients with HIV-associated *Pneumocystis* pneumonia tend to have higher arterial oxygen tension and a lower alveolar-arterial oxygen gradient than non-HIV patients [Limper *et al.* 1989]. In contrast, neutrophil and inflammatory cell numbers, rather than organism burden, appear to correlate with the degree of oxygenation during *Pneumocystis* pneumonia, suggesting that patients that mount a more robust inflammatory response are at higher risk of developing respiratory failure compared with those who exhibit lower levels of lung inflammation [Limper *et al.* 1989].

The lung exam can also vary depending of the degree of illness, ranging from an essentially normal examination to diffuse crackles on auscultation. Extrapulmonary manifestations are not common. However, retinitis, thyroiditis, bone lesions and pneumocystosis of brain, liver, spleen and kidney have all been described rarely [Panos *et al.* 2007; Hagmann *et al.* 2001; Bartlett and Hulette, 1997; Guttler *et al.* 1993; Dieterich *et al.* 1992; Edelstein and McCabe, 1990]. These findings tend to occur more frequently in patients who have been on prophylaxis with aerosolized pentamidine or who were extremely immunocompromised, generally from advanced AIDS. Radiological findings of *Pneumocystis* pneumonia are generally not specific and may range from a normal or near normal chest X-ray to different degrees of bilateral and usually symmetrical pulmonary infiltrates (Figure 1A). However, other less-common patterns have been reported, including: lobar infiltrates, pulmonary nodules, pneumatoceles and other cystic changes, and pneumothorax. These are more frequently found again in very severely immunocompromised individuals and those treated with aerosolized pentamidine [Edelstein and McCabe, 1990]. At times clinical symptomatology, specifically in the absence of significant chest X-ray findings, initiates more sensitive radiological approaches such as high-resolution chest CT. The most typical

findings on chest CT are bilateral ground-glass opacities (Figure 1B). Less-common features can include reticular, granular, and cystic lesions (Figure 1C) [Hidalgo *et al.* 2003; Crans and Boiselle, 1999].

Other radiological techniques such as 18-fluorodeoxyglucose positron emission tomography (FDG-PET) and Ga-67 scintigraphy have been reported as potential tools to assist in the early diagnosis of *Pneumocystis* pneumonia [Zhuang and Alavi, 2002; Tumei *et al.* 1992]. However, these techniques have been most commonly applied for the evaluation of other concomitant problem, with incidental or unexpected uptake in the lung prompting further diagnostic approaches, such as BAL to document *Pneumocystis* pneumonia. In the past, Ga-67 scintigraphy was proposed as an adjunct for early detection of *Pneumocystis*. However, with the implementation of more sensitive molecular techniques, the use Ga-67 scintigraphy no longer appears to be justified (see the diagnosis section).

Diagnosis

The single most important diagnostic tool for *Pneumocystis* infection is a high clinical suspicion. In the right clinical setting, an immunosuppressed patient with new onset of dyspnea or new symptoms of pneumonia, with or without radiological findings, should prompt further evaluation, particularly if they are not receiving chemoprophylaxis. Since *Pneumocystis* cannot be cultured, the gold standard for diagnosis is microscopic visualization of the organism. Traditionally different stains have been used to identify either the trophic form (Gram–Weigert, Wright–Giemsa or modified Papanicolaou stains) or the cyst forms (calcofluor white, cresyl echt violet, Gomori methenamine silver or toluidine blue) (Figure 2). However, the most common technique used currently in the majority of the laboratories is fluorescein-conjugated monoclonal antibodies. Sensitivity of these assays depends on several factors such as the type and quality of the sample (BAL *versus* sputum *versus* tissue), the number of organisms present, and the experience of the laboratory staff with the particular assay. In addition, all of these tests have their own advantages and disadvantages. For instance, calcofluor white has the advantages of being quick, convenient, and can identify the presence of other concomitant fungus. However, the main disadvantage of calcofluor is that this stain requires reviewer expertise for correct interpretation. On the other hand, the immunofluorescent antibody technique is advantageous in that it can detect both life forms of *Pneumocystis* and is also slightly more sensitive. However, immune fluorescence is more time consuming, and nonspecific staining can also occur [Procop *et al.* 2004]. Therefore, for those laboratories that use immunofluorescence as first-line test, confirmation with a second method is generally recommended [Procop *et al.* 2004]. Direct hematoxylin and eosin tissue examination frequently reveals frothy intra-alveolar exudates containing organisms, which can be further confirmed with either methenamine silver stain or immune staining (Figure 3).

All of the direct organism visualization methods can lead to false-negative results, particularly in non-HIV patients. This has been attributed to fewer organisms being present in the collected samples [Limper *et al.* 1989]. Molecular methods are now available which have increased sensitivity and specificity and are particularly useful in these patient populations. In recent years, real-time PCR-based strategies have largely replaced earlier

methods of nested PCR for clinical diagnosis, which may be less specific for active infection leading to higher false-positive rates [Helweg-Larsen *et al.* 1998; Ribes *et al.* 1997; Tamburrini *et al.* 1996; Lu *et al.* 1995; Cartwright *et al.* 1994; Peters *et al.* 1992a]. The use of real-time PCR has also reduced interrater contamination increasing the specificity of the assay. Multiple protocols that use various *Pneumocystis* gene targets have been developed. The main targeted genes include the heat shock protein gene (*HSP70*) [Huggett *et al.* 2008], the dihydrofolate reductase gene (*DHFR*) [Bandt and Monecke, 2007], the dihydropteroate synthase gene (*DHPS*) [Alvarez-Martinez *et al.* 2006], and the cell division cycle 2 gene (*CDC2*) [Arcenas *et al.* 2006]. In our institution, we utilize the *PcCdc2*, a single-copy gene target, and have applied the assay on sputum, BAL or tissue samples. On BAL samples the *PcCdc2*-based real-time PCR assay is 21% more sensitive than calcofluor white staining and has a specificity of 96%, with an analytical sensitivity of 5.6 copies per microliter of positive plasmid control. No cross-reactivity with other pathogens or human DNA was demonstrated using the *PcCdc2* target [Arcenas *et al.* 2006]. False negatives are still possible with PCR-based approaches if fewer copies are present than the lower limit of detection for a given assay.

Despite increased sensitivity and specificity these assays continue to have difficulty discriminating between colonization and infection. Therefore, clinical correlation is always necessary. New techniques that detect messenger RNA (mRNA) have been proposed as surrogate markers for organism viability. The rationale is that mRNA is less stable than DNA. Therefore, if the patient is not actively infected with viable organisms, the mRNA should be largely degraded and no longer detected. In contrast, DNA is far more stable and may still be present even if the organisms are dead. Recent data from HIV patients with suspected *Pneumocystis* pneumonia using reverse-transcriptase PCR targeting a heat shock protein of *Pneumocystis* mRNA (*Phs1*) have yielded a diagnostic sensitivity and specificity of 100% and 86%, respectively, in BAL specimens [de Oliveira *et al.* 2007].

The aforementioned methodologies can be applied to either BAL, sputum or to tissue. However, there are other indirect markers that can be potentially be detected in serum, and serve as an adjunct for diagnosis. One of these assays is based on the detection of 1 $\rightarrow$ 3- β -D-Glucan (BDG) in serum. As mentioned earlier, β -glucans are structural components of the cell walls of most fungi and although this assay is not specific for *Pneumocystis*, substantially increased BDG levels have been found in patients with *Pneumocystis* infection [Fujii *et al.* 2007; Marty *et al.* 2007; Tasaka *et al.* 2007; Yasuoka *et al.* 1996]. In a retrospective case-control study of 295 patients with suspected *Pneumocystis* pneumonia, BDG was compared with microscopic examination of BAL. With an estimated cut-off level of 31.1 pg/ml of BDG, the sensitivity and specificity of the assay were 92.3% and 86.1%, respectively [Tasaka *et al.* 2007]. A more recent study of 35 consecutive retrospective cases diagnosed with *Pneumocystis* pneumonia also indicated that BDG levels might be a reliable diagnostic marker. However, the detection rate of BDG in non-HIV patients was lower than in HIV patients [Nakamura *et al.* 2009]. This could be attributed to the fact that HIV patients have greater numbers of organisms than non-HIV patients. These preliminary studies suggest that in the right clinical setting serum BDG may provide a useful noninvasive diagnostic adjunct for patients with *Pneumocystis* infection. However, additional studies are

needed to address the general specificity of BGD in diagnosing *Pneumocystis* pneumonia versus other fungal infections in diverse immune-suppressed patient populations.

Another proposed serological marker for *Pneumocystis* pneumonia is KL-6, a mucin-like glycoprotein expressed on type II pneumocytes and bronchiolar epithelial cells. Several studies have found elevated levels in patients with *Pneumocystis* pneumonia. However, the reported false-positive rate and level of detection were not as good as for the DBG assay [Nakamura *et al.* 2009; Tasaka *et al.* 2007]. Recent investigations indicate that KL-6 is more a generalized marker for alveolar epithelial injury [Sato *et al.* 2004] and can also be found detected in nonfungal infections such as Legionella, severe tuberculosis, and respiratory syncytial virus bronchiolitis, and even in noninfectious interstitial lung diseases [Kawasaki *et al.* 2009; Sukoh *et al.* 2001; Inoue *et al.* 1995]; Therefore, KL-6 elevation in *Pneumocystis* is thought to be related to lung damage and regeneration of epithelium lining and cannot be used as a specific marker of *Pneumocystis* infection.

Treatment

Historically, the mainstay of treatment for *Pneumocystis* pneumonia has been trimethoprim-sulfamethoxazole (TMP-SMX). Despite the existence of other drugs to treat *Pneumocystis* pneumonia, TMP-SMX is still the recommended first-line therapy for patients with mild, moderate, and severe disease [Huang *et al.* 2006; Benson *et al.* 2004]. The standard dose for both the pediatric (older than 2 months of age) and adult population is 15–20 mg/kg/day of TMP and 75–100 mg/kg/day of SMX administered in divided doses. For severe cases, the intravenous (IV) form is preferred over the oral formulation. However, IV can be switched to oral once clinical improvement is achieved. Dose adjustments are necessary for patients with renal and liver failure since SMX is extensively metabolized in the liver and renally excreted. We recommend that SMX drug levels should be monitored for all patients receiving IV therapy although the literature is controversial and there are no current guidelines about when or how often the levels should be monitored [Klinker *et al.* 1998; Joos *et al.* 1995]. In our experience, levels should be obtained at least once to assure adequate dosage and avoid potential toxicities especially in patients with renal and liver failure. Further monitoring should be individualized according to patient comorbidities and clinical response.

At times, usually due to sulfonamide allergies or other adverse effects, the use of TMP-SMX may not be possible. In past decades, aerosolized pentamidine was an alternative option. However, aerosolized pentamidine has been associated with the development of disseminated disease, likely due to poor systemic levels. Thus, aerosolized pentamidine is now not generally recommended for treatment unless no other drugs are available or the patient has a very mild disease. The IV formulation of pentamidine has better coverage and greater efficacy than the aerosolized route. However, it is at times poorly tolerated due to side effects that include hypotension, hypoglycemia and pancreatitis. A number of alternative regimens have been developed with good success [Colby *et al.* 1999; Wilkin and Feinberg, 1999; Castro, 1998; Masur, 1992; Hughes, 1991]. For instance, regimens with the combination of primaquine with clindamycin have been shown to be valid and better tolerated than the classical alternative treatment with IV pentamidine [Smego *et al.* 2001;

Toma *et al.* 1998]. Atovaquone is an orally available agent that can be used as an alternative therapy in mild to moderately severe cases [Madden *et al.* 2007; Colby *et al.* 1999]. The main limitation for atovaquone is that an IV formulation is not available restricting its use in severe cases. In addition, the absorption of oral atovaquone can be poor unless taken with fatty meals, which may be challenging for some patients. Recommended regimens are summarized in Table 2.

The current recommendation for the duration of treatment with any of these drugs is approximately 21 days. For those cases in which the disease progresses after 1–2 weeks of apparently adequate therapy, drug resistance should be suspected. Several studies have revealed mutations in the DPHS gene, the target for TMP and pyrimethamine, as well as the genes products that are targeted by SMX and dapsone [Crothers *et al.* 2005; Huang *et al.* 2004; Nahimana *et al.* 2004]. Mutations in the cytochrome B gene that confer resistance to atovaquone have also been reported [Kessl *et al.* 2004]. Isolated cases of treatment and prophylaxis failure have been associated with such mutations [Hauser *et al.* 2001; Helweg-Larsen *et al.* 1999]. However, these associations have not been consistently reported in all cases, and whether or not the detection of molecular resistance results in significant clinical resistance and treatment failure has still not yet been firmly established [Navin *et al.* 2001].

Controversy exists as to whether *Pneumocystis* pneumonia responds to echinocandins such as caspofungin. The mechanism of action echinocandins is through the inhibition of a β –1,3-glucan synthase targeting the integrity of fungal cell wall. Several studies in animals have shown that caspofungin has potential activity against *P. carinii* [Kottom and Limper, 2000; Powles *et al.* 1998]. More recently, several cases suggesting successful treatment in patients with *Pneumocystis* pneumonia have been reported as monotherapy or in combination with TMP-SMX [Mu *et al.* 2009; Hof and Schnulle, 2008; Utili *et al.* 2007; Annaloro *et al.* 2006; Beltz *et al.* 2006]. However, no rigorous preclinical animal data and no human randomized controlled trial clinical data are currently available. Thus, no recommendation on the use of these agents to treat human *Pneumocystis* pneumonia can be made at this time.

Another important aspect of *Pneumocystis* treatment is the use of corticosteroids, including prednisone, as adjunctive therapy. This regimen has been shown to be specifically beneficial in HIV patients who are hypoxemic, defined as a partial pressure of arterial oxygen below 70 mmHg or an alveolar-arteriolar oxygen gradient greater than 35 [The National Institutes of Health-University of California Expert Panel for Corticosteroids as Adjunctive Therapy for Pneumocystis Pneumonia, 1990]. A randomized controlled trial of 333 HIV patients with *Pneumocystis* pneumonia demonstrated that the use of corticosteroids reduced the risk of respiratory failure and death in patients with moderate to severe pneumonia. Despite an increase of localized herpetic lesions in the corticosteroid treatment group, no other infectious complications were detected [Bozzette *et al.* 1991]. In addition, a systematic review of adjunctive corticosteroid therapy in HIV-infected patients with *Pneumocystis* pneumonia and hypoxemia that included literature from the early 1980s through 2004 demonstrated a relative risk reduction for overall mortality at 1 month of 44%. It was also shown that the number needed to treat to prevent one death was lower in the period where HAART was not available compared with patients in later time periods that had access to

HAART. They concluded that corticosteroid treatment in HIV-patients with moderate to severe *Pneumocystis* pneumonia was beneficial [Briel *et al.* 2006].

Unfortunately, the data for non-HIV patients are far less clear. For instance, a review of 31 non-HIV patients with histologically confirmed *Pneumocystis* pneumonia and hypoxemia found that those that received higher dose of steroids (prednisone equivalent >60 mg/day) had a shorter duration for mechanical ventilation, ICU stay, and oxygen use [Pareja *et al.* 1998]. However, another similar study was unable to show improvement in survival [Delclaux *et al.* 1999]. Taking the sparsely available data into consideration, the recommendation of adjunctive therapy in non-HIV patient must be individualized. Corticosteroids should not be first-line adjunctive therapy in patients with mild to moderate disease with no respiratory failure. However, adjunctive corticosteroids should be seriously considered in non-HIV-infected patients with severe *Pneumocystis* pneumonia, particularly if hypoxemia is present. A detailed dosage regimen is given in Table 3.

Emerging anti-inflammatory medications for *Pneumocystis* pneumonia are currently under investigation, especially for those severe cases with rapid progression to respiratory failure. Recent studies in a mouse model of *Pneumocystis* pneumonia have shown that depletion of T cells using anti-CD3 antibody improves pulmonary function and survival rate compared with mice receiving the control antibody. This antibody, known as Muromonab-CD3 is currently in clinical use to treat transplant patients with organ rejection [Opelz, 1995]. Other agents such as the glycosphingolipid synthesis inhibitor, PDMP, also have activity as possible anti-inflammatory agents [Hahn *et al.* 2003]. However, further investigations are needed for these potential new adjunctive anti-inflammatory treatments for severe cases of *Pneumocystis* pneumonia.

Prophylaxis

Immunosuppressed patients especially those receiving corticosteroids or other immunosuppressive therapy should be considered candidates for *Pneumocystis* prophylaxis as outlined in the 2006 official American Thoracic Society Workshop on *Pneumocystis* [Huang *et al.* 2006]. Other guidelines about the specific use of prophylaxis in cancer and HIV-infected patients were published in 2008 and 2002, respectively [Segal *et al.* 2008; Kaplan *et al.* 2002]. A concise synopsis of the main recommendations for prophylaxis is given in Table 2 [Moosig *et al.* 2009; Mukhtyar *et al.* 2009; Lapraik *et al.* 2007; Godeau *et al.* 1995; Ognibene *et al.* 1995].

The preferred agent for prophylaxis for those who can tolerate sulfonamides continues to be TMP-SMX [Segal *et al.* 2008; Huang *et al.* 2006; Kaplan *et al.* 2002]. A meta-analysis of 12 randomized controlled trials of patients without HIV demonstrated that TMP-SMX dramatically reduced the number of *Pneumocystis* cases, up to 91%. The number needed to treat to prevent one infection was estimated at 15. However, no differences were detected in terms of all cause mortality. This differs from HIV patients in which a mean survival of 23 months versus 12 months was seen in patient treated with TMP-SMX compared with untreated patients [Fischl *et al.* 1988]. This may, in part, be related to effects of TMP-SMX in preventing other infections in this population such as toxoplasmosis [Mermin *et al.* 2005;

Ioannidis *et al.* 1996; Hirschtick *et al.* 1995]. A comparison of the efficacy of daily with thrice-weekly regimens does not seem to show statistical differences [Green *et al.* 2007]. In HIV patients, the most commonly used regimen is a single strength dose daily (SMX 400 mg/TMP 80 mg), but for those patients who are toxoplasma serology positive, a double strength dose daily (SMX 800 mg/TMP 160 mg) offers them added protection against toxoplasma as well [Kaplan *et al.* 2002].

For patients who do not tolerate TMP-SMX, other regimens are available, which are outlined in Table 4. For instance, HIV-patients may tolerate prophylaxis regimens with either dapsone or aerosolized pentamidine. Both regimens are, however, less effective than TMP-SMX, with dapsone generally being preferred over aerosolized pentamidine in recent years. Another effective option is atovaquone, as it offers similar efficacy. If additional protection for toxoplasma is required, pyrimethamine and leucovorin should be added to dapsone or atovaquone [Kaplan *et al.* 2002].

The duration of prophylaxis varies depending on the underlying risk factors and must be individualized. In the particular case of immune reconstitution after antiretroviral therapy in HIV patients, the recommendation supports discontinuation of therapy when CD4 counts are >200 cells/μl for at least 3 months [Kaplan *et al.* 2002]. For non-HIV patients, prophylaxis can often be discontinued after the related risk factors, such as significant dose systemic corticosteroid or other immune suppressant agents are no longer exerting their effect. However, it should be kept in mind that the immunosuppressed state can persist for months after discontinuation of some cytotoxic agents such as cyclophosphamide. For non-HIV patients, the data to monitor peripheral CD4 counts does not correlate as convincingly with the risk of developing *Pneumocystis* pneumonia, as it does during HIV infection. However, many experts do suggest monitoring CD4 counts and discontinuing prophylaxis if the peripheral blood CD4 cells are greater than 200 cells/μl for at least 6 months [Suryaprasad and Stone, 2008].

Conclusions

Pneumocystis pneumonia remains a common problem and a high index of suspicion is necessary for those caring for individuals who are immunocompromised. *Pneumocystis* pneumonia is a preventable disease if chemoprophylaxis is instituted appropriately and patient remains compliant. It is very important to be aware of the clinical presentation and radiological appearance, since, in the particular case on HIV-infected patients, *Pneumocystis* can be the first presenting symptom of immune suppression even before the patient is aware of his or her HIV status [Walzer *et al.* 2008]. If *Pneumocystis* is suspected, the most widely employed clinical diagnostic approach is tinctorial or antibody staining of sputum or BAL. However, PCR-based assays are becoming increasingly popular and are now available in many institutions. Diagnostic results are rapidly available. If the index of suspicion is high and the initial sputum analysis is negative, BAL is recommended. TMP-SMX is still the preferred drug for treatment and prophylaxis, but other options are available for those individuals that are intolerant. For patients that do not respond to adequate treatment over the first 1–2 weeks of therapy, development of resistance should be suspected. The

Pneumocystis research community continues to investigate this fascinating organism. New drugs are under development that may help treat patients with the severe lung disease.

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References

- Aliouat-Denis CM, Chabe M, Demanche C, Aliouat el M, Viscogliosi E, Guillot J et al. (2008) *Pneumocystis* species, co-evolution and pathogenic power. *Infect Genet Evol* 8: 708–726. [PubMed: 18565802]
- Alvarez-Martinez MJ, Miro JM, Valls ME, Moreno A, Rivas PV, Sole M et al. (2006) Sensitivity and specificity of nested and real-time PCR for the detection of *Pneumocystis jiroveci* in clinical specimens. *Diagn Microbiol Infect Dis* 56: 153–160. [PubMed: 16678378]
- Annaloro C, Della Volpe A, Usardi P and Lambertenghi Delilieri G (2006) Caspofungin treatment of *Pneumocystis pneumonia* during conditioning for bone marrow transplantation. *Eur J Clin Microbiol Infect Dis* 25: 52–54.
- Arcenas RC, Uhl JR, Buckwalter SP, Limper AH, Crino D, Roberts GD et al. (2006) A real-time polymerase chain reaction assay for detection of *Pneumocystis* from bronchoalveolar lavage fluid. *Diagn Microbiol Infect Dis* 54: 169–175. [PubMed: 16423488]
- Baar HS (1955) Interstitial plasmacellular pneumonia due to *Pneumocystis carinii*. *J Clin Pathol* 8: 19–24.
- Bandt D and Monecke S (2007) Development and evaluation of a real-time PCR assay for detection of *Pneumocystis jiroveci*. *Transpl Infect Dis* 9: 196–202. [PubMed: 17605743]
- Bartlett JA and Hulette C (1997) Central nervous system pneumocystosis in a patient with AIDS. *Clin Infect Dis* 25: 82–85. [PubMed: 9243039]
- Beck JM, Warnock ML, Curtis JL, Sniezek MJ, Arraj-Peffer SM, Kaltreider HB et al. (1991) Inflammatory responses to *Pneumocystis carinii* in mice selectively depleted of helper T lymphocytes. *Am J Respir Cell Mol Biol* 5: 186–197.
- Beltz K, Kramm CM, Laws HJ, Schroten H, Wessalowski R and Gobel U (2006) Combined trimethoprim and caspofungin treatment for severe *Pneumocystis jiroveci* pneumonia in a five year old boy with acute lymphoblastic leukemia. *Klin Padiatr* 218: 177–179. [PubMed: 16688676]
- Benson CA, Kaplan JE, Masur H, Pau A and Holmes KK (2004) Treating opportunistic infections among HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association/Infectious Diseases Society of America. *MMWR Recomm Rep* 53(RR-15): 1–112.
- Bozzette SA, Arcia J, Bartok AE, McGlynn LM, McCutchan JA, Richman DD et al. (1991) The impact of concomitant viral pathogens on the course of *Pneumocystis carinii* pneumonia. *J Protozool* 38: 183S–184S.
- Briel M, Bucher HC, Boscacci R and Furrer H (2006) Adjunctive corticosteroids for *Pneumocystis jiroveci* pneumonia in patients with HIV-infection. *Cochrane Database Syst Rev* 3: CD006150.
- Burgess JW, Kottom TJ and Limper AH (2008) *Pneumocystis carinii* exhibits a conserved meiotic control pathway. *Infect Immun* 76: 417–425. [PubMed: 17984205]
- Carmona EM, Vassallo R, Vuk-Pavlovic Z, Standing JE, Kottom TJ and Limper AH (2006) *Pneumocystis* cell wall beta-glucans induce dendritic cell costimulatory molecule expression and inflammatory activation through a Fas-Fas ligand mechanism. *J Immunol* 177: 459–467.
- Cartwright CP, Nelson NA and Gill VJ (1994) Development and evaluation of a rapid and simple procedure for detection of *Pneumocystis carinii* by PCR. *J Clin Microbiol* 32: 1634–1638.
- Castro M (1998) Treatment and prophylaxis of *Pneumocystis carinii* pneumonia. *Semin Respir Infect* 13: 296–303. [PubMed: 9872626]
- Cettomai D, Gelber AC and Christopher-Stine L (2010) A survey of rheumatologists' practice for prescribing *Pneumocystis* prophylaxis. *J Rheumatol*, in press.

- Chen W, Havell EA and Harmsen AG (1992) Importance of endogenous tumor necrosis factor alpha and gamma interferon in host resistance against *Pneumocystis carinii* infection. *Infect Immun* 60: 1279–1284. [PubMed: 1548057]
- Colby C, McAfee S, Sackstein R, Finkelstein D, Fishman J and Spitzer T (1999) A prospective randomized trial comparing the toxicity and safety of atovaquone with trimethoprim/sulfamethoxazole as *Pneumocystis carinii* pneumonia prophylaxis following autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant* 24: 897–902. [PubMed: 10516703]
- Crans CA Jr and Boisselle PM (1999) Imaging features of *Pneumocystis carinii* pneumonia. *Crit Rev Diagn Imaging* 40: 251–284. [PubMed: 10514937]
- Crothers K, Beard CB, Turner J, Groner G, Fox M, Morris A et al. (2005) Severity and outcome of HIV-associated *Pneumocystis pneumonia* containing *Pneumocystis jirovecii* dihydropteroate synthase gene mutations. *Aids* 19: 801–805. [PubMed: 15867494]
- Cushion MT, Ruffolo JJ, Linke MJ and Walzer PD (1985) *Pneumocystis carinii*: growth variables and estimates in the A549 and WI-38 VA13 human cell lines. *Exp Parasitol* 60: 43–54. [PubMed: 3894045]
- de Oliveira A, Unnasch TR, Crothers K, Eiser S, Zucchi P, Moir J et al. (2007) Performance of a molecular viability assay for the diagnosis of *Pneumocystis pneumonia* in HIV-infected patients. *Diagn Microbiol Infect Dis* 57: 169–176. [PubMed: 17049800]
- Delclaux C, Zahar JR, Amraoui G, Leleu G, Lebargy F, Brochard L et al. (1999) Corticosteroids as adjunctive therapy for severe *Pneumocystis carinii* pneumonia in non-human immunodeficiency virus-infected patients: retrospective study of 31 patients. *Clin Infect Dis* 29: 670–672. [PubMed: 10530464]
- Demanche C, Wanert F, Barthelemy M, Mathieu J, Durand-Joly I, Dei-Cas E et al. (2005) Molecular and serological evidence of *Pneumocystis* circulation in a social organization of healthy macaques (*Macaca fascicularis*). *Microbiology* 151: 3117–3125. [PubMed: 16151222]
- Dieterich DT, Lew EA, Bacon DJ, Pearlman KI and Scholes JV (1992) Gastrointestinal pneumocystosis in HIV-infected patients on aerosolized pentamidine: report of five cases and literature review. *Am J Gastroenterol* 87: 1763–1770.
- Dungarwalla M, Marsh JC, Tooze JA, Lucas G, Ouwehand W, Pettengell R et al. (2007) Lack of clinical efficacy of rituximab in the treatment of autoimmune neutropenia and pure red cell aplasia: implications for their pathophysiology. *Ann Hematol* 86: 191–197. [PubMed: 17123083]
- Edelstein H and McCabe RE (1990) Atypical presentations of *Pneumocystis carinii* pneumonia in patients receiving inhaled pentamidine prophylaxis. *Chest* 98: 1366–1369. [PubMed: 2245676]
- Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ and Sogin ML (1988) Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. *Nature* 334: 519–522. [PubMed: 2970013]
- Festic E, Gajic O, Limper AH and Aksamit TR (2005) Acute respiratory failure due to *Pneumocystis pneumonia* in patients without human immunodeficiency virus infection: outcome and associated features. *Chest* 128: 573–579. [PubMed: 16100140]
- Fischl MA, Dickinson GM and La Voie L (1988) Safety and efficacy of sulfamethoxazole and trimethoprim chemoprophylaxis for *Pneumocystis carinii* pneumonia in AIDS. *JAMA* 259: 1185–1189.
- Fishman JA (2001) Prevention of infection caused by *Pneumocystis carinii* in transplant recipients. *Clin Infect Dis* 33: 1397–1405. [PubMed: 11565082]
- Fujii T, Nakamura T and Iwamoto A (2007) *Pneumocystis pneumonia* in patients with HIV infection: clinical manifestations, laboratory findings, and radiological features. *J Infect Chemother* 13: 1–7.
- Giese W (1953) Pathogenese und atologie der interstitiellen plasmazellularen sauglingspneumonie. *Verhandl d deutsch path gesellsch* 36: 284–289.
- Gigliotti F and Wright TW (2005) Immunopathogenesis of *Pneumocystis carinii* pneumonia. *Expert Rev Mol Med* 7: 1–16.
- Godeau B, Mainardi JL, Roudot-Thoraval F, Hachulla E, Guillemin L, Huong Du LT et al. (1995) Factors associated with *Pneumocystis carinii* pneumonia in Wegener's granulomatosis. *Ann Rheum Dis* 54: 991–994. [PubMed: 8546533]

- Goodridge HS, Wolf AJ and Underhill DM (2009) Beta-glucan recognition by the innate immune system. *Immunol Rev* 230: 38–50. [PubMed: 19594628]
- Green H, Paul M, Vidal L and Leibovici L (2007) Prophylaxis of *Pneumocystis pneumonia* in immunocompromised non-HIV-infected patients: systematic review and meta-analysis of randomized controlled trials. *Mayo Clin Proc* 82: 1052–1059. [PubMed: 17803871]
- Guttler R, Singer PA, Axline SG, Greaves TS and McGill JJ (1993) *Pneumocystis carinii* thyroiditis. Report of three cases and review of the literature. *Arch Intern Med* 153: 393–396. [PubMed: 8427542]
- Hagmann S, Merali S, Sitnitskaya Y, Fefferman N and Pollack H (2001) *Pneumocystis carinii* infection presenting as an intra-abdominal cystic mass in a child with acquired immunodeficiency syndrome. *Clin Infect Dis* 33: 1424–1426. [PubMed: 11565084]
- Hahn PY, Evans SE, Kottom TJ, Standing JE, Pagano RE and Limper AH (2003) *Pneumocystis carinii* cell wall beta-glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. *J Biol Chem* 278: 2043–2050.
- Hardy AM, Wajszczuk CP, Suffredini AF, Hakala TR and Ho M (1984) *Pneumocystis carinii* pneumonia in renal-transplant recipients treated with cyclosporine and steroids. *J Infect Dis* 149: 143–147.
- Harigai M, Koike R and Miyasaka N (2007) *Pneumocystis pneumonia* associated with infliximab in Japan. *N Engl J Med* 357: 1874–1876.
- Hauser PM, Sudre P, Nahimana A and Francioli P (2001) Prophylaxis failure is associated with a specific *Pneumocystis carinii* genotype. *Clin Infect Dis* 33: 1080–1082. [PubMed: 11528584]
- Helweg-Larsen J, Benfield TL, Eugen-Olsen J, Lundgren JD and Lundgren B (1999) Effects of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of AIDS-associated *P. carinii* pneumonia. *Lancet* 354: 1347–1351. [PubMed: 10533864]
- Helweg-Larsen J, Jensen JS, Benfield T, Svendsen UG, Lundgren JD and Lundgren B (1998) Diagnostic use of PCR for detection of *Pneumocystis carinii* in oral wash samples. *J Clin Microbiol* 36: 2068–2072.
- Hidalgo A, Falco V, Mauleon S, Andreu J, Crespo M, Ribera E et al. (2003) Accuracy of high-resolution CT in distinguishing between *Pneumocystis carinii* pneumonia and non-*Pneumocystis carinii* pneumonia in AIDS patients. *Eur Radiol* 13: 1179–1184. [PubMed: 12695843]
- Hirschtick RE, Glassroth J, Jordan MC, Wilcosky TC, Wallace JM, Kvale PA et al. (1995) Bacterial pneumonia in persons infected with the human immunodeficiency virus. Pulmonary Complications of HIV Infection Study Group. *N Engl J Med* 333: 845–851.
- Hof H and Schnulle P (2008) *Pneumocystis jirovecii* pneumonia in a patient with Wegener's granulomatosis treated efficiently with caspofungin. *Mycoses* 51(Suppl 1): 65–67. [PubMed: 18471163]
- Hoffman OA, Standing JE and Limper AH (1993) *Pneumocystis carinii* stimulates tumor necrosis factor-alpha release from alveolar macrophages through a beta-glucan-mediated mechanism. *J Immunol* 150: 3932–3940.
- Huang L, Crothers K, Atzori C, Benfield T, Miller R, Rabodonirina M et al. (2004) Dihydropteroate synthase gene mutations in *Pneumocystis* and sulfa resistance. *Emerg Infect Dis* 10: 1721–1728. [PubMed: 15504256]
- Huang L, Morris A, Limper AH and Beck JM (2006) An Official ATS Workshop Summary: Recent advances and future directions in *Pneumocystis pneumonia* (PCP). *Proc Am Thorac Soc* 3: 655–664. [PubMed: 17065370]
- Huggett JF, Taylor MS, Kocjan G, Evans HE, Morris-Jones S, Gant V et al. (2008) Development and evaluation of a real-time PCR assay for detection of *Pneumocystis jirovecii* DNA in bronchoalveolar lavage fluid of HIV-infected patients. *Thorax* 63: 154–159. [PubMed: 17693588]
- Hughes WT (1991) Prevention and treatment of *Pneumocystis carinii* pneumonia. *Annu Rev Med* 42: 287–295. [PubMed: 2035974]
- Icenhour CR, Rebholz SL, Collins MS and Cushion MT (2002) Early acquisition of *Pneumocystis carinii* in neonatal rats as evidenced by PCR and oral swabs. *Eukaryot Cell* 1: 414–419. [PubMed: 12455989]

- Inoue Y, Nishimura K, Shiode M, Akutsu H, Hamada H, Fujioka S et al. (1995) Evaluation of serum KL-6 levels in patients with pulmonary tuberculosis. *Tuber Lung Dis* 76: 230–233. [PubMed: 7548906]
- Ioannidis JP, Cappelleri JC, Skolnik PR, Lau J and Sacks HS (1996) A meta-analysis of the relative efficacy and toxicity of *Pneumocystis carinii* prophylactic regimens. *Arch Intern Med* 156: 177–188. [PubMed: 8546551]
- Jones JL, Hanson DL, Dworkin MS, Alderton DL, Fleming PL, Kaplan JE et al. (1999) Surveillance for AIDS-defining opportunistic illnesses, 1992–1997. *MMWR CDC Surveill Summ* 48: 1–22.
- Joos B, Blaser J, Opravil M, Chave JP and Luthy R (1995) Monitoring of co-trimoxazole concentrations in serum during treatment of *Pneumocystis carinii* pneumonia. *Antimicrob Agents Chemother* 39: 2661–2666. [PubMed: 8592998]
- Kalyoncu U, Karadag O, Akdogan A, Kisacik B, Erman M, Erguven S et al. (2007) *Pneumocystis carinii* pneumonia in a rheumatoid arthritis patient treated with adalimumab. *Scand J Infect Dis* 39: 475–478.
- Kaplan JE, Hanson D, Dworkin MS, Frederick T, Bertolli J, Lindegren ML et al. (2000) Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. *Clin Infect Dis* 30(Suppl 1): S5–S14. [PubMed: 10770911]
- Kaplan JE, Masur H and Holmes KK (2002) Guidelines for preventing opportunistic infections among HIV-infected persons—2002. Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *MMWR Recomm Rep* 51(RR-8): 1–52.
- Kaur N and Mahl TC (2007) *Pneumocystis jirovecii* (carinii) pneumonia after infliximab therapy: a review of 84 cases. *Dig Dis Sci* 52: 1481–1484. [PubMed: 17429728]
- Kawasaki Y, Aoyagi Y, Abe Y, Go H, Imamura T, Kaneko M et al. (2009) Serum KL-6 levels as a biomarker of lung injury in respiratory syncytial virus bronchiolitis. *J Med Virol* 81: 2104–2108.
- Kessl JJ, Hill P, Lange BB, Meshnick SR, Meunier B and Trumpower BL (2004) Molecular basis for atovaquone resistance in *Pneumocystis jirovecii* modeled in the cytochrome bc(1) complex of *Saccharomyces cerevisiae*. *J Biol Chem* 279: 2817–2824.
- Klinker H, Langmann P, Zilly M and Richter E (1998) Drug monitoring during the treatment of AIDS-associated *Pneumocystis carinii* pneumonia with trimethoprim-sulfamethoxazole. *J Clin Pharm Ther* 23: 149–154.
- Kolls JK, Lei D, Vazquez C, Odom G, Summer WR, Nelson S et al. (1997) Exacerbation of murine *Pneumocystis carinii* infection by adenoviral-mediated gene transfer of a TNF inhibitor. *Am J Respir Cell Mol Biol* 16: 112–118.
- Kolstad A, Holte H, Fossa A, Lauritzsen GF, Gaustad P and Torfoss D (2007) *Pneumocystis jirovecii* pneumonia in B-cell lymphoma patients treated with the rituximab-CHOEP-14 regimen. *Haematologica* 92: 139–140. [PubMed: 17229653]
- Komano Y, Harigai M, Koike R, Sugiyama H, Ogawa J, Saito K et al. (2009) *Pneumocystis jirovecii* pneumonia in patients with rheumatoid arthritis treated with infliximab: a retrospective review and case-control study of 21 patients. *Arthritis Rheum* 61: 305–312. [PubMed: 19248121]
- Kottom TJ, Kennedy CC and Limper AH (2008) *Pneumocystis* PCINT1, a molecule with integrin-like features that mediates organism adhesion to fibronectin. *Mol Microbiol* 67: 747–761. [PubMed: 18179594]
- Kottom TJ and Limper AH (2000) Cell wall assembly by *Pneumocystis carinii*. Evidence for a unique gsc-1 subunit mediating beta -1,3-glucan deposition. *J Biol Chem* 275: 40628–40634.
- Kozel H, Eichbaum Q, Kruskal BA, Pinkston P, Rogers RA, Armstrong MY et al. (1998) Reduced binding and phagocytosis of *Pneumocystis carinii* by alveolar macrophages from persons infected with HIV-1 correlates with mannose receptor downregulation. *J Clin Invest* 102: 1332–1344.
- Lahiff C, Khieron OB, Nolan N and Chadwick GA (2007) *Pneumocystis carinii* pneumonia in a patient on etanercept for psoriatic arthritis. *Ir J Med Sci* 176: 309–311.
- Lapraik C, Watts R, Bacon P, Carruthers D, Chakravarty K, D'Cruz D et al. (2007) BSR and BHPR guidelines for the management of adults with ANCA associated vasculitis. *Rheumatology (Oxford)* 46: 1615–1616. [PubMed: 17804455]

- Lasbury ME, Durant PJ, Ray CA, Tschang D, Schwendener R and Lee CH (2006) Suppression of alveolar macrophage apoptosis prolongs survival of rats and mice with *Pneumocystis pneumonia*. *J Immunol* 176: 6443–6453.
- Lebron F, Vassallo R, Puri V and Limper AH (2003) *Pneumocystis carinii* cell wall beta-glucans initiate macrophage inflammatory responses through NF-kappaB activation. *J Biol Chem* 278: 25001–25008.
- Limper AH, Hoyte JS and Standing JE (1997a) The role of alveolar macrophages in *Pneumocystis carinii* degradation and clearance from the lung. *J Clin Invest* 99: 2110–2117.
- Limper AH, Offord KP, Smith TF and Martin II WJ (1989) *Pneumocystis carinii* pneumonia. Differences in lung parasite number and inflammation in patients with and without AIDS. *Am Rev Respir Dis* 140: 1204–1209. [PubMed: 2817582]
- Limper AH, Standing JE, Hoffman OA, Castro M and Neese LW (1993) Vitronectin binds to *Pneumocystis carinii* and mediates organism attachment to cultured lung epithelial cells. *Infect Immun* 61: 4302–4309. [PubMed: 7691747]
- Limper AH, Thomas CF Jr, Anders RA and Leof EB (1997b) Interactions of parasite and host epithelial cell cycle regulation during *Pneumocystis carinii* pneumonia. *J Lab Clin Med* 130: 132–138.
- Lu JJ, Chen CH, Bartlett MS, Smith JW and Lee CH (1995) Comparison of six different PCR methods for detection of *Pneumocystis carinii*. *J Clin Microbiol* 33: 2785–2788.
- Madden RM, Pui CH, Hughes WT, Flynn PM and Leung W (2007) Prophylaxis of *Pneumocystis carinii* pneumonia with atovaquone in children with leukemia. *Cancer* 109: 1654–1658. [PubMed: 17345613]
- Martin SI and Fishman JA (2009) *Pneumocystis pneumonia* in solid organ transplant recipients. *Am J Transplant* 9(Suppl 4): S227–S233.
- Marty FM, Koo S, Bryar J and Baden LR (2007) (1->3)beta-D-glucan assay positivity in patients with *Pneumocystis (carinii) jirovecii* pneumonia. *Ann Intern Med* 147: 70–72.
- Masur H (1992) Prevention and treatment of *Pneumocystis pneumonia*. *N Engl J Med* 327: 1853–1860.
- Merali S, Frevert U, Williams JH, Chin K, Bryan R and Clarkson AB Jr (1999) Continuous axenic cultivation of *Pneumocystis carinii*. *Proc Natl Acad Sci U S A* 96: 2402–2407. [PubMed: 10051654]
- Mermin J, Lule JR, Ekwaru JP and Pitter C (2005) Should cotrimoxazole prophylaxis be taken by all adults with HIV in Africa? *Aids* 19: 845–846. [PubMed: 15867506]
- Moosig F, Holle JU and Gross WL (2009) Value of anti-infective chemoprophylaxis in primary systemic vasculitis: what is the evidence? *Arthritis Res Ther* 11: 253. [PubMed: 19886977]
- Mu XD, Que CL, He B, Wang GF and Li HC (2009) Caspofungin in salvage treatment of severe *Pneumocystis pneumonia*: case report and literature review. *Chin Med J (Engl)* 122: 996–999.
- Mukhtyar C, Guillevin L, Cid MC, Dasgupta B, de Groot K, Gross W et al. (2009) EULAR recommendations for the management of primary small and medium vessel vasculitis. *Ann Rheum Dis* 68: 310–317. [PubMed: 18413444]
- Nahimana A, Rabodonirina M, Bille J, Francioli P and Hauser PM (2004) Mutations of *Pneumocystis jirovecii* dihydrofolate reductase associated with failure of prophylaxis. *Antimicrob Agents Chemother* 48: 4301–4305. [PubMed: 15504856]
- Nakamura H, Tateyama M, Tasato D, Haranaga S, Yara S, Higa F et al. (2009) Clinical utility of serum beta-D-glucan and KL-6 levels in *Pneumocystis jirovecii* pneumonia. *Intern Med* 48: 195–202. [PubMed: 19218768]
- Navin TR, Beard CB, Huang L, del Rio C, Lee S, Pieniazek NJ et al. (2001) Effect of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of *P carinii* pneumonia in patients with HIV-1: a prospective study. *Lancet* 358: 545–549. [PubMed: 11520525]
- Ochs H, Stiehm ER and Winkelstein JA (2004) *Immunological Disorders in Infants and Children*, In: Stiehm E, Ochs HD and Winkelstein JA (eds), Elsevier Saunders: Philadelphia, PA, p. 357.
- Ognibene FP, Shelhamer JH, Hoffman GS, Kerr GS, Reda D, Fauci AS et al. (1995) *Pneumocystis carinii* pneumonia: a major complication of immunosuppressive therapy in patients with Wegener's granulomatosis. *Am J Respir Crit Care Med* 151: 795–799.

- Opelz G (1995) Efficacy of rejection prophylaxis with OKT3 in renal transplantation. Collaborative Transplant Study. *Transplantation* 60: 1220–1224. [PubMed: 8525514]
- Panos GZ, Karydis I, Velakoulis SE and Falagas ME (2007) Multi-skeletal *Pneumocystis jiroveci* (carinii) in an HIV-seropositive patient. *Int J STD AIDS* 18: 134–137.
- Pareja JG, Garland R and Koziel H (1998) Use of adjunctive corticosteroids in severe adult non-HIV *Pneumocystis carinii* pneumonia. *Chest* 113: 1215–1224. [PubMed: 9596297]
- Peglow SL, Smulian AG, Linke MJ, Pogue CL, Nurre S, Crisler J et al. (1990) Serologic responses to *Pneumocystis carinii* antigens in health and disease. *J Infect Dis* 161: 296–306.
- Perruquet JL, Harrington TM and Davis DE (1983) *Pneumocystis carinii* pneumonia following methotrexate therapy for rheumatoid arthritis. *Arthritis Rheum* 26: 1291–1292. [PubMed: 6605149]
- Peters SE, Wakefield AE, Banerji S and Hopkin JM (1992a) Quantification of the detection of *Pneumocystis carinii* by DNA amplification. *Mol Cell Probes* 6: 115–117. [PubMed: 1513340]
- Peters SE, Wakefield AE, Sinclair K, Millard PR and Hopkin JM (1992b) A search for *Pneumocystis carinii* in post-mortem lungs by DNA amplification. *J Pathol* 166: 195–198.
- Phair J, Munoz A, Detels R, Kaslow R, Rinaldo C and Saah A (1990) The risk of *Pneumocystis carinii* pneumonia among men infected with human immunodeficiency virus type 1. Multicenter AIDS Cohort Study Group. *N Engl J Med* 322: 161–165.
- Pifer LL, Hughes WT, Stagno S and Woods D (1978) *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. *Pediatrics* 61: 35–41. [PubMed: 400818]
- Ponce CA, Gallo M, Bustamante R and Vargas SL (2010) *Pneumocystis* colonization is highly prevalent in the autopsied lungs of the general population. *Clin Infect Dis* 50: 347–353. [PubMed: 20047487]
- Powles MA, Liberator P, Anderson J, Karkhanis Y, Dropinski JF, Bouffard FA et al. (1998) Efficacy of MK-991 (L-743,872), a semisynthetic pneumocandin, in murine models of *Pneumocystis carinii*. *Antimicrob Agents Chemother* 42: 985–989. [PubMed: 9687394]
- Procop GW, Haddad S, Quinn J, Wilson ML, Henshaw NG, Reller LB et al. (2004) Detection of *Pneumocystis jiroveci* in respiratory specimens by four staining methods. *J Clin Microbiol* 42: 3333–3335.
- Ribes JA, Limper AH, Espy MJ and Smith TF (1997) PCR detection of *Pneumocystis carinii* in bronchoalveolar lavage specimens: analysis of sensitivity and specificity. *J Clin Microbiol* 35: 830–835.
- Ruggli GM, Weber R, Messmer EP, Font RL, Moll C and Bernauer W (1997) *Pneumocystis carinii* infection of the conjunctiva in a patient with acquired immune deficiency syndrome. *Ophthalmology* 104: 1853–1856. [PubMed: 9373116]
- Sato H, Callister ME, Mumby S, Quinlan GJ, Welsh KI, duBois RM et al. (2004) KL-6 levels are elevated in plasma from patients with acute respiratory distress syndrome. *Eur Respir J* 23: 142–145.
- Segal BH, Freifeld AG, Baden LR, Brown AE, Casper C, Dubberke E et al. (2008) Prevention and treatment of cancer-related infections. *J Natl Compr Canc Netw* 6: 122–174.
- Sepkowitz KA (1996) *Pneumocystis carinii* pneumonia without acquired immunodeficiency syndrome: who should receive prophylaxis? *Mayo Clin Proc* 71: 102–103. [PubMed: 8538221]
- Shellito J, Suzara VV, Blumenfeld W, Beck JM, Steger HJ and Ermak TH (1990) A new model of *Pneumocystis carinii* infection in mice selectively depleted of helper T lymphocytes. *J Clin Invest* 85: 1686–1693.
- Smego RA Jr, Nagar S, Maloba B and Popara M (2001) A meta-analysis of salvage therapy for *Pneumocystis carinii* pneumonia. *Arch Intern Med* 161: 1529–1533. [PubMed: 11427101]
- Stiehm E, Ochs HD and Winkelstein JA (2004) *Immunological Disorders in Infants and Children*, 5th edn, Elsevier Saunders: Philadelphia, PA, p. 289.
- Stringer JR (2002) *Pneumocystis*. *Int J Med Microbiol* 292: 391–404.
- Sukoh N, Yamamoto H, Kikuchi E, Suzuki T, Kamimura A, Watanabe N et al. (2001) [A case of severe *Legionella* pneumonia monitored with serum SP-A, SP-D, and KL-6]. *Nihon Kokyuki Gakkai Zasshi* 39: 126–130. [PubMed: 11321824]

- Suryaprasad A and Stone JH (2008) When is it safe to stop *Pneumocystis jirovecii* pneumonia prophylaxis? Insights from three cases complicating autoimmune diseases. *Arthritis Rheum* 59: 1034–1039. [PubMed: 18576286]
- Tamburrini E, Mencarini P, Visconti E, Zolfo M, De Luca A, Siracusano A et al. (1996) Comparison of two PCR methods for detection of *Pneumocystis carinii* in bronchoalveolar lavage fluid. *J Eukaryot Microbiol* 43: 20S.
- Tasaka S, Hasegawa N, Kobayashi S, Yamada W, Nishimura T, Takeuchi T et al. (2007) Serum indicators for the diagnosis of *Pneumocystis pneumonia*. *Chest* 131: 1173–1180. [PubMed: 17426225]
- The National Institutes of Health-University of California Expert Panel for Corticosteroids as Adjunctive Therapy for *Pneumocystis Pneumonia*. (1990) Consensus statement on the use of corticosteroids as adjunctive therapy for *Pneumocystis pneumonia* in the acquired immunodeficiency syndrome. *N Engl J Med* 323: 1500–1504.
- Thomas CF Jr and Limper AH (2004) *Pneumocystis pneumonia*. *N Engl J Med* 350: 2487–2498.
- Thomas CF Jr and Limper AH (2007) Current insights into the biology and pathogenesis of *Pneumocystis pneumonia*. *Nat Rev Microbiol* 5: 298–308. [PubMed: 17363968]
- Toma E, Thorne A, Singer J, Raboud J, Lemieux C, Trottier S et al. (1998) Clindamycin with primaquine vs. Trimethoprim-sulfamethoxazole therapy for mild and moderately severe *Pneumocystis carinii* pneumonia in patients with AIDS: a multicenter, double-blind, randomized trial (CTN 004). CTN-PCP Study Group. *Clin Infect Dis* 27: 524–530. [PubMed: 9770152]
- Tumeh SS, Belville JS, Pugatch R and McNeil BJ (1992) Ga-67 scintigraphy and computed tomography in the diagnosis of *Pneumocystis carinii* pneumonia in patients with AIDS. A prospective comparison. *Clin Nucl Med* 17: 387–394. [PubMed: 1587045]
- Utili R, Durante-Mangoni E, Basilico C, Mattei A, Ragone E and Grossi P (2007) Efficacy of caspofungin addition to trimethoprim-sulfamethoxazole treatment for severe *Pneumocystis pneumonia* in solid organ transplant recipients. *Transplantation* 84: 685–688. [PubMed: 17893600]
- Van der Meer MG and Brug S (1942) Infection à *Pneumocystis* chez l'homme et chez les animaux. *Amer Soc Belge Méd Trop* 22: 301–309.
- Vanek J and Jírovec O (1952) [Parasitic pneumonia. Interstitial plasma cell pneumonia of premature, caused by *Pneumocystis carinii*.]. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg* 158: 120–127.
- Vassallo R, Standing J and Limper AH (1999a) Beta-glucan from *Pneumocystis carinii* stimulates TNF alpha release from alveolar macrophages. *J Eukaryot Microbiol* 46: 145S.
- Vassallo R, Thomas CF Jr, Vuk-Pavlovic Z and Limper AH (1999b) Alveolar macrophage interactions with *Pneumocystis carinii*. *J Lab Clin Med* 133: 535–540.
- Villegas LR, Kottom TJ and Limper AH (2009) Characterization of PCEng2 a {beta}-1,3-endoglucanase homologue in *Pneumocystis carinii* with activity in cell wall regulation. *Am J Respir Cell Mol Biol*, in press.
- Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER et al. (1990a) Detection of *Pneumocystis carinii* with DNA amplification. *Lancet* 336: 451–453. [PubMed: 1974987]
- Wakefield AE, Stewart TJ, Moxon ER, Marsh K and Hopkin JM (1990b) Infection with *Pneumocystis carinii* is prevalent in healthy Gambian children. *Trans R Soc Trop Med Hyg* 84: 800–802. [PubMed: 2096511]
- Wakefield AE, Stringer JR, Tamburrini E and Dei-Cas E (1998) Genetics, metabolism and host specificity of *Pneumocystis carinii*. *Med Mycol* 36(Suppl 1): 183–193. [PubMed: 9988507]
- Walzer PD (1986) Attachment of microbes to host cells: relevance of *Pneumocystis carinii*. *Lab Invest* 54: 589–592. [PubMed: 2423776]
- Walzer PD, Evans HE, Copas AJ, Edwards SG, Grant AD and Miller RF (2008) Early predictors of mortality from *Pneumocystis jirovecii* pneumonia in HIV-infected patients: 1985–2006. *Clin Infect Dis* 46: 625–633. [PubMed: 18190281]
- Wilkin A and Feinberg J (1999) *Pneumocystis carinii* pneumonia: a clinical review. *Am Fam Physician* 60: 1699–1708. [PubMed: 10537385]

- Wislez M, Bergot E, Antoine M, Parrot A, Carette MF, Mayaud C et al. (2001) Acute respiratory failure following HAART introduction in patients treated for *Pneumocystis carinii* pneumonia. *Am J Respir Crit Care Med* 164: 847–851.
- Wright TW, Gigliotti F, Finkelstein JN, McBride JT, An CL and Harmsen AG (1999) Immune-mediated inflammation directly impairs pulmonary function, contributing to the pathogenesis of *Pneumocystis carinii* pneumonia. *J Clin Invest* 104: 1307–1317.
- Yale SH and Limper AH (1996) *Pneumocystis carinii* pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. *Mayo Clin Proc* 71: 5–13. [PubMed: 8538233]
- Yasuoka A, Tachikawa N, Shimada K, Kimura S and Oka S (1996) (1->3) beta-D-glucan as a quantitative serological marker for *Pneumocystis carinii* pneumonia. *Clin Diagn Lab Immunol* 3: 197–199. [PubMed: 8991635]
- Zelante T, De Luca A, Bonifazi P, Montagnoli C, Bozza S, Moretti S et al. (2007) IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol* 37: 2695–2706.
- Zhuang H and Alavi A (2002) 18-fluorodeoxyglucose positron emission tomographic imaging in the detection and monitoring of infection and inflammation. *Semin Nucl Med* 32: 47–59. [PubMed: 11839069]

Figure 1.

(A) Posteroanterior chest radiograph showing diffuse bilateral predominantly interstitial opacities in a 71-year-old male with *Pneumocystis* pneumonia in the setting of diffuse large B-cell lymphoma on R-CHOP therapy. (B) Selected noncontrast CT axial image revealing bilateral diffuse ground-glass opacities and associated interlobular and intralobular septal thickening. (C) Selected contrast CT axial image revealing diffuse ground-glass pulmonary infiltrates and innumerable cystic changes predominantly in the upper lobes in a 53-year-old male with *Pneumocystis* pneumonia in the setting of recently diagnosed HIV.

Figure 2.
Calcofluor white staining of *Pneumocystis* organisms in a bronchoalveolar lavage (BAL) specimen. A human cell on the right has picked up some of the fluorescent stain. Original size $\times 1000$, scale bar is 20 μm .

Figure 3.

Histological features of *Pneumocystis* pneumonia. (A) *Pneumocystis* pneumonia in a surgical lung biopsy specimen. The characteristic frothy exudates harboring the organisms fills an alveolus (small arrows) lined by reactive type II cells (large arrow). Internal structures of the organisms can be discerned at high magnification (inset). Hematoxylin-Eosin stain; original $\times 400$ and $\times 1000$ (inset), scale bar is 25 μm . (B) Silver stain of *Pneumocystis* organisms in a surgical lung biopsy. The organisms appear round or cup-shaped, some show intracystic bodies (lower right). Gomori's Methenamine Silver stain; original size $\times 400$, scale bar is 25 μm . (C) Immunohistochemical stain for *Pneumocystis* organisms. Surgical lung biopsy; original size $\times 1000$, scale bar is 25 μm . All three images provided courtesy of Dr Frank Schneider, Department of Pathology, Mayo Clinic, Rochester, MN.

Table 1.

Immunosuppressive agents associated with the development of *Pneumocystis* pneumonia.

Corticosteroids
Alkylating agents
Cyclophosphamide
Temozolomide
Antibiotics/Immunosuppressants
Bleomycin
Sirolimus
Tacrolimus
Antimetabolites
Cytarabine
Fluorouracil
Methotrexate
Polypeptides
Cyclosporine
Purine analogs
Azathioprine
Cladribine
Fludarabine
TNF α inhibitors
Adalimumab ^a
Etanercept
Infliximab ^a
Monoclonal antibodies
Alemtuzumab ^a
Rituximab ^a

^a Monoclonal antibodies TNF, tumor necrosis factor.

Table 2.

Main indicators for *Pneumocystis* prophylaxis.

General patients:	
•	Prednisone ^a of at least 20 mg for >4 weeks if patient has underlying immunosuppressive disorder or COPD [Sepkowitz, 1996; Yale and Limper, 1996]
•	TNF α inhibitors specially if on corticosteroids ^a or other intensive immunosuppression [Komano <i>et al.</i> 2009; Kaur and Mahl, 2007]
•	Corticosteroids ^a and steroid-sparing agent (e.g. MTX and AZA) [Moosig <i>et al.</i> 2009]
•	Cyclophosphamide and corticosteroids ^a [Mukhtyar <i>et al.</i> 2009; Lapraik <i>et al.</i> 2007]
Cancer patients:	
•	Receiving corticosteroids ^a [Segal <i>et al.</i> 2008]
•	Alemtuzumab during and for at least 2 months after treatment and CD4 >200 cells/ μ l [Segal <i>et al.</i> 2008]
•	Temozolomide and radiation therapy and until CD4 is >200 cells/ μ l [Segal <i>et al.</i> 2008]
•	Fludarabine and T-cell-depleting agent (e.g. cladribine) until DC4 >200 cells/ μ l [Segal <i>et al.</i> 2008]
•	ALL patients while receiving anti-leukemic therapy [Segal <i>et al.</i> 2008]
HIV positive patients:	
•	History of Pneumocystis pneumonia [Huang <i>et al.</i> 2006]
•	CD4 cell count below 200 cells/ μ l [Huang <i>et al.</i> 2006]
•	Active oropharyngeal candidiasis [Huang <i>et al.</i> 2006]
Rheumatologic patients:	
•	Wegener patients on cyclophosphamide especially if also receiving corticosteroids ^a [Godeau <i>et al.</i> 1995; Ognibene <i>et al.</i> 1995]
Transplant patients:	
•	Allogeneic stem cell recipients for at least 180 days [Segal <i>et al.</i> 2008]
•	Autologous peripheral blood stem cell transplant recipients for 3–6 months after transplant [Segal <i>et al.</i> 2008]
•	Solid organ transplant recipients for at least 6–12 months after transplant [Martin and Fishman, 2009]
Primary immunodeficiencies:	
•	Severe combined immunodeficiency [Ochs <i>et al.</i> 2004; Stiehm <i>et al.</i> 2004]
•	Idiopathic CD4T-lymphocytopenia [Ochs <i>et al.</i> 2004; Stiehm <i>et al.</i> 2004]

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Hyper-IgM syndrome [Ochs *et al.* 2004; Stiehm *et al.* 2004]

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^gPrednisone (or dose equivalent) of at least 16–20 mg for >4 weeks.

COPD, chronic obstructive pulmonary disease; TNF, tumor necrosis factor; MTX, methotrexate, AZA, azathioprine; RT, radiotherapy; Tx, transplant.

Table 3.

Treatment options for *Pneumocystis* pneumonia.

PREFERRED OPTION		
Drug	Dose and route ^a	Indications/other comments
TMP-SMX	15–20 mg/kg/day (TMP)	• IV recommended in severe cases
	75–100 mg/kg/day (SMX)	• Oral for mild and moderate cases
	IV or oral In divided doses	• IV form can be switched to oral after clinical improvement
	(3–4 times a day)	• Levels should be monitored
ALTERNATIVE REGIMENS		
Pentamidine	4 mg/kg/day	• Moderate to severe cases
	IV or IM	• High incidence of toxicity
Primaquine + Clindamycin	30 mg/day orally +	• Mild to moderate cases
	600 mg/three times a day	• Check for G6PD deficiency prior to the use of primaquine
Atovaquone	IV or oral	• Mild to moderate cases
	750 mg twice a day oral	• Mild to moderate case
TMP +	5 mg/kg three times a day +	• Methemoglobinemia with Dapsone
Dapsone	100 mg/day	
Oral		
ADJUNCTIVE THERAPY		
Prednisone	40 mg twice a day for 5 days →	• Moderate to severe cases
	40 mg a day for 5 days →	• Especially if hypoxemia (PaO ₂ 70 or alveolar-arterial oxygen gradient 35 mmHg)
	20 mg a day for 11 days Oral	

TMP-SMX, trimethoprim-sulfamethoxazole; IV, intravenous; IM, intramuscular.

Recommended duration of treatment is 21 days.

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Table 4.

Prophylaxis options for *Pneumocystis* pneumonia.

PREFERRED OPTION	
Drug	Dose and route
TMP-SMX	1 DS ^a tablet daily or
	1 DS ^a tablet 3 times a week or
	1 SS ^b tablet daily
	Oral
ALTERNATIVE OPTIONS	
Dapsone	50 mg twice a day or
	100 mg daily
	Oral
Atovaquone	750 mg twice a day or
	1500 mg daily
	Oral suspension
Pentamidine	300 mg monthly Aerosolized or
	4 mg/kg IV monthly
ALTERNATIVE OPTIONS FOR: HIV with CD4 <100 cells/ μ l and + toxoplasma IgG or non-HIV but risk factors for toxoplasma	
Dapsone +	50 mg/day (D) + 50 mg/week (P) + 25 mg/week (L) or
Pyrimethamine +	200 mg/week (D) + 75 mg/week (P) + 25 mg/week (L)
Leucovorin	Oral

^aDS tablet contains SMX 800 mg/TMP 160 mg.

^bSS tablet contains SMX 400 mg/TMP 80 mg.

TMP-SMX, trimethoprim-sulfamethoxazole; D, dapsone; P, pyrimethamine; L, leucovorin; SS, single strength, DS double strength; IV, intravenous.