Pneumocystis jirovecii Pneumonia in AIDS Patients

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1. Introduction

Pneumocystis pneumonia (PcP) in humans is caused by the opportunistic eukaryotic pathogen *Pneumocystis jirovecii* (previously known as *Pneumocystis carinii* f. sp. *hominis*), which has recently been reclassified as a fungus because its cell wall composition and gene sequences (Edman, 1988, Stringer, 1989). This atypical uncultured fungus remains a major cause of illness and death in patients who have HIV infection. PcP has been the most common AIDS-defining opportunistic infection in the United States and Europe during more than two decades. Before 1989, 60-80% of AIDS patients presented with PcP, and the infection was estimated to be the cause of the death of 20-25% of these patients (Dei-Cas, 2000). Nowadays, despite the introduction of *Pneumocystis* chemoprophylaxis and advances in the treatment of HIV infection, mainly the development of highly active anti-retroviral therapy (HAART), PcP remains as a major opportunistic infection in patients with AIDS. While, the incidence of PcP among individuals with HIV infection has decreased in developed countries, the prevalence of AIDS-related PcP in developing countries remains high and poorly controlled. AIDS-related PcP continues to be an overwhelming illness among individuals who are unaware of their HIV infection, those without access to antiretroviral therapy, among patients who are intolerant or nonadherent to therapy, those who do not comply with prophylactic medications and in cases of failure of prophylaxis, probably relate to the emergence of drug-resistant strains (Calderon, 2010b).

2. Epidemiology

Pneumocystis jirovecii is probably one of the more frequent infectious agents faced by humans in everyday life. Today, it is recognized as an extracellular, obligate, host-specific, yeast-like parasitic fungus virtually restricted to lung tissue that can be directly transmitted among susceptible hosts by the airborne route. It is established that human PcP in not a

zoonotic disease, and this notion has important implications for the epidemiology of *P. jirovecii* (Calderon, 2009). Although early studies reported the isolation of *Pneumocystis* DNA from the air surrounding apple orchards and the surface of pond water, no *Pneumocystis* forms were identified in environmental samples by microscopic analysis, and it is uncertain whether there is an ecological niche for *Pneumocystis* outside mammalian hosts (Wakefield, 1996). Animal sources of *P. jirovecii* can be excluded, because the *Pneumocystis* organisms that infect mammalian species are characterized by strong, close host-species specificity (Aliouat-Denis, 2008). Thus far, the human being is the only known reservoir host for *P. jirovecii*, and humans probably acquire the infection only from other humans (Calderon, 2009).

Serologic studies have shown that specific serum anti-*Pneumocystis* antibody can be detected in most children early in life, indicating frequent exposure to this organism (Respaldiza, 2004). On the basis of this finding, disease in immunocompromised persons has long been thought to result from reactivation of latent infection acquired in childhood. However, animal and human studies have shown that elimination of *Pneumocystis* often occurs after infection, implying that the persistence of latent organisms is limited (Morris, 2002). Recent demonstration of *P. jirovecii* transplacental transmission may explain the accumulating evidence that the primary infection is widely acquired very early in the life and support the commonly held view that human infants are a major natural reservoir for *P. jirovecii*, since they can remain colonized as their immune response matures (Montes-Cano, 2009).

Colonization with *P. jirovecii* in adults has recently gained attention as an important issue for understanding complete cycle of human *Pneumocystis* infection (Calderón, 2010a). In general, colonization is defined as isolation of an infectious agent that does not result in sufficient damage to cause clinical disease, but that may alter host homeostasis. In the specific case of *Pneumocystis,* colonization is currently defined as the detection of the organism or its DNA in respiratory samples from subjects without signs or symptoms of pneumonia (Morris, 2008).

Among adults, *Pneumocystis* colonization has been well documented in both HIV-infected and non–HIV-infected individuals, and certain populations appear to have a higher risk of colonization. Studies have shown that individuals who have an underlying HIV-infection or another cause of immunosuppression and those who are not immunosuppressed but have chronic lung disease may often be colonized by *P. jirovecii* (Calderón, 2009). These groups at risk for carriage probably represent a major species-specific reservoir of infection, although transient *Pneumocystis* colonization has been also identified in healthy individuals that could behave as a sort of dynamic reservoir for future *Pneumocystis* infection in other susceptible subjects (Medrano, 2005).

Several outbreaks of PcP have been reported in hospitals. Molecular analyses of *Pneumocystis* in some of these studies suggested nosocomial acquisition of the infection (de Boer, 2007, Olsson, 2001, Rabodonirina, 2004). In addition, *Pneumocystis* colonization has been found more frequently in health care workers in close occupational contact with patients with PcP than in those who had no occupational exposure (Vargas, 2000, Miller 2001). On the other hand, a recent study has provided molecular evidence that airborne transmission of *P. jirovecii* from colonized immunocompetent carrier hosts to susceptible persons may occur (Rivero, 2008). Therefore, interindividual airborne transmission seems to occur in humans in both hospitals, as a nosocomial infection, and in the community.

3. Pathogenesis

Basic knowledge on *Pneumocystis* has been hampered by the lack of a reliable in vitro culture system. However, through the use of molecular techniques and experimental models of PcP in immunosuppressed animals, many progresses have been made over the last decades in our understanding of the complex pathophysiology and pathogenesis of this fungal infection.

At the histopathological level has been shown that the proliferation of *Pneumocystis* is accompanied by anatomical and physiological changes. In animal models, alterations in alveolar-capillary permeability are followed by degenerative changes in type I pneumocytes, restorative hypertrophy of type II pneumocytes and diffuse alveolar damage leading to pulmonary fibrosis (Walzer, 1993). Studies in humans have also shown changes in the permeability of the alveolar-capillary membrane, pulmonary diffusing capacity and vital capacity in total lung (Coleman et al., 1984). These changes depend on the ability of *Pneumocystis*, demonstrated in animal models to induce in the very early stages of the infection alveolar macrophage activation, increased pro-inflammatory cytokines and changes in pulmonary surfactant even when small amounts of microorganisms are present (Prevost et al., 1998).

In the infected host, *Pneumocystis* organisms dwell almost exclusively within lung alveoli. Within some hours after experimental intra-tracheal infection, Pneumocystis trophic forms attach to the alveolar epithelial cells. The host immune response against the infection involves complex interactions between CD4+ and CD8+ T-cells, alveolar macrophages, neutrophils and soluble mediators that facilitate clearance of the infection. Disease only occurs when cellular and/or humoral immunity is defective.

3.1 Interactions of *Pneumocystis* with alveolar host cells

Trophic forms adhere tightly to alveolar type I cells through interdigitation of their membranes with those of the host. The binding of *Pneumocystis* to the epithelium is facilitated by interactions with proteins of the alveolar fluid, such as fibronectin and vitronectin that bind to the surface of *Pneumocystis* and mediate the attachment to integrin receptors present on the alveolar epithelium. In infected tissues, type I alveolar cells with adherent *Pneumocystis* appear vacuolated and eroded (Benfield et al., 1997). However, studies of cultured lung epithelial cells have shown that the adherence of *Pneumocystis* alone does not disrupt the structure or barrier function of alveolar epithelial cells, although proliferative repair of the epithelium is reduced. It is therefore unlikely that the adherence of *Pneumocystis* to alveolar epithelium is by itself responsible for the diffuse alveolar damage in severe pneumonia (Benfield et al., 1997; Thomas & Limper, 2007). Rather, the inflammatory responses in the host are primarily responsible for the compromise of the alveolar-capillary surface (Thomas & Limper, 2007).

Electron microscopic studies have shown that *Pneumocystis* organisms are embedded in protein-rich alveolar exudates, which contain abundant fibronectin, vitronectin, and hydrophilic surfactant proteins A and D. In contrast, hydrophobic surfactant protein B and C are reduced during PcP. Both surfactant protein A and surfactant protein D interact with the Major Surface Glycoprotein (MSG) components of the surface at *Pneumocystis*. Surfactant protein A modulates the interactions of *Pneumocystis* with the alveolar macrophages. In contrast, surfactant protein D mediates the aggregation of the *Pneumocystis* organisms, but because the aggregated organisms are extremely poorly taken up by macrophages, they may escape elimination. Pulmonary surfactant phospholipids, which contribute to the low surface tension in the alveoli, are reduced during PcP, and abnormalities in the composition

and function of the surfactant are the result of the host's inflammatory response to *Pneumocystis*, rather than direct effects of the organisms on the surfactant components (Wright et al., 2001; Thomas & Limper, 2007).

3.2 Innate immunity (alveolar macrophages and neutrophils)

Although parasite attachment to lung epithelial cells is essential for *Pneumocystis* infection and propagation, invasion of host cells does not occur (Krajicek et al., 2009). Alveolar macrophages are the first line of host defence to control the infection, since they are the principal phagocytes mediating the uptake and direct degradation of both trophic forms and cysts forms in the lung (Kelly & Shellito, 2010). Macrophages display several potential receptors for glucans, including CD11b/CD18 integrin (CR3), dectin-1, and toll-like receptor 2. The activation of macrophages by *Pneumocystis* is augmented by host proteins such as vitronectin and fibronectin that bind the glucan components on the organism (Vassallo et al., 2001). When there are not opsonins in the epithelial-lining fluid, the uptake of *Pneumocystis* is mediated mainly through the macrophage mannose receptors, pattern-recognition molecules that interact with the surface mannoprotein, MSG (also called glycoprotein A). After they have been taken up by macrophages, *Pneumocystis* organisms are incorporated into phagolysosomes and degraded. Macrophages produce a large variety of proinflammatory cytokines, chemokines, and eicosanoid metabolites in response to phagocytosis of *Pneumocystis*. Although these mediators participate in eradicating *Pneumocystis*, they also promote pulmonary injury (Limper et al., 1997).

Neutrophils alone are unable to control the infection. Unlike other opportunistic fungal infections, *Pneumocystis* disease is rare in patients with neutropenia. Neutrophils are associated with inflammation and, therefore, have been implicated in severity of disease. In fact, decreased pulmonary function and local lung inflammation and damage have been correlated with elevated neutrophil counts in HIV-infected patients with PcP (Kelly & Shellito, 2010). The neutrophils recruited into the lungs release reactive oxidant species, proteases, and cationic proteins, which directly injure capillary endothelial cells and alveolar epithelial cells (Thomas & Limper, 2004).

3.3 Adaptive immunity (T cells and B cells)

Both cellular and humoral immune systems are important in defence against *Pneumocystis* infection.

The activity of CD4+ T cells is pivotal in the host's defences against *Pneumocystis*, since most HIV-infected patients with PcP have CD4+ T-cell counts below 200 cells/mm^{3}. CD4+ lymphocytes as memory cells coordinate and orchestrate the host inflammatory responses by means of the recruitment and activation of other immune effector cells, including monocytes and macrophages, which are responsible for elimination of the organism. Macrophage-derived TNF-α and interleukin-1 are believed to be necessary for initiating pulmonary responses to *Pneumocystis* infection that are mediated by CD4+ cells. The cells proliferate in response to *Pneumocystis* antigens and generate cytokine mediators, including lymphotactin and interferon gamma (IFN-γ). Lymphotactin, a chemokine, acts as a potent chemoattractant for further lymphocyte recruitment in PcP.

Although T lymphocytes are essential for the clearance of *Pneumocystis*, experimental data suggest that T-cell responses may also result in substantial pulmonary impairment during pneumonia. For instance, in severe combined immunodeficiency (SCID) mice infected with *Pneumocystis*, normal oxygenation and lung function occur despite active infection until the late stages of the disease. When the immune systems in these animals are reconstituted with

the use of intact spleen cells, an intense T-cell–mediated inflammatory response ensues, resulting in substantially impaired gas exchange (Wright et al., 1999b). In the absence of brisk lung inflammation, *Pneumocystis* has little direct effect on pulmonary function. In a similar manner, in patients who have undergone bone marrow transplantation the clinical onset of PcP and of most marked alterations in lung function occur during engraftment (Thomas & Limper, 2004).

The CD8+ T cells seem to play also an important role in control of *Pneumocystis* infection since in experimental animal models depletion of both CD4+ and CD8+ cells results in a more severe PcP than only depletion of CD4+ cells. However, their role may be less important than CD4+ T cells since CD8+ T-cell-depleted animals can still clear the infection (Lu & Lee, 2008). *Pneumocystis* infection results in the marked accumulation of CD8+ T lymphocytes in the lung. Although not as extensively investigated as CD4+ T cells, insights into the role of CD8+ T cells in host defence against *Pneumocystis* have been achieved, but that is not nearly enough. Recent data provide the concept that CD8+ T cells, most likely those of the Tc1 phenotype, are critical for clearance of some fungal organisms including *Pneumocystis*, particularly in the context of CD4+ T-cell deficiency or dysfunction. CD8+ T cells have also been shown to play a detrimental role in *Pneumocystis* infection. CD8+ T cells are considered to be part of the damaging inflammatory response in CD4+ T-cell-depleted mice. The presence of CD8+ T cells affected surfactant function and it also has been shown to exacerbate TNF-α production (Steele et al., 2005).

Lastly, a significant role of humoral immune response in the host defence against *Pneumocystis* is supported by the observations that SCID animals require B cells to clear the infection (Burns et al., 1990), and that patients with agammaglobulinemia develop PcP despite of an intact cellular immune system (Alibrahim et al., 1998; Lu & Lee, 2008). B lymphocytes appears to play an important role in the generation of CD4+ memory cells in response to *Pneumocystis* (Lu & Lee, 2008).

3.4 Cytokine and chemokine networks

Various pro-inflammatory cytokines including IFN-γ, tumour necrosis factor alpha (TNF-α), interleukin (IL)-8, IL-1 and IL-6 and chemokines such as RANTES (Regulated upon Activation normal T-cell Expressed, and Secreted), macrophage inflammatory protein (MIP)-1α, MIP-1β and MIP-2 release by macrophages, neutrophils, epithelial cells and lymphocytes are involved in the host immune response and lung damage during *Pneumocystis* disease (Calderon et al., 2007).

INF-γ has a critical role for control lung inflammation during PcP, although is not directly toxic to *Pneumocystis* organisms. This cytokine is produced primarily by CD4+ T cells. There is an indirect correlation between IFN-levels and severity of PcP (Kelly & Shellito, 2010). IFN-γ strongly activates the macrophage production of TNF-α, superoxides, and reactive nitrogen species, each of which is implicated in the host defence against *Pneumocystis* (Wright et al., 1999a).

TNF-α is a potent pro-inflammatory cytokine secreted primarily by macrophage that promotes the recruitment of neutrophils, lymphocytes, and monocytes. Although their recruitment is important for clearance of the organisms, these cells injure the lung by releasing oxidants, cationic proteins, and proteases. TNF-α also induces the production of other cytokines and chemokines, including IL-8 and IFN-γ, which stimulate the recruitment and activation of inflammatory cells during *Pneumocystis* infection (Wright et al., 2004). The cell wall of *Pneumocystis* contains abundant beta-glucans, and studies have confirmed that the production of TNF-α by alveolar macrophages is mediated by recognition of the beta-glucan components of *Pneumocystis*. IL-8 is correlated with both

neutrophil infiltration of the lung and impaired gas exchange during severe PcP (Thomas & Limper, 2004).

The most important function of chemokines is to recruit effector cells to the site of injury. Many studies have found an increase in chemokine expression during PcP. In a SCID experimental model of PcP it has been found that expression levels of RANTES, MIP-1α, MIP-1β and MIP-2 were all upregulated after lymphocyte reconstitution of the SCID animal. In addition, the time course of chemokine expression correlates *Pneumocystis* clearance, but also with the lung inflammation (Wright et al., 1999a). Thus, the role of chemokines is essential for the resolution of infection, but overexpression may also result in a hyperinflammatory state and lung damage (Kelly & Shellito, 2010).

4. Clinical presentation and chest radiology

In patients infected with HIV, PcP is a common AIDS-defining illness and occurs most frequently in subjects with a CD4+ count less than 200 cells per mm[3]. The symptoms of PcP are nonspecific and PcP in patients with HIV infection tends to run a more subacute indolent course and tends to present much later, often after several weeks of symptoms, compared with PcP associated with other immunocompromising conditions. A more acute illness with symptoms including a cough productive with purulent sputum should suggest an alternate infectious diagnosis, such as bacterial pneumonia or tuberculosis

Patients with PcP often develop dyspnea (95%), which increases over time; cough productive of clear sputum or non-productive cough; low grade or no fever; malaise, and sometimes chest tightness or pain. However, the clinical picture in individual patients is variable and many infectious and non-infectious processes can present identically. Also, the general hallmarks of this disease such as fever, shortness of breath, and diffuse infiltrates do not invariably occur, especially early in the course while the disease is mild (Thomas & Limper, 2004). Acute dyspnea with pleuritic chest pain may indicate the development of a pneumothorax, which has been presented in 2% to 4% of patients (Sepkowitz et al., 1991). In all cases, a high index of suspicion and a thorough history are key factors in early detection of PcP. Physical examination may reveal tachypnea, tachycardia, and cyanosis. Lung auscultation usually reveals few abnormalities with dry cackles or rhonchi present in less than 50% of patients. Individuals with PcP can be hypoxemic with respiratory alkalosis but can also have normal alveolar-arterial gradients if identified early in the natural history of their disease. Elevated serum levels of lactate dehydrogenase (LDH) have been related with PcP and probably reflects lung parenchymal damage but is not specific and elevations can be seen in many pulmonary and non-pulmonary conditions. In general, laboratory abnormalities are less severe in HIV-infected patients than in non-HIV immunosuppressed patients (Hughes, 2004).

Classically, chest radiographic features of PcP are bilateral, symmetric, fine reticular interstitial infiltrates involving the perihilar areas (figure 1a), becoming more homogenous and diffuse as the severity of the infection increases (Thomas & Limper, 2004). Less frequently, PcP may present with unilateral or asymmetrical opacities. Thin-walled cysts or pneumatocele are seen in 10-20% of cases. Pleural effusions and intrathoracic adenopathy are rare. Patients who receive aerosolized pentamidine have an increased frequency of upper-lobe infiltrates, pneumothorax, or cystic lesions. Early in the course of PcP, the chest radiograph may be normal in up to 25% of cases (Schliep & Yarrish, 1999).

A high-resolution computed tomography scan is more sensitive than chest radiograph and is helpful when the chest radiography findings are equivocal. The typical appearance is patchy areas of ground-glass attenuation with a background of interlobular septal

thickening or cystic lesions predominating in perihilar areas (figure 1b), even then chest radiographic findings are normal (Nyamande et al., 2007). While such findings are suggestive, they are not diagnostic. However, a negative high-resolution computed tomography scan may allow exclusion of PcP in patients with HIV.
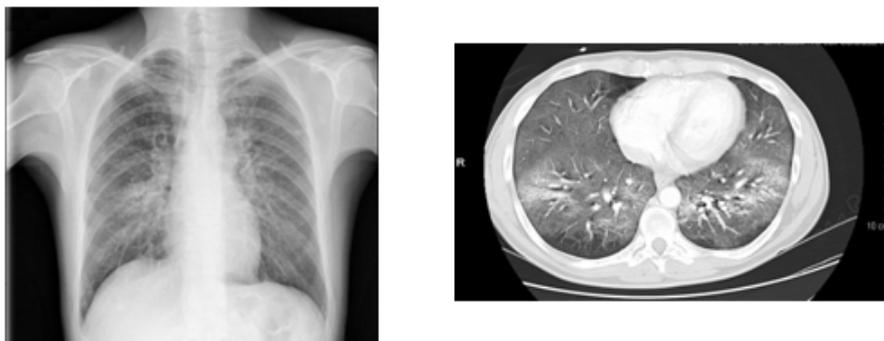

Fig. 1. Radiographic findings of *Pneumocystis* pneumonia in a patient with AIDS. (1a, left) Chest x-ray of a *Pneumocystis* pneumonia showing diffuse infiltrations in both lung fields. (1b, right) Chest high-resolution CT scan of a patient with AIDS revealing diffuse ground glass opacities and thickened alveolar septum in both lungs.

Extrapulmonary manifestations of *P. jirovecii* infection (extrapulmonary pneumocystosis) are distinctly unusual but they has been reported primarily among HIV-infected patients, particularly those who receive aerosolized pentamidine for prophylaxis of PcP and in those with advanced HIV infection who are not taking any prophylaxis. Mainly, during the terminal stage of HIV-related disease *Pneumocystis* organisms may disseminate from the lungs to other organs where they induce secondary visceral lesions. However, sometimes pulmonary infection may not be apparent when extrapulmonary lesions are detected. Lymph nodes, spleen, kidneys, liver, thyroid and bone marrow are the most commonly infected organs, but microorganisms have been also found in the brain, pancreas, skin, heart, muscle and other organs (Ng et al, 1997). For HIV-infected patients, extrapulmonary pneumocystosis limited to the choroid layer or ear (external auditory canal or middle ear) has a better prognosis, with good response to specific treatment, than disseminated pneumocystosis in multiple noncontiguous sites. Lesions are frequently nodular and may contain necrotic material or calcification. Extrapulmonary pneumocystosis in solid organs appears on computed tomography scan as focal, hypodense lesions with well-defined borders and central or peripheral calcification (Schliep & Yarrish, 1999).

Immunorestitution disease (IRD) is defined as an acute symptomatic or paradoxical deterioration of a (most probably) preexisting infection that is temporally related to the recovery of the immune system and it is due to immunopathological damage associated with the reversal of immunosuppressive processes. PcP manifesting as a form of IRD has been described in both HIV and non-HIV immunosuppressed patients (Cheng et al., 2004; Jagannathan et al., 2009; Mori et al., 2009). Among HIV-infected patients, PcP manifesting acutely during the initiation of HAART is a well-recognized phenomenon (Wislez et al., 2001). AIDS-related PcP patients seem to be at risk of clinical deterioration due to IRD if antiretroviral therapy is started within one to two weeks after the initiation of treatment for PcP (Wislez et al., 2001). The onset of clinical deterioration is associated with an increase in the CD4+ lymphocyte count and a reduction in the HIV viral load (Wislez et al., 2001).

5. Prognosis

Despite treatment, mortality of PcP still remains high. Several studies highlight that mortality rates are declining in patients with PcP. However, in other studies, PcP has remained the leading cause of death among those nor receiving o failing to comply with HAART or PcP prophylaxis. Predictors of mortality include older age, recent injection drug use, increased total bilirrubin, low serum albumin, and alveolar-arterial oxygen gradient >50 mm Hg (Fei et al, 2009).

6. Management PcP

There is no universally agreed approach on to the initial management of patients with suspected PcP. Many institutions treat patients with suspected PcP empirically, while others pursue a definitive microbiological diagnosis (Huang, 2004). Since PcP can be rapidly progressive and the mortality rate remains high, early therapy is essential (Calderon et al, 2004; Roblot, 2005). Identification of patients having PcP into mild, moderate or severe disease allows to guide the choice of the drug for the treatment of PcP, as well as, to decide if adjuvant corticosteroids are indicated (table 1) (Miller et al., 1996). In AIDS-related PcP, the typical duration of therapy is at least 21 days because of the risk for relapse with shorter treatment duration. Patients generally improved after 4 to 8 days of therapy. Although the overall prognosis of patients whose degree of hypoxemia requires intensive care unit (ICU) admission or mechanical ventilation remains poor, survival in up to 50% of patients requiring ventilatory support has been reported. Patients with reasonable functional status and severe PcP should be offered ICU admission or mechanical ventilation (CDC, 2009).

	Mild	Moderate	Severe
Symptoms and signs	Dyspnoea on exertion, with or without cough and sweats	Dyspnoea on minimal exertion and occasionally at rest. Cough and fever	Dyspnoea and tachypnoea at rest. Persistent fever and cough
Arterial oxygen tension (PaO2) at rest	> 11.0 kPa (82.7 mmHg)	8.0 to 11.0 kPa (60-82.7 mmHg)	< 8.0 kPa (60 mmHg)
Arterial oxygen saturation (SaO2) at rest	> 96%	91 to 96%	< 91%
Chest radiograph	Normal, or minor perihiliar shadowing	Diffuse interstitial shadowing	Extension interstitial shadowing with or without diffuse alveolar shadowing

Modified of Miller RF, et al., 1996.

Table 1. Grading of severity of *Pneumocystis* pneumonia.

7. Diagnosis of PCP

7.1 Microscopic detection of *Pneumocystis*

The single most important diagnostic tool for *Pneumocystis* infection is a high clinical suspicion. However, specific diagnosis of PcP requires documentation of the microorganism in respiratory specimens. Since *Pneumocystis* cannot be cultured, the diagnosis of PcP relies on microscopic detection of *Pneumocystis* organisms on stained respiratory specimens. Conventional stains such as toluidine blue O (TBO), Grocott's methenamine silver nitrate

(GMS), or methanol Giemsa methods (figure 2) can be used to identify the organism (cysts or trophic forms) but immunofluorescent staining is the most common technique currently used. TBO or GMS stains facilitate rapid parasite detection, even at low magnification, in all kinds of clinical specimens. However, these dyes also stain the cell wall of yeasts or other fungi. For this reason, a good strategy to identify *Pneumocystis* organisms accurately in clinical specimens is to systematically associate the examination of both TBO- or GMS-stained smears and methanol-Giemsa–stained smears from the same specimen (table 2). Actually, methanol-Giemsa (or other equivalent panoptical Giemsa-like stains) makes it possible, on the one hand, to distinguish *Pneumocystis* organisms from other microorganism and, on the other hand, to identify the different *Pneumocystis* life-cycle stages. In fact, Giemsa and other stains with similar cytological affinities, such as Diff Quick or RAL-555, cause the parasite nuclei to stain pinkish purple and the cytoplasm to stain blue (Dei-Cas et al, 1998). They do not stain cystic or sporocytic walls, which appear like a clear peripheral halo around cystic forms. These polychrome stains make it possible accurately to distinguish *Pneumocystis* trophic or cystic forms from other fungi and also from host cells or cell debris. On the whole, the biggest advantage of methanol-Giemsa or Giemsa-like stain methods consists in staining trophic forms and sporocytes, which remain unidentified in TBO- or GMS-stained smears (Dei-Cas et al, 1998).
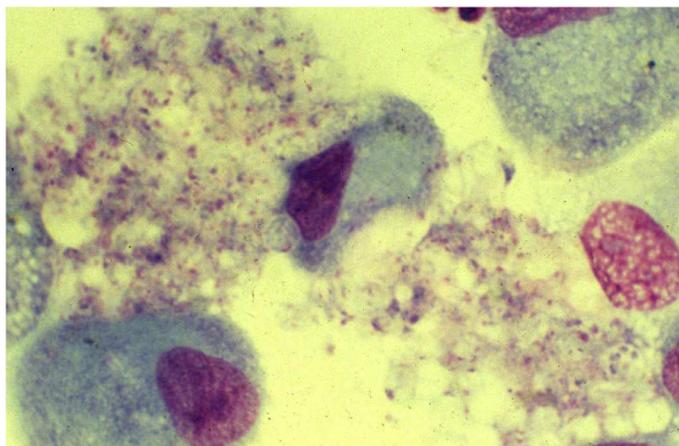

Fig. 2. Stain with methanol–Giemsa stain

Efficiency and cost-effectiveness of the different microscopic stains evoked here vary according to experience of groups, technical protocols, type and quality of the samples, local incidence of PcP, and the number of organisms present (Chouaid et al., 1995) (table 2). It is generally accepted, however, that association of methods that stain cystic cell wall (e.g. TBO or GMS) with panoptical techniques (methanol-Giemsa or analogous staining methods) is usually required (Dei-Cas et al., 2006). Moreover, it is usually recognized that specific antibody staining is mainly helpful to detect *Pneumocystis* organisms in non-BAL smears (e.g. induced sputum, expectorated sputum, gastric wash) and to clarify conflicting light microscopic observations (Aderaye et al., 2008; Cruciani et al., 2002; Kovacs et al., 1998; Limawongpranee et al., 2007).

Typically, the respiratory specimens are obtained by sputum induction or fiberoptic bronchoscopy with bronchoalveolar lavage (BAL). Sputum induction by inhalation of a

hypertonic saline solution is the quickest and least-invasive method for definitively diagnosing PcP with a sensitivity between 50-90% and specificity of 99-100%. Sputum induced may also be less sensitive in patients receiving aerosolized pentamidine for prophylaxis. All of the direct organism visualization methods can lead to false-negative results, consequently, a negative sputum induction cannot rule out a diagnosis of PcP. If sputum induction is nondiagnostic or cannot be performed, then bronchoscopy with BAL is the next step. A BAL that is negative for *Pneumocystis* rules out the diagnosis of PcP.

In order to detect *Pneumocystis* organisms in histological sections from lung or other organs, pathologists target usually the cystic forms, since trophic ones are uneasily identifiable in paraffin-embedded tissues. They use therefore GMS and, less frequently, TBO staining procedures adapted to tissue sections. Trophic forms can however be identified in epon-embedded semi-thin sections stained with toluidine blue or other stains (Dei-Cas et al., 1998; Durand-Joly et al., 2000). Furthermore, *Pneumocystis*-specific fluorescein-, phosphatase or peroxidase-labeled monoclonal antibodies available from many suppliers may help to identify *Pneumocystis* organisms in BAL, induced sputum or tissue samples (table 2).

Technique	Suitable kind of sample	Needed experience	Sensitivity	Specificity	Advantages	Drawbacks	Recommended combination with:
Microscopy:							
PC/IC	BALF wet smear	very good	variable	good	rapidity	needs confirmation by other methods	panoptical stain
GMS/TBO	BALF air-dried cytospin smear or biopsy (histological section)	average	high	average	cost; rapidity	false positive (poor experienced staffs); identifies only the cystic stages	panoptical stain
Panoptical stains*	BALF air-dried cytospin smear	very good	average	very high	cost; rapidity; identify all *Pneumocystis* stages	limited sensitivity (poor experienced staffs)	GMS/TBO
FL Mab	BALF, IS or sputum air-dried cytospin smear	good	high	good	good sensitivity/ specificity	cost; time-consuming	-
IP/AP Mab	biopsy (histological section), air-dried cytospin smear	good	good	good	good specificity	cost; time-consuming	-
PCR	BALF, IS, OW, NPA, biopsy	average	very high	very high	Helpful in HIV-negative patients; rapidity (real-time PCR assays); non-invasive	cost; positive in colonized patients	-

Technique	Suitable kind of sample	Needed experience	Sensitivity	Specificity	Advantages	Drawbacks	Recommended combination with:
					sampling; genotyping		
BG	serum	average	good	low	rapidity; post-therapeutic control	positive in other deep fungal infections	other tests
KL-6	serum	average	good	low	-	positive in other pulmonary infections	
Serum *Pneumocystis* antibody assay	serum	average	depending on antigen and assay	depending on antigen and assay	helpful in epidemiology studies	positive in people without PcP	other tests

*Giemsa or Giemsa-like stains. BALF: Bronchoalveolar lavage fluid; BG: serum beta-1,3-glucan; FL Mab: fluorescein-labeled Pneumocystis monoclonal antibody; GMS: Grocott-methenamine silver stain; IP/AP Mab: immunoperoxidase/alkaline-phosphatase labeled monoclonal antibody; IS: induced sputum; PC/IC: phase contrast/interference contrast; TBO: toluidine blue stain. KL-6: Mucin like glycoprotein.

Table 2. Laboratory diagnostic methods for *Pneumocystis* pneumonia.

7.2 Molecular detection of *Pneumocystis*

Many *Pneumocystis* PCR assays have been developed during the last two decades. PCR tool revealed highly efficacious to amplify *Pneumocystis* DNA from diverse kinds of clinical specimens (BALF, IS, expectorated sputum, oropharyngeal or nasopharyngeal wash samples, biopsy specimens) (figure 3) (de la Horra et al., 2006; Durand-Joly et al., 2005; Olsson et al., 1993; Wakefield et al., 1990). In the clinical laboratory, the use of molecular methods is mainly warranted to increase the sensitivity of *P. jirovecii* detection in clinical specimens in order to establish earlier PcP diagnosis, detecting low parasite rates, mainly in non-HIV infected patients with PcP, and detecting *Pneumocystis* DNA in noninvasive samples (Durand-Joly et al., 2005; Respaldiza et al., 2006) (table 2). Moreover, PCR assays followed by direct sequencing or other strategies were used for typing *Pneumocystis* isolates in order to identify parasite strains and to explore correlation between specific genotypes and virulence, transmissibility or drug susceptibility. PCR, especially nested PCR assays applied to noninvasive samples, have also been used to detect *Pneumocystis* colonization either in susceptible individuals or in apparently healthy people, including health care workers in hospitals (Durand-Joly et al., 2003; Medrano et al., 2005; Nevez et al., 2008).
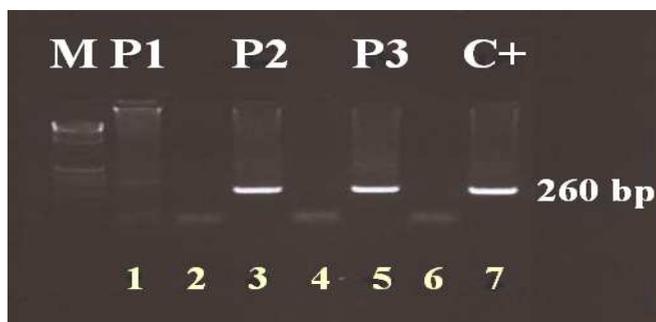

Fig. 3. Nested PCR (mtLSU rRNA region) results.

M: molecular mass marker. Lane 1 (P1) negative specimen. Lanes 3 and 5 (P2, P3) positive specimens of oral wash in cystic fibrosis patients. Lane 7 (C+) positive control. Lanes 2, 4 and 6 negative controls (water).

For PcP diagnosis in humans, conventional or real-time PCR assays based on the amplification of the large subunit of mitochondrial ribosomal DNA (mtLSUrDNA) (Wakefield et al., 1990) are the most commonly used, but many other sequences have been targeted (Major Surface Glycoprotein, Internal Transcribed Spacers, Thymidylate Synthase, Dihydrofolate Reductase, heat-shock protein 70, etc.) (Durand-Joly et al., 2005; Hugett et al., 2008). Comparative evaluating studies are uneasy to perform because of different clinical contexts, sampling methods, laboratory reagents or technical strategies used to DNA extraction, amplification or analysis of results (Durand-Joly et al., 2005).

In general, conventional or real-time *Pneumocystis* PCR assays have represented a significant advance in PcP laboratory diagnosis. Actually, highly sensitive and specific PCR tools, especially real-time PCR assays, improved the clinical diagnosis of PcP allowing an accurate, early diagnosis of *Pneumocystis* infection (Durand-Joly et al., 2005), which should lead to a decreased duration from onset of symptoms to treatment. This period has a recognized impact on prognosis since PcP-associated respiratory failure requiring mechanic ventilation entails significant mortality (Huang, 2004). In addition, PCR assay may reveal PcP in patients with negative microscopic test. For instance, among 62 HIV-negative patients with clinical PcP diagnosed in the Lille University Hospital between 1998 and 2001, 30 patients (48%) had positive PCR results with negative microscopic tests (Durand-Joly, 2002).

Notably, molecular techniques play a significant role when they are applied to noninvasive specimens as IS, oropharyngeal wash (OW, obtained by gargling 10 ml of 0.9% NaCl for >60 seconds) (Respaldiza et al., 2006) or nasopharyngeal aspirates (NPA) (Richards et al. 1994). When DNA sequences used as primers or probe have been adequately defined, the analytical specificity of *Pneumocystis*-PCR assays applied to noninvasive or to BALF samples should usually be of 100% (Durand-Joly et al., 2005). With regard to sensitivity, *Pneumocystis*-mtLSUrDNA PCR showed high analytical sensitivity for the detection of *Pneumocystis* organisms on BALF samples from AIDS patients, with a detection threshold of 0.5–1 organism/μl^{-1} (Tamburrini et al., 1998). The sensitivity of PCR assays applied to OW (or other noninvasive samples) is certainly lower (<80%) than that of PCR on BALF samples (>95%) (Tsolaki et al., 2008). However, OW can be easily repeated in order to monitor the evolution of infection and, potentially, the therapeutic response (Tsolaki et al., 2008).

A significant problem of *Pneumocystis* PCR assays is raised by *Pneumocystis* colonization (Calderon, 2009). Actually, a positive PCR result associated with a negative microscopic test may result from either *Pneumocystis* colonization or PcP. In common practice, this difficulty is often solved on the basis of a careful clinical, radiological and laboratory assessment of the patient pathological condition, as it is usually done to other infectious diseases, especially when their agents are opportunistic pathogens. However, the alternative of quantifying parasite rates was also explored (Larsen et al., 2002). Thus, a quantitative real-time PCR assay that targeted *Pneumocystis* Major Surface Glycoprotein (MSG) multigene family was applied to OW samples, and revealed significant differences between PcP patients and *Pneumocystis* colonized subjects in the number of MSG copies. The authors suggested a cutoff value of 50 MSG gene fragment copies/tube for distinguishing between the two conditions (Larsen et al., 2002). However, quantitative PCR results seemed difficult to use on the field. The main problem was inability to control the volume of the sample. Another

difficulty is related with the kind of patients. Actually, it seems difficult to apply a same cutoff to AIDS patients, patients with other underlying diseases or individuals receiving chemoprophylaxis against *Pneumocystis*.

There is no formal agreement about an unequivocal definition of *Pneumocystis* colonization. The notion may however be characterized on the basis of clinical and experimental observations. In clinical practice, the diagnosis of *Pneumocystis* colonization or subclinical carriage is usually retained when *Pneumocystis* DNA is detected by PCR methods in respiratory samples from immunodepressed or immunocompetent subjects without symptoms or signs of *Pneumocystis* infection, and who do not progress to PcP (Morris et al., 2008) In these subjects, *Pneumocystis* organisms are only exceptionally detected by microscopy (Vidal et al., 2006). Interestingly, recent experimental data strengthened the biological significance of *Pneumocystis* colonization (Chabe et al., 2004). They demonstrated that *Pneumocystis* organisms can replicate in the lungs of immunocompetent carriers, stimulate an antibody response and be efficiently transmitted by airborne route to either naive immunocompetent hosts, who will develop a primary infection, or to immunosuppressed hosts, who may then develop PcP (Chabe et al., 2004). In addition, many evidences suggest that beyond PcP, *Pneumocystis* colonization may induce local or systemic inflammation, a condition that could aggravate chronic pulmonary diseases. For instance, *P. jirovecii* pulmonary carriage in patients with chronic obstructive pulmonary disease (COPD) could favor the progression of this disease (Calderon et al., 2007; Morris et al., 2008).

Efforts have been made to associate specific *P. jirovecii* genotypes with virulence, drug susceptibility or other medically important biological properties of parasite strains. Some studies reported some correlation between polymorphism and clinical features (Miller & Wakefield, 1999; Totet et al., 2003). Polymorphism of internal transcribed spacer (ITS1/ITS2) sequences was quite frequently used and more than 30 ITS1 genotypes and 40 ITS2 genotypes with more than 90 haplotypes (combinations of ITS1 and ITS2 types) have been reported (Beard, 2004).

Most polymorphism studies targeted mutations of the *P. jirovecii* dihydropteroate synthase (DHPS) gene, which could potentially be linked with sulfa resistance. Regarding this issue, and since effective *P. jirovecii* culture systems are unavailable, several groups have assessed putative trimethoprim-sulfamethoxazole (TMP-SMX) drug resistance by detecting *Pneumocystis* DHPS mutations. Indeed nonsynonymous DHPS point mutations at nucleotide positions 165 and 171 entail an amino acid change at positions 55 (Thr to Ala) and/or 57 (Pro to Ser) (Friaza et al., 2009). Such mutations confer resistance to sulfa drugs in other organisms, including *Escherichia coli*, *Streptococcus pneumoniae* and *Plasmodium falciparum*. The *P. jirovecii* DHPS mutant form has also been shown to be more resistant to sulfamethoxazole in a *Saccharomyces cerevisiae* model (Iliades et al., 2004), but it is still uncertain if *Pneumocystis* DHPS mutations lead to drug resistance in patients (Huang et al., 2000, 2004; Nahimana et al., 2004). Such mutations were shown to be associated with the use of TMP-SMX or dapsone (two DHPS inhibitors), the duration of sulfa or dapsone prophylaxis and with geographic areas in which sulfamethoxazole or dapsone were commonly used for PcP prophylaxis (Huang et al., 2004; Kazanjian et al., 2000). However, results of studies searching specifically to establish an association between the presence of *P. jirovecii* DHPS mutations and clinical outcomes, such as treatment failure or death, are contradictory (Alvarez-Martinez et al., 2008, Helweg-Larsen et al., 1999; Huang et al., 2004,

Stein et al., 2004; van Hal et al., 2009). Outstandingly, most PcP patients carrying *P. jirovecii* isolates with DHPS mutations responded well to TMP-SMX treatment and survived probably because these mutations may confer a low-level of resistance to sulfa-drugs that is overcome by high drug concentration achieved in lung tissues by sulfamethoxazole (Calderon et al., 2004; Huang et al., 2004).

7.3 Other laboratory diagnostic methods
7.3.1 Beta-D-glucan assay

β-1,3-glucan (BG) is the main structural component of the cell wall of all fungi, including *Pneumocystis* cysts (Thomas & Limper, 2007). Interestingly, high serum BG levels have been reported in patients with PcP (Desmet et al., 2009; Nakamura et al., 2009; Teramoto et al., 2000). Consistently, such levels decreased with effective anti-*Pneumocystis* treatment (Teramoto et al., 2000). Serum BG appeared therefore as a good marker of *Pneumocystis* infection. The potential utility of this assay was analyzed in a retrospective case-control study of patients with suspected PcP comparing BG with microscopic examination on BAL. The BG assay had a sensitivity of 92% and a specificity of 86% for detecting PcP for a ut-off level of 31.1 pg/ml (Tasaka et al., 2007). In a recent study, it has been observed that BG levels in HIV patients with PcP are higher than in non-HIV patients. This could be attributed to the fact that HIV patients have greater numbers of microorganisms that non-HIV patients (Nakamura et al., 2009).

However, BG levels could not be correlated with PcP prognosis, and false positive results could exceed more than 30% (Nakamura et al., 2009). False positive results were reported in patients undergoing bacterial septicemia, hemodialysis with cellulose dialysis membranes, treatment with immunoglobulin, glucan-containing antitumor drugs, amoxicillin-clavulanate, piperacillin-tazobactam or contact with gauze or surgical sponges containing BG (Ponton, 2009). Furthermore, since invasive fungal infections induce also an increase of serum BG, the test should often be associated with laboratory assays aiming at detecting such infections (Desmet et al., 2009). These preliminary studies suggest that in the right clinical setting serum BG may provide a useful noninvasive diagnostic adjunct for patients with *Pneumocystis* infection. However, additional information is necessary to address the general specificity of BG in diagnosing PcP versus other fungal infections in diverse immune-suppressed patient populations and to differentiate among patients with PcP and patients with *Pneumocystis* colonization.

7.3.2 KL-6

KL-6 is a mucin-like glycoprotein expressed on type II pneumocytes and bronchiolar epithelial cells. This marker has reached elevated levels in several studies in patients with PcP. However, the reported false-positive rate and level of detection were not as good as for the BG 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 high levels have also be found in non-fungal infections such as *legionellosis*, severe tuberculosis and respiratory syncythial virus bronchiolitis, and even in noninfectious interstitial lung disease (Inou et al., 1995; Kawasaki et al., 2009; Sukoh et al., 2001). Therefore, KL-6 elevation in PcP is thought to be related to lung damage and regeneration of epithelium lining and cannot be used as a specified marker of *Pneumocystis* infection.

7.3.3 *S*-adenosylmethionine (SAM)

Some observations suggested that S-adenosylmethionine (SAM), which is a universal methyl donor synthesized from methionine and ATP by SAM synthetase, could stimulate *Pneumocystis* in vitro growth (Clarkson et al., 2004). Since SAM was depleted from both the culture medium and the plasma of rats with PcP, it was hypothesized that *Pneumocystis* cells could scavenge SAM from host fluids due to the lack of SAM synthetase (Clarkson et al., 2004) Consistently, plasma SAM levels were found to be low in patients with PcP and to increase gradually with treatment (Skelly et al., 2003, 2008). These findings strengthened the idea of using plasma SAM levels as a non-invasive PcP diagnostic method. However, recent data showed that SAM-related issue could be more complex than previously thought. Firstly, differences in SAM levels between laboratories could be influenced by the method of measurement. Thus, Wang and colleagues using Chromatography Tandem Mass Spectrometry found generally higher plasma SAM levels than those reported before (Wang et al., 2008). The same group was unable to distinguish patients with acute PcP from the ones without PcP on the basis of plasma SAM levels, though these levels increased significantly with effective anti-*Pneumocystis* treatment. Indeed, the concern needs to be further explored because fasting status, dietary intake of methionine and other medications can affect plasma SAM concentration (Wang et al, 2008). Secondly, and contrarily to the results of previous works (Clarkson et al., 2004), *P. carinii*, *P. murina* and *P. jirovecii* have genes that encoded SAM synthetase (Sam1) (Kutty et al., 2008) . Moreover, the corresponding *Sam1* mRNA is transcribed, and the protein, which is enzymatically active, was immuno-localized in *P. murina* cells. Such data suggest strongly that *Pneumocystis* species do not depend on an exogenous source of SAM to survive (Kutty et al., 2008).

7.3.4 Serological tests

Serum antibody detection constitutes an adjunctive strategy currently used to diagnose systemic fungal infections, even in immunosupressed patients. This strategy was however only rarely used to PcP diagnosis because healthy subjects have frequently significant levels of serum anti-*Pneumocystis* antibody. Moreover, the antibody response against *Pneumocystis* infection is currently highly variable and the results reported by diverse groups are contradictory (Walzer, 2004). In contrast, *Pneumocystis* antibody assays, especially those using recombinant *Pneumocystis* antigens, constitutes an interesting tool in epidemiology (Daly et al., 2009).

8. Treatment

The recommended treatment of PcP has remained unchanged for many years, being Co-trimoxazole, an association of trimethoprim and sulfamethoxazole, the drug of choice as first line of treatment. Regarding which agent of second line must be choice preferably, data are limited (table 3). Drug related toxicities are increasing in HIV-infected patients and organ transplant recipients. Because of the potential for additive or synergistic toxicities associated with anti-*Pneumocystis* and antiretroviral therapies, certain health-care providers delay initiation of HAART until after the completion of anti-*Pneumocystis* therapy, or until at least 2 weeks after initiating anti-*Pneumocystis* therapy, despite some suggestion of potential benefit of early HAART in the treatment of patients with AIDS-related opportunistic infections (CDC, 2009; Zolopa et al., 2009). In order to a correct management of PcP is important to distinguish between progressive PcP, drug toxicity and concomitant infection if clinical deterioration is detected.

Trimethoprim-sulfamethoxazole (TMP-SMX)

TMP and SMX target sequential steps in the folate synthesis pathway. TMP inhibits dihydrofolate reductase and SMX inhibits dihydropteroate synthetase. TMP-SMX is the treatment of choice for PcP in all patients who tolerate this drug, and it achieves the most rapid clinical response of the anti-*Pneumocystis* agents (CDC, 2009; Helweg-Larsen et al., 2009). The recommended dose of TMP-SMX for adults (or children aged > 2 months) is 15 to 20 mg/kg/day of TMP and 75 to 100 mg/kg/day of SMX intravenously every 6 or 8 hours. With renal dysfunction, dosing must be reduced. The bioavailability of TMP-SMX from oral therapy is comparable to parenteral administration (CDC, 2009; Mofenson et al., 2009).

Patients who have PcP despite the use of TMP-SMX prophylaxis, are usually successfully treated with TMP-SMX. In this way, the presence of mutations in the DHPS gene of *P. jirovecii* has been associated with resistance to sulfa drugs, although the clinical outcome is uncertain (Crothers et al. 2005; Huang et al., 2004; Stein et al., 2004). Drug related toxicities from TMP-SMX are greater than that from therapy with other anti-*Pneumocystis* agents. The side effects of TMP-SMX are: rash (30-55%), (including Stevens-Johnson syndrome), fever (30-40%), leukopenia (30-40%), hepatitis (20%), thrombocytopenia (15%), azotemia (1-5%), and hyperkaliemia (Eeftinck et al., 1990; Gordin et al., 1984; Hughes et al., 1995). Nephrotoxicity occurs frequently in the renal transplantation recipient receiving full-dose of TMP-SMX. Liver transplant recipients are particularly susceptible to TMP-SMX toxicity. Leucovorin to prevent myelosuppression is not recommended because its uncertain efficacy and a higher rate of failure (CDC, 2009).

Pentamidine

Pentamidine is an aromatic diamidine that has broad spectrum anti-protozoal activity. This drug inhibits metabolism of *P* amino benzoic acid, interferes with anaerobic glycolysis, inhibits oxidative phosphorylation and impairs nucleic acid and protein synthesis. It was the first drug reported to treat PcP successfully and subsequent reports have confirmed the efficacy of intravenous pentamidine. Although intravenous pentamidine has been recommended as the main alternative to TMP-SMX for moderate to severe PcP (Gordin et al., 1984), a recent study has found a greater risk of death when pentamidine was used as first and second-line therapy for HIV-associated PcP with compared with TMP-SMX and clindamycin-primaquine (Helweg-Larsen et al., 2009). These findings could be due to toxicities related to pentamidine and the absence of an antibacterial effect, in contrast to TMP-SMX or clindamycin-primaquine, which might act against concomitant bacterial co-infection (Helweg-Larsen et al., 2009).

Pentamidine for children and adults is administered once a day at 4 mg/kg (maximum 300 mg daily) intravenously, infused slowly 1 to 2 hr in 5% glucose; due to its toxicity the dose can be reduced to 3 mg/kg. Aerosolized pentamidine should not be used because of limited efficacy and more frequent relapse, and intramuscular administration is not used due to the related complications (Conte et al., 1990). Side effects of pentamidine include azotemia, pancreatitis, hypo- or hyperglycemia, pancytopenia, electrolyte abnormalities, cardiac dysrhythmia and renal dysfunction (Conte et al., 1990). Pentamidine should be avoided in pancreas transplant recipients due to the potential for islet cell necrosis.

Clindamycin-primaquine

Clindamycin is a lincosamide antibiotic used to treat infections with anaerobic bacteria but can also be used to treat some protozoan diseases. Primaquine is an 8-aminoquinoline anti-protozoan agent. This combination is effective in adult patients with mild to moderate PcP, but

data for children are not available (Toma et al., 1998). Clindamycin is given at 600 to 900 mg intravenously or 300-450 mg orally every 6 to 8 hours and primaquine is given orally at 15 to 30 mg/day. Clindamycin component can be administered intravenously in severe cases; primaquine is only available orally. Recently, clindamycin-primaquine appeared superior to pentamidine as second-line therapy for PcP in patients failing or developing toxicity with TMP-SMX (Helweg-Larsen et al., 2009). Side effects of clindamycin include rash, anemia, neutropenia and the development of *Clostridium difficile* colitis. The main toxicity of primaquine is methemoglobinemia, thus, patients should be tested for glucose-6-phosphate dehydrogenase deficiency before administration of primaquine (Larsen, 2004).

Dapsone

Dapsone is a sulfone drug that inhibits DHPS and it is used as alternative therapeutic regimen for mild-to-moderate PcP. Dapsone must be taken with TMP (Medina et al., 1990). Although this association might have similar efficacy and fewer side effects than TMP-SMX, is less recommended due to the number of pills. The dosage of dapsone for adolescents and adults is 100 mg orally once daily (among children aged < 13 years, 2 mg/kg/day). The dosage of TMP for children and adults taken orally is 15 mg/kg/day divided into three doses (CDC, 2009; Mofenson et al., 2009). The most common adverse effects associated to dapsone are methemoglobinemia and hemolysis, especially in those with glucose-6-phosphate dehydrogenase deficiency. Thus, patients should be tested for glucose-6-phosphate dehydrogenase deficiency (Larsen et al. 2004).

Atovaquone

Atovaquone is a unique naphthoquinone that target the cytochome B complex and, thus, inhibits mitochondrial electron transport. This drug was developed clinically in the 1990s and it is available only as oral agent. It is used as second-line agent for treatment of mild to moderate PcP if TMP-SMX cannot be used. The standard dosing regimen for adults is atovaquone 750 mg orally twice a day with food for increasing gastrointestinal absorption (30-40 mg/kg/day for children < 3 months and > 24 months of age; between 3-24 months of age, 45 mg/kg/day are required) (Medina et al., 1990; Mofenson et al., 2009). Mutations of the cytochrome *b* gene have occurred in atovaquone-resistant isolates of *Pneumocystis*, but the clinical significance of gene mutations has not been determined (Kazanjian et al., 2001). The advantages of atovaquone include oral administration and fewer side effects. Disadvantages are its high cost and its bioavailability, although it has been improved with the micronized suspension formulation (Baggish & Hill, 2002). The most frequently reported adverse effects are rash, nausea, diarrhea, elevation of liver enzyme levels and headache. Atovaquone does not cause bone marrow suppression (Larsen et al., 2004).

Trimetrexate

Trimetrexate is an analogue of methotrexate that is an inhibitor of dihydrofolate reductase, and *in vitro* it is 1500 times more potent than trimethoprim (Kovacs et al., 1988). This drug is effective for treating PcP but is available only in an intravenous formulation. Because this drug also inhibits human folate metabolism, leucovorin must be administered concomitantly to prevent cytopenias (Larsen et al., 2004). A clinical trial showed that trimetrexate is less effective but better tolerate than TMP-SMX against AIDS-related PcP (Sattler et al., 1994). Trimetrexate with folinic acid have been approved for use in patients with moderately severe PcP, however, it is not longer available commercially. The dosage recommended for treatment of PcP is trimetrexate, 45 mg/m^2 intravenously once daily, plus

leucovorin 20 mg/m^2 orally or intravenously four times daily (Sattler et al., 1994).. Leucovorin therapy must extend for 72 hours past the last dose of trimetrexate. For adults, trimetrexate may alternatively be dosed on a mg/kg basis, depending on the patient's body weight: <50 kg, 1.5 mg/kg; 50-80 kg, 1.2 mg/kg, and >80 kg, 1.0 mg/kg. Also, leucovorin may be dosed on a mg/kg basis (<50 kg, 0.6 mg/kg, and >50 kg 0.5 mg/kg) administered every 6 hours. Despite the suggestion that leucovorin impairs the efficacy of TMP-SMX, there is no indication that the coadministration of leucovorin impairs the efficacy of trimetrexate for PcP (Larsen et al., 2004). In some cases trimetrexate plus leucovorin could be used as salvage treatment for PcP (Short et al., 2009).

Adjunctive therapies

The use of corticosteroids may reduce pulmonary inflammation response caused by the lysis of *Pneumocystis* in the lung after initiating treatment of PcP. Corticosteroids have been related with a significant benefit in terms of preventing deterioration in oxygenation in the first seven days of therapy, mortality, and reduction of intubations in AIDS patients (Briel et al., 2005). Corticosteroids are indicated in HIV-infected patients with a moderate-to-severe PcP who have hypoxemia (the partial pressure of arterial oxygen under 70 mm Hg with the patient breathing room air or an alveolar-arteriolar gradient greater than 35). In these cases, corticosteroids should be administered as early as possible within 72 hours after starting anti-*Pneumocystis* therapy (Thomas & Limper, 2004; CDC, 2009). Recommended dose are showed in table 3.

Therapeutic use	Moderate to severe *Pneumocystis* pneumonia		
	Drug	Dose	Route
First line	Trimethoprim-Sulfamethoxazole	15-20 mg/Kg daily divided into 3 or 4 doses 75-100 mg/Kg daily divided into 3 or 4 doses	Intravenous
Second line	Primaquine plus Clindamycin	30 mg daily 600-900 mg three times daily	Oral Intravenous
Second line	Pentamidine	4 mg/Kg daily (3 mg/Kg if toxicities)	Intravenous
Salvage therapy	Trimetrexate plus Leucovorin	45 mg/m^2 daily 20 mg/m^2 four times daily	Intravenous Intravenous or oral
Adjunctive therapy	Prednisone	Days 1–5: 80 mg daily divided into 2 doses Days 6–10: 40 mg daily Days 11–21: 20 mg daily	Oral
	Methylprednisolone	75% of prednisone dose	Intravenous
	Mild to moderate *Pneumocystis* pneumonia		
First line	Trimethoprim-Sulfamethoxazole	15-20 mg/Kg daily divided into 3 doses 75-100 mg/Kg daily divided into 3 doses	Oral
Second line	Dapsone plus Trimethoprim	100 mg daily 15-20 mg/Kg daily divided into 3 doses	Oral Oral or intravenous
Second line	Primaquine plus Clindamycin	15-30 mg daily 300-450 mg 3 or 4 times daily	Oral Oral
Second line	Atovaquone	750 mg two times daily	Oral with food

Table 3. Drugs therapy for treatment of *Pneumocystis* pneumonia in adults according to severity

Novel agents

Novel agents undergoing clinical investigation include echinocandins and pneumocandins, which target synthesis of beta 1,3 glucan, a cell wall compound of *Pneumocystis* and other fungi.

Caspofungin is an echinocandin that acts on the cell wall by inhibiting β-1,3-glucan synthesis and it has been approved for several fungal infections as *Candida* and *Aspergillus* species. Caspofungin has shown activity against *Pneumocystis* in experimental animal models and it has strong activity on cyst forms and weak activity on trophic forms (Powles et al., 1998). Due to TMP-SMX affects only the trophic forms, it has been suggested that the association of TMP-SMX and caspofungin by fully inhibiting the organism life cycle, may provide a synergistic activity against *Pneumocystis*. According to this, it has been reported cases of PcP where the association of caspofungin and TMP-SMX achieved a complete cure of PcP (Utili et al., 2007). However, this promising therapeutic approach needs to be assessed by controlled clinical trials.

9. Prevention

Many studies have demonstrated that PcP can largely be prevented by administration of chemoprophylaxis to susceptible individuals (Di Cocco et al., 2009; Green et al., 2007; Podzamcser et al., 1995; Rodriguez & Fishman, 2004) and according with the American Thoracic Society recommendations patients infected with HIV (Huang et al., 2006) need to receive prophylaxis to prevent disease depending on specific risks to the patient's system. Recommendations for chemoprophylaxis should be based on weighing the efficacy against the risk of adverse events, the risk of developments of antimicrobial resistance, and the cost of the intervention (Roblot et al., 2005). Medications recommended for chemoprophylaxis against PcP are listed in table 4.

9.1 Primary prophylaxis

The majority of recommendations are based in studies performed in HIV-infected patients. Guidelines recommend starting primary prophylaxis against PcP in HIV-infected adolescents and adults, including pregnant and patients under HAART, when the CD4 cell count is less than 200 cells/mm^3 or the patient has a history of oropharyngeal candidiasis. Patients with a CD4 cell percentage of <14% or a history of an AIDS-defining illness should be considered for chemoprophylaxis (CDC, 2009). Prophylaxis recommendations for HIV-infected children are age-based. Chemoprophylaxis should be provided for children 6 years or older based on adults guidelines, for children aged 1 to 5 years if CD4 counts are less than 500 cells/mm^3 or CD4 percentage is less than 15%, and for all HIV-infected infants younger than 12 months (Zolopa et al., 2009).

TMP-SMX is the recommended prophylactic agent in both primary and secondary prophylaxis for PCP, because of its high efficacy, relative safety, low cost, and broad antimicrobial spectrum (CDC, 2009; Di Cocco et al., 2009; Roblot et al., 2005; Rodriguez & Fishman, 2004). TMP–SMX also is effective in preventing *Toxoplasma gondi, Isospora belli, Cyclospora cayetanensis* and some bacterial infections such us, *Streptococcus pneumoniae*, *Salmonella*, *Haemophilus*, *Staphylococcus,* common gram-negative gastrointestinal and urinary pathogens (Rodriguez & Fishman, 2004). Either one single-strength tablet daily or one double-strength tablet daily are the preferred regimens, but the first regimen might be better

tolerated than the second (CDC, 2009). An alternative choice can be one double-strength tablet three times per week (CDC, 2009; Roblot et al., 2005). TMP-SMX at a dose of one double-strength tablet daily confers cross-protection against toxoplasmosis and selected common respiratory bacterial infections. Lower doses of TMP-SMX also likely confer such protection (CDC, 2009; Di Cocco et al., 2009).

For patients who have an adverse reaction that is not life threatening, prophylaxis with TMP-SMX should be reinstituted. These patients might better tolerate reintroduction of the drug with a gradual increase in dose or reintroduction of TMP-SMX at a reduced dose or frequency (CDC, 2009). If TMP-SMX is not tolerated, a second choice would be dapsone given 100 mg daily, dapsone 50 mg daily plus pyrimethamine 50 mg weekly plus leucovorin 25 mg weekly or dapsone 200 plus pyrimethamine 75 mg plus leucovorin 25 mg weekly, aerosolized pentamidine 300 mg monthly administered by an ultrasonic or jet-nebulizer, and atovaquone 1500 mg daily (CDC,2009). Dapsone is effective and inexpensive but

Drug	Dose for adults	Dose for children	Route	Comments
Trimethoprim-Sulfamethoxazole	160/800 mg (DS tablet) per day or 3 times per week 80/400 mg (SS tablet) per day	150/750 mg/m² body surface area (max: 320/1600 mg) as single or 2 divided doses 3 times per week	Oral	First choice Weekly regimen is recommended if daily therapy in not tolerated
Dapsone	100 mg per day	2 mg/Kg body weight (max: 100 g) per day 4 mg/Kg body weight (max: 200 g) per week	Oral	Alternative choice Ensure patient does not have Glucose-6 phosphate dehydrogenase deficiency
Pentamidine	300 mg per month	300 mg per month (aged ≥ 5 years)	Aerosol	Alternative choice
Atovaquone	1500 mg per day	30-45 mg/Kg body weight according to age per day	Oral	Alternative choice Take with high-fat meals for maximal absorption
Dapsona + Pyrimethamine + Leucovorin	50 mg per day 50 mg per week 25 mg per week		Oral Oral Oral	Alternative choice Ensure patient does not have Glucose-6 phosphate dehydrogenase deficiency Effective in preventing toxoplasmosis
Dapsona + Pyrimethamine + Leucovorin	200 mg per week 75 mg per week 25 mg per week		Oral Oral Oral	Alternative choice Ensure patient does not have Glucose-6 phosphate dehydrogenase deficiency Effective in preventing toxoplasmosis

Table 4. Prophylaxis regimens for *Pneumocystis* pneumonia.

associated with more serious adverse effects than atovaquone (El-Sadr et al., 1998). Atovaquone is effective, safe and it is effective against *Toxoplasma gondii* but it is more expensive (Rodriguez & Fishman, 2004). The widespread concept that TMP-SMX is contraindicated for prophylaxis in patients treated with methotrexate might be obsolete because the safety of one single-strength tablet daily or one double-strength tablet thrice-weekly has been proved in clinical studies (Langford et al., 2003). However, these patients need to receive folate supplementation besides blood counts and liver-function tests should be closely monitored (Roblot, 2005).

Primary prophylaxis should be discontinued for HIV-infected adult and adolescent patients who have responded to HAART with an increase in CD4 counts major than 200 cells/mm^3 during more than 3 months (Lopez Bernaldo et al., 2001). Prophylaxis should be reintroduced if the CD4 cell count decreases to less than 200 cells/mm^3.

9.2 Secondary prophylaxis

HIV-infected adults and adolescents patients who have developed previous episodes of PcP should receive secondary prophylaxis (Thomas & Limper, 2004). Chemoprophylaxis should be discontinued for adult and adolescent patients when CD4 cell count increases to more than 200 cells/mm^3 for a period of 3 months as a result of HAART (Lopez Bernaldo et al., 2001). Prophylaxis should be reintroduced if the CD4 count decreases again to less than 200 cells/mm^3. If PcP recurs at a CD4 count higher than 200 cells/mm^3, continuing PcP prophylaxis for life would be prudent (CDC, 2009).

10. Conclusions

Pneumocystis jirovecii is an atypical fungus that causes PcP mainly in HIV-infected individuals. Today, PcP is still a major cause of morbidity and mortality among AIDS patients, and constitutes a worldwide problem to public health. While the incidence of PcP among HIV infected individuals has decreased in developed countries, the prevalence of AIDS-related PcP in developing countries remains high and poorly controlled. The epidemiology of this infection is only beginning to be understood. The accumulating evidence suggests that *P. jirovecii* is a highly infectious organism with low virulence that takes advantage of hosts as temporary reservoirs of infection. In this sense, colonization with *P. jirovecii* (that is infection without disease) has recently gained attention as a important issue for understanding the complete cycle of human *Pneumocystis* infection. The clinical presentation in HIV-infected patients may differ from that in other immunosuppressed patients and its diagnosis continues to be challenging. Clinicians must be familiar with its presentation and management because mild cases are sometimes difficult to diagnose. The emergence of highly sensitive and specific molecular methods for PcP diagnosis have represented a significant advance in order to establish earlier PcP diagnosis, detect low parasite rates, and detect *Pneumocystis* DNA in non-invasive samples. Co-trimoxazole is the most effective medication for its prevention and treatment but other alternative medications are also available. Future clinical research should include studying the transmission and epidemiology of PcP in populations worldwide, improving the diagnosis of PcP, improving regimens for prophylaxis and treatment in various patient populations, and determining the significance of the DHPS mutations in various populations and in different geographic locations. Furthermore, the threat of emerging

resistance to available anti-*Pneumocystis* drugs highlights the need to continue investigating the biology of this organism in the hope of developing novel treatment strategies.

11. References

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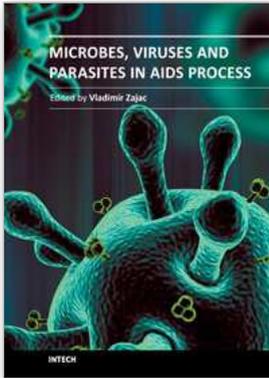
Microbes, Viruses and Parasites in AIDS Process

Edited by Prof. VladimÃr Zajac

The main goal in compiling this book was to highlight the situation in Africa in terms of AIDS and opportunistic diseases.Â Several chapters reveal great poverty, an apocalyptic situation in many parts of Africa.Â Global migration of people resulted in their exposure to pathogens from all over the world. This fact has to be acknowledged and accepted as African reality. New, unconventional hypotheses, not determined by established dogmas, have been incorporated into the book, although they have not yet been sufficiently validated experimentally.Â It still applies that any dogma in any area of science, and medicine in particular, has and always will hinder progress. According to some biologists, in the future, AIDS is very likely to occur in a number of variations, as a direct result of the ongoing processes in the global human society. Thus, we urgently need a comprehensive solution for AIDS, in order to be ready to fight other, much more dangerous intruders.

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Jose M. Varela, Francisco J. Medrano, Eduardo Dei-Cas and Enrique J. Calderón (2011). Pneumocystis jirovecii Pneumonia in AIDS Patients, Microbes, Viruses and Parasites in AIDS Process, Prof. VladimÃr Zajac (Ed.), ISBN: 978-953-307-601-0, InTech, Available from: http://www.intechopen.com/books/microbes-viruses-and-parasites-in-aids-process/pneumocystis-jirovecii-pneumonia-in-aids-patients

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