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Pneumocystis pneumonia in the twenty-first century: HIV-infected versus HIV-

uninfected patients

Catia Cillóniz¹, Cristina Dominedò², Míriam J Álvarez-Martínez³, Asunción Moreno⁴, Felipe García⁴, Antoni Torres*¹, José M Miro⁴

¹ Department of Pneumology, Hospital Clinic, Barcelona; August Pi i Sunyer Biomedical Research Institute (IDIBAPS); University of Barcelona; SGR 911; Biomedical Research Networking Centres in Respiratory Diseases (CIBERES), Barcelona, Spain

² Department of Anesthesiology and Intensive Care Medicine, Fondazione Policlinico Universitario A. Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy

³ Department of Microbiology, Hospital Clinic, Barcelona; University of Barcelona, Barcelona, Spain

⁴ Department of Infectious Diseases, Hospital Clinic, Barcelona; IDIBAPS; University of Barcelona, Barcelona, Spain

*Corresponding author:

Antoni Torres

Department of Pneumology, Hospital Clinic, Barcelona

C/ Villarroel 170, 08036 Barcelona, Spain

Phone: 0034 932275549

Email: atorres@clinic.cat

Abstract

Introduction: *Pneumocystis pneumonia* (PcP) has classically been described as a serious complication in patients infected with the human immunodeficiency virus (HIV). However, the emerging number of conditions associated with immunosuppression has led to its appearance in other patient populations, such as those receiving chronic corticosteroid therapy, those with hematological or solid malignancies, transplant recipients and those who receive immunomodulatory or biological therapy.

Areas covered: This article reviews the most recent publications on PcP in the HIV-infected and HIV-uninfected population, focusing on epidemiology, diagnostic, therapy and prevention. The data discussed here were mainly obtained from a non-systematic review using Medline and references from relevant articles including randomized clinical trials, meta-analyses, observational studies and clinical reviews. Eligible studies were selected in two stages: sequential examination of title and abstract, followed by full text.

Expert opinion: Widespread use of antiretroviral and prophylactic therapy in HIV-infected patients has decreased the incidence of PcP in this population. However, the growing incidence of *Pneumocystis* infection in the HIV-uninfected population suggests the need for new global epidemiological studies in order to identify the true scale of the disease in this population. These data would allow us to improve diagnosis, therapeutic strategies, and clinical management. It is very important that both patients and physicians realize that HIV-uninfected patients are at risk of PcP and that rapid diagnosis and early initiation of treatment are associated with better prognosis. Currently, in-hospital mortality rates are very high: 15% for HIV-infected patients and 50% in some HIV-uninfected patients. Therefore, adequate preventive measures should be implemented to avoid the high mortality rates seen in recent decades.

Article highlights

- *Pneumocystis* is prevalent in the lungs of immunocompetent individuals, suggesting that asymptomatic healthy adult carriers provide a reservoir for *Pneumocystis* infection.
- In HIV-infected patients the risk for PcP increases exponentially when the CD4+ cell count is below 200 cells/ μ l. Patients on ART who develop PcP typically have low CD4+ cell levels due to poor adherence to ART or possible resistance to ART.
- There is a growing incidence of *Pneumocystis* infection in the HIV-uninfected population due to the emerging number of conditions associated with immunosuppression. These include hematological malignancies, solid tumors, solid-organ transplantation, autoimmune diseases, steroids therapy, immunosuppressive or biological therapy.
- There is strong evidence of the presence of *Pneumocystis* in the air of areas where infected patients reside and of a transmission through interpersonal contacts. It is reasonable to isolate infected patients until respiratory symptoms resolution or discharge from hospital; when patients need to be moved a mask should be worn.

1. Introduction

In the last decade the number of immunocompromised patients has increased globally[1,2]. Patients with immunosuppression show an increased risk of pulmonary infectious complications like *Pneumocystis pneumonia* (PcP). Indeed, the risk of PcP and other fungal infections is elevated in patients with human immunodeficiency virus (HIV) infection, chronic corticosteroid use, hematological or solid malignancies, organ transplantation and immunomodulatory or biological therapy, although the data regarding prevalence come from a small number of patients in single-center or single-country studies [3–5]. (Figure 1).

Pneumocystis jirovecii is probably transmitted via environment aerosols from infected patients or colonized individuals[6]. The global incidence of PcP is unclear due to its non-specific symptoms, the difficulty to obtain good respiratory samples and to perform microbiological diagnosis. Delayed or missing PcP diagnosis may compromise the early initiation of an adequate therapy, thus increasing the risk of poor outcomes. Because of the widespread use of antiretroviral and prophylactic therapy in HIV-infected patients, the incidence and mortality of PcP has fallen in recent decades[7]. However, several studies still report high mortality rates among HIV-uninfected patients probably due to the greater intensity of the inflammatory response, the acuteness of symptoms, the more frequent progression to respiratory failure requiring tracheal intubation [8], and also the influence of different management practices[9–13].

The aim of this review is to present and discuss recent advances in our understanding of PcP in HIV-infected and HIV-uninfected patients. We focus principally on its epidemiology, clinical presentation, diagnosis, colonization and risk factors. We believe

that this information will help to identify patients at risk and will help in the diagnosis of PcP, especially in HIV-uninfected patients, in order to initiate prompt therapy.

2. Transmission

Pneumocystis jirovecii is an obligate fungal organism with high tropism for the alveolar epithelium of the lung. Generally, it attaches to cells without invading them, thus showing a parasitic behavior[9]. Trophic and cystic forms have been identified in its life cycle, with trophic forms predominating over the cystic ones during lung infections[14]. Unfortunately, the inability to culture *Pneumocystis* outside the lung limits the complete comprehension of its life cycle [15,16]. *Pneumocystis* rarely causes disseminated infections, although some cases have been reported in patients with AIDS[17,18].

Infection generally occurs in immunosuppressed patients due to the acquisition of a new infection rather than the reactivation of a latent one. In a rat model of prior PcP episodes, animals did not present reactivation of *Pneumocystis* infection when immune suppression was induced, unless they have been exposed to other rats with active infection[19]. Interestingly, the latency model of infection in humans by Wakefield et al.[15] (which used 47 bronchoalveolar lavage samples from 16 HIV-infected patients) showed that after a PcP episode in HIV-infected patients asymptomatic carriage of *Pneumocystis* did not last longer than 9.5 months.

Pneumocystis transmission has been understood to be interindividual. It has been hypothesized that *P. jirovecii* is generally transmitted via the airborne route (17–20),

although no established reservoir has been identified so far and several possible routes of transmission have been considered. Nosocomial interhuman transmission is one possibility, with Rabodonirina et al.[24] demonstrating the pathogen transmission between HIV-infected patients with PcP and hospitalized renal transplant recipients. Yazaki et al.[25] investigated the environmental exposure as a possible route of *Pneumocystis* transmission following an outbreak of PcP in renal transplant recipients. The authors detected in outpatients consulting rooms *P. jirovecii* deoxyribonucleic acid (DNA) with the same genotypes as in clinical samples obtained two months earlier. Finally, data on *Pneumocystis* colonization in immunocompetent[26–29] or physiologically immunosuppressed individuals (e.g., pregnant woman)[30] have led to the hypothesis that these groups may act as contagious sources for susceptible persons, making them possible reservoirs in the community[14].

Mother-to-infant transmission of *P. jirovecii* has also been described. In a pilot study of 33 third-trimester, pregnant, asymptomatic healthy women (median age 26 years) and 28 controls (healthy women within 15 days of a menstrual period; median age 28 years). Vargas et al.[30] detected *P. jirovecii* DNA in deep nasal swab samples in five (16%) of the 33 pregnant women but in none (0%) of the controls. Thus explaining why primary *Pneumocystis* infection can be acquired early in life (i.e., through vertical transmission). Montes-Cano et al.[31] analyzed placentas and lungs of 20 fetuses at 28 ± 8 weeks of gestation. *P. jirovecii* was observed in lung tissue samples from 7 (35%) fetuses, thus confirming the possibility of a trans placental transmission in humans. Interestingly, the study by Rojas et al.[32]. that investigated the prevalence of *P. jirovecii* colonization in 128 preterm infants and its possible association with medical complications reported that *Pneumocystis* DNA was presented in 26% of the newborns

studied. The authors suggested that *P. jirovecii* colonization could be a risk factor for ARDS in preterm infants.

3. Focusing on colonization

Colonization or subclinical infection with *P. jirovecii* is defined as the isolation of *P. jirovecii* in respiratory samples in patients with no specific symptoms or history of PCP. Interstitial pneumonia is the major clinical manifestation in patients with active infection for *P. jirovecii* [6].

Several studies have identified possible colonization in both HIV-infected and HIV-uninfected patients[6,14,26,28,29]. Colonization influences interindividual transmission of the pathogen, appearance of lung inflammation and damage, progression of the disease and development of resistance.

3.1. Colonization in immunocompetent individuals

A prospective Spanish study [33] was designed to determine whether *P. jirovecii* can be detected in the normal, healthy population. It investigated oropharyngeal wash samples from 50 persons who had not been exposed to patients in a hospital environment within the year before the study, or who had not been diagnosed with, or were not suspected to have, chronic lung disease, neoplasm, or immunosuppression of any cause. *P. jirovecii* DNA was present in 24% of cases. All carriers were asymptomatic, HIV-uninfected and had normal total lymphocyte and CD4+ cell counts. Similarly, in a study of the detection of *P. jirovecii* DNA in oropharyngeal wash and nasal swab samples from 110 immunocompetent older adults (>65 years), Vargas et al.[34] reported that *P. jirovecii* DNA was detected in 13% of oropharyngeal wash samples and in 11% of the nasal swab samples. However, taking the oropharyngeal

wash and nasal swab samples together, the rate of detection rose to 22%.

Interestingly, the authors reported that none of the participants developed PcP within 1 year of study completion. As the above studies reported, *Pneumocystis* is frequently detected in the respiratory tract of immunocompetent individuals, suggesting that asymptomatic healthy adult carriers provide a reservoir for *Pneumocystis* infection[33,34].

3.2. Colonization in patients with Chronic Lung Diseases

Several studies have reported *Pneumocystis* colonization in individuals with chronic lung diseases (chronic obstructive pulmonary disease [COPD], cystic fibrosis, chronic bronchitis, and asthma)[35,36]. Some of these studies have also found an association between lung disease progression and colonization with *Pneumocystis*. In a study of *Pneumocystis* colonization rates in lung tissue obtained during lung resection or transplantation in smokers with a range of airway obstruction severity and in a control group with lung diseases other than COPD, Morris et al.[35] found *Pneumocystis* colonization in 36.7% of patients with very severe COPD and in 5.3% of smokers with normal lung function or less severe COPD. Colonized individuals presented more severe airway obstruction (median FEV₁ = 21% predicted versus 62% in non-colonized subjects, p = 0.006). The authors found that the Global Health Initiative on Obstructive Lung Diseases (GOLD) GOLD IV classification[37] was the strongest predictor of *Pneumocystis* colonization (odds ratio: 7.3, 95% confidence interval = 2.4 to 22.4, p < 0.001) and was independent of smoking history.

An interesting study on the distribution of *Pneumocystis* colonization in the lungs of patients with COPD was published in 2011[36]. The authors examined the explanted

lung tissue of 19 patients with COPD and without HIV infection who underwent lung transplantation. *Pneumocystis* colonization was detected in 42% of patients with advanced COPD. Colonization was more frequent in the lower and middle lobes than in upper lobes. According to the authors, these differences in the distribution of colonization may be due to the lower sensitivity of *Pneumocystis* detection in the upper lobes of COPD patients because the disease has already become end-stage in these areas of the lung, or because these areas have lower tissue content. The authors also tentatively attributed the relative propensity for lower lobe colonization to the greater ventilation in the lower regions of the lung than in the upper regions. The authors suggested that the use of a single sample from an individual might underestimate the prevalence of *Pneumocystis* colonization[36].

3.3. Colonization in patients with Immunosuppression

There is significant variability regarding the rate of *Pneumocystis* colonization in patients with HIV, with studies reporting rates between 6% and 68%[6,38–41]. This variation reflects differences in the study populations, biological samples and microbiological tests. Gutierrez et al.[42] analyzed the prevalence of *P. jiroveci* colonization in oropharyngeal wash samples in a cohort of 20 young HIV-infected patients and found colonization in 40% of the population; after 50 weeks of follow-up, only one colonized patient with advanced immunosuppression developed PcP.

Mekinian et al.[43] analyzed *Pneumocystis* colonization in 67 patients with systemic autoimmune diseases and 28 controls (healthy subjects). None of the selected patients had respiratory or general signs of current *Pneumocystis* infection but the authors found that 16% (n=11) of the patients with systemic autoimmune diseases had

evidence of colonization, compared with 7% (n=2) in the control group. The authors also analyzed the risk factors for colonization in patients with systemic autoimmune diseases, finding that male gender was the only risk factor. However, after a new analysis with nested models the authors were able to attribute the difference in the *P. jirovecii* colonization to the higher level of induced immunosuppression in the male patients, as suggested by lymphocyte counts and corticosteroid volume.

4. Infection

4.1. Risk Factors in HIV-infected patients

Incidence of PcP infection in HIV-infected patients fell substantially with the widespread use of active antiretroviral therapy (ART), especially in developing countries[44]. Most cases of PcP now occur in patients who have undiagnosed HIV infection, who are not receiving ART or who are not adhering to ART at the time of PcP diagnosis, or in cases of sub-optimal immune recovery [45–47]. The risk for PcP increases exponentially when the CD4+ cell count is below 200 cells/ μ l[17]. Patients on ART who develop PcP typically have low CD4+ cell levels due to poor adherence to ART or possible resistance to ART[46]. When in patients on ART the CD4+ cell count (> 200 cells/ μ l) increases, the risk for PcP decreases sufficiently to safely discontinue both primary and secondary prophylaxis[17,48].

Recent global estimates report that approximately more than 400,000 cases of PcP in patients with acquired immunodeficiency syndrome (AIDS) occur each year[49,50]. The case fatality rate is approximately 15% in patients with AIDS, despite optimal treatment [51]. Interestingly, a systematic review and meta-analysis of the prevalence of PcP in HIV-infected patients in sub-Saharan Africa between 1995 and 2015[52]

showed a fall in the prevalence of PcP from 28% to 9% after 2005.

The overall annual incidence of PcP in HIV-infected adults ranges from 0.9 to 5.4 cases per 1000 person-years [5,53,54]. In the United States and Canada, a recent study of 16 large cohorts of HIV-infected patients showed a fall in the incidence of PcP from 0.92 events/100 person-years in 2000–2003 to 0.39 events/100 person-years in 2008–2010 [9]. Similarly, a Spanish study [45] of 136 PcP cases indicated a significant reduction in the annual incidence of PcP from 13.4 to 3.3 cases per 1000 HIV-infected patients per year during the period between 2000 and 2013. However, the proportion of patients with PcP before a diagnosis of HIV also rose from 48% in 2000 to 67% in 2013, confirming that PcP remains an important health problem in patients with HIV[45].

Certainly, the use of ART has influenced the incidence of PcP. Reporting trends in AIDS-defining opportunistic illnesses over a 25 year period (1987–2012) in Brazil, Coelho et al.[56] found a significant reduction in the incidence of PcP from 87.2 to 2.84 cases/1000 person-years between the periods 1987–1990 and 2009–2012. The authors observed that the use of ART increased over these years, with almost 80% of patients receiving ART in the latter period.

The mortality rate associated with PcP in HIV-infected patients is reported to be 15%, ranging from 4% to 32% on the basis of the population studied, the underlying chronic diseases, the immunological status and the precocity of diagnosis [7,51]. Several risk factors have been related to mortality in HIV-infected patients: older age, subsequent episodes of PcP, low haemoglobin level and low basal PaO₂ at hospital admission, pulmonary Kaposi sarcoma and pre-existing comorbidities[57,58]. However, recent studies indicate a decrease in PcP-related mortality [7,59].

4.2. Risk Factors in HIV-uninfected patients

PcP is emerging as an important infectious disease in HIV-uninfected persons [4,13]. It is estimated that the annual incidence of PcP in this population exceeds 100,000 cases globally, that the case fatality rate is about 50% and that there are more than 50,000 related deaths[49]. The conditions most frequently associated with an increased risk of PcP are:

*Hematological malignancies (especially leukemia and lymphomas): in this population risk factors related to PcP include the use of corticosteroids, monoclonal antibody therapy, or T-cell dysfunction related to underlying disease or therapy.

*Solid tumors (e.g., brain, breast, lung, and renal): it is reported that the main risk factors in this population are the high-dose chemotherapy and marrow transplantation, and the prolonged use of corticosteroid therapy.

*Solid-organ transplantation: in this population, the main risk for Pneumocystis infection is CD4+ lymphocytopenia.

*Autoimmune diseases (e.g., rheumatoid arthritis, inflammatory bowel diseases and ankylosing spondylitis): in this population, low CD4+ cell count and the use of anti-TNF α , rituximab or cyclophosphamide may increase the risk.

*Patients receiving steroid, immunosuppressive or biological therapy (anti-tumor necrosis factor [TNF], anti-interleukin 6, anti-interleukin 1, anti-cd52, or anti-CD20) are also at increased risk [60–62].

Hematologic populations at highest risk of PcP are those with acute lymphoblastic leukemia and those who have received allogeneic hematopoietic stem cell

transplantation (HSCT)[63]. The nationwide study in the UK by Maini et al.[5] analyzed data from 2258 cases of PcP over a decade (2000–2010) and showed an increase in the incidence of PcP, particularly in patients who had an hematologic malignancy or who had undergone transplantation. The number of cases rose from 157 in 2000 to 352 in 2010 (a mean annual increase of 9%). The authors attribute this increase to the improvements in diagnostic methods over the study period. With regard to the increased transmission of the *P. jirovecii* organism between susceptible persons, the authors suggest that the levels of exposure of susceptible persons to infectious persons may rise as a result of changes in the delivery of health care. Other possible reasons mentioned are the increase in the number of potentially vulnerable patients who did not receive appropriate prophylactic therapy, and an increase in the use of potent immunosuppressant agents in several medical conditions.

In 2014 an interesting French study on the incidence of PcP was published by Fillatre et al.[64]. The study was carried out between 1990 and 2010 and 293 cases of PcP were detected, 154 (52%) of which in HIV-uninfected patients. The main underlying conditions associated with PcP were hematological malignancies (32%), solid tumors (18%), inflammatory diseases (15%), solid-organ transplantation (12%) and vasculitis (10%) (Figure 1). The authors grouped the risk and the incidence of PcP into three categories according to the underlying diseases or conditions:

- Low risk (incidence <25 cases/100,000/patient-years): inflammatory diseases, Hodgkin lymphoma, and other solid tumors.
- Intermediate risk (incidence 25–45 cases/100,000/patient-years): Waldenström macroglobulinemia, multiple myeloma, and central nervous system cancer.

- High risk (incidence >45 cases/100,000/patient-years): polyarteritis nodosa, granulomatosis with polyangiitis, polymyositis/dermatomyositis, acute leukemia, chronic lymphocytic leukemia, and non-Hodgkin lymphoma.

Recently, a French study [65] proposed a score to predict the risk of *Pneumocystis* infection in patients with hematological malignancies and acute respiratory failure (ARF) requiring ICU admission. Variables included in the score were age (<50 [1 point]; 50-70 [-1.5 points]; >70 [-2.5 points]), lymphoproliferative disease (2 points), anti-*Pneumocystis* prophylaxis (1 point), respiratory symptom duration (<3 days [0 point]; 3 to 5 days [3 points]; >5 days [3 points]), shock (-1.5 points), chest radiograph pattern (2.5 points) and pleural effusion (-2 points). The cut-off score for the definition of pneumocystis was three points. The score showed a specificity of 88% and a negative predictive value of 97% for PcP. The authors expect this score to accelerate the initiation of anti-PcP therapy.

Mortality remains higher in HIV-uninfected patients than in HIV-infected patients (40%–60% [62,64] and 5%–15% [7,66] respectively), probably due to the higher inflammatory response, the acute presentation of symptoms, the rapid progression to respiratory failure with the need for invasive mechanical ventilation [8] and the different management practices. Mortality in intensive care unit (ICU) is very high for HIV-uninfected patients with PcP. A study of 82 patients with PcP admitted to ICU showed a mortality rate of 76%, with the main risk factors being increased age, raised white blood cell counts and the presence of a pneumomediastinum [12].

5. Clinical manifestation and outcomes of PcP

Table 1 summarizes the clinical presentation, radiographic features and outcomes of PcP in HIV-infected and HIV-uninfected patients. HIV-infected patients typically show a sub-acute onset of the infection, longer symptoms duration (3 weeks), low-grade fever with progressive dyspnea and less severe course with a mortality rate of 15% [46,51,67]. HIV-uninfected individuals, by contrast, typically have a rapid onset, fast progression to respiratory failure, shorter symptoms duration (4-7 days), are more likely to require ICU admission and fulminant course with a mortality rate of 30%-60%. The different clinical presentation is not associated with the pulmonary fungal load; in fact, it seems to be related to pneumonia severity and the degree of lung inflammation. Limper et al. found that patients with PcP and AIDS have more *Pneumocystis* organisms and fewer neutrophils in the lung than immunocompromised patients with PcP without AIDS (with the exception of organ transplant patients), and that inflammation in the lower respiratory tract (as measured by the number and percentage of lavage neutrophils and not by microorganism number) is associated with greater impairment of gas exchange and lower patient survival in PcP with and without AIDS [68].

Roux et al. [51] reported clear differences in the clinical features of PcP between 223 patients with AIDS and 321 patients with other immunosuppressive disorders (non-AIDS patients). Patients without AIDS had significantly shorter median times from symptom onset to presentation (5 days, compared with 21 days for patients with AIDS; $p < 0.001$), hypoxemia was more severe; ICU admission was more common (50% vs. 35%; $p = 0.001$), and both non-invasive (16% vs. 8%; $p = 0.005$), invasive (30% vs. 11%; $p = 0.001$) mechanical ventilation were required more often and lower in-hospital mortality (4% vs. 27%; $p = 0.001$). These results were in accordance with a recent

published study from Portugal that assessed the differences between 75 HIV and 54 non-HIV patients with PcP. The authors reported that the clinical presentation in non-HIV patients was more tenuous (respiratory complaints: 67% vs 37%, $p = 0.002$), but with worse outcomes than in HIV-infected patients (in-hospital mortality: 10% vs 20%, $p = 0.002$)[69].

6. ART and the Immune Reconstitution Inflammatory Syndrome in PcP

The immune reconstitution inflammatory syndrome in PcP (PcP-IRIS) is an exaggerated pulmonary immune reaction occurring in HIV-infected patients after ART starting in response to *Pneumocystis* infection, mediated by the immune system recovery [70]. PcP-IRIS has been associated with high morbidity and ICU admission but mortality rates are very low; the reason for this lower mortality could be the fact that the patients received corticosteroids, which have immunomodulatory properties and may regulate the inflammatory storm of IRIS, or that these patients are on PcP therapy which reduces the *Pneumocystis* load [71–74]. The timing of PcP-IRIS presentation in relation to ART initiation dictates its further definition: “paradoxical IRIS” refers to a worsening of symptoms secondary to *Pneumocystis* infection after ART starting and treatment for PcP, whereas “unmasked IRIS” refers to *Pneumocystis* infection that is undiagnosed before ART. Although the onset of PcP-IRIS is variable, it usually occurs within 3 months of ART starting [70].

The true incidence of PcP-IRIS remains unclear because most data have come from case reports[71,75]. A retrospective study in the USA by Achenbach et al.[76] on patients suffering opportunistic infections after ART starting reported that IRIS occurred in 4% of patients with *Pneumocystis* infection within 1 year.

Early initiation of ART does not affect the incidence of PcP-IRIS[77]. Also, according to clinical trial data for the optimal timing of ART initiation in AIDS-related opportunistic infections (OI), it is recommended to start ART therapy within two weeks of OI treatment initiation when there is co-infection with PcP [78].

Clinically, PcP-IRIS presents as a worsening of existing PcP or an unmasking of PcP[72]. Most patients display an inflammatory response upon starting therapy directed against *Pneumocystis*, which is tempered by treatment with corticosteroids; however, some patients develop a worsening of pulmonary disease after starting ART, presenting fever, dyspnea and worsening hypoxia. The common radiological manifestations in PcP IRIS are the presence of ground glass opacities, but in some cases atypical radiological manifestations such as consolidations, organization pneumonia, and absence of effusion and nodes have been reported [70]. Currently, the consensus is to continue with ART due to the possibility that HIV replication progresses, but to start specific treatments for any co-infection[17].

7. Radiographic features

HIV-infected patients generally present bilateral interstitial and alveolar infiltrates in perihilar areas (Figure 2, a, b). Conversely, HIV-uninfected patients generally show diffuse lung infiltrates and evident consolidations, with few cases presenting pulmonary cysts or pneumatoceles (Figure 2, c, d). Radiographic chest patterns are not-specific in general. Several studies have described the role of the High Resolution Computed Tomography (HRCT) of the chest in the diagnosis of PcP in HIV-infected and HIV-uninfected patients[79–82].

The study by Fuji et al.[79] described the clinical features of 34 episodes of PcP in 32 HIV-infected patients. HRCT of the lung showed ground-glass opacities (GGO) sparing the lung periphery in 41% of episodes, or displaying a mosaic pattern (29%), or being nearly homogeneous (24%), GGO associated with air-space consolidation (21%), associated with cystic formation (21%), associated with linear-reticular opacities (18%), patchily and irregularly distributed (15%), associated with solitary or multiple nodules (9%) and associated with parenchymal cavity lesions (6%).

In HIV-uninfected patients consolidations and GGO have been reported more frequently [80]. Interestingly, the study by Mu et al.[83] investigated the relationship between radiological stages (*early*: early normal or nearly normal on chest x-ray or bilateral diffuse GGO on chest CT; *mild*: mild bilateral infiltrates on chest x-ray or bilateral diffuse GGO and patchy consolidations on chest CT; *late*: late bilateral consolidations on chest x-ray or bilateral predominant consolidations on chest CT) and PcP prognoses in 105 HIV-uninfected PcP immunocompromised patients. According to the chest HRCT, 40 cases were at early stage, showing bilateral diffuse GGO, and the case fatality rate (CFR) was 20.0% ($P > 0.05$); 34 cases were at mid stage, showing bilateral diffuse GGO and patchy consolidations, and the CFR was 47.1% ($P > 0.05$); finally, 10 cases were at late stage, showing predominant consolidations in bilateral lung fields, and the CFR was 80.0% ($P < 0.05$). The authors described other radiographic findings such as bilateral diffuse GGO with mosaic signs and consolidations, pleural effusions, small nodules, arothorax, pneumomediastinum, pneumohypoderma, cystic lesions and thickened lobular septa.

8. Microbiological Diagnosis

P. jirovecii is difficult to isolate or culture with conventional microbiological methods.

However, in a recent study by Schildgen et al.[84] it was successfully cultured and propagated from a positive BAL sample. Propagation was possible in CuFi-8 cells, derived from the bronchus of a patient with cystic fibrosis, and EpiAirway cells, derived from human tracheal/bronchial epithelial cells. Unfortunately, no other study has been able to replicate this method of propagation[16].

The most frequently used respiratory samples for PcP diagnosis in HIV-infected patients are induced sputum (less invasive but with low sensitivity) or BAL (higher diagnostic yield). It is known that the immunosuppression profile influences the *Pneumocystis* load in the infection[51,68], HIV-uninfected patients have a lower burden of organisms and so may present false negative results. PcP diagnosis in HIV-uninfected patients requires invasive samples such as BAL or lung biopsies. Other noninvasive samples such as spontaneous sputum, nasopharyngeal aspirate (NA), and oropharyngeal washing (OW) may be useful in the diagnosis of pneumocystis. Of course, their sensibility raises to 75% when analysed together with molecular tests such as PCR compared to microscopy[85–87].

8.1. Microscopy

The gold standard for the microbiological diagnosis of *P. jirovecii* is the microscopic observation of the microorganism in respiratory samples. Staining methods include Grocott-Gomori methenamine silver, toluidine blue-O, calcofluor white, Giemsa stain, and Diff-Quik. Cystic forms are more often observed with the first three methods, whereas trophic forms with the last two methods [88].

In HIV-infected patients with higher microorganism burden, rapid detection by indirect immunofluorescence assay (IFA) with monoclonal antibodies shows high sensitivity

and specificity in BAL (99% and 100% respectively) or induced sputum (50% and 100% respectively) using other staining methods as a gold standard. This assay facilitates the identification of both cystic and trophic forms [89]. However, in HIV-uninfected patients with a low microorganism burden, microscopy of BAL is less sensitive; these patients could benefit from the use of molecular techniques[90].

Figure 3 shows a representative microscopic observation of a typical BAL sample.

8.2. Molecular Diagnosis

Conventional PCR and real-time PCR assays can detect *P. jirovecii* DNA in various samples, including induced sputum, oral washings, nasopharyngeal aspirates, nasal swabs, BAL, and lung tissue these techniques provide a rapid diagnosis of PcP and help in the initiation of prompt therapy [91]. Molecular assays can also quantify *Pneumocystis* in clinical samples, and may help in monitoring the effectiveness of antifungal therapy and in differentiating colonization from infection[92]. The established method involves the detection of the mitochondrial large-subunit ribosomal RNA of *P. jirovecii* [93], both in HIV-infected and HIV-uninfected individuals. The main limitation of molecular techniques is the lack of standardization. Further studies are needed before these assays can be incorporated into routine clinical practice. However, molecular techniques improve the diagnostic yield for PcP, provide rapid diagnosis and have been applied to minimally invasive clinical specimens.

Figure 4 shows a tentative algorithm for the microbiological diagnosis of PcP.

8.3. Differentiation between Colonization and Active Infection

Several studies have tried to discriminate between colonization and active infection.

Luois et al. [39] carried out a composite diagnosis of PcP based on clinical, radiological

patterns and microbiological results (microscopic examination and quantitative PCR (qPCR) performed on the BAL fluids) from 1003 patients with pneumonia. The authors divided the BAL sample into four groups: a) definite PcP (microscopic demonstration of trophic or cyst forms); b) probable PcP (clinical PcP including outcome after anti-*Pneumocystis* therapy and radiological pattern supporting the diagnosis of PcP and positive qPCR but negative microscopic observation of *Pneumocystis* forms; c) *Pneumocystis* colonization (absence of clinical and radiological evidence of PcP but positive qPCR and negative microscopic observation of *Pneumocystis* forms; d) lack of *Pneumocystis* infection (absence of clinical data supporting the diagnosis of PcP and absence of positive qPCR or a positive microscopy observation). The rate of colonization was 4% among 180 HIV-infected patients and 12% among 823 HIV-uninfected patients. The authors suggested using a qPCR cut-off of 1.5×10^4 copies/mL (100% sensitivity and specificity) to distinguish colonization from infection in HIV-infected patients. For HIV-uninfected patients, cut-off values of 2.87×10^4 copies/mL and 3.39×10^4 copies/mL obtained 100% specificity and sensitivity respectively. HIV-infected patients developed PcP with a lower fungal burden compared to HIV-uninfected patients.

More recently, Fauchier et al. [38] evaluated the cycle threshold (C_T) values (the number of PCR cycles after which the reaction is considered positive) obtained by qPCR, which are correlated with the fungal burden in the sample, in order to differentiate between colonization and PcP on respiratory samples (sputum, induced sputum, bronchoalveolar lavage [BAL] fluid) from immunocompromised patients with respiratory symptoms. Considering the receiver operating characteristic curve (ROC), the authors found that:

- a C_T value of 32 discriminated between colonization and pneumonia with 72% of sensitivity and 75% of specificity (area under the ROC curve [AUC], 0.88);
- in HIV-infected patients, a C_T value of 27 excluded colonization with a sensitivity of 74% and a specificity of 100% (AUC, 0.90);
- in HIV-uninfected patients, a C_T value of 35 excluded colonization with a sensitivity of 80% and a specificity of 60% (AUC, 0.72).

Although these data suggest the utility of qPCR cut-offs to distinguish colonization from infection, they cannot be applied universally. Clinicians should always consider the fungal load variability due to patients characteristics (HIV-infected, HIV-uninfected or degree of immunosuppression), specimen type (invasive or non-invasive) and sample quality.

9. Biochemical Diagnosis

9.1. Serum detection of (1-3)- β -D-Glucan

The main component of the cell wall in *P. jirovecii* cysts is the (1-3)- β -D-glucan (BG), this polysaccharide is released into the circulation during *Pneumocystis* infection in detectable quantities. The study by Son et al.[94] that compared blood levels of BG in patients with PcP, patients with other diseases (like candidemia, chronic disseminated candidiasis, invasive aspergillosis, mucormycosis and tuberculosis) and in healthy volunteers found that at a cut-off value > 31.25 pg/mL, which is highly sensitive for PcP versus tuberculosis plus healthy volunteers at the expense of specificity, BG had a sensitivity of 92% and a specificity of 55%. More recently, Lahmer et al.[95] investigated the usefulness of serum BG in mechanically ventilated HIV-uninfected patients with ARDS suspected of having PcP. The detection of serum BG showed an

overall sensitivity of 92%, and specificity of 84% for PcP diagnosis, with a high negative predictive value. Interestingly, a bivariate meta-analysis and systematic review published in 2015 analyzed 33 studies on the role of serum BG in the diagnosis of PcP in HIV-infected and HIV-uninfected patients. The authors found that in HIV-infected patients a negative serum BG was sufficient for ruling out PcP, as confirmed in other studies[96,97]. Conversely, in HIV-uninfected patients results of serum BG should be interpreted in parallel with clinical and radiological findings[98].

Although these studies reported that the detection of this polysaccharide in the serum has a specificity of 96% and a sensitivity of 87% [99], it is not a specific biomarker of PcP. Its use is limited by the lack of a standardized detection method, the fact that extraneous factors (e.g., immunoglobulins) may cause false-positive results and the fact that high values cannot distinguish between different fungal infections [100]. For these reasons, the use of BG is recommended only in combination with other diagnostic tests.

Figure 4 shows a tentative algorithm for the diagnosis of PcP.

9.2. S-adenosyl methionine and other biomarkers

S-adenosyl methionine (SAM) is a molecule that *P. jirovecii* cannot synthesize, but necessary for its metabolism. It is an essential intermediate for protein and nucleic acid methylation, folate metabolism, and polyamine synthesis. *P. jirovecii* therefore requires exogenous SAM and in a study of rats it was observed that plasma SAM concentration was inversely correlated with the lung *P. jirovecii* count [101].

Subsequent testing in human PcP also revealed low plasma SAM concentrations during infection with return to normal values after treatment [102,103].

Recently, the study by Esteves et al.[104] investigated the usefulness of four serologic biomarkers [(Krebs von den Lungen-6 antigen (KL-6), BG, lactate dehydrogenase (LDH) and SAM] for the diagnosis of PcP. The authors found that BG was the most promising biomarker, followed by KL-6, LDH and SAM. The combination of BG and KL-6 showed the highest accuracy, with 94.3% sensitivity and 89.6% specificity. The authors recommended their use both in HIV-infected and HIV-uninfected patients. Conversely, despite SAM concentration was slightly decreased in PcP cases, the sensitivity and specificity analysis demonstrated that SAM failed to discriminate between patients with and without PcP, showing low diagnostic accuracy.

In clinical practice, we recommend the use of biomarkers in association with other specific diagnostic tests for *Pneumocystis* that complement the interpretation of clinical and radiological data. Although biomarkers and molecular assays are important tools for identifying PcP, the lack of standardized methods makes their implementation difficult in routine clinical practice.

10. Therapy

Table 2 summarizes the main recommendations for PcP treatment in HIV-infected and HIV-uninfected patients. Furthermore, details regarding PcP therapy (first line and alternative treatments) and prophylaxis in HIV-infected patients have been provided by Huang et al. [105] in a recently published review covering the main areas of the topic.

Some points regarding additional or alternative therapies in PcP are worthy of importance:

- Adjunctive corticosteroid therapy should be started as early as possible in HIV-

infected patients with moderate-to-severe PcP due to its benefits in terms of survival [106]. However, adjunctive corticosteroid therapy is not indicated in HIV-infected patients with mild-to-moderate PcP.

- Due to the conflicting results regarding their benefit, glucocorticoids are not recommended in HIV-uninfected patients with PcP. According to current guidelines, the decision to add corticosteroids in HIV-uninfected patients with PcP and respiratory failure should be made on a case by case basis[107].
- Folinic acid supplements are contraindicated in HIV-infected patients with PcP because of the increased risk of treatment failure and death [17,108].
- Clinical experience regarding the use of caspofungin in severe PcP is limited, controversial and based on case reports of severe PcP successfully treated with a combination of low-dose cotrimoxazole and caspofungin in different populations[109–112]. These data suggest the need for future randomized clinical trials.
- Cases of treatment failure with cotrimoxazole and dapsone have been associated with mutations in the DHPS gene of *P. jirovecii* [113–117]. However, research results are unclear and there is only limited information regarding these mutations and their association with outcomes and therapy. Moreover, prophylaxis with sulfamethoxazole may be a risk factor for DHPS mutations [118,119].
- Corticosteroids are recommended for the treatment of paradoxical or unmasked PcP-IRIS [70].
- The use of clindamycin and primaquine as salvage therapy is not currently recommended because the data regarding their efficacy are insufficient.

However, they may be recommended in cases in which the first and alternative therapies cannot be administered or are not tolerated[17].

11. Prevention of exposure and disease

Although there is evidence of the presence of *P. jirovecii* in the air in areas where infected patients reside and of transmission through interpersonal contact[21,22], and although contact between severely immunocompromised patients and patients with confirmed *Pneumocystis* infection should be avoided, there are insufficient data to support the practice of isolation of patients with PcP in routine clinical practice[17].

11.1.- Primary and secondary prophylaxis

Table 3 summarizes the primary and secondary prophylaxis in HIV-infected and HIV-uninfected patients.

- Primary prophylaxis should be discontinued in HIV-infected patients when the CD4 count increases from <200 cell/mm³ to ≥ 200 cell/mm³ for at least 3 months in response to ART therapy or when the CD4 count is 100–200 cell/mm³ and the viral load suppressed[120].
- Secondary prophylaxis should be discontinued in HIV-infected patients when the CD4 count increases from <200 cell/mm³ to ≥ 200 cell/mm³ for at least 3 months in response to ART and HIV-viral load is undetectable over 3 months. [121].
- There is no consensus about secondary prophylaxis in HIV-uninfected patients. However, it is recommended to continue secondary prophylaxis until immunosuppression ends in patients with autoimmune disease, solid-organ

transplantation, hematological malignancies, and in stem cell transplant recipients [90].

12. Conclusion

Although the incidence of PcP is decreasing in patients with HIV, principally because of ART introduction, several major concerns persist in different areas. Notably, there are many patients with unknown HIV infection at the time of PcP diagnosis; many patients do not receive ART or are non-compliant with the therapy; finally there is a rising incidence of patients who are immunocompromised for reasons other than HIV infection. These concerns are compounded by difficulties with PcP diagnosis.

13. Expert opinion

During recent years, the scientific community has made significant progress in the epidemiology, diagnosis and management of PcP. However, many questions remain unresolved regarding our understanding of the pathophysiology of *Pneumocystis* infection, especially in relation to the microorganism-host interaction. The reservoir of *Pneumocystis* infection has not yet been identified and the mechanism of transmission and infection are not entirely clear; nor has an ideal diagnostic strategy been defined. For this reason, it is important that the research topics listed below be explored in the coming years.

First, epidemiological studies are needed to investigate the global burden of PcP and its attributable mortality. Due to the differences in the study populations, biological samples, and microbiological tests, observational studies should be carried out to assess the rate of *Pneumocystis* colonization in HIV-infected and HIV-uninfected

patients. Moreover, prospective studies are required to identify risk factors for PcP, mostly in the “new” group of HIV-uninfected patients, and to recognize patients who would truly benefit from prophylaxis.

The main issue regarding *P. jirovecii* diagnosis is the difficulty of isolating or culturing the pathogen with conventional microbiological methods. Studies on accelerated diagnosis, through rapid and non-invasive molecular techniques, would make it possible to differentiate *P. jirovecii* colonization from infection, and thus allow prompt initiation of targeted therapies. A comparison between current treatment strategy and an approach integrating clinical stratification with old and new biomarkers could provide important strategic insights. Finally, the role of caspofungin in severe PcP should be assessed in randomized clinical trials.

13.1 Five-year view

The widespread use of antiretroviral and prophylactic therapy in HIV-infected patients has reduced the incidence of PcP in this population. However, the growing incidence of *Pneumocystis* infection in HIV-uninfected patients (those receiving chronic corticosteroids, those with hematological or solid malignancies, transplant recipients, and those receiving immunomodulatory or biological therapy) suggests the need for new studies to define the true scale of the disease. Currently, in-hospital mortality rates are very high: 15% for HIV-infected patients and 50% in some HIV-uninfected patients. Early identification of patients at risk for PcP and the development of standardized microbiological diagnostic methods for early *Pneumocystis* detection could facilitate patient management and thus improve their short and long term-outcomes.

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Table 1. Clinical manifestations, radiographic features and outcomes of *Pneumocystis* pneumonia in HIV-infected and HIV-uninfected patients

HIV-infected patients	HIV-uninfected patients
Sub-acute onset	Rapid onset
Longer symptoms duration (3 weeks)	Shorter symptom duration (4–7 days)
Low-grade fever with progressive dyspnea	High grade fever and rapid progression to respiratory failure
Bilateral interstitial and alveolar infiltrates involving perihilar areas	Consolidation with ground-glass opacities
10-20% require ICU admission	Approximately 50% require ICU admission
Less severe course with a mortality rate of 15% (range 10%–20%)	Fulminant course with a mortality rate of 30%–60%
<p><u>Prognostic factors:</u></p> <p>Acute respiratory failure (PaO₂ <60 mmHg, A-aO₂ gradient) at admission, delay in ICU admission, need of mechanical ventilation, marked chest radiographic abnormalities, pneumothorax, low serum albumin; low hemoglobin, high LDH, high APACHE II score, increased age, injection drug use, previous PCP, pulmonary KS, and coinfections and comorbidities.</p>	<p><u>Prognostic factors:</u></p> <p>Respiratory failure, pre-existing lung disease, pneumothorax, lymphopenia, increased BUN, high lactate dehydrogenase, low serum albumin, adjunctive steroid therapy, longer time from onset of symptoms to diagnosis.</p>

Abbreviations: HIV, human immunodeficiency virus; ICU, intensive care unit

Table 2. Recommendation for *Pneumocystis pneumonia* treatment (5, 87)

Population	Recommendation
<p>HIV-infected patients with moderate-to-severe PCP</p>	<p>*First line: TMP-SMX: 15–20 mg/kg (TMP); 75–100 mg/kg (SMX) per day, in 3 or 4 divided doses for 21 days (The dose must be adjusted if there is abnormal renal function).</p> <p>* Alternatives:</p> <ul style="list-style-type: none"> • Pentamidine 4 mg/kg IV once daily (may be reduced to 3 mg/kg IV once daily if toxicities arise). • Primaquine 30 mg (base) once daily + (Clindamycin 600/6h or 900 mg/8) or (450 mg/ 6h or 600 mg/8h). <p>Adjunctive corticosteroid: start as early as possible if PaO₂ <70 mmHg in room air <i>or</i> alveolar-arterial O₂ gradient ≥35 mmHg.</p> <p>Doses recommended: days 1–5: 40mg (oral) twice daily; days 6–10:40mg (oral) daily; days 11–15/20:20 mg (oral) daily.</p>
<p>HIV-infected patients with</p>	<p>*First line: TMP-SMX: 15–20 mg/kg (TMP); 75–100 mg/kg (SMX) per day, divided in 3 doses for 21 days (oral)</p>

<p>mild-to-moderate PCP</p>	<p>* Alternatives:</p> <ul style="list-style-type: none"> • Dapsone 100mg daily + TMP 15mg /kg/day, divided in 3 doses (oral) • Primaquine 30 mg (base) once daily + (Clindamycin 600/8h or 450 mg/ 6h) (oral) • Atovaquone 750 mg twice daily with food (oral) <p>Adjunctive therapy with corticosteroids is not indicated</p>
<p>HIV-uninfected patients: haematological malignancies, solid organ transplants, autoimmune diseases, inflammatory diseases.</p>	<p>*First line: TMP-SMX: 15–20 mg/kg (TMP); 75–100 mg/kg (SMX) per day, in 3 or 4 divided doses for ≥14 days.</p> <p>*Alternatives:</p> <ul style="list-style-type: none"> • Pentamidine 4 mg/kg/day (iv). Primaquine + clindamycin 30 mg/day (oral) + 600 mg × 3/day (iv/oral). Atovaquone 750 mg in 2 or 3 doses per day (oral). <p>Adjunctive therapy with corticosteroids (on a case-by-case basis)</p>

Abbreviations: HIV, human immunodeficiency virus; ICU, intensive care unit; PCP, *Pneumocystis pneumonia*; SMX, sulfamethoxazole; TMP, trimethoprim.

Table 3. Prophylaxis of PCP

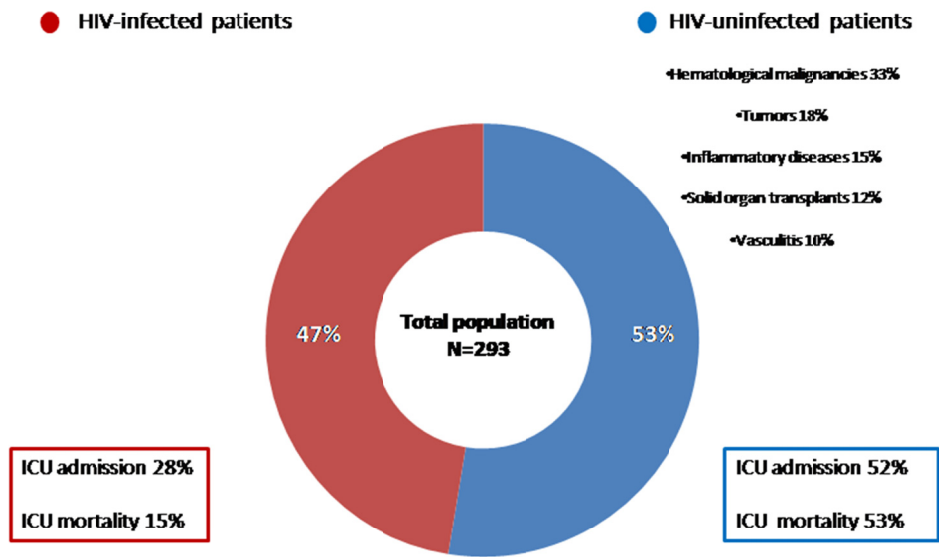
Primary prophylaxis	
HIV-infected patients	
Initiate: if CD4 count < 200 cells/ μ L, CD4 percentage < 14%, oral thrush or relevant concomitant immunosuppression	
Stop: if CD4 count > 200 cells/ μ L over 3 months or CD4 count 100-200 cells/ μ L and HIV-VL undetectable over 3 months	
First line	Dose
Trimethoprim/sulfamethoxazole	1 single-strength tablet (80 mg/400 mg) daily or 1 double-strength tablet (160 mg/800 mg) daily.
Second line	
Dapsone	1 x 100 mg/day p.o.
Atovaquone	1 x 1500 mg/day p.o. (with food)
Pentamidine aerosols	300 mg in 6 mL sterile water, 1 inhalation/month
HIV-uninfected patients	
First line	Dose
Trimethoprim/sulfamethoxazole	1 single-strength tablet (80 mg/400 mg) daily or 1 double-strength tablet (160 mg/800 mg) daily.
Second line	
Dapsone	2 x 50 mg/day p.o.
Atovaquone	1 x 1500 mg/day p.o. (with food)
Pentamidine aerosols	300 mg in 6 mL of sterile water, 1 inhalation/month
Secondary prophylaxis	
HIV-infected patients	
Stop: if CD4 count > 200 cells/ μ L and HIV-VL undetectable over 3 months	
Trimethoprim/sulfamethoxazole	1 single-strength tablet (80 mg/400 mg) daily or 1 double-strength tablet (160 mg/800 mg) daily.
Atovaquone	1 x 1500 mg/day p.o. (with food)
Pentamidine aerosols	300 mg in 6 mL of sterile water, 1 inhalation/month

HIV-uninfected patients

No general consensus: continuation of the secondary prophylaxis is recommended until the end of the immunosuppression condition.

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Figure 1. PCP in the French population (1990-2010)
Data from a single country study



Modified from Filatre et al. Am J Med. 2014 Dec;127(12):1242.e11-17

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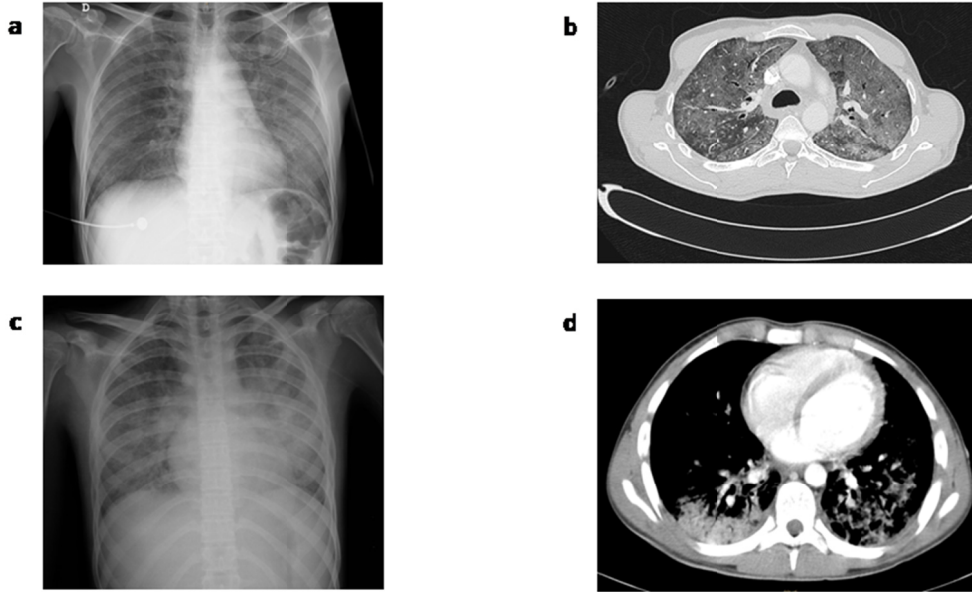


Figure 2. Radiographic imaging of *Pneumocystis pneumonia*.

(a) A 41-year old man with recent diagnosis of HIV and 4 CD4+ T cells/mm³; chest-x-ray on day 1 do not show abnormalities.

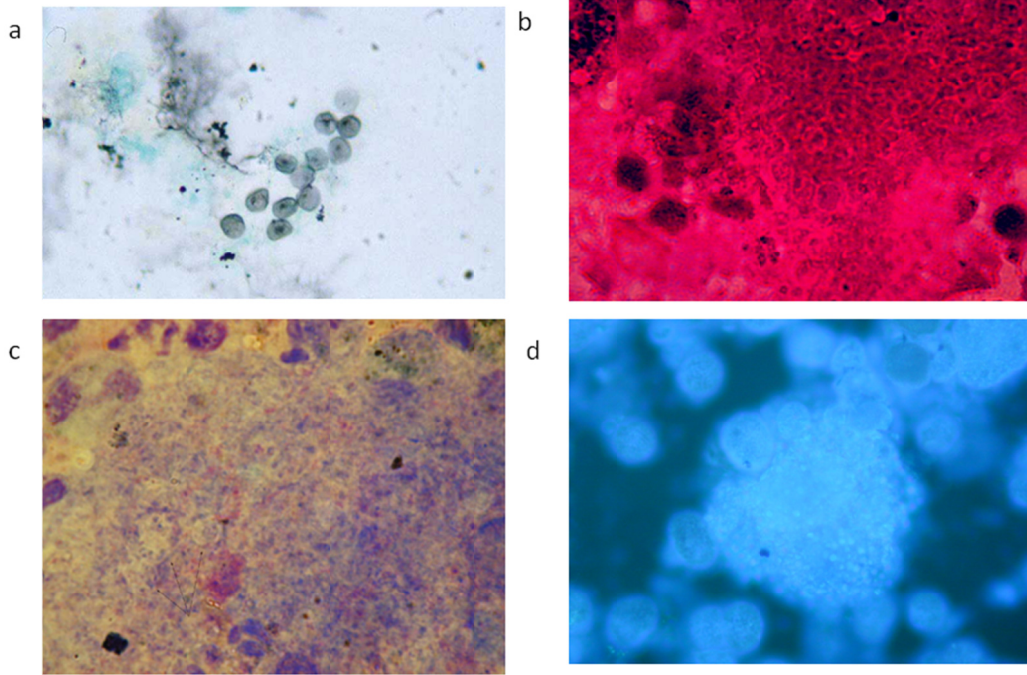
(b) CT scan on day 1 shows bilateral interstitial infiltrates and ground-glass opacities.

(c) A 16-year old patient with acute myeloid leukemia; chest-x-ray on day 2 shows a bilateral interstitial pattern, cardiomegaly and a consolidation in the mid-basal left lung.

(d) CT scan on day 3 shows multiple pulmonary infiltrates with a diffuse and bilateral distribution, consolidations in both lower lobes (LL/RL) and a minimum bilateral pleural effusion .

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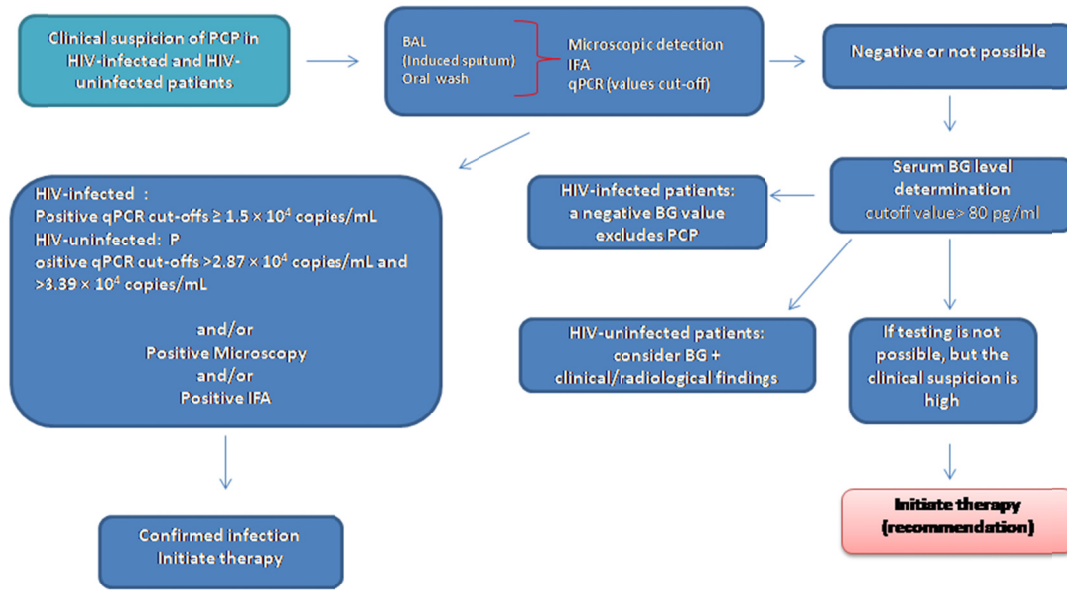
Figure 3. Microscopic observation of bronchoalveolar lavage sample



a) Gomori methamine silver stain; b) Gram stain; c) May-Grunwald Giemsa stain c) Fluorescent Calcofluor-white stain: Cysts are observed in all stains

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Figure 4. Proposed flow algorithm for the microbiological diagnosis of *Pneumocystis pneumonia* in HIV-infected and HIV-uninfected patients



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