Original Article

Pneumocystis jirovecii pneumonia in AIDS and non-AIDS immunocompromised patients – an update

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Abstract

Introduction: *Pneumocystis jirovecii* (PJ) pneumonia (PJP) is an important opportunistic infection affecting various types of immunocompromised patients and is associated with an increased risk of mortality. PJ is a unique fungal pathogen which is increasingly common and maybe associated with a higher mortality rate in patients without AIDS. We present the characteristics of PJP, diagnosis, and treatment outcomes between AIDS and non-AIDS patients.

Methodology: We conducted a review of studies of AIDS and non-AIDS patients with PJP using PubMed to search for studies until December 2017.

Results: The annual incidence of AIDS-PJP decreased from 13.4 to 3.3 per 1000 person-years in industrialized countries, while the incidence of non-AIDS-PJP varied widely. Both groups had similar clinical manifestations and radiological features, but the non-AIDS-PJP group potentially had a more fulminant course, more diffuse ground glass opacities, and fewer cystic lesions. The mortality rate decreased in the AIDS-PJP group after the advent of antiretroviral therapy; however, the mortality rate remained high in both groups. A laboratory diagnosis was usually nonspecific; CD4+ T-cell < 200 cells/mL or < 14% favored AIDS-PJP. Serum 1,3-β-D-glucan (BDG) had a high diagnostic odds ratio. Combining BDG and lactic dehydrogenase improved the diagnosis of AIDS-PJP. Histopathological staining and polymerase chain reactions could not discriminate infection from colonization when the result was positive. The use of antibiotics, prophylaxis, and adjunctive corticosteroids was controversial.

Conclusions: Early diagnosis and treatment can be achieved through vigilance, thereby improving the survival rate for PJP in immunocompromised patients.

Key words: human immunodeficiency virus; acquired immunodeficiency syndrome; immunocompromised host; *Pneumocystis jirovecii* pneumonia; mortality.

Introduction

Pneumocystis jirovecii (PJ) pneumonia (PJP) is an opportunistic fungal infection that is associated with a high mortality rate in immunocompromised patients, including those with acquired immunodeficiency syndrome (AIDS) and those without AIDS (non-AIDS). The non-AIDS group includes patients with innate or acquired immunodepression such as those receiving long-term or high-dose corticosteroids or immune suppression treatment (monoclonal antibody therapy) for autoimmune diseases, those with solid-organ or hematologic malignancies receiving hematopoietic stem cell transplantation, and those with bone marrow or solid-organ transplantations [1,2].

Good syndrome [3], DiGeorge syndrome and common variable immunodeficiency [4], severe combined immunodeficiency [5], and protein-calorie malnutrition [6] are also associated with PJP.

In the case of PJP, clinical studies have shown that the clinical course of non-AIDS-PJP is potentially fulminant, and that the prognosis can be poor if the diagnosis of PJP is delayed, thereby resulting in poor outcomes [1,2]. Therefore, we conducted this review of studies of immunocompromised patients with and without AIDS with PJP. We used the PubMed database to search for studies until December 2017. The aim of this review was to raise the awareness of clinicians and intensivists so that they can make an early diagnosis of

PJP by comparing the differences in clinical manifestations and pathogenesis of hypoxemia between patients with AIDS- and non-AIDS-related PJP. We also discuss the challenges of making a diagnosis and the treatment of PJP.

Methodology

Search Strategy

We conducted a systematic review of studies of immunocompromised patients with and without AIDS with PJP. Data collected from the studies included patient demographics, methods of diagnosis, treatment details, duration of treatment, microbiologic response, presence of respiratory failure, cause of death related pneumocystis, and mortality. We used the PubMed database to search for studies using the following combinations of search items: pneumocystis AND (classical article OR clinical study OR clinical trial OR controlled clinical trial OR editorial OR journal article

Figure 1. Flowchart of studies screened and included.

OR guideline OR letter OR meta-analysis OR review OR scientific integrity review OR systematic OR validation studies) AND (free full text AND full text) AND (humans) AND (English) AND (Medline) AND studies published from January, 1970 to December, 2017. A total of 5913 articles were identified. Of these, a total 487 items were included based on the following criteria.

Inclusion and exclusion criteria

The inclusion criteria for the review were: 1) all classical articles, clinical studies, clinical trials, journal articles, meta-analyses, multicenter studies, observational studies, scientific integrity reviews, systematic reviews, randomized controlled trials or practice guidelines; 2) studies evaluating AIDS or non-AIDS patients with the diagnosis of PJP; 3) studies in which patients were treated with PJ therapy; 4) studies in which microbiologic response was measured; and 5) studies which reported the presence of respiratory failure, cause of death related to pneumocystis, and mortality. The exclusion criteria were studies published in the form of a letter, comment, editorial, case report, proceedings, and personal communications, or studies without quantitative primary outcomes. We reviewed and eliminated 440articles, and retained 48 articles (Figure 1). Of the 48 articles, we screened the references listed in the review papers to find further relevant studies. Another 12 references were selected. A total of 60 publications were identified (Figure 1).

Results

Organism and transmission route

The PJ organism, previously known as *Pneumocystis carinii,* was thought to be a primary infection occurring in young children. PJP occurred due to a reactivation of a latent infection when the host's immune system was compromised [7]. The primary route of PJ transmission has yet to be determined conclusively [8]. It is also possible that PJ infection can occur due to airborne transmission from the respiratory tract of other mammals and humans [7,9]. A study stated that PJ isolates were found in the air surrounding PJP patients and from the environment of hospitals where PJP patients were admitted [9]. Several outbreaks of PJP have been reported among organ transplant recipients or non-AIDS immunocompromised patients [8]. Interhuman transmission of PJ may lead to nosocomial outbreak infections of varying prevalence and the spread of sulfa drug-resistant strains [7].
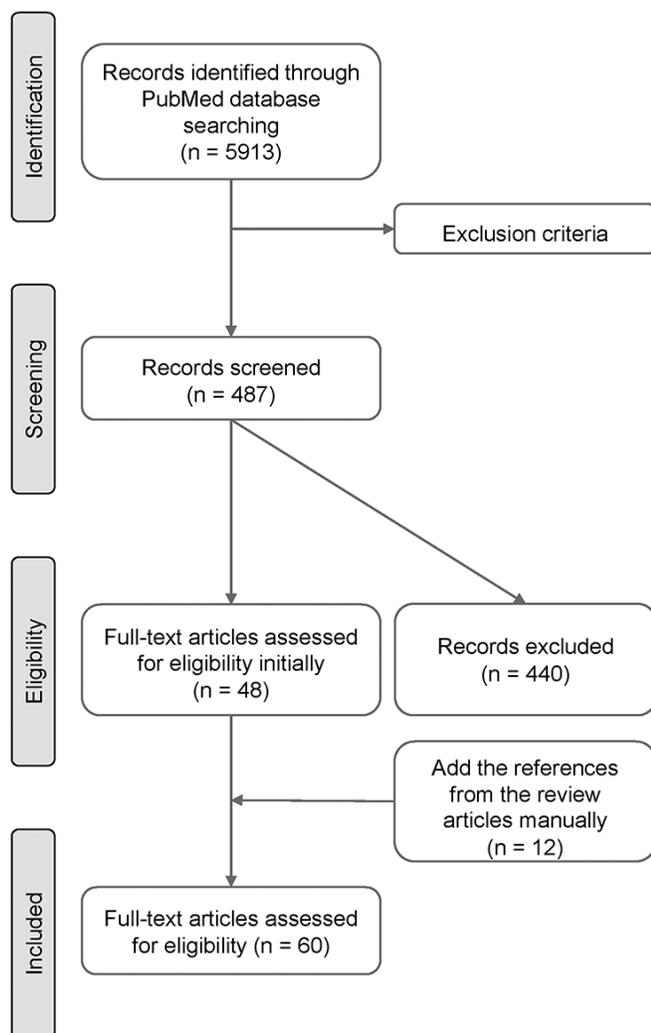
Epidemiology and risk factors for Pneumocystis jirovecii pneumonia

The global incidence of PJP is estimated to be more than 400,000 cases per year [10]. With antiretroviral therapy (ART) and the use of anti-pneumocystis prophylaxis, the incidence of AIDS-PJP significantly declined from 95 to 8.4 per 1000 person-years from 1993 to 2008 [11]. In industrialized countries, the annual incidence of AIDS-PJP decreased from 13.4 cases/1000 AIDS patients to 3.3 cases/1000 AIDS patients (p < 0.001) from 2000 to 2013 [12]. One meta-analysis reported a positive relationship between the gross domestic product and the diagnosis of AIDS-PJP in tropical and middle income countries due to a lack of resources to report such cases in poor countries [13]. The authors also indicated that prophylaxis appeared to reduce the risk of developing PJP in adults [13].The associated risk factors for AIDS-PJP included CD4+ T-cell count ≤ 200 cells/mL or ≤ 14%, history of oropharyngeal candidiasis, previous episode of PJP, history of unexplained fever, recurrent bacterial pneumonia, unintentional weight loss, and high levels of plasma HIV RNA [14] (Table 1).

With the increasing use of corticosteroids, chemotherapy, and other immunosuppressive treatments, the incidence of non-AIDS-PJP varies widely [15]. An observational cohort study on 293 PJP patients including 154 non-AIDS-PJP patients reported that 32.5% had hematological malignancies, 18.2% had solid tumors, 14.9% had inflammatory diseases, 12.3% had solid organ transplants, and 9.7% had vasculitis. The annual incidence rate was estimated and stratified into three groups: 1) high risk, defined as > 45/100,000 patients with polyarteritis nodosa, granulomatosis with polyangiitis, polymyositis/dermatopolymyosis, acute leukemia, chronic lymphocytic leukemia, or non-Hodgkin lymphoma; 2) intermediate risk, defined as 25-45/100,000 patients with Waldenström macroglobulinemia, multiple myeloma, or central nervous system cancer; and 3) low risk, defined as < 25/100,000 patients with other solid tumors, inflammatory diseases, or Hodgkin lymphoma [15]. This stratification may be used to guide anti-pneumocystis prophylaxis for non-AIDS-PJP [15].

Pathogenesis of infection and hypoxemia

Both groups had similar clinical manifestations and radiological features but different courses and outcomes. The life cycle of PJ remains elusive [16], but it was presumed to play a particularly critical role in the pathogenesis in one clinical study [17]. This is may be due to the different pathogenesis of infection and hypoxemia.

Infection

A large number of neutrophils was noted in the non-AIDS-PJP group, which may have been caused by trophic forms of PJ, in which the capability to bind type I alveolar cells is strong enough to cause overwhelming alveolar inflammation [18]. There were few PJ cysts inside the alveoli [17] because there was not enough time for the cysts to grow. In contrast, there were fewer neutrophils in the alveoli of the patients with AIDS-PJP

Table 1. Comparisons of *Pneumocystis jirovecii* pneumonia with regards to the epidemiology, risk factors, clinical characteristics, and pathogenesis between the patients with and without AIDS.

Variables	AIDS	Non-AIDS	Reference
Incidence, per 1000 person-years	Decline from 95 to 8.4; Decline 13.4 to 3.3 in the industrialized countries	45-25	[11], [12], [15]
Clinical characteristics	Fever, respiratory symptoms (dry cough, dyspnea), gradual, insidious progression	Similar clinical manifestations, fulminant course	[1], [2], [16], [20], [22], [23]
Pathogenesis	Significant *Pneumocystis* cyst formation within the alveoli of the lung and fewer neutrophils recovered in BAL fluid	Few *Pneumocystis* cysts and abundant neutrophils, reflecting more severe lung inflammation	[16-19]
Risk factors	CD4 lymphocyte count ≤ 200 cells/mL or ≤ 14%, history of oropharyngeal candidiasis, previous episode of PJP, history of unexplained fever, recurrent bacterial pneumonia, unintentional weight loss, and higher plasma HIV viral load levels	CD4 lymphocyte counts variety, long-term or high-dose corticosteroids use, immune suppression treatment for autoimmune disease, solid or hematologic malignancies, BMT or SOT, also seen in Good syndrome, DiGeorge syndrome, common variable immunodeficiency, severe combined immunodeficiency, and Protein-calorie malnutrition	[1-6], [14], [15]

BAL: Broncho alveolar lavage; BMT: bone marrow transplantations; HIV: human immunodeficiency virus; PJP: *Pneumocystis jirovecii* Pneumonia; SOT: solid-organ transplantations.

so that the alveolar inflammation was not so fulminant, thereby allowing cysts to grow over time [17].

Hypoxemia

Several studies reported that hypoxemia was induced by true intrapulmonary shunting after alveolar inflammation [18,19]. Since the infection caused different levels of pulmonary inflammation between the non-AIDS and AIDS patients [17-19], it was not surprising that the patients with non-AIDS-PJP had more severe hypoxemia than those with AIDS-PJP. Respiratory failure ensued within 5-7 days of symptom onset [18], thereby requiring a higher oxygen flow and more frequent invasive mechanical ventilation [17]. In the patients with AIDS, hypoxemia progressed slowly at approximately 3 weeks after symptom onset [19], and mechanical ventilation was required less frequently, and thus more people survived [19]. Further studies are needed to confirm these pathophysiological assumptions.

Clinical manifestations

The constitutional and respiratory symptoms and signs and findings of chest radiography (CXR) and computed tomography (CT) were nonspecific and similar in the early stage between the two groups [1,2,19]. However, there were some clues that tended to favor one over the other (Figure 2).

Symptoms

The symptoms of PJP ranged from a subtle onset of progressive dyspnea, nonproductive cough, low-grade fever, and loss of body weight to acute onset of dyspnea and pleuritic chest pain [2,19]. Bienvenu *et al*. reported that the most common symptoms in both patients with AIDS-PJP and non-AIDS-PJP were dyspnea (66%), fever (65%), and cough (55%) [2]. Approximately one third of these patients presented with these three symptoms, one third presented with two symptoms, and one third had one symptom. Of note, approximately one tenth of the patients did not develop any of the three typical symptoms [2]. The frequency of fever was similar between the two groups; however, the AIDS-PJP group developed a more gradual onset of dry cough and dyspnea [2], while the non-AIDS-PJP group presented with a more fulminant course and atypical manifestations [1,16,20]. The time from symptom onset to seeking medical consultation was 21 days for the patients with AIDS and 5 days for those without AIDS, with times to a diagnosis of PJP of 30 and 7 days, respectively [1]. The time delay to initiate treatment for

PJP was 2 days [95% confidence interval (CI), 0-6] and 1 day [95% CI, 0-2], respectively [1] (Table 1).

Signs

The signs associated with PJP were also nonspecific in both groups, and may have included fever, tachypnea, and inspiratory crackles. Of note, chest physical examinations were unremarkable in half of the patients [19]. Oropharyngeal candidiasis was a common co-infection and indicated the immunodeficiency status and the risk of developing PJP, thereby indicating the need to start primary prophylaxis for PJP [14,21]. There were some unusual extrapulmonary features including retinitis, digital necrosis, and space occupying lesions of the liver, spleen, kidney, and brain [22]. The incidence of

Figure 2. Flowchart of clinical characteristics, diagnosis, management, and outcome of *Pneumocystis jiroveci* pneumonia in the immunocompromised patients.
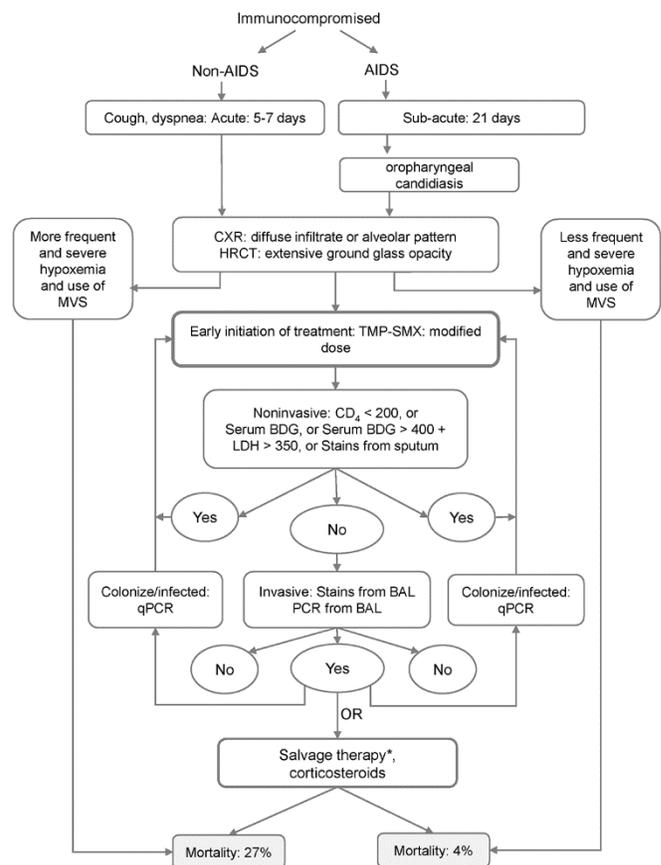
AIDS: acquired immunodeficiency syndrome, BAL: Broncho alveolar lavage, BDG: 1,3-β-D-glucan, CXR: chest radiography, HRCT: High-resolution computed tomography, LDH: lactic dehydrogenase, MVS: mechanical ventilatory support, qPCR: quantitative polymerase chain reaction, TMP-SMX: trimethoprim-sulfamethoxazole. Solid arrowed lines: continues, solid line box: diagnosis elements, heavy line box: treatment modalities, dashed line box: outcomes. *Salvage therapy: pentamidine, Corticosteroids: high-dose steroid therapy ≥ 1 mg/kg/day.

extrapulmonary features was < 2.5% in the AIDS-PJP group and even lower in the non-AIDS-PJP group [22,23].

Radiological features
Chest radiography

The patterns of CXR were similar for PJP in both groups [1,2]. The typical CXR pattern was bilateral symmetric perihilar interstitial infiltrates or extensive ground glass opacity (GGO) [23,24]. As the disease progressed, the lesions emanated from the hila to the periphery forming a butterfly pattern. The other presentations included alveolar consolidation, asymmetrical infiltrates, finely granular, reticular, and military lesions, cavities, intrathoracic adenopathy, pleural effusions, and pneumothorax [25]. Nevertheless, PJP often spared apical regions except in the cases of prophylactic pentamidine inhalation [22]. A typical pattern of extensive GGO and a presentation of oropharyngeal candidiasis and/or CD4+ T-cell count ≤ 250 cells/mL or ≤ 14% should raise the suspicion of AIDS-PJP [21]. Comparing the CXR in the two groups, the AIDS-PJP group had a more diffuse pattern, large-sized ground glass nodules (5-10 mm), and intrathoracic adenopathy [26]. Of note, a normal CXR did not eliminate the diagnosis of PJP, as it was noted in approximately 40% of the AIDS-PJP group in the early stage [24]. This may be related to a temporal effect.

Chest computed tomography

In general, chest high-resolution computed tomography (HRCT) is the most reliable diagnostic tool in detecting and differentiating intestinal or diffuse parenchymal lung diseases in immunocompromised hosts [27,28]. In the AIDS-PJP group, HRCT typically demonstrated the characteristic GGOs [25]. The atypical manifestations included sub-pleural nodules, focal condensation, cavitations, and marked pleural effusions [1]. The incidence of atypical chest CT findings was rare (5%), and the number of cases who were eventually confirmed to have co-infection with bacteria was even lower (1%) with other opportunistic microorganisms. Hence, atypical radiographic findings indicate the need for advanced diagnostic investigations and the concomitant administration of broad-spectrum antimicrobials [1,29]. Nevertheless, HRCT had a high negative predictive value, thereby excluding PJP should the findings be normal, equivocal, or nonspecific [29].

In the non-AIDS-PJP group, GGOs were more centrally distributed while relatively sparing the periphery in 43% (n = 36) of the patients, apical predominance in 86% (n = 71) [27], a mosaic pattern in 57% (n = 48), parenchymal consolidations in 17% (n = 14), and fine reticulation and traction bronchiectasis in only 6% of the patients (n = 5). Compared with the AIDS-PJP group, the non-AIDS-PJP group had more frequent diffuse GGO lesions (86% versus 44%, p = 0.02) and less frequent cystic lesions (3% versus 56%, p = 0.015) [30]. This may also have been related to a temporal effect. In the non-AIDS group, the severity of PJP could be assessed by HRCT, thereby predicting survival [28].

Laboratory diagnosis
CD4+ T-cell count

Immune status as assessed by CD4+ T-cell count could also serve as a clue for the suspicion of PJP in the patients with AIDS, but not in those without AIDS [1,16]. A CD4+ T-cell count < 200 cells/mL indicated the potential development and progression of PJP [16,21]. Therefore, the CD4+ T-cell count may guide clinicians as to whether to use or discontinue PJP prophylaxis.

Histopathology staining methods

Since PJ organisms are not cultivatable, a definitive diagnosis relies on positive staining for PJ using conventional staining methods such as the Gomori-Grocott test, toluidine blue stain, or a direct immunofluorescence assay (DFA) from induced sputum, bronchoalveolar lavage (BAL) fluid, or lung biopsy specimens [1]. These staining methods for PJP are more suitable for patients with AIDS than those without AIDS due to the number of PJ cysts in the alveoli [17]. However, these staining methods cannot discriminate infection from colonization when the result is positive [20].

Polymerase chain reaction (PCR)

The first study to use a real-time PCR assay to diagnose PJP used a semi-quantitative PCR, and the results showed that it could distinguish colonization, sub-clinical infection, and PJP, and the authors suggested that PJP could be excluded if the PCR results were negative, and that this could be used instead of DFA [31]. PCR-based methods had a higher sensitivity than conventional staining methods and were therefore good tools to exclude PJ in BAL fluid and induced sputum. However, they were still unable to discriminate infection from colonization when the results were positive [32]. Therefore, these methods appear to be more suitable for patients without AIDS than those with AIDS, as the number of PJ cysts in the alveoli is small

[33]. Another study reported that a positive real-time PCR result may indicate the development of long-term emphysema [7].

Quantitative real-time PCR (qPCR) assays were very sensitive to detect PJ in BAL fluid [32], and they were able to distinguish between infection and colonization [32]. However, the definition of the threshold value of fungal load for the diagnosis of PJP was problematic, and the accuracy of qPCR was affected by underlying diseases [32]. Moreover, the timing of the collection of sputum or BAL fluid affected the accuracy of the PCR test. Samples collected during or after anti-pneumocystis therapy may have caused false negative results. Hence, samples should be collected in a timely manner [33].

Serum 1,3-β-D-glucan (BDG)

Serum BDG assays appeared to be a promising alternative approach to diagnose PJP [34-37], and they could be used as a screening tool for PJP to avoid unnecessary invasive procedures such as BAL in patients with AIDS [34,36]. Two meta-analyses of the use of BDG to diagnose PJP in patients with and without AIDS reported that the average sensitivity and specificity were 95%-96% and 84%-86%, respectively, with a diagnostic odds ratio of 102.3, positive/negative likelihood ratios of 6.9 and 0.06, respectively, and the area under the summary receiver operating characteristic curve of 0.96 [35,36]. However, it should be noted that BDG is: (1) a common cell-wall component of many fungi including *Candida*, *Aspergillus*, and *Pneumocystis* (except for *Zygomycetes*), so it can be difficult to discriminate between fungal species [38]; (2) confounded by the concurrent use of intravenous antimicrobials such as ampicillin-sulbactam, cefazolin, cefotaxime, cefepime, trimethoprim-sulfamethoxazole (TMP-SMX), colistin, and ertapenem [38]; and (3) also confounded by cross-reacting with *Pseudomonas aeruginosa* or bacterial bloodstream co-infections [39].

Lactic dehydrogenase (LDH)

Serum LDH was often reported to be elevated, but a normal LDH level did not rule out the diagnosis [7]. Zaman and White reported a level > 450 U/L could predict PJP, a normal LDH value could exclude the diagnosis, and a progressive increase in the level of LDH during treatment could predict a poor prognosis but that is was also nonspecific [40]. However, combining BDG and LDH could further improve the diagnosis of AIDS-PJP. One study demonstrated that a BDG level > 400 pg/mL plus an LDH level > 350 U/L

and a clinical presentation of PJP could improve the diagnostic rate of AIDS-PJP (92.8% sensitivity, 83.9% specificity, 92.8% positive predictive value, 83.9% negative predictive value, 5.764/0.086 positive/negative likelihood ratios, p < 0.001) [37]. In addition, Grover *et al.* reported that dyspnea and an LDH level ≥ 220 U/L could predict PJP in patients with AIDS with a sensitivity and specificity of 94% and 78%, respectively [41]. However, further studies are needed to investigate the use of serum LDH in the diagnosis of non-AIDS-PJP.

Other laboratory findings

Laboratory findings including high C-reactive protein, low albumin, and high blood urea nitrogen levels were common in PJP, but none could differentiate AIDS from non-AIDS [42]. Hypoxemia was the most characteristic laboratory finding [17-20], and ranged from mild to severe with room air breathing (mild: PaO_2 predicted value ≥ 70 mm Hg or alveolo-arterial O2 gradient (A-aDO$_2$) < 35 mm Hg, moderate: PaO2 < 70 mm Hg or A-aDO$_2$ ≥ 35 and < 45 mm Hg, and severe: PaO_2 < 70 mm Hg or A-aDO$_2$ ≥ 45 mm Hg) [21]. Oxygen desaturation with exercise was also common but non-specific [21].

Mortality

The overall mortality rate ranged from 4% to 60% [1,2,42-45]. The mortality rate in the AIDS-PJP group improved after the advent of ART [43,44]. In a recent study of 544 patients with PJP, the overall in-hospital mortality rates were 4% for AIDS patients and 27% for non-AIDS patients (p < 0.0001) [1]. However, in the intensive care unit, the mortality rate of PJP remained higher in both groups (AIDS 17% versus non-AIDS 48%) [45]. The main causes of mortality in both groups were acute respiratory failure, using mechanical ventilation, and/or the development of pneumothorax [1,42,43]. In the AIDS-PJP group, pneumothorax was thought to occur because PJ had enough time to grow in the sub-pleural spaces and was thus difficult to eradicate by treatment [46]. In contrast, the most common causes of death in the patients with non-AIDS-PJP were hypoxemia and microbial co-infection with shock (odds ratio 3.09 [95% CI 1.44-6.68], p = 0.004) [1].

The factors related to mortality were non-AIDS patients, higher Simplified Acute Physiology Score, use of mechanical ventilation, and the development of pneumothorax [45]. The early detection of pneumothorax and appropriate treatment of the disease could reduce PJP-associated complications and

outcomes [26]. Using multivariate analyses, a high A-aDO2, bacteremia, preexisting lung disease, and elevated blood urea nitrogen were associated with mortality [42]. It was suggested that to improve the outcomes of the non-AIDS-PJP patients, clinicians could use invasive diagnostic techniques during the early stage to identify the pathogens [33].

Treatment
Trimethoprim-sulfamethoxazole (TMP-SMX)
    TMP-SMX was used as a first-line treatment for PJP in patients with AIDS and non-AIDS [19,21]. Despite the indications being well established for patients with AIDS [21], most of the data for patients without AIDS were derived from the experience with patients with AIDS (Table 2). Hence, there was no consensus on the indications, duration, and discontinuation of primary prophylaxis for non-AIDS-PJP patients. Some concerns regarding anti-pneumocystis were also raised as below.

TMP-SMX can be administered intravenously for patients with severe illnesses and gastrointestinal dysfunction. However, the use of TMP/SMX in the treatment or prophylaxis for AIDS-PJP was reported to cause adverse effects including severe asthenia (8%), severe abdominal pain (20%), nausea/vomiting (11.5%), severe rash (16%; including Stevens-Johnson syndrome), fever (30%-40%), anemia (31%), leukopenia (21.8-59%), hepatitis (16.4%), acute psychosis (11.9-13.3%), hyperkalemia (21.1%), hyponatremia (70.7%), thrombocytopenia (7%), azotemia (1-15%) and acute pancreatitis (3%) [21,47,48]. Although a daily dose of TMP/SMX of 15-20/75-100 mg/kg was recommended by Lee *et al.,* this caused 11.9% of 135 patients with AIDS to develop acute psychosis [47]. They therefore recommended a lower daily TMP dose of 12 mg/kg or lower [47]. Life-threatening immune reconstitution inflammatory syndrome was also noted after PJP in AIDS patients, and the U.S. Department of Health and Human Services

Table 2. Comparison of *Pneumocystis jirovecii* pneumonia in treatment guideline recommendations between the patients with and without AIDS.

Variables	AIDS	Non-AIDS	Reference
Treatment			
Time to initiation	Early after admission	Delayed initiation	[1]
TMP- SMX Dose, mg/kg/day Modified dose* Duration, days	Guideline Preferring therapy 15–20, IV every 6–8 hours ≤ 12 21	Preferred therapy based on AIDS guidelines	[16], [18 - 21], [47], [57]
Pentamidine Dose, mg/kg/day Duration, days	Alternative therapy, more adverse effects 4, IV infused over ≥ 60 minutes 21	Alternative therapy, based on AIDS guidelines	[16], [18-21], [48], [52], [53], [57]
Echinocandins	Animal studies and no large randomized studies	Case reports as salvage therapy only, failure of salvage also reported	[49-51]
Microbial co-infections, Bacterial infection, %	80/223 (35.8%) 18.3	121/331 (36.5%) 11.1**	[1]
Chemoprophylaxis			
TMP-SMX Indications Dose Duration Benefit	First-choice CD4$^+$ T-cell count < 200/mL or oropharyngeal candidiasis TMP-SMX, 1 DS, or 1 SS PO daily, or 1 DS PO three times weekly# Decided by CD4+ T-cell count§ Increase survival	First-choice No consensus Undecided regarding the immunosuppressive conditions¶ No consensus Limited data	[16], [18 - 21], [47], [53], [54], [57]
Adjunctive corticosteroids			
Indications Dose Duration, days Outcome	Moderate to severe disease⸸ Start 40 mg of prednisone or equivalent twice daily 21 Increase survival	No randomized studies high-dose steroid therapy ≥ 1 mg/kg/day No consensus increase death rate	[19], [21], [54-57]

TMP- SMX = Trimethoprim-sulfamethoxazole; *if acute psychosis or acute hepatitis; **P = 0.018; ⸸Moderate to severe disease defined as arterial oxygen pressure ≤ 70 mm Hg or alveolar-arterial oxygen gradient ≥ 35 mm Hg; #TMP-SMX single strength (SS) = 80 mg TMP/400 mg SMX; TMP-SMX double strength (DS) = 160 mg TMP/800 mg SMX; ¶All solid organ transplant recipients at least 6-12 months post transplant; §CD4+ T-cell count increased from < 200 cells/mm3 to > 200 cells/mm3 for > 3 months antiretroviral treatment or CD4+ T-cell count 100-200 cells/µL and HIV viral load below limits of detection lasting 3-6 months.

guidelines recommended that ART should be initiated within 2 weeks of a diagnosis of PJP [21].

Other alternatives

Salvage therapy with echinocandins was also reported in PJP patients with and without AIDS [49-51] after encountering the significant adverse effects of TMP-SMX and intravenous pentamidine [47,48,52]. PJP does not respond to standard antifungal treatment such as the azole family and amphotericin B because of the lack of ergosterol biosynthesis in PJ. In contrast, echinocandins were reported to inhibit BDG [49], and caspofungin was reported to improve overall mortality in patients with AIDS-PJP [49]. However, some studies reported failure of salvage therapy using echinocandins to improve survival among non-AIDS patients [50,51].

Pentamidine administered parenterally was as effective as TMP-SMX, however severe adverse effects including tremors, rash, leucopenia, thrombocytopenia, interstitial nephritis, impaired renal function, hyperkalemia, torsade de pointes, prolonged rate corrected QT interval, and acute pancreatitis may limit its use [48,52,53]. Other treatment regimens included clindamycin-primaquine, atovaquone, and dapsone-TMP [21]; however, most lacked clinical trials to compare their efficacy with TMP-SMX for the treatment of patients with AIDS-PJP [21].

Adjunctive corticosteroid therapy (ACT)

Indications for ACT for the patients with AIDS included those with moderate to severe PJP [21], and it was reported to potentially be beneficial for adult AIDS-PJP patients with substantial hypoxemia [54]. The recommended initial dose of ACT was 80 mg daily of oral prednisone divided into two doses on day 1 through 5, 40 mg of prednisone daily on day 6 through 10, and 20 mg of prednisone daily on day 11 through 21 [19,21]. The recommended dose of intravenous methylprednisolone was equal to 75% of the prednisone dose. However, Gallant *et al.* reported that this could increase the risk of oropharyngeal candidiasis among the patients with AIDS-PJP [55]. The authors also reported that there were no significant differences in the incidence of other opportunistic infections, including cytomegalovirus disease, *Mycobacterium avium* complex, cryptococcal meningitis, toxoplasmosis, Kaposi's sarcoma, herpes simplex and herpes zoster between the patients who did and did not receive steroid treatment [55]. Moreover, ACT did not increase the risk of mortality in the patients with AIDS [55].

Relatively few studies reported the use of corticosteroids for non-AIDS-PJP [56,57]. Guidelines

for the treatment of solid-organ transplantation recipients with PJP recommend an ACT protocol that may improve survival, such as 40-60 mg of prednisone or equivalent twice daily within 72 hours of initiating antimicrobial therapy for 5-7 days, followed by tapering over a period of at least 7-14 days [57]. High-dose ACT was reported to have a detrimental effect on survival for both the patients with AIDS-PJP and non-AIDS-PJP [56].

Prophylaxis

Trimethoprim-sulfamethoxazole

TMP-SMX was used as the first-line prophylaxis regimen for PJP in patients with and without AIDS [19,21]. This prophylaxis regimen is well established for AIDS patients [21], however, most of the data for non-AIDS patients were extrapolated from experience with those with AIDS. Prophylaxis with TMP-SMX could reduce the occurrence of PJP among non–AIDS patients.

Huang *et al.* reported that the widespread and long-term prophylaxis with TMP-SMX led to the development of sulfa drug-resistant PJ [58]. In addition, drug-resistant PJ may result in the failure of standard therapy and prophylaxis, and may cause interhuman transmission [7,32]. Interhuman transmission and severity of PJP were reported to be related to specific PJ genotypes [59,60]. Susceptibility testing for PJ would seem to be pertinent to counter this issue, however, this is not possible in routine practice as PJ is not cultivatable in vitro [**7**].

Aerosolized Pentamidine

Intravenous pentamidine treatment for AIDS-PJP was reported to be more toxic than TMP-SMX [48, 52,53]. Compared with TMP-SMX, aerosolized pentamidine was reported to have a better tolerance rate and survival rate (11% versus 7.4%) [53]. However, aerosolized pentamidine was not suggested as first-line treatment for AIDS-PJP due to more frequent relapses [21,53], although it could be an alternative choice for primary and secondary prophylaxis despite causing respiratory adverse effects (bronchospasm, cough or dyspnea). Of note, prophylaxis with aerosolized pentamidine for PJP was reported to potentially cause atypical presentations and make the diagnosis difficult [24]. The recommended dose was 300 mg monthly via a Respigard II™ nebulizer (manufactured by. Marquest, Englewood. Colorado) [21]. For the non-AIDS-PJP patients, aerosolized pentamidine may also be an alternative prophylactic option [48,57]. Other options include dapsone,

dapsone/pyrimethamine/leucovorin, atovaquone, or atovaquone/pyrimethamine/leucovorin for primary or secondary prophylaxis in AIDS or non-AIDS-PJP patients with PJP (Table 2) [21, 57].

Conclusion

Comparing the two groups of patients, the clinical course of PJP is potentially more acute and the frequency of PJP seems to be higher in immunocompromised patients without AIDS. Once acquired, the patients have greater fulminant progression, possibly due to a stronger immune reaction. This results in more severe hypoxemia, more frequent use of mechanical ventilation, and a higher mortality rate. The dilemma of diagnosis is differentiating between active infection and colonization of PJ. At present, neither conventional staining nor PCR-based methods can resolve this problem. Staining method is not sensitive enough to detect colonization as the fungal load is low. The best treatment strategy for PJP has yet to be determined in immunocompromised patients without AIDS, however, early initiation of therapy and early invasive investigation seem appropriate. For patients with AIDS, prophylaxis and early treatment with an optimal dose of TMP-SMX for PJP and adjunctive corticosteroid therapy for moderate to severe PJP may achieve a better prognosis. Invasive procedure (BAL-qPCR-based therapeutic strategies) may help identify the pathogens earlier thereby offering additional benefits in the management of non-AIDS subjects.

References

1. Roux A, Canet E, Valade S, Gangneux-Robert F, Hamane S, Lafabrie A, Maubon D, Debourgogne A, Le Gal S, Dalle F, Leterrier M, Toubas D, Pomares C, Bellanger AP, Bonhomme J, Berry A, Durand-Joly I, Magne D, Pons D, Hennequin C, Maury E, Roux P, Azoulay É (2014) *Pneumocystis jirovecii* pneumonia in patients with or without AIDS, France. Emerg Infect Dis 20: 1490-1497.
2. Bienvenu A-L, Traore K, Plekhanova I, Bouchrik M, Bossard C, Picot S (2016) *Pneumocystis* pneumonia suspected cases in 604 non-HIV and HIV patients. Int J Infect Dis 46: 11-17.
3. Jian L, Bin D, Haiyun W (2004) Fatal *Pneumocystis* pneumonia with Good Syndrome and pure red cell aplasia. Clin Infect Dis 39: 1740-1741.
4. Cunningham-Rundles C, Bodian C (1999) Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol 92: 34-48.
5. Roths JB, Sidman CL (1992) Both immunity and hyperresponsiveness to *Pneumocystis carinii* result from transfer of CD4+ but not CD8+ T cells into severe combined immunodeficiency mice. J Clin Microbiol 90: 673-678.
6. Hughes WT, Price RA, Sisko F, Havron WS, Kafatos AG, Schonland M, Smythe P (1974) Protein-calorie malnutrition: a host determinant for *Pneumocystis carinii* infection. Am J Dis Child 128: 44-52.
7. Morris A, Norris KA (2012) Colonization by *Pneumocystis jirovecii* and its role in disease. Clin Microbiol Rev 25: 297-317.
8. Yiannakis EP, Boswell TC (2016) Systematic review of outbreaks of *Pneumocystis jirovecii* pneumonia: evidence that *P. jirovecii* is a transmissible organism and the implications for healthcare infection control. J Hosp Infect 93: 1-8.
9. Choukri F, Menotti J, Sarfati C, Lucet JC, Nevez G, Garin YJF, Totet A (2010) Quantification and spread of *Pneumocystis jirovecii* in the surrounding air of patients with Pneumocystis pneumonia. Clin Infect Dis 51: 259-265.
10. Armstrong-James D, Meintjes G, Brown GD (2014) A neglected epidemic: fungal infections in HIV/AIDS. Trends Microbiol 22: 120-127.
11. Schwarcz L, Chen M-J, Vittinghoff E, Hsu L, Schwarcz S (2013) Declining incidence of AIDS-defining opportunistic illnesses: results from 16 years of population-based AIDS surveillance. AIDS 27: 597-605.
12. López-Sánchez C, Falcó V, Burgos J, Navarro J, Martín MT, Curran A, Miguel L, Ocaña I, Ribera E, Crespo M, Almirante B (2015) Epidemiology and long-term survival in HIV-infected patients with *Pneumocystis jirovecii* pneumonia in the HAART era: experience in a university hospital and review of the literature. Medicine 94: e681.
13. Lowe DM, Rangaka MX, Gordon F, James CD, Miller RF (2013) *Pneumocystis jirovecii* pneumonia in tropical and low and middle income countries: a systematic review and meta-regression. PLoS ONE 8: e69969.
14. Kaplan JE, Hanson DL, Navin TR, Jones JL (1998) Risk factors for primary *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected adolescents and adults in the United States: reassessment of indications for chemoprophylaxis. J Infect Dis 178: 1126-1132.
15. Fillatre P, Decaux O, Jouneau S, Revest M, Gacouin A, Robert-Gangneux F, Fresnel A, Guiguen C, Le Tulzo Y, Jégo P, Tattevin P (2014) Incidence of *Pneumocystis jirovecii* pneumonia among groups at risk in HIV-negative patients. Am J Med 127: 1242.e11-1242.e17.
16. Kovacs JA, Gill VJ, Meshnick S, Masur H (2001) New insights into transmission, diagnosis, and drug treatment of *Pneumocystis carinii* pneumonia. JAMA 286: 2450-2460.
17. Limper AH, Offord KP, Smith TF, Martin WJ (1989) *Pneumocystis carinii* pneumonia: differences in lung parasite number and inflammation in patients with and without AIDS. Am Rev Respir Dis 140: 1204-1209.
18. Thomas CF, Limper AH (2007) Current insights into the biology and pathogenesis of *Pneumocystis* pneumonia. Nat Rev Micro 5: 298-308

19. Thomas CF, Limper AH (2004) *Pneumocystis* Pneumonia. N Engl J Med 350: 2487-2498.
20. Kovacs JA, Hiemenz, JW, Macher AM, Stover D, Murray HW, Shelhamer J, Lane HC, Urmacher C, Honig C, Longo DL, Parker MM, Natanson C, Parrillo JE, Fauci AS, Pizzo PA, Masur H (1984) *Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med 100:663-671.
21. AIDSinfo (2018) Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Available: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed 15 October 2018.
22. Ng VL, Yajko DM, Hadley WK (1997) Extrapulmonary pneumocystosis. Clin Microbiol Rev 10: 401-418.
23. Kovacs JA, Masur H (2009) Evolving health effects of pneumocystis: One hundred years of progress in diagnosis and treatment. JAMA 301: 2578-2585.
24. Opravil M, Marincek B, Fuchs WA, Weber R, Speich R, Battegay M, Russi EW, Lüthy R (1994) Shortcomings of chest radiography in detecting *Pneumocystis carinii* pneumonia. J Acquir Immune Defic Syndr 7: 39-45.
25. Castañer E, Gallardo X, Maria Mata J, Esteba L (2004) Radiologic approach to the diagnosis of infectious pulmonary diseases in patients infected with the human immunodeficiency virus. Eur J Radiol 51: 114-129.
26. Ebner L, Walti LN, Rauch A, Furrer H, Cusini A, Meyer AMJ, Weiler S, Huynh-Do U, Heverhagen J, Arampatzis S, Christe A (2016) Clinical course, radiological manifestations, and outcome of *Pneumocystis jirovecii* pneumonia in HIV Patients and renal transplant recipients. PLoS ONE 11: e0164320.
27. Vogel MN, Vatlach M, Weissgerber P, Goeppert B, Claussen CD, Hetzel J, Horger M (2012) HRCT-features of *Pneumocystis jirovecii* pneumonia and their evolution before and after treatment in non-HIV immunocompromised patients. Eur J Radiol 81: 1315-1320.
28. Chou CW, Chao HS, Lin FC, Tsai HC, Yuan WH, Chang SC (2015) Clinical usefulness of HRCT in assessing the severity of *Pneumocystis jirovecii* pneumonia: a cross-sectional study. Medicine 94: e768.
29. Gruden JF, Huang L, Turner J, Webb WR, Merrifield C, Stansell JD, Gamsu G, Hopewell PC (1997) High-resolution CT in the evaluation of clinically suspected *Pneumocystis carinii* pneumonia in AIDS patients with normal, equivocal, or nonspecific radiographic findings. AJR Am J Roentgenol 169: 967-975.
30. Hardak E, Brook O, Yigla M (2010) Radiological features of *Pneumocystis jirovecii* pneumonia in immunocompromised patients with and without AIDS. Lung 188: 159-163.
31. Fillaux J, Malvy S, Alvarez M, Fabre R, Cassaing S, Marchou B, Linas M-D, Berry A (2008) Accuracy of a routine real-time PCR assay for the diagnosis of *Pneumocystis jirovecii* pneumonia. J Microbiol Methods 75: 258-261.
32. Botterel F, Cabaret O, Foulet F, Cordonnier C, Costa J-M, Bretagne S (2012) Clinical significance of quantifying *Pneumocystis jirovecii* DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. J Clin Microbiol 50: 227-231.
33. Azoulay É, Bergeron A, Chevret S, Bele N, Schlemmer B, Menotti J (2009) Polymerase chain reaction for diagnosing Pneumocystis pneumonia in non-HIV immunocompromised patients with pulmonary infiltrates. Chest 135: 655-661.
34. Sax PE, Komarow L, Finkelman MA, Grant PM, Andersen J, Scully E, Powderly WG, Zolopa AR (2011) Blood $(1{\rightarrow}3)$-β-D-Glucan as a diagnostic test for HIV-related *Pneumocystis jirovecii* pneumonia. Clin Infect Dis 53: 197-202.
35. Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME (2013) Accuracy of β-D-glucan for the diagnosis of *Pneumocystis jirovecii* pneumonia: a meta-analysis. Clin Microbiol Infect 19: 39-49.
36. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, Morinobu A, Nishimura K, Kumagai S (2012) Diagnostic accuracy of serum 1,3-β-d-Glucan for *Pneumocystis jirovecii* pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol 50: 7-15.
37. Esteves F, Lee CH, de Sousa B, Badura R, Seringa M, Fernandes C, Gaspar JF, Antunes F, Matos O (2014) $(1–3)$-Beta-D-glucan in association with lactate dehydrogenase as biomarkers of *Pneumocystis* pneumonia (PcP) in HIV-infected patients. Eur J Clin Microbiol Infect Dis 33: 1173-1180.
38. Marty FM, Lowry CM, Lempitski SJ, Kubiak DW, Finkelman MA, Baden LR (2006) Reactivity of $(1{\rightarrow}3)$-β-d-Glucan assay with commonly used intravenous antimicrobials. Antimicrob Agents Chemother 50: 3450-3453.
39. Mennink-Kersten MASH, Ruegebrink D, Verweij PE (2008) *Pseudomonas aeruginosa* as a cause of 1,3-β-d-Glucan assay reactivity. Clin Infect Dis 46: 1930-1931.
40. Zaman MK, White DA (1988) Serum lactate dehydrogenase levels and *Pneumocystis carinii* pneumonia: diagnostic and prognostic significance. Am Rev Respir Dis 137: 796-800.
41. Grover SA, Coupal L, Suissa S, Szentveri T, Falutz J, Tsoukas C, Battista RN, Gilmore N (1992) The clinical utility of serum lactate dehydrogenase in diagnosing *Pneumocystis carinii* pneumonia among hospitalized AIDS patients. Clin Invest Med 15: 309-317.
42. Kim SJ, Lee J, Cho YJ, Park YS, Lee CH, Yoon HI, Lee SM, Yim JJ, Lee JH, Yoo CG, Lee CT, Kim YW, Han SK, Kim HB, Park JS (2014) Prognostic factors of *Pneumocystis jirovecii* pneumonia in patients without HIV infection. J Infect 69: 88-95.
43. Miller RF, Allen E, Copas A, Singer M, Edwards SG (2006) Improved survival for HIV-infected patients with severe *Pneumocystis jirovecii* pneumonia is independent of highly active antiretroviral therapy. Thorax 61: 716-721.
44. Curtis JR, Yarnold PR, Schwartz DN, Weinstein RA, Bennett CL (2000) Improvements in outcomes of acute respiratory failure for patients with human immunodeficiency virus-related *Pneumocystis carinii* pneumonia. Am J Respir Crit Care Med 162: 393-398.
45. Monnet X, Vidal-Petiot E, Osman D, Hamzaoui O, Durrbach A, Goujard C, Miceli C, Bourée P, Richard C (2008) Critical care management and outcome of severe *Pneumocystis* pneumonia in patients with and without HIV infection. Critical Care 12: R28.
46. Azoulay É, Parrot A, Flahault A, Cesari D, Lecomte I, Roux P, Saidi F, Fartoukh M, Bernaudin J-F, Cadranel J, Mayaud C (1999) AIDS-related *Pneumocystis carinii* pneumonia in the era of adjunctive steroids. Am J Respir Crit Care Med 160: 493-499.

47. Lee KY, Huang CH, Tang HJ, Yang CJ, Ko WC, Chen YH, Lee YC, Hung CC (2012) Acute psychosis related to use of trimethoprim/sulfamethoxazole in the treatment of HIV-infected patients with *Pneumocystis jirovecii* pneumonia: a multicentre, retrospective study. J Antimicrob Chemother 67: 2749-2754.

48. Hardy WD, Feinberg J, Finkelstein DM, Power ME, He W, Kaczka C, Frame PT, Holmes M, Waskin H, Fass RJ, Powderly WG, Steigbigel RT, Zuger A, Holzman RS (1992) A controlled trial of trimethoprim–sulfamethoxazole or aerosolized pentamidine for secondary prophylaxis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. N Engl J Med 327: 1842-1848.

49. Armstrong-James D, Stebbing J, John L, Murungi A, Bower M, Gazzard B, Nelson M (2011) A trial of caspofungin salvage treatment in PCP pneumonia. Thorax 66: 537-538.

50. Kamboj M, Weinstock D, Sepkowitz KA (2006) Progression of *Pneumocystis jiroveci* pneumonia in patients receiving echinocandin therapy. Clin Infect Dis 43: e92-e94.

51. Kim T, Hong HL, Lee YM, Sung H, Kim SH, Choi SH, Kim YS, Woo JH, Lee SO (2013) Is caspofungin really an effective treatment for *Pneumocystis jirovecii* pneumonia in immunocompromised patients without human immunodeficiency virus infection? Experiences at a single center and a literature review. Scand J Infect Dis 45: 484-48853.

52. Eisenhauer MD, Eliasson AH, Taylor AJ, Coyne PE, Wortham DC (1994) Incidence of cardiac arrhythmias during intravenous pentamidine therapy in HIV-infected patients. Chest 105: 389-395.

53. Montgomery AB, D W Feigal J, Sattler F, Mason GR, Catanzaro A, Edison R, Markowitz N, Johnson E, Ogawa S, Rovzar M (1995) Pentamidine aerosol versus trimethoprim-sulfamethoxazole for *Pneumocystis carinii* in acquired immune deficiency syndrome. Am J Respir Crit Care Med 151: 1068-1074.

54. Ewald H, Raatz H, Boscacci R, Furrer H, Bucher HC, Briel M (2015) Adjunctive corticosteroids for *Pneumocystis jirovecii* pneumonia in patients with HIV infection. Cochrane Database Syst Rev 4: (CD006150).

55. Gallant JE, Chaisson RE, Moore RD (1998) The effect of adjunctive corticosteroids for the treatment of *Pneumocystis carinii* pneumonia on mortality and subsequent complications. Chest 114: 1258-1263.

56. Lemiale V, Debrumetz A, Delannoy A, Alberti C, Azoulay É (2013) Adjunctive steroid in HIV-negative patients with severe *Pneumocystis* pneumonia. Respir Res 14: 87.

57. Martin SI, Fishman JA, the AST Infectious Diseases Community of Practice (2013) *Pneumocystis* pneumonia in solid organ transplantation. Am J Transplant 13: 272-279.

58. Huang L, Crothers K, Atzori C, Benfield T, Miller R, Rabodonirina M, Helweg-Larsen J (2004) Dihydropteroate synthase gene mutations in *Pneumocystis* and sulfa resistance. Emerg Infect Dis 10: 1721.

59. Hauser PM, Sudre P, Nahimana A, Francioli P, the Study G (2001) Prophylaxis failure is associated with a specific *Pneumocystis carinii* genotype. Clin Infect Dis 33: 1080-1082.

60. Rabodonirina M, Vaillant L, Taffé P, Nahimana A, Gillibert R-P, Vanhems P, Hauser PM (2013) *Pneumocystis jirovecii* genotype associated with increased death rate of HIV-infected patients with pneumonia. Emerg Infect Dis 19: 21.

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Conflict of interests: No conflict of interests is declared.