Egyptian Journal of Medical Microbiology Volume 33 / No.3 / July 2024 11-18 Online ISSN: 2537-0979

REVIEW ARTICLE

Spotlights on *Pneumocystis jiroveci*

Asmaa S. Hegab*, Somaia A. Eissa, Iman E. Wali, Noha M. Gohar
Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo11562, Egypt

Key words:
Pneumocystis jiroveci, opportunistic, HIV/AIDS

****Corresponding Author:***
Asmaa Sayed Hegab, Department of Medical Microbiology & Immunology, Cairo University, Egypt
Tel.: 01005195939
asmahgab@yahoo.com, asmahgab@kasralainy.edu.eg, asmahgab@cu.edu.eg,
ORCID:0000-0002-6304-652X

ABSTRACT

Pneumocystis jiroveci (P. jiroveci), previously referred to as Pneumocystis carinii (P. carinii), is the upmost encountered opportunistic infection in individuals with HIV/AIDS. Although the occurrence of Pneumocystis jiroveci pneumonia (PJP) has declined in HIV patients as a result of antiretroviral therapy, its prevalence has increased among individuals with hematologic malignancies, autoimmune diseases, recipients of stem-cell or solid-organ transplants, and those undergoing systemic chemotherapy. While diagnosis of PJP typically requires cytological staining, it is crucial not to delay treatment. In high-risk and critically ill patients, PJP treatment can be initiated before completing the diagnostic workup. The alveolar-arterial gradient is utilized to assess the severity of illness at the time of diagnosis. Antibiotics are recommended for managing PJP. Treatment of preference is trimethoprim/sulfamethoxazole (TMP-SMX). Primaquine with clindamycin is the recommended combination for individuals who have a history of intolerance to this regimen. Corticosteroids are employed as a supplementary treatment only in Patients with HIV who have severe PJP. While prophylaxis has decreased the frequency of PJP, its usage raised concerns regarding the emergence of resistant organisms. Due to the inability to cultivate human Pneumocystis in a standardized culture system, conducting routine susceptibility testing and detecting drug resistance is not feasible.

INTRODUCTION

Pneumocystis jiroveci pneumonia (PJP) is an infection that can be fatal for individuals with impaired immune systems. To differentiate it from the rodent-infecting species, the nomenclature for the *Pneumocystis* species infecting humans has been altered from *P. carinii* to *P. jiroveci*[1,2].

Patients with HIV and a low CD4 level are the most vulnerable to PJP. Individuals undergoing stem cell and solid organ transplants, those receiving chemotherapy, glucocorticoids, other immunosuppressive medications, and patients with hematologic malignancies are among the other groups who are seriously at risk. The frequency of PJP is rising as the number of persons receiving immunosuppressive drugs increases[3].

However, "PCP" as an abbreviation is still used to refer to the clinical entity of "***P****neumo****c****ystis* **p**neumonia"; this preserves the accuracy of the abbreviation in earlier published studies and permits the continuation of the widely used abbreviation among physicians[4].

Epidemiology

Before the global HIV/AIDS epidemic, the majority of cases of PJP were observed in undernourished or premature infants, as well as individuals with cellular immunodeficiencies caused by conditions including T cell insufficiency, hematological malignancies, and other severe illnesses requiring corticosteroid therapy. In the 1980s, PJP significant morbidity among HIV-positive patients concealed the condition prevalence in non-HIV patients. The occurrence of PJP in HIV-positive patients has dramatically dropped since the late 1990s because of the widespread use of prophylaxis and the highly active antiretroviral therapy (HAART) that followed, nonetheless PJP still affects individuals with other immunodeficiencies[5,6].

It is more challenging to diagnose PJP promptly in a non-HIV immunocompromised population because of its shorter incubation period, increased risk of respiratory failure and fatality, and rapid illness development. This rapidly progressing disease in non-HIV patients is probably caused by a stronger immunological response to PJP in the lungs than in those with HIV, which leads to marked hypoxia, a larger alveolar-arterial gradient, respiratory failure, and a generally bad prognosis[7].

In a recent list of invasive fungal infections, the World Health Organization (WHO) included PJP and recommended increased study and public health measures[8].

Life cycle:

During the early twentieth century, Carlos Chagas recognized *P. jiroveci* as a protozoan that was thought to be a component of *Trypanosoma cruzi* life cycle. In 1988, a phylogenetic link to the fungal kingdom was discovered through genomic analysis of the small rRNA

subunit, leading to the reclassification of *Pneumocystis* as a fungus. There are now only six officially recognized species of *Pneumocystis*: *P. jiroveci* (human), *P. murina* (house mice), *P. carinii* (Brown or Norway rats), *P. wakefieldiae* (Brown or Norway rats), *P. oryctolagi* (rabbits), and *P. canis* (dogs) [9].

In the lungs, *Pneumocystis* has a biphasic life cycle featuring (a) an asexual phase, which consists of the binary fission of haploid trophozoites, and (b) a sexual phase, during which haploid trophozoites fuse, leading to the formation of cysts. Haploid trophozoites conjugate into diploid early sporozoites, which undergo meiotic division followed by mitotic replication. This results in the formation of late sporozoites containing eight nuclei (or spores). The maturation of late sporozoites leads to the evolution of thick-walled cysts (ascus). There is still a lot we don't know about *P. jiroveci* because of its significantly difficult *in vitro* development[10].

Co-housing studies in immunocompromised rats and mice suggest that the ascus is the transmissible and environmental living form since these animals were unable to spread the illness after being treated with cyst depleting medications such as echinocandins. By using transmission electron microscopy at a magnification of 5000, the cyst thick wall with double electron-dense layers encircling eight intracystic spores, can be observed. *P. jiroveci* cyst wall is abundant in (1,3) β-D-glucan (BDG), a target for binding, activation, and phagocytosis for macrophages[10].

The trophozoite forms are discharged into the lungs upon inhalation of the cysts. *Pneumocystis* trophozoites adhere to type I alveolar epithelium as the infection progresses. Trophozoite life forms are far more prevalent than cysts after infection and are usually present in a ratio of at least 10:1. In immunocompetent hosts, alveolar macrophages and functional CD4 T cells are necessary for the effective clearance of both life forms; nevertheless, an excessive immune response may result in lung injury and respiratory compromise[11].

Pathophysiology:

There is strong evidence that humans act as only reservoirs for this fungus. Potential human reservoirs include patients with PJP and those with competent or weakened immunity who are colonized or have a subclinical infection with *P. jiroveci* including children (particularly neonates) and adults. The respiratory tract undoubtedly serves as *Pneumocystis* infection entrance and exit points[12].

Transmission of Pneumocystis:

It is presently believed that the mode of transmission is airborne. Evidence is supplied by molecular analysis of *Pneumocystis* isolated from hospital outbreak patients[13].

Risk factors for Pneumocystis jiroveci pneumonia:

Groups at risk for PJP include:

- HIV patients with a CD4 cell count less than 200 cells/mm^3 and who are not getting PJP prophylaxis (regardless of CD4 cell count, the presence of other opportunistic infections [e.g., oral thrush] raises the liability of PJP) [14,15]
- Persons suffering from primary immunological deficiencies, such as severe combined immunodeficiency (SCID) and hypogammaglobulinemia [16]
- Persons using immunosuppressive medication for autoimmune disorders, or organ transplantation [17]
- Persons with cancer, such as solid tumors and lymphomas [18]
- COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can impair the host immune system and raise the risk of secondary infections with bacterial and fungal pathogens, including *P. jiroveci* [7]

Development of Pneumocystis jiroveci pneumonia:

Immunocompetent people can control and remove *Pneumocystis* without symptoms, however people with weakened immune systems are more susceptible to serious and even deadly pneumonia. The risk of PJP is determined by the interaction between the type and the severity of immunocompromising diseases and epidemiologic *P. jiroveci* exposure. When humoral and cellular immunity are ineffective, disease results. *P. jiroveci* can multiply uncontrollably due to host immunological defects. Alveolar macrophages without T helper lymphocytes fail to eliminate *Pneumocystis* infection. Electron microscopy shows increased capillary permeability. Physiological alterations include: respiratory alkalosis, hypoxemia with increased oxygen gradient, reduced diffusion capacity and modifications in overall lung and vital capacities[14].

The respiratory tract is the primary site of COVID-19 infection, which results in dysregulated and increased pro-inflammatory as well as inflammatory responses and lung tissue damage. These responses have a broad influence on the adaptive immune system and the innate defensive mechanisms. Significant decreases in CD4 T cells, CD8 T cells, B lymphocytes, and natural killer cells are observed in severe COVID-19 patients. Exhausted T lymphocytes that are less capable to proliferate with an overabundance of pro-inflammatory cytokines typically accompany these cell number declines. Invasive fungal infections like PJP can arise in COVID-19 patients because of their low immunity, and the use of corticosteroids and other immunomodulatory medications. PJP has been described in numerous reports, with a recent study revealing a 9.3% of severely ill COVID-19 patients had *P. jiroveci* infection[7].

CLINICAL PRESENTATION

Patient history:

PJP symptoms are nonspecific. PJP in HIV patients runs a subacute course compared to PJP in patients with other immunocompromising diseases. PJP symptoms consist of: dyspnea with progressive exercise (95%), dry cough (95%), fever (>80%), chest pain, weight loss, chills and hemoptysis (infrequent) [19,20].

Clinical examination:

PJP physical examination results are vague and consist of tachypnea, fever, and tachycardia. Chest examination may show slight crackles and rhonchi, although in 50% of the patients, the results may be normal. Children presenting with severe illness also have intercostal retractions, cyanosis, and nasal flaring[1].

Extrapulmonary manifestations:

Less than 3% of patients get extrapulmonary lesions. *P. jiroveci* infection may involve any of the following organ systems: Nervous system, bone marrow (necrosis may occur, resulting in pancytopenia), lymphadenopathy, eyes (may show retinal spots), thyroid (may show a quickly growing thyroid tumor) and the digestive tract[1].

Complications:

Severe PJP patients with may encounter acute respiratory distress syndrome (ARDS), requiring intubation. This has a significant negative impact on the prognosis[21].

Prognosis:

PJP has a mortality incidence of 10%-20% in HIV patients, depending on the degree of disease severity at onset of presentation. PJP has a poor prognosis in people who do not have HIV. Mortality rates of 30%-50% have been reported. PJP with patients who experience pneumothorax or need mechanical breathing have a worse prognosis[22].

Differential Diagnosis:

PJP differential diagnoses includes: acute respiratory distress syndrome (ARDS), legionellosis, tuberculosis, pulmonary embolism, lymphocytic interstitial pneumonia (LIP), mycoplasma infections, viral pneumonia, mycobacterium avium complex (MAC) infections[23].

Diagnosis:

I. Specimens:

Sputum

The least invasive technique for PJP diagnosis is sputum induction. *P. jiroveci is usually identified in sputum produced by inhaling hypertonic saline.* Expectorated sputum should not be used for diagnosis because of its low sensitivity[24].

Sputum induction sensitivity varies greatly (from less than 50% to more than 90%), depending on the laboratory experience and proficiency skills. There is excellent specificity (99%–100%). Due to the reduced alveolar burden of *P. jiroveci*, limited sensitivity is observed in both HIV-negative patients and those using pentamidine aerosol as a prophylactic therapy[1].

Bronchoalveolar lavage

When the induced sputum results are negative, bronchoalveolar lavage (BAL) is strongly advised. In uncooperative patients, BAL is beneficial. It has a diagnostic accuracy of more than 90%. The more lobes sampled during BAL, the greater the microbiological yield[25]. Because BAL is less sensitive in patients using pentamidine aerosol, a transbronchial biopsy is carried out additionally to BAL[26].

Lung biopsy

The most invasive method is an open lung biopsy, yielding 100% sensitivity and specificity due to the large quantity of tissue available for diagnosis. But this process is only applied when the results of bronchoscopy cannot be applied to make a diagnosis[1].

II. Direct detection:

Cytological staining

As *P. jiroveci* is not cultivable *in vitro* and because clinical as well as radiological signs are not unique for PJP, histopathologic examination is necessary for establishing a diagnosis. Wright, Giemsa, Diff-Quik, and crystal violet stains can identify trophozoite and cyst forms but not the *Pneumocystis* cyst wall. The cyst wall is selectively stained with methenamine silver, Gram-Weigert and toluidine blue. *Pneumocystis* in the Papanicolaou smear is surrounded by eosinophils. Since direct immunofluorescence may be more sensitive than cytological labelling, some institutions utilize it to detect *Pneumocystis*[1].

Quantitative polymerase chain reaction (qPCR)

PCR of respiratory fluid, particularly BAL, is increasingly used to diagnose PJP in HIV-infected and non-infected individuals. The gold standard for identifying *P. jiroveci* is molecular techniques, which have demonstrated extremely high sensitivity and specificity. In patients who are already in respiratory failure, sputum as a specimen for PCR testing for *P. jiroveci* might be a less risky option than bronchoscopic evaluation[19,27,28,29].

β-D-Glucan

Employing less invasive diagnostic biomarkers, such as BDG, to identify PJP has gained more attention recently. Several pathogenic fungi produce BDG, a structural polysaccharide, during the production of their fungal cell walls. Assays designed and approved primarily based on the detection of BDG for the diagnosis of invasive candidiasis and pulmonary aspergillosis have been developed. Although the BDG assay is not a perfect diagnostic tool, it can assist in identifying patients who are more likely to have PJP when invasive procedures are not appropriate for a conclusive diagnosis[30,31].

Lactate dehydrogenase

The level of lactate dehydrogenase (LDH) is measured. Patients with PJP had high LDH levels (>220

U/L). It is raised in 90% of PJP patients who test positive for HIV. The test sensitivity ranges from 78% to 100%, but because many different illnesses can induce elevated LDH levels, its specificity is lower. LDH level appears to be related to the severity of lung damage. It should decrease if the treatment is successful. A persistently increased LDH level throughout therapy may suggest treatment failure and a bad outcome[32].

III. Other Tests:

Pulmonary function tests

Pulmonary function tests are conducted on individuals who may have PJP. It is possible that the results indicate a lowered diffusion capacity of carbon monoxide (DLCO) of less than 75%. Dropped DLCO has a low specificity of 53% and a high sensitivity of 89%–100%. With normal DLCO, PJP is improbable[1].

Pulse oximetry

Pulse oximetry is to be assessed in all patients. Both at rest and during exercise, oxygen saturation should be measured. If hypoxemia (O2 saturation 90%) is detected, an arterial blood gas (ABG) level had to be acquired to determine the necessity for supplementary corticosteroids[1].

IV. Imaging:

Chest Radiography

An immunocompromised patient with fever and/or respiratory signs or symptoms should get a chest radiograph. Chest radiography results could be normal in those who have a minor illness at the beginning. Diffuse bilateral infiltrates that radiate from the perihilar region are present in the majority of PJP patients. Patchy asymmetrical infiltrates, pneumothorax, and pneumatoceles are unusual findings. Intrathoracic adenopathy and pleural effusions are quite uncommon. Patients receiving prophylactic pentamidine aerosol may develop pneumothorax or apical illness[33].

Computed Tomography

When the results of chest radiography are confusing, high resonance computed tomography (HRCT) of the chest may assist. In HIV patients, HRCT has a high sensitivity for PJP. The background of interlobular septal thickening combined with patches of ground-glass attenuation is indicative of PJP[1].

Treatment:

General considerations:

Based on clinical findings, characteristic radiography, and occasionally abnormal laboratory results, systemic antibiotic treatment is initiated promptly in patients who are liable for PJP. The first line of treatment is TMP-SMX. Pentamidine, dapsone (usually combined to pyrimethamine), or atovaquone are second-line medicines. Echinocandins like capsofungin, which are effective in inhibiting BDG from being incorporated into the fungal cell wall, may also be beneficial against *Pneumocystis*, however clinical data are insufficient. In circumstances where a patient is unresponsive, it might be utilized as a therapeutic option, but further studies are evidently needed[34].

Although cytological staining required for diagnosis, therapy should begin as soon as possible. In severe PJP patients, treatment can begin before the workup is completed. Following the initiation of treatment, histopathologic testing is utilized to confirm the diagnosis of PJP. After therapy is started, *P. jiroveci* remains in the host for days to weeks, giving time for the appropriate workup to be completed[1].

The severity of PJP is defined by the alveolar-arterial gradient and is classified as mild (35 mm Hg), moderate (35-45 mm Hg), or severe (>45 mm Hg). A partial pressure of 70 mm Hg of oxygen in room air is also suggestive of a serious illness[1].

In patients without HIV infection, response to medications should start in four to five days. The therapeutic response in HIV patients happens within the first 8 days. Treatment success should be evaluated at this point, and if clinical non-response is observed, an alternative regimen should be applied. The danger of developing respiratory impairment should prompt hospital admission for all individuals requiring corticosteroids[35].

Antibiotic therapy:

Antibiotics are used for the treatment of mild, moderate, or severe PJP. It has been demonstrated that TMP-SMX is more efficacious than alternative therapy choices and equally effective as intravenous pentamidine. Patients with serious illness or those experiencing gastrointestinal side effects are advised to choose the parenteral route[5, 36].

Pregnancy-related published standards stated that TMP-SMX is the recommended first therapy. Because of the possibility of hyperbilirubinemia and kernicterus, the patient neonatologist should be informed when this medication is used near to delivery. Clindamycin plus primaquine is expected to work better for infections resistant to TMP-SMX than intravenous pentamidine[37, 38].

Atovaquone is given twice daily orally as an alternative option, but It's contraindicated during pregnancy[1].

The recommended treatment period for PJP in HIV patients is 21 days, and 14 days in all other patients. Individuals with HIV require longer therapy durations due to their higher organism burden and slower response to treatment compared to non-infected individuals[1].

Adjunctive corticosteroid therapy:

Only HIV patients with severe PJP, which is characterized by an arterial-alveolar O2 gradient greater than 35 mm Hg or a room air arterial oxygen pressure less than 70 mm Hg, are treated with corticosteroids as an extra medication. Patients without HIV are not recommended to use adjuvant steroids[1]. Microbial degradation can cause a strong inflammatory reaction in

the lungs, which frequently worsens with the onset of therapy. Adjunctive corticosteroid therapy can improve oxygenation, minimize the inflammatory response, and lower the risk of respiratory failure. [39, 40]

Outpatient care:

Close medical monitoring after hospital release is required to monitor illness resolution and to implement prophylactic regimen. TMP-SMX given orally has demonstrated remarkable efficacy. Oral treatment is only considered in individuals with mild to moderate PJP who have consistent outpatient follow-up care[1].

Prevention:

Currently, there is no vaccine for PJP. Chemoprophylaxis is quite effective in avoiding PJP in HIV/AIDS patients and other high-risk immunocompromised individuals[7].

Smoking cessation:

Immunocompromised patients are highly recommended to stop smoking since, in addition to the typical harmful effects of tobacco use, smokers have a higher risk of developing PJP and require a more complicated treatment regimen[41].

Chemoprophylaxis in patients with HIV infection:

Two categories of outpatient chemoprophylactic guidelines exist. Immunocompromised HIV patients without a history of PJP are prescribed primary prophylaxis. For HIV patients who have had prior PJP, secondary prophylaxis is used[1].

The Infectious Disease Society of America and the US Public Health Service have released protocols for prophylactic PJP in adult and pediatric HIV patients. The following categories benefit from chemoprophylaxis[1]:

- Prophylaxis should be given to adults, adolescents, and pregnant patients with a CD4 count less than 200/ mm^3. Oropharyngeal candidiasis, an unexplained fever of more than 37.7° C for more than two weeks, or a prior episode of PJP are indications of chemoprophylaxis regardless of CD4 level.
- Children born to HIV-positive mothers should start TMP-SMX prophylaxis at 4-6 weeks, if HIV infection has not been excluded by two negative HIV DNA PCR tests (often one at birth and one at 4 weeks).
- All children with HIV should get prophylaxis for the first year of life, then as decided by age specific CD4 levels.

When four negative HIV DNA PCRs are performed on children born to HIV-positive mothers (one after four months) or a negative HIV antibody test is performed after six months, the child is considered HIV-uninfected and PJP prophylaxis can be stopped[1].

HIV patients whose CD4 count rises beyond 200/ mm^3 for three consecutive months during HAART may no longer get prophylaxis. Resuming prophylaxis is necessary if the CD4 count is less than 200/ mm^3. Prophylaxis should continue in patients who got PJP while their CD4 level was more than 200/ mm^3 for life[42].

Chemoprophylaxis in patients without HIV infection:

In contrast to individuals who have HIV infection, immunocompromised individuals without HIV infection do not have specific criteria for PJP prophylaxis. In the following patients, chemoprophylaxis should be taken into consideration[1]:

- Patients suffering from a primary immunological deficiency (for example, hypogammaglobulinemia or SCID)
- Patients who maintain a CD4 level below 200/ mm^3
- Recipients of solid organ transplants
- Patients undergoing hematopoietic stem cell transplantation (HSCT), with prophylaxis administered either (1) for a period of six months following engraftment or (2) for a period of more than six months following HSCT in patients who continue immunosuppressive medication (prednisone, cyclosporine, etc.) or who suffer from chronic graft *versus* host disease.
- Individuals receiving systemic corticosteroids on a daily basis
- Patients using immunosuppressive or cytotoxic drugs, such as cyclosporine or the purine analogs fludarabine or cladribine, as well as those with collagen vascular disorders, malignancy, or vasculitis.

Chemoprophylactic regimens:

For prophylaxis, TMP-SMX is the recommended medication unless there is a contraindication. For individuals who are intolerant to TMP-SMX, alternative options include pentamidine aerosol, atovaquone, dapsone, and dapsone plus pyrimethamine[2].

The recommended daily dosage for TMP-SMX is one double-strength tablet. Another useful medication is a single-strength tablet used once daily. Taking one tablet of double strength three times a week is an additional alternative. On the other hand, a daily dosage schedule offers further defense against bacterial infections and *Toxoplasma gondii* (*T. gondii*) infections[2].

Long-term prophylaxis with TMP-SMX showed no influence on the incidence of infection by drug-resistant bacteria like *Staphylococcus aureus* or *Streptococcus pneumoniae*. Certain strains of *P. jiroveci* have been found to be resistant to TMP-SMX. Sulfa medication resistance has been linked to certain point mutations in the dihydropteroate synthase (DHPS) gene. According to animal studies, approximately all TMP-SMX anti-*Pneumocystis* action is attributed to sulfamethoxazole. Sulfonamide resistance could lead to the failure of sulfamethoxazole as well as dapsone, a sulfone antibacterial drug used in treatment and prevention of PJP[43].

The recommended daily dosage of dapsone is 100 mg. Dapsone prevents *T. gondii* infections but not bacterial infections when combined with pyrimethamine. Atovaquone 1500 mg should be taken once daily, preferably with food. If the patient is unable to tolerate dapsone or TMP-SMX, this medication is an option due to its minimal toxicity profile. Atovaquone, however, is very costly[2].

Pentamidine aerosol compared to dapsone or TMP-SMX is better tolerated. But compared to other prophylactic treatments, it is more costly and troublesome. Coughing and bronchospasm are possible side effects. Extrapulmonary *P. jiroveci* symptoms and apical lung illness are possible. Furthermore, sputum induction and bronchoalveolar lavage diagnostic sensitivity have been demonstrated to be decreased by pentamidine aerosol[1].

RECOMMENDATIONS

The ongoing failure of *Pneumocystis* propagation has limited study on many aspects of this organism, such as immune evasion, medication resistance, life cycle, and antimicrobial susceptibility testing. Improved knowledge of the infection process and mode of transmission will probably drive the development of practical methods for managing PJP in immunocompromised individuals and providing clinical care. Additionally, as the WHO has recently recommended, it is critical to step up efforts to investigate the epidemiology and implications of genetic alterations associated with medication resistance in PJP in light of recent reports of growing resistance. The development of a protective vaccine for PJP is crucial in the upcoming days.

CONCLUSIONS

As a potentially fatal opportunistic illness, PJP not only costs a lot to treat, but also has a big impact on the health and well-being of people who have weakened immune systems all over the world. Compared to HIV/AIDS patients, PJP in non-HIV individuals is typically more severe and challenging to diagnose. To apply timely and efficient preventive and treatment measures, clinicians and other healthcare professionals need to be aware of and recognize various immunodeficient disorders as well as the risk factors for PJP at an early stage.

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