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Pneumocystis jiroveci **in Solid Organ Transplantation -** Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice

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Abstract

These updated guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation review the diagnosis, prevention and management of *Pneumocystis jiroveci* fungal infection transplant recipients. Pneumonia (PJP) may develop via airborne transmission or reactivation of prior infection. Nosocomial clusters of infection have been described among transplant recipients. PJP should not occur during prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX). Without prophylaxis, PJP risk is greatest in the first 6 months after organ transplantation but may develop later. Risk factors include low lymphocyte counts, cytomegalovirus infection (CMV), hypogammaglobulinemia, treated graft rejection or corticosteroids, and advancing patient age (> 65). Presentation typically includes fever, dyspnea with hypoxemia, and cough. Chest radiographic patterns generally reveal diffuse interstitial processes best seen by CT scans. Patients generally have $PO_2 < 60$ mmHg, elevated serum lactic dehydrogenase (LDH), and elevated serum $(1\rightarrow3)$ β-D-glucan assay. Specific diagnosis uses respiratory specimens with direct immunofluorescent staining; invasive procedures may be required. Quantitative PCR is a useful adjunct to diagnosis. TMP-SMX is the drug of choice for therapy; drug allergy should be documented before resorting to alternative therapies. Adjunctive corticosteroids may be useful early. Routine PJP prophylaxis is recommended for at least 6-12 months posttransplant, preferably with TMP-SMX.

Introduction

Pneumocystis jiroveci, formerly *P. carinii*, remains an important opportunistic fungal pathogen in immunocompromised patients including solid organ and stem cell recipients not receiving effective prophylaxis[1-4]. With prolonged survival and increasingly intense immunosuppressive regimens in transplantation, *Pneumocystis jiroveci* pneumonia (PJP) remains an important consideration in solid organ transplant recipients [5]. Major advances include improved diagnostic assays which are useful in the setting of atypical presentations or incompletely effective prophylaxis.

Etiology – The Organism

The cyst form of *Pneumocystis* was first described in 1909 by Chagas and again in 1910 by Carinii as a parasite in the life cycle of *Trypanosoma cruzi*. The organism was first associated with human disease in 1952 described as "plasma cell interstitial pneumonitis" among malnourished children and neonates[6]. The increasing incidence of PJP in patients receiving corticosteroids and chemotherapeutic drugs led to studies by the Centers for Disease Control (CDC) in the 1970's, but it was not until the 1980s when *P. carinii* gained global recognition as being the first disease-defining illness associated with AIDS, causing over one-fourth of community-acquired pneumonias in HIV-infected persons and more than 200,000 cases of PJP since 1979. The species causing infection in humans was renamed *P. jiroveci* after the Czech parasitologist Otto Jírovec to recognize host-specificity of the organism[7].

Based on conserved mRNA sequences *P. jiroveci* is identified with the taxonomic kingdom of fungi (*Rhizopoda, Myxomycetes, Zygomycota, Schizosaccharomyces, Neurospora, Candida,* and the red yeasts), rather than the parasitic kingdom of protozoa. In humans and animals, three forms of the organism have been identified: trophozoite, cyst, and sporozoite (or intracystic bodies). The trophozoite, 2 to 5 μ in diameter, is either round or sickle-shaped and contains a nucleus, mitochondria, and vacuoles. It includes pseudopodia and filopodia. The cyst usually measures between 3 and 6 μ in diameter. Its cell wall consists of three layers, and its cytoplasm contains up to eight small pleomorphic intracystic oval bodies called sporozoites. Two other cystic forms have been described, but these are probably intermediates including empty or developing cysts. Many small surface projections, or tubular expansions, form a branching network over the surfaces of the cysts and the trophozoites. In the alveolus, *Pneumocystis* are covered with glycoproteins derived from both the organism and the host. The organism produces a relatively limited array of surface glycoproteins; the organism adapts to the host with both shared and unique antigenic epitopes in animal- and human-derived organisms[8,9]. The cell wall contains cholesterol but no ergosterol and does not synthesize sterols de novo which accounts for the lack of susceptibility to the azole and polyene anti-fungal antibiotics. The surface of *P. jiroveci* is carbohydrate-rich with glucose, mannose, and β-1,3-glucan which may play roles in attachment to epithelial or surfactant layers, in phagocytosis of the organism, and as a diagnostic target. The life cycle of *Pneumocystis* is incompletely understood; human-derived organisms have been grown inconsistently in vitro. It seems that the sporozoites, or daughter forms, emerge from the cyst to develop into trophozoites. These trophozoites mature to form cysts and repeat the cycle. Both sexual and asexual intermediate stages have been postulated. It is likely that some differences exist in *Pneumocystis* growth in different hosts and with different immune defects.

Different strains of *Pneumocystis* have been demonstrated using pulsed-field gel electrophoresis and DNA sequencing; multiple strains may coexist in any individual. *P. jiroveci* expresses both unique and common antigens in different host species. Surface antigens have been characterized at the glycoprotein and molecular levels. The major surface glycoprotein (MSG) represents the main humoral immunogen in the rat model; although other antigens (gp45–55) also have importance in human infection. The MSG is a large family of related genes, many located in tandem repeated arrays in the subtelomeric regions and may contribute to the generation of the variety of antigenic types, possibly to evade human immune responses.

Epidemiology and risk factors

Some studies suggest that *Pneumocystis jiroveci* may exist in the environment[10] however no definitive environmental reservoir for human disease has been identified. Serologic studies suggest that exposure occurs commonly in childhood [11], though symptomatic disease is almost entirely limited to individuals with identified immune deficits. Animal models and studies in humans of pneumocystis suggest that disease may arise de novo via airborne transmission or from reactivation of previously established and/or undertreated infection[10,12-15]. Nosocomial outbreaks of infection, including molecular typing, have been described among heart, renal and hepatic transplant recipients [16-19], supporting interhuman transmission in the hospital environment with incubation periods of up to 150 days. Based on studies prior to the broad implementation of prophylaxis, the risk of developing infection among solid organ transplant recipients is approximately 5-15% [20]. Incidence varies with the organ transplanted, geography and center-specific approaches to prophylaxis and immunosuppression.

PJP should not occur in patients receiving effective prophylaxis with TMP-SMX[21,22]. Without prophylaxis, the risk of *Pneumocystis* pneumonia is greatest in the first 6 months after solid organ transplantation, most notably after lung transplantation, during periods of intensified immune suppression such as bolus corticosteroids or lymphocyte-depleting antibody therapies for treatment of graft rejection, and with corticosteroid therapy (3 to 6 months of 15-20 mg prednisone equivalent)[20,23]. Of note, 25% of patients develop PJP after 8 weeks or less of corticosteroid therapy[24,25]. In patients receiving lung or heart-lung transplants, asymptomatic isolation of *P. jiroveci* can exceed 10%[26]. Recent reports have shown that despite effective prophylaxis for 6-12 months post-transplantation, PJP may emerge beyond 12 months post-transplant[22]; risk factors include low total and CD4+ lymphocyte counts, cytomegalovirus infection (CMV), hypogammaglobulinemia, graft rejection, and patient age[23]. PJP has also complicated the syndrome of rapamycin lung, the

idiosyncratic syndrome of diffuse pulmonary infiltrates in solid organ transplant recipients receiving sirolimus-based immune suppression. PJP infection in persons not in these categories should suggest an excess overall net state of immunosuppression engendered by multiple factors (metabolic factors, neutrophil and lymphocyte counts and function, viral coinfection (CMV), underlying lung disease), exposure to infected persons, malignancy, or HIV infection. Table 1 outlines some well-described risk factors for PJP among non-HIV-infected individuals.

Clinical Manifestations

The symptomatic progression of PJP in HIV-negative patients can be quite variable but is classically more acute than in AIDS. In the setting of transplantation, symptoms often develop over the course of a few days up to 1 to 2 weeks, usually dominated by hypoxemia out of proportion to plain radiographic imaging. Corticosteroids, calcineurin inhibitors, and sirolimus may mask the signs and symptoms of PJP. The typical signs and symptoms are outlined in Table 2[27] and include fever, dyspnea in the majority often accompanied by nonproductive cough, but the latter is inconsistently present. In addition to more rapid onset, the resulting pneumonia and lung involvement is often more severe in HIV-negative PJP, with lower arterial-oxygen tension and more frequent respiratory failure[18,23].

Diagnostic Strategies

The Chest Radiograph

No radiographic pattern is pathognomonic for *Pneumocystis* infection. PJP is a diffuse interstitial processes on chest radiograph that presents with hypoxemia and fever, but without significant sputum production[28]. The radiographic pattern ultimately depends on the patient's underlying or accompanying disease, state of immunosuppression, and duration of infection. Early PJP is manifested by fine, bilateral, perihilar, diffuse infiltrates that progress to an interstitial alveolar butterfly pattern; from the hilar region, the infiltrates often spread to the apices or bases. This pattern often progresses despite therapy with progressive consolidation over 3 to 5 days. Unusual patterns are common including nodules, unilateral infiltrates, pleural effusions, pneumothoraces, lymphadenopathy, or lobar consolidations. *P. jiroveci* can superinfect fungal or mycobacterial cavities. Breakthrough disease in patients receiving aerosolized pentamidine classically presents largely or solely in the upper lobes on chest radiograph. In lung transplant patients, graft rejection, infection (PJP, CMV), and combinations of these events can produce similar chest radiographs. Chest computed tomography (CT) often demonstrate abnormalities not appreciated on routine chest

radiography and should be obtained, notably in the face of normal plain chest radiographs with a consistent clinical presentation[29,30]. Nuclear medicine scans are nonspecific and add little to the diagnosis of pulmonary pneumocystosis except a normal scan will generally exclude pulmonary infection due to *Pneumocystis*. Abnormal PET scan images may be observed early in disease.

Laboratory Evaluation

Nonspecific indicators of pulmonary processes are useful in the presumptive diagnosis of PJP (Table 3). In general, the patient will have a PO_2 less than 60 mmHg and a respiratory alkalosis. The serum lactic dehydrogenase (LDH) enzyme will be elevated in almost all cases of PJP (over 300 IU/ml)[31]. The marked hypoxemia of PJP is accompanied by an alveolar-arterial PAO_2–PaO_2 gradient rise; gradients over 30 mmHg at the start of therapy are associated with a high mortality (and may be an indication for use of adjunctive corticosteroid therapy based on studies in HIV infected individuals with PJP[32]). Both LDH and the arterial oxygenation gradient will return to normal with successful therapy. The angiotensin-converting enzyme (ACE) level (also non-specific) can be detected in the serum of patients with PJP. The serum (1→3) β-D-glucan assay carries a high sensitivity (>90%) with lower specificity (less than 80%) and poor tracking with disease resolution[33-37]. However, the (1→3) β-D-glucan assay has a high negative predictive value. Serologic testing may be complicated by inadequate antibody production in many immunosuppressed patients and low sensitivity in acute disease.

Microbiology

A definitive diagnosis of PJP is made by demonstration of organisms in lung tissue or respiratory tract secretions (Table 3)[38,39]. Acceptable samples include specimens obtained through bronchial alveolar lavage (BAL), sputum (spontaneous or induced) and transbronchial or open lung biopsy. Induced sputum may be collected after 20 to 30 minutes of exposure to aerosolized hypertonic saline or water, or after oral hydration. Smears are prepared from the mucoid, nonpurulent portion of the specimen. Toluidine blue O or Gomori methenamine-silver stain only the cyst wall which represent 5-10% of the organism burden. Giemsa, Wright's or Diff-Quik stains detect intracystic bodies, or sporozoites, and trophozoites, the most common form of the organism in the alveolus. Calcofluor white and silver stains are most predictive for routine use when monoclonal antibody staining is not available[38].

Specimen choice considerations include diagnostic sensitivity and the invasiveness of diagnostic procedures. The sensitivity of induced sputum is only 30-55%, compared with 80-95% with BAL[18,23]. An induced sputum examination coupled with direct immunofluorescent staining for *P. jiroveci* is preferred as an initial step, followed by more invasive testing. In children not able to undergo induced sputum examination, BAL should be performed. Because the organism burden is lower in the non-HIV infected host with PJP, sputum may be less revealing and bronchoscopy with BAL may have a higher yield. Lavage should be performed from the upper lobes if diffuse disease is present. Invasive diagnosis should be considered in transplant recipients with pneumonia without a microbiological diagnosis (Table 3). BAL (with biopsies if possible), radiologically guided needle aspiration (for accessible cystic or mass lesions), or open lung biopsy may be required. The choice of the specific test depends on the clinical condition of the patient and the expertise available at the institution (Table 3). A negative smear from any single respiratory specimen cannot be used to exclude PJP.

Early nucleic acid amplification (NAT) techniques increased the sensitivity of *Pneumocystis* detection with some loss in specificity, limiting the ability to distinguish between asymptomatic colonization and infection. Increasingly, paradigms are used with real-time quantitative PCR with BAL samples (better than induced specimens), often with other modalities including the (1→3) β-D-glucan assay [40-42]. In general, patients with low LDH or low serum (1→3) β-D-glucan levels are unlikely to have PJP. Conversely, transplant patients with compatible syndromes and positive, validated NAT assays are likely to have PJP[1,43-45]. Positive β-D-glucan assays do not exclude other fungal infections. These assays are not well studied in solid organ recipients.

NAT has been used to demonstrate the presence of multiple *Pneumocystis* strains in infected individuals and in investigation of outbreaks of PJP including among organ transplant recipients [9,43,46],[13,16,17,47] NAT assays may be adapted to the detection of dihydropteroate synthase mutations; however, this is rarely important clinically given clinical response to full dose TMP-SMX even in the presence of apparent resistance and DHPS mutations [48]. Use of NAT with blood samples has not yet been validated.

Histopathology and Invasive Diagnosis

The histopathology of *Pneumocystis*-infected lung is distinctive. The airspaces are filled with a foamy eosinophilic exudate and appear honeycombed; the intra-alveolar exudate consists of organisms, large amounts of surface glycoprotein, proteinaceous exudate from the lungs, and debris of macrophages and inflammatory cells. At the same time, the alveolar interstitium is infiltrated by

polymorphonuclear leukocytes and lymphocytes. Patchiness in the distribution of disease within the lungs is common.

Attempts to avoid the use of invasive procedures by resorting to empiric therapy risks inappropriate medications and undesirable side effects, missing coinfections, and delaying effective therapy.

- Radiographs alone should not be used to make a diagnosis since no radiographic pattern is pathognomonic for *Pneumocystis* infection and unusual patterns are common. (strong, high). Radiography is often complicated by other processes
- Nonspecific indicators are useful including hypoxia, elevated serum lactic dehydrogenase (LDH), and elevated serum (1→3) β-D-glucan assay, which carry a high negative predictive value (strong , low).
- Nucleic acid amplification (NAT) assays may be useful adjuncts to diagnosis but specificity for disease remains challenging. Transplant patients with compatible syndromes and positive, validated NAT assays are likely to have PJP (strong, low)
- A definitive diagnosis of PJP is made by demonstration of organisms in lung tissue or respiratory tract secretions
- Initial diagnosis should be attempted using induced sputum examination coupled with direct immunofluorescent staining for *P. jiroveci* (strong, moderate).
- Invasive diagnosis should be considered if induced sputum not feasible (as is the case in younger children) or unrevealing and in transplant recipients with pneumonia without a microbiological diagnosis (strong , moderate).

Treatment

For the established or presumed diagnosis of PJP, therapeutic options are outlined in Table 4. Practice recommendations regarding the treatment of PJP in transplant recipients include the use of TMP-SMX as the first line agent and drug of choice. No agent has been shown to have better outcomes than TMP-SMX. In severe infections, intravenous pentamidine probably remains the second-line agent after TMP-SMX. Pentamidine therapy can be complicated by numerous toxicities including pancreatitis, hypo- and hyperglycemia, bone marrow suppression, renal failure and electrolyte disturbances (Table 4). Most experts recommend avoiding it in pancreas transplant recipients because of the potential for islet cell necrosis. Echinocandins are effective against only the cyst form of the organism. While adjunctive corticosteroids show a survival advantage in HIV infected individuals, the studies in non-HIV PJP infections are contradictory[22,49,50]. However, given poor outcomes, in patients with hypoxemia (pAO$_2$ < 70mmHg on room air), use of adjunctive corticosteroids remains controversial but are recommended regardless of the antimicrobial agent selected. Corticosteroid therapy should be considered early for maximum benefit, ideally within 72 hours of initiating antimicrobial therapy. The optimal dose of corticosteroids has not been

established, but 40-60 mg of prednisone (or equivalent) in adults given two to three times daily and 1mg/kg twice daily in children for 5-7 days before gradual tapering over 7-14 days is recommended to avoid rebound pneumonitis. The duration of antimicrobial therapy has not been fully established in this this population, however should be at least 14 days, though longer courses are often required based on response to therapy, severity of disease, and studies in HIV infected individuals who have a greater risk of relapse with 14 versus 21 days of therapy despite low infectious burdens[27]. Thus, unless the clinical presentation and initial work support mild disease, 21 days is preferred to avoid disease progression and relapse. Adjunctive measures such as colony stimulating factors (G- or GM-CSF) and reduction of immunosuppression have not been subjected to clinical trials.

Coinfection with other pathogens is a consideration in the setting of poor response to therapy or atypical presentations. Simultaneous cytomegalovirus (CMV) pneumonitis may necessitate antiviral therapy; CMV is generally detected by blood studies and cannot be diagnosed based on the presence of CMV in pulmonary secretions alone [5],[2].

- Trimethoprim-sulfamethoxazole (TMP-SMX) is the first line therapeutic agent and drug of choice for documented PJP (strong, high).
- Alternative agents are less effective and include intravenous pentamidine isethionate, atovaquone, primaquine and clindamycin (strong, high).
- Pentamidine therapy may cause pancreatitis, hypo- and hyperglycemia, and electrolyte disturbances and should generally be avoided in pancreas recipients. (strong, moderate)
- Adjunctive corticosteroids are best administered within 72 hours of presentation in the setting of hypoxia (pAO_2 < 70mmHg) (strong , low).
- The duration of antimicrobial therapy should be at least 14 days; longer courses are often required (strong, low).

Prevention

Routine anti-*Pneumocystis* prophylaxis is generally recommended at most transplant centers. Historically, an incidence of at least 3-5% of PJP among transplant recipients was considered a threshold for using prophylaxis [51]. Now with widespread use of prophylaxis and changing immunosuppressant regimens, the incidence post-transplant is uncertain but appears to range from 0.3 to 2.5%[23,52,53]. For those who have risk factors such as the intensive immunosuppression for graft rejection, infection with CMV, higher-dose corticosteroid therapy (e.g. >20 mg daily of prednisone for at least 2 weeks), prolonged neutropenia, or flares of autoimmune disease, continued or

reinstituted prophylaxis is generally indicated for periods of increased susceptibility. Lung transplant recipients are considered at high risk for PJP[2]. In any transplant population, the risk is considered highest within the first 6 months post-transplant, though features outlined above may prolong or shift that risk as for late onset disease. For patients receiving immunosuppressive drugs or corticosteroids pre-transplant (as in the case of certain autoimmune diseases), the risk for PJP may be acute after transplant, occurring in the first few weeks rather than after 1 month.

In general, anti-*Pneumocystis* prophylaxis is recommended for all solid organ transplant recipients for at least 6-12 months posttransplant[51,54,55]. For lung and small bowel transplant recipients, as well as any transplant patient with a history of prior PJP infection, lifelong prophylaxis is often indicated. Lifelong prophylaxis is recommended by some experts in the setting of heart and liver transplantation as well, depending on perceived overall risk and intensity of immunosuppression [51,55,56]. Agents used for prophylaxis are outlined in Table 5.

Practice recommendations regarding prophylaxis would include TMP-SMX as the drug of choice for prophylaxis of PJP. All other prophylactic agents should be considered second line agents due to drug intolerances, cost, and efficacy issues that are not as favorable overall compared to TMP-SMX. TMP-SMX also has the potential advantage of being effective at preventing other opportunistic pathogens after transplantation. The most commonly cited example is *Toxoplasma gondii*, though TMP-SMX may also have a role in preventing some community-acquired respiratory pathogens, gastrointestinal infections, and some urinary tract pathogens.

The side effects of TMP-SMX require monitoring. Bone marrow suppression may occur and be potentiated by concomitant administration of other myelosuppressive agents. Rash can occur from benign reactions to Stevens-Johnson syndrome. Other potential effects include hepatitis, interstitial nephritis, aseptic meningitis, and pancreatitis. Trimethoprim has the capacity to inhibit potassium and creatinine secretion resulting in hyperkalemia, with elevation of serum creatinine that does not necessarily reflect true renal function.

Dapsone is often used as a second-line agent for PJP prophylaxis. Some reports of daily dapsone use have included it in combination with pyrimethamine at 25-50 mg once weekly, which may also be an effective agent at prophylaxis for *T. gondii* [57], thus should be used in combination if prophylaxis for both organisms is desired. Monotherapy with dapsone may be sufficient for PJP prophylaxis. Although it may be tolerated in transplant patients who cannot receive TMP-SMX, it is generally not recommended in those who have documented severe side effects with TMP-SMX including desquamation, neutropenia, severe nephritis or hepatitis, or in cases of documented

glucose-6-phosphate dehydrogenase (G6PD) deficiencies. The most commonly associated side effects of dapsone include hemolytic anemias and methemoglobinemia. Classically these symptoms are associated with G6PD enzyme deficiency, though G6PD deficiency is not a prerequisite. These side effects are often unrecognized [58]. Thus, periodic screening including complete blood count may be beneficial.

Atovaquone is well-studied in the HIV population and has also been studied in small prospective trials of adult solid organ transplant recipients at a dose of 1500 mg orally once daily[51]. Available only in suspension, atovaquone acts by inhibiting mitochondrial electron transport in susceptible *Pneumocystis*. Absorption is enhanced by fatty foods and decreased in the setting of diarrhea. Rash and gastrointestinal complaints are the most common side effects. Increased hepatic transaminases are rarely noted. Although ideal dosing may be unclear, breakthrough infections have been well-documented in patients taking 1000 mg or less daily [5,51] and a recent failure has been reported due to resistance[59]. Atovaquone may also have activity against the bradyzoites of toxoplasmosis, like TMP-SMX and dapsone.

Infection control issues

Airborne transmission of *Pneumocystis jiroveci* is likely based on animal studies while a reservoir in nature remains elusive. Some experts have suggested the possibility of person-to-person spread[60]. If so, asymptomatic immunocompetent carriers of *Pneumocystis* or infected immunocompromised individuals could serve as a reservoir[61]. While uncommon, nosocomial clusters of infection have been described among renal transplant recipients at individual care centers [10,13,14]. Older studies demonstrated *Pneumocystis* in air samples from hospital patient care rooms using polymerase chain reaction (PCR) techniques [62,63]. Molecular genotyping studies in outbreaks demonstrate limited numbers of strains, strengthening the argument for nosocomial acquisition; comparisons with community-acquired strains are lacking[17,64,65]. Although these data support the role of transmission of PJP in the nosocomial setting, the role of environmental contamination vs. direct person-to-person spread remains unclear. These events have led some authors to recommend strict hospital segregation of immunocompromised patients with PJP and the use of facemask filtering to prevent transmission among infected individuals [13]. It should be noted that use of effective prophylaxis should prevent infection; without more definitive data, formal recommendations regarding infection control in the hospital cannot be made.

- Anti-*Pneumocystis* prophylaxis is recommended for all solid organ transplant recipients for at least 6-12 months posttransplant (strong, moderate) and for programs with an incidence of at least 3-5% of PJP among transplant recipients (strong, moderate).
- For lung and small bowel transplant recipients, with higher intensity of immunosuppression, or with history of prior PJP infection or chronic CMV infections, prolonged prophylaxis may be indicated (strong, low).
- Trimethoprim-sulfamethoxazole (TMP-SMX) is the drug of choice for prophylaxis of PJP (strong, high).
- Outbreaks of *Pneumocystis* may occur in nosocomial settings, possibly due to person-to-person spread.

Future Directions and Research

The environmental reservoir for *Pneumocystis* remains unknown. This has hindered epidemiologic studies which have been advanced by genome analysis in outbreaks and in various geographic regions [9,66-69]. The mechanisms underlying adaptation of the organism to various hosts remain to be elucidated and may provide new targets for prophylaxis and therapy [9,70-72]. In addition, studies better defining high risk periods and the potential need for prolonged or reinstitution of prophylaxis are needed especially in light of several reports of late on-set disease[22]. New, more rapid diagnostic assays for invasive disease are required to advance prevention and epidemiologic investigations. Enhanced efforts to understand the immune response to Pneumocystis are warranted and will likely assist in further defining high risk populations and to provide effective vaccines which are currently only in the early phases of development[73].

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Table 1. Risk Factors for the Development of *Pneumocystis* pneumonia

Risk Factor	Comments
Immunosuppressive therapies	
Corticosteroids	Retrospective case series on non-HIV-infected patients with PJP have identified corticosteroids as a common feature in up to 90%. The median dose and duration of therapy in one series equivalent to 30 mg/day of prednisone for 12 weeks [24,25].
Chemotherapy	PJP reported with numerous chemotherapeutic agents including methotrexate, fluorouracil, and bleomycin. Risk for infection related to intensity and duration of neutropenia [5]. Purine analogs, fludarabine and cladribine, and the anti-metabolite cytarabine are independent risk factors for PJP[74,75].
Antibody therapies	Antilymphocyte antibodies for graft rejection or in induction are linked to the highest risk of PJP being in the 1 to 6 month posttransplant time period [76]. Alemtuzumab may confer the highest risk for PJP of antibody therapies [77].
Mycophenolate mofetil	Mycophenolate may have anti-*Pneumocystis* effects in animal models and uncontrolled human data, leading to theories about it being protective [78,79]; definitive data are lacking.
Calcineurin inhibitors	Limited data suggest greater risk with cyclosporine A compared to azathioprine in renal transplantation[80]. Retrospective study with higher incidence of PJP among renal transplant recipients on tacrolimus-based regimens compared to cyclosporine A[3].
Sirolimus	Sirolimus associated with a clinical syndrome of interstitial pneumonitis that may be confused with PJP[81].
Other clinical factors	
CMV disease	Systemic immunosuppressive effects of CMV is an independent risk factor for PJP [51]; CMV and PJP coinfection well-reported [23,82,83].
Allograft rejection	PJP has been related to the degree and intensity of immunosuppression in

	transplant recipients [3], and directly linked to treatment for and number of episodes of acute rejection [83].
GVHD	Patients greater than 6 months out from HSCT are more likely to develop PJP when still being maintained on immunosuppressant therapies for ongoing GVHD [84].
Low CD4+ T cell counts	In HIV infection, the risk for PJP is directly linked to the fall of CD4+T cell counts to <200 cells/mL, or <20% of the total circulating lymphocytes[85]. 73% of PJP diagnosis in SOT recipients occurred in patients with CD 4+ T cell counts of < 200 cells/ml and associated with absolute lymphocyte count < 500×10^6 cells [86,87]. Lymphopenia and decreased CD4+T cell counts is a risk factor for PJP in HSCT recipients [88], solid tumor patients receiving chemotherapy [89], and autoimmune disease and hematological malignancy[90]. Low CD4+ T cell counts may reflect other processes such as viral coinfection or exogenous immunosuppression[23,51].
Neutropenia	Prolonged neutropenia has been suggested as a potential risk factor for the development of PJP in transplant recipients [51].

HIV=Human immunodeficiency virus, PJP=*Pneumocystis* pneumonia, CMV=Cytomegalovirus,

HSCT=Hematopoietic stem cell transplant, GVHD=Graft vs. host disease

Table 2. Signs and Symptoms of *Pneumocystis* pneumonia

Sign or Symptom of PJP	Incidence
Fever	81-87%
Dyspnea	66-68%
Cough	71-81%
Chest pain	23-24%
Abnormal lung auscultation on exam	30-34%
Abnormal chest radiography	92-96%
Hypoxemia	78-91%

Table 3. Recommended diagnostic approach to PJP in patients with hematological

malignancies, stem cell transplant and solid organ transplant recipients*

SPECIMEN/TECHNIQUE	RECOMMENDED USAGE	STRENGTH OF RECOMMENDATION	QUALITY OF EVIDENCE
Diagnostic specimen			
Bronchoalveolar Lavage	Allows detection of multiple etiologies; yield $\geq$80% [43,91]	Strong	High
Transbronchial biopsy	Increases yield of BAL, other lung pathology	Strong	Moderate
Open Lung biopsy or video-assisted thorocoscopy (VATS)	Gold standard for diagnosis, generally not required[92,93 94]	Strong	Low
Induced Sputum	Alternative specimen to BAL, yield $\geq$50% [94,95]	Strong	High
Other Respiratory specimens[a]	Not a good alternative, low organism burden [96].	Strong	Low
Diagnostic technique			
Immunofluorescence assays	Most sensitive microscopic diagnostic method; increased yield over other stains	Strong	High
Real-time quantitative PCR, nucleic acid testing	Quantification in BAL; cannot distinguish infection from carriage [40,46,97]	Strong	Low
Silver, polychrome, or calcofluor stains	Exclusion of PJP by negative BAL only	Strong	High
Serum			
Lactic dehydrogenase (LDH)	Not specific, generally positive in PJP[98]	Weak	Low
β-D-glucan	Not specific, useful as adjunctive diagnostic tool; β-D-Glucan is component of *P.*	Weak	Moderate

	jiroveci cell wall [40,98]		
Genotyping, sequencing	Investigation of suspected outbreaks	Strong	Low
Dihydropteroate synthase mutations	Not recommended	Weak	Low

[a]Includes sputa and upper respiratory samples (nasopharyngeal aspirates, nasal or oral washes).

*PJP diagnosis should not rely solely on clinical criteria or imaging.

Table 4. Therapeutic options for treating *Pneumocystis* pneumonia

Agents	Dosing	Comments
Trimethoprim-sulfamethoxazole (TMP-SMX)	Adults/Adolescents:15-20 mg/kg/day of the TMP component given IV in divided doses every 6-8 hours; lower doses may be sufficient. In milder disease, two double-strength tablets can be given po tid Children: >2 mo 3.75 -5 mg/kg/dose of the TMP component and 19 to 25 mg/kg/dose of the SMX component given IV every 6 hours. In milder disease the same daily dosing can be given po tid-qid	TMP-SMX remains the **drug of choice;** most effective systemic therapy for PJP. Correct for renal function and maintain hydration. May consider adjunctive corticosteroids (below). (strong recommendation, moderate evidence).
Pentamidine	All ages: 4 mg/kg/day IV initially with dose reduction to 2-3 mg/kg/day if needed	Infusions given over 1-2 hour period once daily; prolonged half-life may complicate amelioration of side effects after cessation of therapy; side effects include pancreatitis, hypoglycemia, hyperglycemia, bone marrow suppression, renal failure and electrolyte disturbances. Avoid in setting of pancreas transplant. (strong recommendation, moderate evidence).
Atovaquone	Adults/Adolescents: 750 mg po bid (higher doses to 1500 mg bid commonly used) Children: 1 -3 mo and 24 mo-12 y: 30–40 mg/kg (max 1500 mg), orally, daily or divided into 2 daily doses. 4 -24 mo: 45 mg/kg (maximum 1500 mg), orally, daily or divided into 2 daily doses.	Atovaquone available in oral suspension only with variable oral absorption and tested only in mild and moderate PJP infection. (strong recommendation, low evidence).
Primaquine and clindamycin	Primaquine 15-30 mg po qd in combination with clindamycin 600-900	This combination tested in patients with mild to moderate PJP infections in AIDS.

	mg IV or po q6-8 hours	Long-term use of clindamycin can increase infection by *Clostridium difficile*. Primaquine should be avoided in the setting of G6PD deficiency.
(not studied adequately in children to recommend)		(strong recommendation, moderate evidence).
Dapsone and trimethoprim (not studied adequately in children to recommend)	Dapsone 100 mg po qd used in combination with trimethoprim 15 mg/kg/day po divided tid Children: dapsone: 2 mg/kg/dose po qd with TMP 5 mg/kg /day/dose po tid	Combination has been used in some patients with sulfa allergies; dapsone may elicit allergic reactions in sulfa intolerant. Check G-6-PD deficiency. (weak recommendation, low evidence).
Pyrimethamine and sulfadiazine (not studied adequately in children to recommend)	Pyrimethamine load of 100-200 mg po, followed by 50-100 mg po qd in combination with sulfadiazine 4 g po qd in divided doses	Limited data available; supplement with folinic acid 10mg po qd to reduce toxicity. (weak recommendation, low evidence).
Macrolide and SMX	Macrolides such as clarithromycin or azithromycin in combination with sulfamethoxazole may be synergistic in vivo [99]	Few data to support this combination. (weak recommendation, low evidence).
Caspofungin and TMP-SMX	Adults/Adolescents: 70 mg IV loading dose of caspofungin on day 1, followed by 50 mg IV qd after in combination with TMP-SMX (dose reduced in the setting of moderate to severe hepatic dysfunction) Children: caspofungin: 0- <3mo : 25 mg/m2 IV qd: 3mo-17y: 70 mg/m2 on day 1 (max: 70 mg/dose); followed by 50 mg/m2 (max: 50 mg/dose) IV qd in combination with TMP-SMX (dose reduced in the setting of moderate to severe hepatic dysfunction)	Echinocandins have activity against *Pneumocystis* in animal models [100,101]. Case reports in combination with TMP-SMX for PJP in solid organ and bone marrow transplantation [102,103]. Clinical efficacy compared to TMP-SMX unknown. (weak recommendation, low evidence).
Trimetrexate with folinic acid (*no longer available*)	Trimetrexate 45 mg/m^2/day IV (or 1.5 mg/kg/day IV in patients <50 kg) administered concomitantly with folinic acid 20 mg/m^2 po or IV every 6 hours (80 mg/m^2 total daily); Folinic acid	Severe bone marrow suppression without folinic acid supplementation; inferior outcomes compared to TMP-SMX.

	therapy should extend for at least 3 days beyond cessation of trimetrexate	
Adjunctive agents		
Corticosteroids	Adolescents and Adults: 40 mg-60 mg of prednisone (or equivalent) po/iv bid-tid with taper after 5-7 days over a period of 1-2 weeks Children: 1mg/kg po bid for 5 days, then 0.5mg/kg po bid for 5 days, then 0.5mg/kg po qd for 10 days	Corticosteroids are best administered within 72 hours of patient presentation in the setting of hypoxia (pAO$_2$ < 70mmHg). (strong recommendation, low evidence).
Colony-stimulating factors	Standard dosing	Use of GM-CSF as an adjuvant has been tried in animal models of PJP with some success [104]. No clinical data in humans are available.

TMP-SMX=Trimethoprim-sulfamethoxazole, PJP=*Pneumocystis* pneumonia, AIDS=Acquired immunodeficiency syndrome, G6PD=Glucose-6-phosphate dehydrogenase, GM-CSF=Granulocyte/macrophage colony stimulating factor, po=orally, qd=daily, bid= twice a day, tid= three times a day; qid=four times a day

Table 5. Specific prophylactic agents for prevention of *Pneumocystis*

Agents	Dosing	Comments
Trimethoprim-sulfamethoxazole (TMP-SMX, cotrimoxazole)	Adults/Adolescents: Can be given as either 80 mg TMP/400 mg SMX (single strength) or 160 mg TMP/800 mg SMX (double strength) po (double strength) either daily or three times weekly Children: trimethoprim, 5–10 mg/kg and sulfamethoxazole, 25–50 mg/kg orally (max dose 320 mg TMP and 1600 mg SMX) given once daily 7 days a week or daily dose divided and given twice daily twice or three times weekly	TMP-SMX remains the **drug of choice** for PJP prophylaxis [57]. (strong recommendation, high evidence).
Dapsone	Adults/Adolescents 50-100 mg po qd Children: 2 mg/kg (max 100 mg), orally, once daily or 4 mg/kg (max 200 mg), orally, every week	Dapsone is a second-line agent for prophylaxis in HIV-infected patients [105]. The hematologic side effects of dapsone underappreciated in SOT[58]. (weak recommendation, low evidence).
Atovaquone	Adults/Adolescents 1500 orally, daily Children: 1 -3 mo and 24 mo-12 y: 30–40 mg/kg (max 1500 mg), orally, daily 4 -24 mo: 45 mg/kg (maximum 1500 mg), orally, daily	In HIV patients intolerant of TMP-SMX, atovaquone was equal to dapsone in preventing PJP [106]. Data in SOT recipients show it well-tolerated [51]. Failures of atovaquone reported at doses of 1000 mg or less daily [51,107]. (strong recommendation, moderate evidence).
Pentamidine	All ages: 300 mg administered through aerosolized nebulizer q 3-4 weeks	Aerosolized pentamidine administration requires experienced personnel with nebulizer for droplets 1-3 μ. Side effects include cough and bronchospasm. Higher incidence of breakthrough infection compared to TMP-SMX and dapsone. (strong recommendation, moderate evidence).
Clindamycin and	Up to 300 mg of clindamycin po qd with 15 mg of pyrimethamine	Prophylaxis somewhat efficacious in AIDS patients, though clearly less effective than TMP-SMX or

pyrimethamine	po qd (some clinicians have administered this regimen 3 times weekly instead of daily)	dapsone comparitors [108]. Failure rate also higher than for aerosolized pentamidine. Gastrointestinal intolerance may be limiting.
(not studied adequately in children to recommend)		(weak recommendation, low evidence).