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SUPPLEMENT ARTICLE

Pneumocystis jirovecii Disease: Basis for the Revised EORTC/MSGERC Invasive Fungal Disease Definitions in Individuals Without Human Immunodeficiency Virus

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Background. *Pneumocystis jirovecii* pneumonia (PCP) causes substantive morbidity in immunocompromised patients. The EORTC/MSGERC convened an expert group to elaborate consensus definitions for *Pneumocystis* disease for the purpose of interventional clinical trials and epidemiological studies and evaluation of diagnostic tests.

Methods. Definitions were based on the triad of host factors, clinical-radiologic features, and mycologic tests with categorization into probable and proven *Pneumocystis* disease, and to be applicable to immunocompromised adults and children without human immunodeficiency virus (HIV). Definitions were formulated and their criteria debated and adjusted after public consultation. The definitions were published within the 2019 update of the EORTC/MSGERC Consensus Definitions of Invasive Fungal Disease. Here we detail the scientific rationale behind the disease definitions.

Results. The diagnosis of proven PCP is based on clinical and radiologic criteria plus demonstration of *P. jirovecii* by microscopy using conventional or immunofluorescence staining in tissue or respiratory tract specimens. Probable PCP is defined by the presence of appropriate host factors and clinical-radiologic criteria, plus amplification of *P. jirovecii* DNA by quantitative real-time polymerase chain reaction (PCR) in respiratory specimens and/or detection of β-D-glucan in serum provided that another invasive fungal disease and a false-positive result can be ruled out. Extrapulmonary *Pneumocystis* disease requires demonstration of the organism in affected tissue by microscopy and, preferably, PCR.

Conclusions. These updated definitions of *Pneumocystis* diseases should prove applicable in clinical, diagnostic, and epidemiologic research in a broad range of immunocompromised patients without HIV.

Keywords. cancer; transplantation; definitions; *Pneumocystis*; clinical trials; consensus; adults; children.

The EORTC/MSGERC consensus definitions of invasive fungal diseases (IFDs) published in 2002 [1] and updated in 2008 [2] have evolved into essential documents for research in clinical mycology. The definitions have fostered comparison of clinical research in patients with cancer and solid-organ and hematopoietic stem cell transplantation (HSCT) [3, 4]; they have been adopted by regulatory agencies for antifungal agents [4–7] and used to evaluate diagnostic tests [8] and for epidemiologic studies [9–12]. As such, they are specifically intended for research only, and not to direct patient care.

The 2008 revised definitions had their limitations, including poor applicability to patients treated in intensive care units (ICUs), lack of thresholds of positivity, and validation of, fungal biomarkers, and a focus on opportunistic mold infections [2]. Notably, no definitions were provided for diseases caused by *Pneumocystis jirovecii* including life-threatening pneumonia (PCP). *Pneumocystis jirovecii* pneumonia is particularly relevant in patients with profound impairment of T-cell–mediated immunity.

To overcome this limitation in a time of evolving anticancer immunotherapies, change in composition of immunocompromised patient populations, and new diagnostic tools, the EORTC/MSGERC established definitions for *P. jirovecii* disease in their second revision of IFD definitions [13]. The definitions were based on the established triad of host factors, clinical features, and mycologic tests with categorization into probable and proven disease, and were applicable to immunocompromised adults and children without human immunodeficiency virus

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(HIV). Here we present the scientific rationale behind these disease classifications.

METHODS

The *Pneumocystis* subcommittee of the EORTC/MSGERC was asked to restrict their purview to immunocompromised individuals without HIV [13]. This restriction was based on important differences in the biology and presentation of *Pneumocystis* disease between patients with advanced HIV infection and immunocompromised patients without HIV with attendant impact on applicability of diagnostic criteria [14–19].

Under the senior author (A. H. G.), host factors and diagnostic clinical and microbiologic criteria of *Pneumocystis* disease were evaluated through systematic literature review. Medical subject heading (MESH) terms were used as keywords to search articles published in English in PubMed. Host factors and clinical and microbiologic criteria were formulated and adapted after discussion within the group. The process for review and formulation of consensus is detailed in the 2019 revised IFD definitions [13].

DIAGNOSTIC CRITERIA

Host Factors

Pneumocystis jirovecii is a transient fungal colonizer of human pulmonary alveoli [20–22]. Although not completely elucidated, the mode of acquisition of infection likely occurs by the airborne route and person-to-person spread [23]. Seroepidemiologic studies suggest primary contact with the organism occurs in infancy [24, 25], with asymptomatic or mild upper respiratory tract infection [26]. There is no molecular evidence for a truly latent infection, and disease is believed to arise from prior colonization or by new infection [26, 27].

In immunocompromised patients, the organism may proliferate and cause lung disease through interaction with type I alveolar cells. Extrapulmonary manifestations are rare [28] and may be associated with atypical forms of *P. jirovecii* [29]. Pulmonary disease or PCP is typically diffuse with alveolar damage, an eosinophilic intra-alveolar foamy matrix, and interstitial inflammatory response, resulting in restrictive pulmonary disease, progressive hypoxemia, and death if untreated [26, 30]. Alveolar macrophages are the central effector cells in host defense against *P. jirovecii*, and PCP is exclusively associated with qualitative or quantitative impairment of T-cellular immunity [31–33].

Apart from institutionalized neonates with functional immaturity of cellular immunity, the principal populations at risk include children with primary T-cell immunodeficiencies, patients with very low CD4+ lymphocyte counts (eg, those undergoing intensive immunosuppressive therapy with glucocorticosteroids and other agents affecting CD4+ lymphocyte counts), and patients undergoing chemotherapy for cancer, or solid-organ transplantation or allogeneic HSCT prior to immune-reconstitution [26, 30, 34–36]. Historical incidence rates of PCP in immunocompromised patients without HIV not receiving prophylaxis are more than 20% for children with acute lymphoblastic leukemia [37], non-Hodgkin's lymphoma [38], or soft tissue sarcoma [39]; and for adults, rates are 20–30% in non-Hodgkin's lymphoma [40, 41], 5–15% in allogeneic HSCT recipients [42], 5–15% in solid-organ transplant recipients [43], and 6% in brain tumor patients with irradiation receiving glucocorticosteroids [44]. Compliance with trimethoprim/sulfamethoxazole renders *Pneumocystis* disease unlikely [39].

In case series, 80–90% of immunocompromised patients with PCP without HIV received corticosteroids [36, 45, 46] and, similar to patients with HIV [36, 47], a systematic review concluded that a CD4+ cell count of less than 200 was a sensitive biomarker of "high risk" in immunocompromised patients without HIV [48]. The strong association between *Pneumocystis* disease and immunosuppression suggests that risk should be focused on the net state of immunosuppression as opposed to underlying disease, including the use of glucocorticosteroids and therapies and conditions that specifically compromise T-cell–mediated immunity (Table 1).

Clinical Criteria

Symptoms and Signs

Unlike in patients with HIV where the onset of PCP is usually gradual and insidious, with few physical or radiologic findings, in immunocompromised patients without HIV, clinical presentation tends to be more acute with rapid onset of respiratory symptoms and faster progression to respiratory failure, higher ICU admission rates, and mortality exceeding 50% [16, 36, 49–51]. Clinical features include fever, progressive dyspnea, nonproductive cough, chest pain, circulatory failure, pneumothorax, and, very rarely, hemoptysis [36, 50–61]. The differences in clinical presentation between patients with and without HIV appear to be related to differences in severity of pneumonia and degree of lung inflammation. Patients with HIV have a higher organismal load and fewer granulocytes in the lung than do patients without HIV, with greater impairment of gas exchange [15] (Table 1). There is a paucity of findings at auscultation. Serum lactate dehydrogenase levels are not typically elevated in patients without HIV [49–51].

Very rarely, *Pneumocystis* disease may spread to other body sites and cause extrapulmonary manifestations whose signs and symptoms are nonspecific and will depend on the site involved [28, 29].

Table 1. Host Factors, Clinical Criteria, and Microbiologic Criteria Used for the Definition of *Pneumocystis jirovecii* Pneumonia

	Description
Host factors	• Use of therapeutic doses of ≥0.3 mg/kg prednisone equivalent for ≥2 weeks in the past 60 days • Low CD4+ lymphocyte counts (observed or expected; <200 cells/mm^3) induced by a medical condition, anticancer, anti-inflammatory, and immunosuppressive treatment, including but not limited to: - Primary immunodeficiencies with numeric/functional T-cell deficiency - Acute leukemia, non-Hodgkin's lymphoma, solid tumors, allogeneic HSCT - Solid-organ transplantation - Autoimmune- and hyperinflammatory disorders, including treatment with agents that lead to functional T-cell deficiencies
Clinical criteria	• Fever • Respiratory symptoms including cough, dyspnea, or hypoxemia • Bilateral or diffuse GGO on X-ray with interstitial infiltrates as the predominant feature; alveolar, alveolar-interstitial, and unilateral infiltrates are less frequent • Extensive, mostly diffuse GGO on CT scans, which typically has an upper lobe and perihilar predominance, sometimes with peripheral sparing or a mosaic pattern; consolidations, small nodules, and unilateral infiltrates are less frequent
Microbiologic criteria	• Visualization of *P. jirovecii* by microscopy using conventional staining methods (Gomori methenamine silver, Toluidine Blue O, Giemsa, Calcofluor White) or immunofluorescence staining in tissue, BAL fluid, induced sputum, expectorated sputum, or oral wash • Amplification of *P. jirovecii* DNA by quantitative real-time PCR in BAL fluid, induced sputum, or oral wash • Detection of β-D-glucan in serum if another invasive fungal infection and a false-positive result can be ruled out

Abbreviations: BAL, bronchoalveolar lavage; CT, computed tomography; GGO, ground-glass opacity; HSCT, hematopoietic stem cell transplantation; PCR, polymerase chain reaction.

Radiographic Patterns

Between 10% and 15% of immunocompromised patients with PCP without HIV have normal chest radiographs and, among those with abnormalities, close to 30% have nonspecific findings. Typical findings are bilateral, diffuse ground-glass opacity (GGO) with interstitial infiltrates. Alveolar infiltrate patterns, unilateral involvement, lung nodules, or pleural effusions are less frequent. In mild or early presentations, opacities are usually perihilar. With advancing disease, opacities become diffuse and are in a butterfly pattern [19, 36].

Using high-resolution pulmonary computed tomography scans, extensive GGO is the main feature, representing exudate formation from alveolitis [15, 53]. Ground-glass opacity is usually symmetric, predominant in the perihilar regions and apices, with peripheral sparing (~20% of cases). A mosaic pattern has also been reported in 60% of cases, reflecting more severe disease [19, 62]. *Pneumocystis jirovecii* pneumonia treatment results in radiologic improvement, while ineffective therapy is associated with evolution to the mosaic pattern with architectural distortion and increasing pulmonary infiltrates [62] (Table 1). Because of host immune-mediated lung damage, GGO may be associated with rapid onset of lung consolidation [19]. Nodules and/or septal thickening are other findings [63]. Pulmonary cysts are rare and are attributed to longstanding, low-intensity inflammation, resulting in tissue destruction. Occasionally, pneumothorax or pneumomediastinum occurs [29, 64].

Microbiologic Criteria

Microbiologic diagnosis of PCP is hampered by the inability to cultivate the organism and little utility of serologic approaches using *P. jirovecii*–specific antibody tests. Although antibodies to *P. jirovecii* may be detected in up to 80% of individuals [25, 65], no commercial tests are available, results are variable, and the natural history of antibody persistence poorly understood. Many immunosuppressed patients are unable to produce antibodies.

The diagnosis of PCP hinges upon the visualization and/or detection of *P. jirovecii* in respiratory tract samples by (1) microscopy, (2) antigen detection, and (3) nucleic acid amplification tests (NAATs) (Table 1).

Microscopy

Definitive diagnosis of PCP has traditionally relied on microscopic visualization of *P. jirovecii* in respiratory specimens using optical brighteners, silver stains, and toluidine blue [66, 67]. Bronchoalveolar lavage (BAL) fluid or washings, with/without transbronchial biopsy, induced sputum (IS), and expectorated sputum are most often submitted for examination, but other upper tract specimens (eg, oral rinses, to avoid invasive sampling procedures) also have utility. Immunofluorescent staining for all the above specimen types exhibits superior sensitivity to conventional microscopy [66, 68, 69]. Today, conventional stains may be used (1) in laboratories that do not offer NAAT or immunofluorescent staining and (2) to visualize the cyst/trophic forms in histologic or cytologic specimens.

The use of mouse anti–*P. jirovecii* monoclonal antibodies to detect cysts and trophic forms in an immunofluorescent assay (IFA) format is the preferred method of microscopic diagnosis [70]. Direct antigen-detection formats identify both morphotypes while indirect IFAs detect only cysts. Although it may be an advantage to detect both forms, direct IFAs suffer from more artefact than those that detect cysts only. Rath and colleagues [66] recommend an IFA that detects only cysts as the most useful assay in contemporary routine diagnostics. The main limitations of IFAs are cost and need for a fluorescent microscope. Notably, detection of *Pneumocystis* microscopically in tissue, BAL fluid, or expectorated sputum remains the

criterion for proven PCP. Due to suboptimal sensitivity, negative microscopy does not rule out infection.

Nucleic Acid Amplification Test Approaches

Polymerase chain reaction (PCR) and other NAAT methods are more sensitive than microscopic examination for the detection of *P. jirovecii*; however, their high sensitivity does not allow for easy distinction between PCP and colonization with *P. jirovecii*. Hence, quantification of the fungal load is essential to interpret PCR results. Purely qualitative endpoint PCR tests (single round or nested) are not recommended for PCP diagnosis.

Instead, real-time PCR is now preferred, as this approach provides quantitative results, is rapid, and allows inclusion of a PCR inhibition control. The *Pneumocystis* multicopy mitochondrial large-subunit ribosomal RNA (*mtLSU*) gene is most commonly targeted, but assays targeting the mitochondrial small-subunit ribosomal RNA (*mtSSU*) gene, multi-copy major surface antigen (*MSG*) gene, 18S ribosomal RNA (rRNA), internal transcribed spacer (ITS), 5S rRNA, *DHPS, B*-tubulin, and *HSP70* genes have been developed. Overall, the performance of in-house PCR tests is similar to commercial tests [66].

Attempts have been made to define a quantitative PCR (qPCR) threshold. Based on literature data and including the results of a prospective multicenter laboratory evaluation over 4 years [71], consideration was given to defining 2 types of PCR "thresholds": a "high" threshold that would diagnose PCP with 100% specificity and a "low" threshold that would exclude PCR with a high degree of certainty (eg, where PCR is performed on BAL fluid). Inevitably, there will be patients with results in the gray zone in-between the 2 thresholds. Therefore, these results should be interpreted in the context of the patient's underlying disease, immunosuppressive therapies, and other treatments to inform decisions of whether or not to institute anti-*Pneumocystis* therapy. Indeed, all *Pneumocystis* qPCR assays (in-house or commercial) should be validated in the appropriate clinical context (eg, non–HIV-positive patients) to define the aforementioned thresholds.

Perret and colleagues [72] have suggested a single cutoff of 5×10^3 copies/mL to discriminate PCP from colonization while assessing an in-house qPCR on BAL samples from 71 patients with positive PCR results including 62 patients without HIV. However, test variability observed due to master mix and thermocycler parameters prevented the application of a consensual threshold and test standardization is essential [73]. The *Pneumocystis* Working Party of the Fungal PCR Initiative (www.fpcri.eu) has been working towards such a consensus method. A 16-laboratory international study confirmed the large (10 000-fold) variation between qPCR assays for a given sample. Assays targeting whole nucleic acid and the *mtSSU* gene were the most sensitive and have been put forward as a basis for standardizing *P. jirovecii* loads [74].

When using qPCR, IS and BAL fluid are equally appropriate as samples to diagnose PCP, and potentially the same interpretive cutoff values can be used; however, the number of patients with PCP in whom both IS and BAL fluid have been tested remains small [75]. Sensitivity of qPCR on upper respiratory tract samples is lower than on BAL fluid. Such positive results can be used as a microbiologic criterion to diagnose PCP, but negative results cannot exclude PCP.

Antigen Detection

Because of the invasiveness of BAL sampling and imperfect specificity of PCR, the utility of the fungal biomarker, 1,3-β-D-glucan (BDG), while not specific for *P. jirovecii*, has been studied in PCP diagnosis [66, 76–78].

BDG is concentrated in the cell wall of the cyst (but not the trophic) form of *P. jirovecii* [76] Several BDG commercial assays can be used—these differ in their cutoff value to call a "positive" test and hence affect study comparability. The most commonly studied are the Fungitell (Associates of Cape Cod, Inc, East Falmouth, MA) test and the Wako β-D-glucan Test (FUJIFILM Wako Chemicals, Osaka, Japan).

A systematic review and meta-analysis of the utility of serum BDG testing provided data studying 997 patients with PCP and 3062 controls [79]. Pooled sensitivity and specificity for PCP were 91% (95% confidence interval [CI], 87–94%) and 79% (95% CI, 72–84%), respectively. The sensitivity in patients with HIV was higher than in those without HIV (94% vs 86%; $P = .02$) with similar specificity. The authors concluded that a negative BDG test is only associated with a low post-test probability of PCP (≤5%) when the pre-test probability was low (≤20%) in patients without HIV. The moderate specificity can be explained by the positivity due to other fungal infections (eg, candidiasis) and the false positivity seen in patients with hemodialysis, receipt of immunoglobulins, and certain medications. A positive BDG result should hence trigger tests to exclude other IFDs (Table 1).

The BDG assay may also be able to distinguish *P. jirovecii* colonization from infection [80]. In 166 immunocompromised patients with pulmonary infiltrates, the results of BAL fluid PCR and serum BDG (Wako; FUJIFILM) were compared. BDG levels in patients with definite PCP were significantly higher than those in patients with probable infection, colonization, and patients without PCP (all $P < .001$). BDG levels in patients with definite/probable PCP (173.1 ± 18.8 pg/mL) were also higher than those in colonized patients who had PCR-positive results ($P < .002$). The cutoff level for discrimination was estimated at 33.5 pg/mL (positive-predictive value, 0.925).

The combination of qPCR and serum BDG testing may result in greater diagnostic performance. In 1 study, patients considered to have PCP (by qPCR on BAL fluid) had BDG levels of 100 pg/mL or higher (Fungitell; Associates of Cape Cod) compared with colonized patients (BDG <100 pg/mL), suggesting

that qPCR on BAL fluid plus serum BDG testing can differentiate between PCP and colonization [81]. In patients with unexplained lung infiltrates who underwent evaluation for suspected PCP with bronchoscopy, higher BDG values (>200 pg/mL; Fungitell; Associates of Cape Cod) were associated with clinical PCP among PCR-positive patients [82]. If BAL sampling is not feasible, combined BDG measurement with qPCR on nasopharyngeal aspirates has been an alternative [83]. It remains uncertain whether serum BDG can inform treatment response or prediction of the outcome [84]. There are no supporting data for BDG detection in BAL fluid.

BDG detection for PCP diagnosis has adequate sensitivity. Requiring 2 consecutive positive results improves specificity [85]. More experience is needed with commercial assays other than the Fungitell assay, with assignment of an optimal cutoff value. One study evaluating 116 PCP cases revealed the performance of the Wako β-glucan assay (FUJIFILM; cutoff 11 pg/mL) to be similar to the Fungitell (Associates of Cape Cod) assay with lower inter- and intra-run variability [86].

DISEASE DEFINITIONS

The criteria for the diagnosis of PCP by the 2019 Update of the EORTC/MSGERC Consensus Definitions of Invasive Fungal Disease are summarized in Table 1, and the disease definitions based on this triad of criteria are in Table 2.

The diagnosis of proven PCP is based on clinical and radiologic criteria plus demonstration of *P. jirovecii* by microscopy using conventional or immunofluorescence staining in tissue or respiratory tract specimens. The diagnosis of proven PCP does not require a host factor; however, in the absence of a host factor at the time of diagnosis, investigations for a predisposing host factor should be initiated. Quantitative PCR is not accepted as a microbiologic criterion for proven PCR because of the lack of standardized methodology and clear interpretation rules to distinguish colonization from infection.

Table 2. Diagnostic Criteria for Definition of Proven and Probable *Pneumocystis jirovecii* Pneumonia

	Description
Proven PCP	• Clinical and radiologic criteria, plus: - Demonstration of *P. jirovecii* by microscopy using conventional or immunofluorescence staining in tissue or - Demonstration of *P. jirovecii* by microscopy using conventional or immunofluorescence staining in respiratory specimens
Probable PCP	• Appropriate host factors and clinical and radiologic criteria, plus: - Amplification of *P. jirovecii* DNA by quantitative real-time PCR in respiratory specimen or - Detection of β-D-glucan in serum (alternative method; another IFD and a false-positive result should be ruled out)

Abbreviations: IFD, invasive fungal diseases; PCP, *Pneumocystis jirovecii* pneumonia; PCR, polymerase chain reaction.

Probable PCP is defined by the presence of appropriate host factors and clinical and radiologic criteria, plus detection of *P. jirovecii* by qPCR in respiratory tract specimens (BAL fluid, induced sputum, or oral wash) and/or detection of BDG in serum, provided that another IFD and a false-positive result can be ruled out. Two types of PCR "thresholds" for distinguishing colonization by *P. jirovecii* from disease have been proposed: a "high" threshold that would diagnose PCP with 100% specificity and a "low" threshold that would exclude PCR with a high degree of certainty, at least on BAL fluid; however, thresholds have not been defined by consensus. The inclusion of the serum BDG test is based on high sensitivity and excellent negative-predictive value; uniformly accepted thresholds, however, have not been defined.

Whereas the definitions of proven and probable IFDs are reliable for research purposes, a diagnosis of possible IFD per se is inconclusive due to lack of a microbiologic criterion but may be upgraded during the diagnostic workup if an appropriate microbiologic test result becomes positive. For PCP, possible disease is defined by appropriate host factors and clinical and radiologic criteria but absence of microbiologic confirmation by microscopy and PCR in tissue or respiratory specimens and BDG in serum, respectively (not done or negative result). *Pneumocystis jirovecii* pneumonia is highly unlikely in cases of failure to demonstrate *P. jirovecii* by microscopy in lung or to demonstrate *P. jirovecii* by PCR in BAL material and a negative BDG in serum immediately prior to or within 3–5 days after the start of appropriate treatment [82].

Finally, the diagnosis of extrapulmonary *Pneumocystis* disease requires demonstration of the organism in involved tissue by microscopy and, preferentially, by NAAT.

SUMMARY

These definitions represent consensus expert opinion based on current evidence. They will need regular review for relevance, particularly regarding the role of qPCR in supporting the definition of proven disease. However, this is more a consideration of scientific accuracy than of practical relevance, as probable and proven disease is usually grouped as 1 entity in clinical research.

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References

1. Ascioglu S, Rex JH, de Pauw B, et al.; Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis **2002**; 34:7–14.
2. De Pauw B, Walsh TJ, Donnelly JP, et al.; European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis **2008**; 46:1813–21.
3. Herbrecht R, Patterson TF, Slavin MA, et al. Application of the 2008 definitions for invasive fungal diseases to the trial comparing voriconazole versus amphotericin B for therapy of invasive aspergillosis: a collaborative study of the Mycoses Study Group (MSG 05) and the European Organization for Research and Treatment of Cancer Infectious Diseases Group. Clin Infect Dis **2015**; 60:713–20.
4. Maertens JA, Raad II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by *Aspergillus* and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. Lancet **2016**; 387:760–9.
5. Herbrecht R, Denning DW, Patterson TF, et al; Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med **2002**; 347:408–15.
6. Maertens J, Raad I, Petrikkos G, et al.; Caspofungin Salvage Aspergillosis Study Group. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. Clin Infect Dis **2004**; 39:1563–71.
7. Cornely OA, Maertens J, Bresnik M, et al.; AmBiLoad Trial Study Group. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). Clin Infect Dis **2007**; 44:1289–97.
8. Kovanda LL, Kolamunnage-Dona R, Neely M, Maertens J, Lee M, Hope WW. Pharmacodynamics of isavuconazole for invasive mold disease: role of galactomannan for real-time monitoring of therapeutic response. Clin Infect Dis **2017**; 64:1557–63.
9. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis **2010**; 50:1101–11.
10. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. Clin Infect Dis **2010**; 50:1091–100.
11. Park BJ, Pappas PG, Wannemuehler KA, et al. Invasive non-*Aspergillus* mold infections in transplant recipients, United States, 2001–2006. Emerg Infect Dis **2011**; 17:1855–64.
12. Kauffman CA, Freifeld AG, Andes DR, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the Transplant-Associated Infection Surveillance Network (TRANSNET). Transpl Infect Dis **2014**; 16:213–24.
13. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. **2020**; 71:1367–76.
14. Kovacs JA, Hiemenz JW, Macher AM, et al. Pneumocystis carinii pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med **1984**; 100:663–71.
15. Limper AH, Offord KP, Smith TF, Martin WJ 2nd. *Pneumocystis carinii* pneumonia: differences in lung parasite number and inflammation in patients with and without AIDS. Am Rev Respir Dis **1989**; 140:1204–9.
16. Monnet X, Vidal-Petiot E, Osman D, et al. Critical care management and outcome of severe *Pneumocystis* pneumonia in patients with and without HIV infection [published correction appears in Crit Care 2009; 13(2)407]. Crit Care **2008**; 12:R28.
17. Helweg-Larsen J, Benfield T, Atzori C, Miller RF. Clinical efficacy of first- and second-line treatments for HIV-associated *Pneumocystis jirovecii* pneumonia: a tri-centre cohort study. J Antimicrob Chemother **2009**; 64:1282–90.
18. Hardak E, Brook O, Yigla M. Radiological features of *Pneumocystis jirovecii* pneumonia in immunocompromised patients with and without AIDS. Lung **2010**; 188:159–63.
19. Tasaka S, Tokuda H, Sakai F, et al. Comparison of clinical and radiological features of *Pneumocystis* pneumonia between malignancy cases and acquired immunodeficiency syndrome cases: a multicenter study. Intern Med **2010**; 49:273–81.
20. Armbruster C, Hassl A, Kriwanek S. *Pneumocystis carinii* colonization in the absence of immunosuppression. Scand J Infect Dis **1997**; 29:591–3.
21. Maskell NA, Waine DJ, Lindley A, et al. Asymptomatic carriage of *Pneumocystis jiroveci* in subjects undergoing bronchoscopy: a prospective study. Thorax **2003**; 58:594–7.
22. Morris A, Norris KA. Colonization by *Pneumocystis jirovecii* and its role in disease. Clin Microbiol Rev **2012**; 25:297–317.
23. Thomas CF Jr, Limper AH. *Pneumocystis* pneumonia. N Engl J Med **2004**; 350:2487–98.
24. Pifer LL, Hughes WT, Stagno S, Woods D. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. Pediatrics **1978**; 61:35–41.
25. Vargas SL, Hughes WT, Santolaya ME, et al. Search for primary infection by *Pneumocystis carinii* in a cohort of normal, healthy infants. Clin Infect Dis **2001**; 32:855–61.
26. Harris JR, Balajee SA, Park BJ. *Pneumocystis jirovecii* pneumonia: current knowledge and outstanding public health issues. Curr Fungal Infect Rep **2010**; 4:229–37.
27. Hughes WT. Current issues in the epidemiology, transmission, and reactivation of *Pneumocystis carinii*. Semin Respir Infect **1998**; 13:283–8.
28. Ng VL, Yajko DM, Hadley WK. Extrapulmonary pneumocystosis. Clin Microbiol Rev **1997**; 10:401–18.
29. Groll AH, Keul HG, Brodt R, Schneider M. AIDS-associated atypical *Pneumocystis carinii* pneumonia revisited. Clin Infect Dis **1998**; 26: 1005–6.
30. Groll AH, Ritter J, Müller FM. [Guidelines for prevention of *Pneumocystis carinii* pneumonitis in children and adolescents with cancer.] Klin Padiatr **2001**; 213:A38–49.
31. Limper AH, Hoyte JS, Standing JE. The role of alveolar macrophages in *Pneumocystis carinii* degradation and clearance from the lung. J Clin Invest **1997**; 99:2110–7.
32. Beck JM, Harmsen AG. Lymphocytes in host defense against *Pneumocystis carinii.* Semin Respir Infect **1998**; 13:330–8.
33. Gigliotti F, Limper AH, Wright T. Pneumocystis. Cold Spring Harb Perspect Med **2014**; 4:a019828.
34. Mackall CL, Stein D, Fleisher TA, et al. Prolonged CD4 depletion after sequential autologous peripheral blood progenitor cell infusions in children and young adults. Blood **2000**; 96:754–62.
35. Fillatre P, Decaux O, Jouneau S, et al. Incidence of *Pneumocystis jirovecii* pneumonia among groups at risk in HIV-negative patients. Am J Med **2014**; 127:1242.e11–e17.
36. Roux A, Canet E, Valade S, et al. *Pneumocystis jirovecii* pneumonia in patients with or without AIDS, France. Emerg Infect Dis **2014**; 20:1490–7.
37. Hughes WT, Feldman S, Aur RJ, Verzosa MS, Hustu HO, Simone JV. Intensity of immunosuppressive therapy and the incidence of Pneumocystis carinii pneumonitis. Cancer **1975**; 36:2004–9.
38. Cyklis R, Zielińska A. [Pneumocystis carinii infection in children with acute leukemia and non-Hodgkin malignant lymphoma.] Pediatr Pol **1983**; 58:337–40.
39. Hughes WT, Kuhn S, Chaudhary S, et al. Successful chemoprophylaxis for Pneumocystis carinii pneumonitis. N Engl J Med **1978**; 297:1419–26.
40. Browne MJ, Hubbard SM, Longo DL, et al. Excess prevalence of Pneumocystis carinii pneumonia in patients treated for lymphoma with combination chemotherapy. Ann Intern Med **1986**; 104:338–44.
41. Byrd JC, Hargis JB, Kester KE, Hospenthal DR, Knutson SW, Diehl LF. Opportunistic pulmonary infections with fludarabine in previously treated patients with low-grade lymphoid malignancies: a role for Pneumocystis carinii pneumonia prophylaxis. Am J Hematol **1995**; 49:135–42.
42. Meyers JD, Pifer LL, Sale GE, Thomas ED. The value of Pneumocystis carinii antibody and antigen detection for diagnosis of Pneumocystis carinii pneumonia after marrow transplantation. Am Rev Respir Dis **1979**; 120:1283–7.
43. Gryzan S, Paradis IL, Zeevi A, et al. Unexpectedly high incidence of Pneumocystis carinii infection after lung-heart transplantation: implications for lung defense and allograft survival. Am Rev Respir Dis **1988**; 137:1268–74.
44. Slivka A, Wen PY, Shea WM, Loeffler JS. Pneumocystis carinii pneumonia during steroid taper in patients with primary brain tumors. Am J Med **1993**; 94:216–9.

45. DeVita VT Jr, Goodell B, Hubbard S, Geelhoed GW, Young RC. Pneumocystis pneumonia in patients with cancer: clinical setting. Natl Cancer Inst Monogr **1976**; 43:41–7.
46. Sepkowitz KA. Pneumocystis carinii pneumonia in patients without AIDS. Clin Infect Dis **1993**; 17:S416–22.
47. Masur H, Ognibene FP, Yarchoan R, et al. CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection. Ann Intern Med **1989**; 111:223–31.
48. Messiaen PE, Cuyx S, Dejagere T, van der Hilst JC. The role of CD4 cell count as discriminatory measure to guide chemoprophylaxis against Pneumocystis jirovecii pneumonia in human immunodeficiency virus-negative immunocompromised patients: a systematic review. Transpl Infect Dis **2017**; 19. doi: 10.1111/tid.12651.
49. Salzer HJF, Schäfer G, Hoenigl M, et al. Clinical, diagnostic, and treatment disparities between HIV-infected and non-HIV-infected immunocompromised patients with Pneumocystis jirovecii pneumonia. Respiration **2018**; 96:52–65.
50. Cillóniz C, Dominedò C, Álvarez-Martínez MJ, et al. Pneumocystis pneumonia in the twenty-first century: HIV-infected versus HIV-uninfected patients. Expert Rev Anti Infect Ther **2019**; 17:787–801.
51. Rego de Figueiredo I, Vieira Alves R, Drummond Borges D, et al. Pneumocystosis pneumonia: a comparison study between HIV and non-HIV immunocompromised patients. Pulmonology **2019**; 25:271–4.
52. McKinnell JA, Cannella AP, Kunz DF, et al. Pneumocystis pneumonia in hospitalized patients: a detailed examination of symptoms, management, and outcomes in human immunodeficiency virus (HIV)-infected and HIV-uninfected persons. Transpl Infect Dis **2012**; 14:510–8.
53. Pagano L, Fianchi L, Mele L, et al. Pneumocystis carinii pneumonia in patients with malignant haematologic diseases: 10 years' experience of infection in GIMEMA centres. Br J Haematol **2002**; 117:379–86.
54. Martin-Garrido I, Carmona EM, Specks U, Limper AH. Pneumocystis pneumonia in patients treated with rituximab. Chest **2013**; 144:258–65.
55. Cerón I, Rabagliati R, Langhaus J, Silva F, Guzmán AM, Lagos M. [Pneumocystis jiroveci pneumonia: comparative study of cases in HIV-infected patients and immunocompromised non-HIV-infected patients.] Rev Chilena Infectol **2014**; 31:417–24.
56. Kofteridis DP, Valachis A, Velegraki M, et al. Predisposing factors, clinical characteristics and outcome of Pneumonocystis jirovecii pneumonia in HIV-negative patients. J Infect Chemother **2014**; 20:412–6.
57. Fily F, Lachkar S, Thiberville L, Favennec L, Caron F. Pneumocystis jirovecii colonization and infection among non HIV-infected patients. Med Mal Infect **2011**; 41:526–31.
58. Guo F, Chen Y, Yang SL, Xia H, Li XW, Tong ZH. Pneumocystis pneumonia in HIV-infected and immunocompromised non-HIV infected patients: a retrospective study of two centers in China. PLoS One **2014**; 9:e101943.
59. Li MC, Lee NY, Lee CC, et al. Pneumocystis jiroveci pneumonia in immunocompromised patients: delayed diagnosis and poor outcomes in non-HIV –infected individuals. J Microbiol Immunol Infect **2014**; 47:42–7.
60. Ko Y, Jeong BH, Park HY, et al. Outcomes of Pneumocystis pneumonia with respiratory failure in HIV-negative patients. J Crit Care **2014**; 29:356–61.
61. Lemiale V, Debrumetz A, Delannoy A, Alberti C, Azoulay E. Adjunctive steroid in HIV-negative patients with severe Pneumocystis pneumonia. Respir Res **2013**; 14:87.
62. Vogel MN, Vatlach M, Weissgerbe P, et al. HRCT-features of Pneumocystis jiroveci pneumonia and their evolution before and after treatment in non-HIV immunocompromised patients. Eur J Radiol **2012**; 81:1315–20.
63. Beigelman-Aubry C, Godet C, Caumes E. Lung infections: the radiologist's perspective. Diagn Interv Imaging **2012**; 93:431–40.
64. Cereser L, Dallorto A, Candoni A, et al. Pneumocystis jirovecii pneumonia at chest high-resolution computed tomography (HRCT) in non-HIV immunocompromised patients: spectrum of findings and mimickers. Eur J Radiol **2019**; 116:116–27.
65. Fong S, Daly KR, Tipirneni R, et al. Antibody responses against *Pneumocystis jirovecii* in health care workers over time. Emerg Infect Dis **2013**; 19:1612–9.
66. Rath PM, Steinmann J. Update on diagnosis of *Pneumocystis* pulmonary infections. Curr Fungal Infect Rep **2014**; 8:227–34.
67. Calderón EJ, Gutiérrez-Rivero S, Durand-Joly I, Dei-Cas E. *Pneumocystis* infection in humans: diagnosis and treatment. Expert Rev Anti Infect Ther **2010**; 8:683–701.
68. Kovacs JA, Ng VL, Masur H, et al. Diagnosis of *Pneumocystis carinii* pneumonia: improved detection in sputum with use of monoclonal antibodies. N Engl J Med **1988**; 318:589–93.
69. Tiley SM, Marriott DJ, Harkness JL. An evaluation of four methods for the detection of *Pneumocystis carinii* in clinical specimens. Pathology **1994**; 26:325–8.
70. Alanio A, Hauser PM, Lagrou K, et al.; 5th European Conference on Infections in Leukemia (ECIL-5), a joint venture of The European Group for Blood and Marrow Transplantation (EBMT), The European Organization for Research and Treatment of Cancer (EORTC), the Immunocompromised Host Society (ICHS) and The European LeukemiaNet (ELN). ECIL guidelines for the diagnosis of *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. J Antimicrob Chemother **2016**; 71:2386–96.
71. Robert-Gangneux F, Belaz S, Revest M, et al. Diagnosis of *Pneumocystis jirovecii* pneumonia in immunocompromised patients by real-time PCR: a 4-year prospective study. J Clin Microbiol **2014**; 52:3370–6.
72. Perret T, Kritikos A, Hauser PM, et al. Ability of quantitative PCR to discriminate *Pneumocystis jirovecii* pneumonia from colonization. J Med Microbiol **2020**; 69:705–11.
73. Dellière S, Gits-Muselli M, White PL, et al. Quantification of *Pneumocystis jirovecii*: cross-platform comparison of one qPCR assay with leading platforms and six master mixes. J Fungi (Basel) **2019**; 6:9.
74. Gits-Muselli M, White PL, Mengoli C, et al. The Fungal PCR Initiative's evaluation of in-house and commercial Pneumocystis jirovecii qPCR assays: toward a standard for a diagnostics assay. Med Mycol **2019**; 58:779–88. doi: 10.1093/mmy/myz115.
75. Alanio A, Desoubeaux G, Sarfati C, et al. Real-time PCR assay-based strategy for differentiation between active *Pneumocystis jirovecii* pneumonia and colonization in immunocompromised patients. Clin Microbiol Infect **2011**; 17:1531–7.
76. Obayashi T, Negishi K, Suzuki T, Funata N. Reappraisal of the serum (1–>3)-beta-D-glucan assay for the diagnosis of invasive fungal infections—a study based on autopsy cases from 6 years. Clin Infect Dis **2008**; 46:1864–70.
77. Onishi A, Sugiyama D, Kogata Y, et al. Diagnostic accuracy of serum 1,3-β-D-glucan for *Pneumocystis jiroveci* pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol **2012**; 50:7–15.
78. Finkelman MA. *Pneumocystis jirovecii* infection: cell wall (1→3)-β;-D-glucan biology and diagnostic utility. Crit Rev Microbiol **2010**; 36:271–81.
79. Del Corpo O, Butler-Laporte G, Sheppard DC, et al. Diagnostic accuracy of serum (1-3)-β-D-glucan for *Pneumocystis jirovecii* pneumonia: a systematic review and meta-analysis. Clin Microbiol Infect **2020**; 26:1137–43. doi: 10.1016/j.cmi.2020.05.024.
80. Tasaka S, Kobayashi S, Yagi K, et al. Serum (1 → 3) β-D-glucan assay for discrimination between *Pneumocystis jirovecii* pneumonia and colonization. J Infect Chemother **2014**; 20:678–81.
81. Damiani C, Le Gal S, Da Costa C, Virmaux M, Nevez G, Totet A. Combined quantification of pulmonary *Pneumocystis jirovecii* DNA and serum (1->3)-β-D-glucan for differential diagnosis of pneumocystis pneumonia and Pneumocystis colonization. J Clin Microbiol **2013**; 51:3380–8.
82. Morjaria S, Frame J, Franco-Garcia A, Geyer A, Kamboj M, Babady NE. Clinical performance of (1,3) Beta-D glucan for the diagnosis of pneumocystis pneumonia (PCP) in cancer patients tested with PCP polymerase chain reaction. Clin Infect Dis **2019**; 69:1303–9.
83. Desoubeaux G, Chesnay A, Mercier V, et al. Combination of β-(1,3)-D-glucan testing in serum and qPCR in nasopharyngeal aspirate for facilitated diagnosis of *Pneumocystis jirovecii* pneumonia. Mycoses **2019**; 62:1015–22.
84. Urabe N, Sakamoto S, Sano G, Ito A, Sekiguchi R, Homma S. Serial change in serum biomarkers during treatment of non-HIV pneumocystis pneumonia. J Infect Chemother **2019**; 25:936–42.
85. Mercier T, Guldentops E, Patteet S, et al. Beta-d-glucan for diagnosing pneumocystis pneumonia: a direct comparison between the Wako β-glucan assay and the Fungitell assay. J Clin Microbiol **2019**; 57:e00322-19.
86. Lamoth F, Cruciani M, Mengoli C, et al.; Third European Conference on Infections in Leukemia (ECIL-3). β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). Clin Infect Dis **2012**; 54:633–43.