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Clinical Performance of (1,3) Beta-D Glucan for the Diagnosis of *Pneumocystis* Pneumonia (PCP) in Cancer Patients Tested With PCP Polymerase Chain Reaction

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(See the Editorial Commentary by Theel on pages 1310–2.)

Background. Serum (1,3)-beta-D glucan (BDG) is increasingly used to guide the management of suspected *Pneumocystis* pneumonia (PCP). BDG lacks specificity for PCP, and its clinical performance in high-risk cancer patients has not been fully assessed. Polymerase chain reaction (PCR) for PCP detection is highly sensitive, but cannot differentiate between colonization and infection. We evaluated the diagnostic performance of serum BDG in conjunction with PCP PCR on respiratory samples in patients with cancer and unexplained lung infiltrates.

Methods. We performed a retrospective analysis of adult patients evaluated for PCP at our institution from 2012 to 2015, using serum BDG and PCP PCR. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the serum BDG at different thresholds were evaluated using PCP PCR alone or in conjunction with clinical presentation in PCP PCR–positive patients.

Results. With PCP PCR alone as the reference method, BDG (≥80 pg/mL) had a sensitivity of 69.8%, specificity of 81.2%, PPV of 34.6%, and NPV of 95.2% for PCP. At ≥200 pg/mL in patients with a positive PCR and a compatible PCP clinical syndrome, BDG had a sensitivity of 70%, specificity of 100%, PPV of 100%, and NPV of 52.0% for PCP.

Conclusions. Patients negative by both BDG and PCR were unlikely to have PCP. In patients with a compatible clinical syndrome for PCP, higher BDG values (>200 pg/mL) were consistently associated with clinically-significant PCP infections among PCP PCR–positive oncology patients.

Keywords. *Pneumocystis jiroveci*; polymerase chain beaction; beta-D glucans; diagnostic accuracy; cancer.

Pneumocystis jiroveci (PCP) is a common cause of opportunistic lung infection and is associated with high mortality in immunocompromised patients [1, 2]. Despite the use of targeted prophylaxis, PCP is frequently encountered in patients with hematologic malignancies and recipients of hematopoietic cell transplantation (HCT) [2, 3]. As a presumptive or suspected diagnosis, PCP is also encountered during the course of cancer treatment in a wide range of patients with solid tumors and in association with novel cancer and immune therapeutics, with clinical outcomes that ultimately hinge on a timely diagnosis [3–7].

The existing literature on PCP diagnoses is largely derived from human immunodeficiency virus (HIV)-infected patients [8–10]. However, there are distinct differences between the clinical presentation of PCP and the burden of infecting organisms in non–HIV-infected patients, which renders any direct extrapolations unreliable. For example, non–HIV-infected patients with PCP have a more sudden onset of pulmonary symptoms, whereas symptoms are more progressive in HIV-positive patients [11]. Most importantly, conventional direct visualization techniques of PCP cysts have inferior diagnostic sensitivity in non–HIV-infected patients [12].

For decades, the diagnostic gold standard for detecting PCP has been direct microscopic identification of cysts in tissue samples. In recent years, molecular assays (eg, polymerase chain reaction [PCR]) on respiratory samples have become available for the direct detection of target organism DNA [13–15]. Due to the lower sensitivity of conventional methods, PCP PCR is appealing, especially in non–HIV-infected patients. However, PCR may be prone to misinterpretation in cases where PCP is a colonizer (ie, present without causing disease) rather than the cause of disease (ie, present in the context of clinical signs or symptoms of acute pneumonia) [16–18].

Serum (1,3)-beta-D Glucan (BDG) is often used as a non-invasive means to support the diagnosis of PCP, especially in situations where critical illness precludes invasive diagnostic procedures [19]. BDG is an antigenic component of the cell wall

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of many fungi, including PCP, and is considered to be a valuable diagnostic adjunct for PCP [19, 20]; however, the application of BDG as a stand-alone test has been challenged, due to its lack of specificity [21, 22].

To date, there are no studies evaluating the accuracy of BDG when combined with PCP PCR in a large cancer-patient population. The objective of this study was to evaluate the diagnostic performance of serum BDG to diagnose PCP in at-risk cancer patients, when combined with PCP PCR obtained from bronchoscopy samples.

METHODS

Study Design and Settings

This was a retrospective cohort study performed at Memorial Sloan Kettering Cancer Center, a 478-bed tertiary care cancer center in New York City with 22 000 annual admissions, where approximately 400 HCT are performed annually.

Study Population and Inclusion Criteria

The study population included all hospitalized adult patients (≥18 years old) with unexplained lung infiltrates and clinical suspicion for PCP who underwent an evaluation with bronchoscopy between January 2012 to December 2015. Individuals included in the study had a bronchoalveolar lavage fluid (BAL), bronchial washings, or a lung tissue sample collected for the diagnosis of PCP using PCR. Results of serum BDG collected within 7 days of bronchoscopy were used for the analysis. If multiple serum BDG tests were obtained during this 7-day window, the highest value was included in the analysis. Patients were excluded if they (1) received empiric treatment (therapeutic doses) for PCP for at least 3 days prior to PCP PCR testing; (2) had PCP PCR performed on specimens other than BAL, bronchial washings, or lung tissues; or (3) had serum BDG performed more than 7 days from the PCP PCR. The following patient demographics and clinical variables were collected: age, sex, underlying disease, absolute lymphocyte count, HIV co-infection, receipt of immunosuppressive medications, receipt of PCP prophylaxis, and serum lactate dehydrogenase (LDH) values obtained within 2 weeks before PCP PCR were collected.

The study was reviewed and approved by the Memorial Sloan Kettering Cancer Center Institutional Review Board.

Definitions

Lymphopenia was defined as having an ACL <0.5 K/μL on the day closest to when a PCP PCR was obtained on a bronchoscopy or biopsy sample. When PCP was suspected, corticosteroid use was defined as use within 60 days of admission date, at an equivalent dose of prednisone 20 mg or greater, and for at least 3 consecutive days. If a patient received temozolamide, fludarabine, or alemtuzumab at least once in the 180 days before PCP testing, they were considered to have received a T-cell suppressing agent; receipt of Ibrutnib or check-point blockade therapy (Ipilumab, Nivolumab, Pembrolizumab) were similarly defined. Patients receiving trimethoprim-sulfamethoxazole (Bactrim), Atovaquone, Pentamidine (inhaled or intravenously), or Dapsone for more than 6 days before PCP PCR was checked were considered to have received a PCP prophylaxis.

PCP infections were defined as either definite, probable, possible, or not PCP, as described for other invasive fungal diseases, with some modifications (Table 1) [23]. Other non-PCP invasive fungal infections (eg, invasive Aspergillosis) were defined as described previously [23]. All patients with a negative PCP PCR who did not receive empiric treatment for PCP were considered negative for PCP infection. Chart reviews for all patients with a positive PCP PCR were performed independently by 2 infectious disease physicians (S. M. and A. F.-G.).

Pneumocystis Pneumonia Laboratory Methods

Samples were tested by a previously-described real-time PCP PCR assay [24, 25]. The assay is a qualitative, real-time PCR performed on the LightCycler instrument (Roche Molecular Diagnostics), with primers and probes targeting the cyclin-dependent protein kinase *cdc2* gene of *P. jiroveci*. BDGs were performed using the Fungitell BDG test (Associates of Cape Cod). Numerical values for the BDG tests range from 31 pg/mL to

Table 1. Definitions

Infection	Definite	Probable	Possible	Not PCP
Pneumocystis jirovecii pneumonia	1. Suggestive respiratory symptoms[a]; and 2. PCP PCR, detected in BAL and/or BW; and 3. Documented PCP in histopathology, GMS cytology, and/or DFA	1. Compatible risk factors with clinical presentation[a]; and 2. PCP PCR, detected in BAL and/or BW	1. Nonspecific clinical presentation without predisposing risk factors; and 2. PCP PCR, detected in BAL and/or BW	1. Negative histopathology/cytology/PCP PCR with or without an established alternate diagnosis

Abbreviations: BAL, bronchoalveolar lavage; BDG, beta-D glucan; BW, bronchial washing; DFA, direct fluorescent antibody; GMS, Gomori methenamine silver stain; PCP, *Pneumocystis* pneumonia; PCR, polymerase chain reaction.

[a]Shortness of breath, dyspnea on exertion, increased O_2 requirements, dry or productive cough, and hemoptysis.

500 pg/mL. The test was interpreted using the following values as interpretive criteria: negative for values <60 pg/mL; indeterminate for values 60–79 pg/mL; and positive for values >80 pg/mL. For this study, all values <80 pg/mL were interpreted as negative.

Statistical Methods

We applied receiver operating characteristic methodology to evaluate the accuracy of BDG at different thresholds in the context of PCP PCR results. For evaluating BDG test performance, we combined and analyzed the definite subgroup with the probable subgroup and the possible subgroup with the not-PCP subgroup. Serum BDG was assessed with respect to sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Analyses were performed in GraphPad Prism 7 and R, version 3.3.2.

RESULTS

A total of 697 unique patients over the 3-year study period underwent bronchoscopy with evaluation for PCP by PCR. Of those, 438 patients met all requirements for inclusion in the study (Figure 1). There were 53 patients who had a PCP PCR–positive test, and among these, 40 patients had definite (n = 11) or probable (n = 29) PCP. Another 13 patients were classified as possible PCP (Supplementary Table S1). All PCP PCR–negative patients (n = 385) were classified as not PCP (Table 1).

The demographics and clinical characteristics of patients included in the study are summarized in Table 2. Cancer diagnoses for all patients included 322 hematologic malignancies, including recipients of HCT (124 patients; 27 autologous HCT, 82 allogeneic HCT, and 15 double umbilical cord transplants). Co-infection with HIV was found to be 6% and 4% in PCP PCR–positive and PCP PCR–negative patients, respectively. There were very few patients (<5%) in each group that received immunomodulating agents, such as immunotherapy, T-cell suppressing agents, and Ibrutinib. Most patients (85.8%) were not on PCP prophylaxis and had no exposure to steroids (64%). Lastly, 70% of PCP PCR–positive patients had an elevated BDG (≥80 pg/ml), while 82% of PCP PCR–negative patients had a BDG value of ≤79 pg/ml (Table 2).

We first evaluated the performance characteristics of BDG using the manufacturer's threshold of >80 pg/mL to designate those positive, with PCP PCR as the reference method. This showed a sensitivity of 69.8% (95% confidence interval [CI] 56.5–80.5%), specificity of 81.2% (95% CI 77.7–85.4%), PPV of 34.6% (95% CI 26.2–44.0%), and NPV of 95.2% (95% CI 92.3–97.0%; Table 3). At a threshold BDG value of 200 pg/mL, the sensitivity was much lower but had an improved specificity of 90.4% (95% CI 87.7–93.1%). The PPV was poor using the higher threshold, but the NPV was high, at 93.6% (95% CI 90.6–95.8%; Table 4). The diagnostic accuracy as shown by the area under the curve was 0.82 (Figure 2).

Next, we focused on the subset of 53 patients positive for PCP DNA. Using clinical data as described in Table 1, 40 patients were considered to have definite or probable PCP and were treated. The other 13 patients did not have convincing clinical evidence for PCP, and were grouped as those with possible PCP infections: among these, 12 were treated. The proportion of patients with BDG values for each of these categories is shown in Figure 3 and Supplementary Table S1. Ground Glass Opacity (GGO; seen on chest imaging), elevated serum LDH,

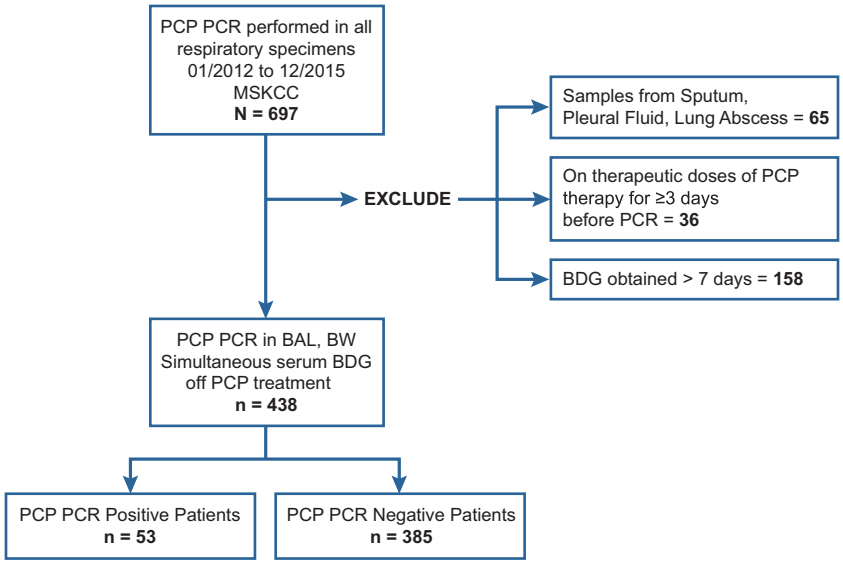

Figure 1. Diagram of inclusion and exclusion criteria for the study population. Abbreviations: BAL, bronchoalveolar lavage; BDG, beta-D glucan; BW, bronchial washings; MSKCC, Memorial Sloan Kettering Cancer Center; PCP, *Pneumocystis* pneumonia; PCR, polymerase chain reaction.

Table 2. Patient Characteristics

	PCP PCR Positive	PCP PCR Negative	Total
Patients	53	385	438
Mean age	57	56	57
Sex (% male)	38 (72%)	221 (57%)	259 (60%)
Underlying disease:			
Transplant type			
Autologous	1 (2%)	26 (7%)	27
Allogeneic	11 (20%)	71 (18%)	82
DUCT	0	15 (4%)	15
Underlying hematologic malignancy (non transplant)			
Lymphoma	12 (23%)	49 (13%)	61
Lymphoid leukemia	5 (9%)	33 (9%)	38
Myeloid leukemia	5 (9%)	79 (21%)	84
Multiple myeloma/aamyloidosis	1 (2%)	14 (4%)	15
Solid tumor	18 (34%)	92 (24%)	110
No cancer	0	6 (2%)	6
HIV co infection	3 (6%)	16 (4%)	6
Steroid exposure	19 (36%)	139 (36%)	158
Other T-cell–suppressing regimens	1 (2%)	4 (1%)	5
Immunotherapy	1 (2%)	9 (2%)	10
Ibrutinib	4 (8%)	6 (2%)	10
PCP prophylaxis			
Bactrim	2 (4%)	25 (6%)	27
Atovaquone	6 (11%)	28 (7%)	34
Pentamidine	0	1 (0.3%)	1
Other	0	0	0
LDH (Units/L)			
<250	9 (17%)	77 (20%)	86
250–500	23 (43%)	116 (30%)	139
>500	11 (21%)	66 (17%)	77
Not obtained	10 (19%)	126 (33%)	136
ALC (K/mcL)[a]			
<0.5	27 (51%)	169 (44%)	196
≥0.5	26 (49%)	216 (56%)	242
BDG (picogram/ml)			
≤79	16 (30%)	315 (82%)	331
80–200	9 (17%)	33 (9%)	42
201–499	8 (15%)	19 (5%)	27
≥500	20 (38%)	18 (5%)	38

Abbreviations: ALC, absolute lymphocyte count; BDG, beta-D glucan; DUCT, double umbilical cord transplant; HIV, human immunodeficiency virus; LDH, lactate dehydrogenase; PCP, *Pneumocystis* pneumonia; PCR, polymerase chain reaction.

[a]Thousands of cells per microliter of blood.

and steroid use were more common in the definite and probable groups (Supplementary Figure S1 and Supplementary Table S1).

A threshold of 80 pg/mL resulted in BDG with sensitivity of 87.5% (95% CI 73.2–95.8%), specificity of 84.6% (95% CI 54.6–98.1%), PPV of 94.6% (95% CI 81.8–99.3%), and NPV of 68.8% (95% CI 41.3–89.0%; Table 5). Using a threshold of ≥200 pg/mL, BDG had a sensitivity of 70% (95% CI 53.5–83.4%), specificity of 100% (95% CI 75.3–100%), PPV value of 100% (95% CI 87.7–100%), and NPV value of 52.0% (95% CI 31.3–72.2%; Table 6).

A total of 70 patients had an elevated BDG (>80 pg/mL), but a negative PCP PCR (Table 3). For 57.1% (40/70) of these patients, an alternative diagnosis other than PCP was found. Of these patients, 52.5% (21/40) patients had an invasive fungal infection with *Aspergillus spp.* or other molds. *Candida* colonization explained the elevated BDG in 40% (16/40) of patients. The remaining 7.5% (3/40) of cases were secondary to intravenous immunoglobulin (IVIG) use (Supplementary Figure S2). For the remaining 42.9% (30/70) patients, no clear reasons could be identified in the electronic health record to explain the elevated BDG value in the setting of a negative PCP PCR. A total of 11 of these 30 patients were treated for PCP.

DISCUSSION

In this study, we evaluated the performance characteristics of serum BDG in conjunction with PCP PCR from bronchoscopy specimens, in a cancer patient population at high risk for PCP

Table 3. Beta-D Glucan Performance at 80 pg/mL Threshold in All *Pneumocystis* Pneumonia Polymerase Chain Reaction Patients

	PCP PCR +	PCP PCR –
BDG + (>80 pg/mL)	37	70
BDG – (<80 pg/mL)	16	315
Total	53	385
Sensitivity	69.8%	95% CI (56.5–80.5%)
Specificity	81.2%	95% CI (77.7–85.4%)
PPV	34.6%	95% CI (26.2–44.0%)
NPV	95.2%	95% CI (92.3–97.0%)

Abbreviations: BDG, beta-D glucan; CI, confidence interval; NPV, negative predictive value; PCP, *Pneumocystis* pneumonia; PCR, polymerase chain reaction, PPV, positive predictive value.

infection. We found this combination to be a useful diagnostic tool. Our study findings, derived from over 400 patients with lower airway disease who underwent evaluations for PCP, suggest that a serum BDG level of <80 pg/mL has a high NPV (95.2%). With the careful review of clinical features, this cutoff value could be used to discontinue anti-PCP therapy. This is consistent with other studies, primarily in HIV patients [10, 26], and could be especially useful in situations where patients are unable to undergo a bronchoscopy evaluation. Our second main finding is that, for patients with a positive BAL PCP PCR, a serum BDG of >200 pg/mL favors infection over colonization (specificity 100% and PPV 100%). The proposed criteria offer support to the probabilistic approach applied by clinicians in interpreting newer diagnostic tests for PCP in clinical practice, especially in oncology patient populations, where an assessment of underlying immune function can be complex and lung infiltrates pose a wide range of diagnostic possibilities.

When clinically feasible, the evaluation of BAL fluid for PCP detection should be pursued. The sensitivity of conventional direct visualization methods (direct fluorescent antibody, cytology) is suboptimal in non-HIV patients [12]. PCR for PCP DNA has high analytical sensitivity and specificity, with a NPV approaching 100% [27–29]. This method is increasingly being used for target organism detection in BAL fluid and is now preferred over conventional techniques by many laboratories.

Table 4. Beta-D Glucan Performance at 200 pg/mL Threshold in All *Pneumocystis* Pneumonia Polymerase Chain Reaction Patients

	PCP PCR +	PCP PCR -
BDG + (>200 pg/mL)	28	37
BDG – (<200 pg/mL)	25	348
Total	53	385
Sensitivity	52.8%	95% CI (38.6–66.7%)
Specificity	90.4%	95% CI (87.0–93.1%)
PPV	43.9%	95% CI (31.7–56.7%)
NPV	93.3%	95% CI (90.3–95.6%)

Abbreviations: BDG, beta-D glucan; CI, confidence interval; NPV, negative predictive value; PCP, *Pneumocystis* pneumonia; PCR, polymerase chain reaction; PPV, positive predictive value.

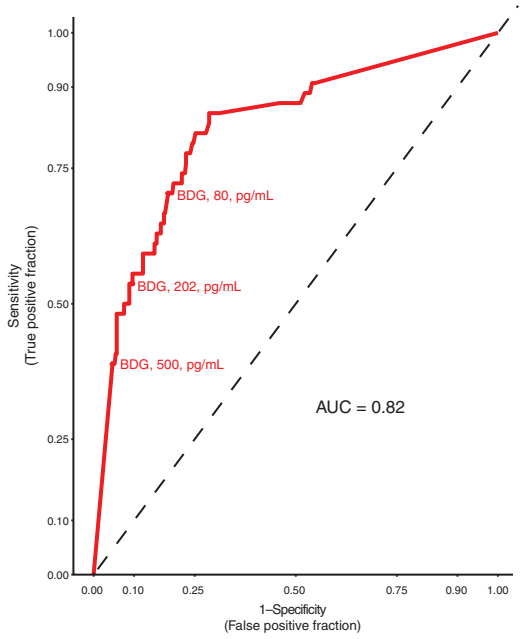

Figure 2. Receiver operating characteristic curve for serum BDG performance, using PCR as the reference method. BDG shown at thresholds of 80 pg/ml, 200 pg/ml, and 500 pg/ml. Abbreviations: AUC, area under the curve; BDG, beta-D glucan; PCR, polymerase chain reaction.

Studies evaluating the clinical utility of PCP PCR for diagnosing PCP have rendered mixed results and raised concerns around the clinical specificity of the test [16–18, 25]. While some of these differences are related to the patient populations evaluated, the analytical performance of the test varies with gene targets (eg, cyclin-dependent kinase 2 vs large subunit ribosomal ribonucleic acid) and assay design (nested PCR and real-time PCR) [29]. Collectively, these studies highlight the limitation of PCR in distinguishing between PCP colonization and infection. In addition, since we identified a subset of patients with negative PCP PCRs and elevated BDGs for reasons that were unclear, the possibility of false-negative PCP PCRs could be considered in patients with clinical syndromes consistent with PCP. BDG testing also demonstrates high sensitivity for the diagnosis of fungal infections but lacks specificity for PCP [30]. However, in

Table 5. Beta-D Glucan Performance at 80 pg/mL Threshold in *Pneumocystis* Pneumonia Polymerase Chain Reaction–Positive Patients

	Definite/Probable PCP	Possible PCP
BDG + (>80 pg/mL)	35	2
BDG – (<80 pg/mL)	5	11
Total	40	13
Sensitivity	87.5%	95% CI (73.2–95.8%)
Specificity	84.6%	95% CI (54.6–98.1%)
PPV	94.6%	95% CI (81.8–99.3%)
NPV	68.8%	95% CI (41.3–89.0%)

Abbreviations: BDG, beta-D glucan; CI, confidence interval; NPV, negative predictive value; PCP, *Pneumocystis* pneumonia; PPV, positive predictive value.

Table 6. Beta-D Glucan Performance at 200 pg/mL Threshold in *Pneumocystis* Pneumonia Polymerase Chain Reaction–Positive Patients

	Definite/Probable PCP	Possible PCP
BDG + (>200 pg/mL)	28	0
BDG – (<200 pg/mL)	12	13
Total	40	13
Sensitivity	70.0%	95% CI (53.5–83.4%)
Specificity	100.0%	95% CI (75.3–100.0%)
PPV	100.0%	95% CI (87.7–100.0%)
NPV	52.0%	95% CI (31.3–72.2%)

Abbreviations: BDG, beta-D glucan; CI, confidence interval; NPV, negative predictive value; PCP, *Pneumocystis* pneumonia; PPV, positive predictive value.

patients with positive PCP PCRs, the PPV of BDG for PCP was excellent (94.2%), and further increased to 100% when a higher threshold value (≥200 pg/mL compared to 80 pg/mL) was used to define a positive result. We conclude that an elevated BDG value of ≥200 pg/mL, in conjunction with a positive PCR result, strongly supports a diagnosis of PCP infection.

We also evaluated a possible relationship between GGOs on chest imaging and higher mean LDH values in the context of PCP PCR. Older studies from HIV patients reported that GGOs on chest imaging and an elevated LDH value could be used as indirect evidence for PCP [31–34]. Although this observation was also seen in our cohort of cancer patients, additional studies will be necessary to confirm this finding.

There are several limitations to our study. First, the retrospective design did not allow for the evaluation of the pre-test probability of PCP to help determine whether the BDG and PCP PCR lab tests were clinically indicated. Second, although we evaluated over 400 patients, only 9% had probable or definite PCP. Third, the quantification of BDG could not be performed for values outside of the reported threshold ranges of 80–500 pg/mL. Similarly, the quantification of fungal burden by PCR was not performed.

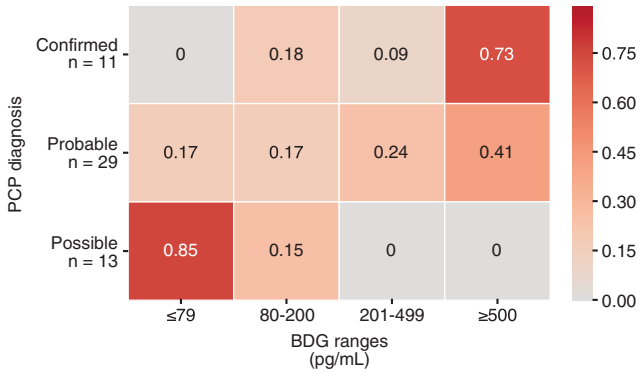

Figure 3. PCP patients and BDG value heat map. This figure illustrates the proportion of patients in each PCP diagnostic category and the corresponding BDG value. The intensity of the red color increases with of a greater proportion of patients falling within the corresponding BDG range and diagnostic category. Abbreviations: BDG, beta-D glucan; PCR, polymerase chain reaction.

In summary, conventional direct visualization techniques are often inadequate for establishing a diagnosis of PCP. Serum BDG and PCR overcome these limitations, but are not specific enough to independently become the current gold standard. Our study findings offer guidance to clinicians on the interpretation of newer tests, alone and in combination, and in various commonly-encountered clinical situations among oncology patients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Sepkowitz KA. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. Clin Infect Dis **2002**; 34:1098–107.
2. Williams KM, Ahn KW, Chen M, et al. The incidence, mortality and timing of *Pneumocystis jiroveci* pneumonia after hematopoietic cell transplantation: a CIBMTR analysis. Bone Marrow Transplant **2016**; 51:573–80.
3. Bollée G, Sarfati C, Thiéry G, et al. Clinical picture of *Pneumocystis jiroveci* pneumonia in cancer patients. Chest **2007**; 132:1305–10.
4. Chamilos G, Lionakis MS, Kontoyiannis DP. Call for action: invasive fungal infections associated with ibrutinib and other small molecule kinase inhibitors targeting immune signaling pathways. Clin Infect Dis **2018**; 66:140–8.
5. Kulke MH, Bergsland EK, Ryan DP, et al. Phase II study of recombinant human endostatin in patients with advanced neuroendocrine tumors. J Clin Oncol **2006**; 24:3555–61.
6. Reinwald M, Boch T, Hofmann WK, Buchheidt D. Risk of infectious complications in hemato-oncological patients treated with kinase inhibitors. Biomark Insights **2015**; 10:55–68.
7. Varughese T, Taur Y, Cohen N, et al. Serious infections in patients receiving ibrutinib for treatment of lymphoid cancer. Clin Infect Dis **2018**; 67:687–92.
8. Salerno D, Mushatt D, Myers L, et al. Serum and bal beta-D-glucan for the diagnosis of *Pneumocystis* pneumonia in HIV positive patients. Respir Med **2014**; 108:1688–95.
9. Wood BR, Komarow L, Zolopa AR, Finkelman MA, Powderly WG, Sax PE. Test performance of blood beta-glucan for *Pneumocystis jirovecii* pneumonia in patients with AIDS and respiratory symptoms. AIDS **2013**; 27:967–72.
10. Sax PE, Komarow L, Finkelman MA, et al; AIDS Clinical Trials Group Study A5164 Team. Blood (1->3)-beta-D-glucan as a diagnostic test for HIV-related *Pneumocystis jirovecii* pneumonia. Clin Infect Dis **2011**; 53:197–202.
11. Cordonnier C, Alanio A, Cesaro S, et al; Fifth 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). *Pneumocystis jirovecii* pneumonia: still a concern in patients with haematological malignancies and stem cell transplant recipients-authors' response. J Antimicrob Chemother **2017**; 72:1266–8.
12. 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.
13. Fan LC, Lu HW, Cheng KB, Li HP, Xu JF. Evaluation of PCR in bronchoalveolar lavage fluid for diagnosis of *Pneumocystis jirovecii* pneumonia: a bivariate meta-analysis and systematic review. PLOS One **2013**; 8:e73099.
14. Lu Y, Ling G, Qiang C, et al. PCR diagnosis of *Pneumocystis* pneumonia: a bivariate meta-analysis. J Clin Microbiol **2011**; 49:4361–3.

15. Tia T, Putaporntip C, Kosuwin R, Kongpolprom N, Kawkitinarong K, Jongwutiwes S. A highly sensitive novel PCR assay for detection of *Pneumocystis jirovecii* DNA in bronchoalveloar lavage specimens from immunocompromised patients. Clin Microbiol Infect **2012**; 18:598–603.
16. Maskell NA, Waine DJ, Lindley A, et al. Asymptomatic carriage of *Pneumocystis jirovecii* in subjects undergoing bronchoscopy: a prospective study. Thorax **2003**; 58:594–7.
17. Nevez G, Raccurt C, Vincent P, Jounieaux V, Dei-Cas E. Pulmonary colonization with *Pneumocystis carinii* in human immunodeficiency virus-negative patients: assessing risk with blood CD4+ T cell counts. Clin Infect Dis **1999**; 29:1331–2.
18. Khodadadi H, Mirhendi H, Mohebali M, Kordbacheh P, Zarrinfar H, Makimura K. *Pneumocystis jirovecii* colonization in non–HIV-infected patients based on nested-PCR detection in bronchoalveolar lavage samples. Iran J Public Health **2013**; 42:298–305.
19. Theel ES, Doern CD. β-D-glucan testing is important for diagnosis of invasive fungal infections. J Clin Microbiol **2013**; 51:3478–83.
20. Theel ES, Jespersen DJ, Iqbal S, et al. Detection of (1, 3)-β-D-glucan in bronchoalveolar lavage and serum samples collected from immunocompromised hosts. Mycopathologia **2013**; 175:33–41.
21. Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME. Accuracy of β-D-glucan for the diagnosis of *Pneumocystis jirovecii* pneumonia: a meta-analysis. Clin Microbiol Infect **2013**; 19:39–49.
22. 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.
23. 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.
24. Arcenas RC, Uhl JR, Buckwalter SP, et al. A real-time polymerase chain reaction assay for detection of *Pneumocystis* from bronchoalveolar lavage fluid. Diagn Microbiol Infect Dis **2006**; 54:169–75.
25. Wilson JW, Limper AH, Grys TE, Karre T, Wengenack NL, Binnicker MJ. *Pneumocystis jirovecii* testing by real-time polymerase chain reaction and direct examination among immunocompetent and immunosuppressed patient groups and correlation to disease specificity. Diagn Microbiol Infect Dis **2011**; 69:145–52.
26. Held J, Koch MS, Reischl U, Danner T, Serr A. Serum (1 → 3)-β-D-glucan measurement as an early indicator of *Pneumocystis jirovecii* pneumonia and evaluation of its prognostic value. Clin Microbiol Infect **2011**; 17:595–602.
27. Torres J, Goldman M, Wheat LJ, et al. Diagnosis of *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected patients with polymerase chain reaction: a blinded comparison to standard methods. Clin Infect Dis **2000**; 30:141–5.
28. Durand-Joly I, Chabé M, Soula F, Delhaes L, Camus D, Dei-Cas E. Molecular diagnosis of *Pneumocystis* pneumonia. FEMS Immunol Med Microbiol **2005**; 45:405–10.
29. Botterel F, Cabaret O, Foulet F, Cordonnier C, Costa JM, Bretagne S. Clinical significance of quantifying *Pneumocystis jirovecii* DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. J Clin Microbiol **2012**; 50:227–31.
30. Egger M, Prüller F, Raggam R, et al. False positive serum levels of (1-3)-ß-D-glucan after infusion of intravenous immunoglobulins and time to normalisation. J Infect **2018**; 76:206–10.
31. Zaman MK, White DA. Serum lactate dehydrogenase levels and *Pneumocystis carinii* pneumonia. Diagnostic and prognostic significance. Am Rev Respir Dis **1988**; 137:796–800.
32. Crans CA Jr, Boiselle PM. Imaging features of *Pneumocystis carinii* pneumonia. Crit Rev Diagn Imaging **1999**; 40:251–84.
33. Boudes P. Practical utility of lactate dehydrogenase in the diagnosis of human immunodeficiency virus-related *Pneumocystis carinii* pneumonia. Arch Intern Med **1991**; 151:198.
34. Butt AA, Michaels S, Kissinger P. The association of serum lactate dehydrogenase level with selected opportunistic infections and HIV progression. Int J Infect Dis **2002**; 6:178–81.