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Identifying optimal serum 1,3-β-D-Glucan cut-off for diagnosing *Pneumocystis Jirovecii* Pneumonia in non-HIV patients in the intensive care unit

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Abstract

Background Serum (1,3)-β-D-glucan (BDG) detection for diagnosis of *Pneumocystis jirovecii* pneumonia (PJP) in non-human immunodeficiency virus (HIV) immunocompromised patients lacks intensive care unit (ICU)-specific data. We aimed to assess its performance and determine the optimal cutoff for PJP in ICU population.

Methods This retrospective study included critically ill non-HIV immunocompromised patients admitted to a medical ICU with suspected pneumonia, undergoing simultaneous microbiological testing for *P. jirovecii* on lower respiratory tract specimens and serum BDG. Confounders affecting BDG positivity were explored by multivariable logistic regression. Optimal cut-offs were derived from Youden's index for the entire cohort and subgroups stratified by confounders. Diagnostic performance of serum BDG was estimated at different cutoffs.

Results Of 400 patients included, 42% were diagnosed with PJP and 58.3% had positive serum BDG. Serum BDG's area under the receiver operating characteristic curve was 0.90 (0.87–0.93). At manufacturer's 150 pg/ml cut-off, serum BDG had high sensitivity and negative predictive value (94%), but low specificity and positive predictive value (67%). Confounders associated with a positive serum BDG in PJP diagnosis included IVIG infusion within 3 days (odds ratio [OR] 9.24; 95% confidence interval [CI] 4.09–20.88, $p < 0.001$), other invasive fungal infections (OR 4.46; 95% CI 2.10–9.49, $p < 0.001$) and gram-negative bacteremia (OR 29.02; 95% CI 9.03–93.23, $p < 0.001$). The application of optimal BDG cut-off values determined by Youden's index (252 pg/ml, 390 pg/ml, and 202 pg/ml) specific for all patients and subgroups with or without confounders improved the specificity (79%, 74%, and 88%) and corresponding PPV (75%, 65%, and 85%), while maintaining reasonable sensitivity and NPV.

Conclusions Tailoring serum BDG cutoff specific to PJP and incorporating consideration of confounders could enhance serum BDG's diagnostic performance in the ICU settings.

Keywords *Pneumocystis Jirovecii* pneumonia, (1,3)-β-D-glucan, Intensive care unit, Diagnosis, Cut-off value

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Background

Pneumocystis jirovecii pneumonia (PJP) is an opportunistic infection that represents a considerable threat to immunocompromised populations, including patients with human immunodeficiency virus (HIV) infection and non-HIV patients receiving chemotherapy, immunosuppressants and corticosteroids for hematological malignancies, solid-organ transplantation, and systemic inflammatory diseases [1, 2]. Non-HIV patients with PJP often have more severe clinical courses and worse outcomes [1, 3] especially those requiring intensive care unit (ICU) admission, with high mortality ranging from 58 to 75% [2, 4, 5]. Accurate and prompt diagnosis of PJP in critically ill non-HIV patients is crucial yet challenging due to difficulties in obtaining high-quality respiratory samples amid severe clinical conditions, exacerbated by the low sensitivity of conventional microscopic examination, which is attributed to low fungal burdens. The serum (1,3)-β-D-glucan (BDG) assay has emerged as a promising adjunct for the diagnosis of PJP. BDG is a polysaccharide found in the cell wall of most pathogenic fungi, including *Pneumocystis jirovecii* (*P.j*). Despite lacking mycological specificity, the serum BDG exhibits superior diagnostic performance for PJP when compared to other invasive fungal infections (IFIs) [6, 7]. Therefore, the serum BDG has been recently incorporated into the 2019 updated European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) diagnostic criteria of probable PJP in non-HIV patients [8]. Notably, PJP is increasingly prevalent in critically ill patients, particularly among non-HIV patients. Nonetheless, the ICU working group has not yet established specific criteria for the diagnosis of PJP in this population [9]. The scarcity of data regarding the performance of serum BDG in the diagnosis of PJP in the ICU poses a significant challenge. Studies have demonstrated variations in the diagnostic performance of BDG across different populations and clinical settings [10, 11]. For instance, the sensitivity of BDG was reportedly lower in patients with hematological malignancies than the non-specific non-HIV PJP patients [10]. Recently, a multicenter retrospective study investigated PJP in ICU patients and evaluated the performance of serum BDG for the diagnosis of PJP. The study revealed that using serum BDG alone yielded a high negative predictive value (NPV) of 96% but had a low positive predictive value (PPV) of 39% [12].

Therefore, we conducted a retrospective study with the aim of assessing the diagnostic performance of the serum BDG assay for PJP in non-HIV immunocompromised patients in the ICU setting and exploring potential strategies to improve diagnostic accuracy.

Methods

Study design and population

This retrospective study was conducted at Peking Union Medical College Hospital Medical ICU from January 1st, 2015, to June 30th, 2021. Non-HIV immunocompromised patients with suspected pneumonia were included if they underwent polymerase chain reaction (PCR) testing and Gomori methenamine silver (GMS) staining for Pneumocystis on lower respiratory tract (LRT) specimens and had serum BDG testing within three days before or after microbiology examination for *P.j* on LRT specimens. Patients who had received treatment for PJP for more than one week before serum BDG testing were excluded.

Ethics statement

The Institutional Review Board of the Peking Union Medical College Hospital approved the study under number K23C3112. Given that the study was noninterventional and retrospective in nature with anonymized data, written informed consent had been waived.

Definitions

In this study, non-HIV immunocompromised patients were defined as those who met any of the following criteria: (1) were diagnosed with hematologic malignancies; (2) had solid organ tumors and received chemotherapy within three months before ICU admission; (3) had undergone hematopoietic stem cell or solid organ transplantation; and (4) had systemic autoimmune or inflammatory diseases (SAIID) and were treated with corticosteroids at a therapeutic dose of ≥0.3 mg/kg of prednisone equivalent for ≥2 weeks in the past three months or patients exposed to cytotoxic drugs, immunosuppressants, anti-inflammatory biological agents, or rituximab in the past three months.

The diagnosis of PJP was based on the 2019 EORTC/MSGERC Consensus definitions, with a modification to exclude BDG as a mycological criterion [8]. Briefly, PJP diagnosis required the fulfilment of the following criteria: (1) the presence of respiratory symptoms such as cough, dyspnoea, or hypoxia; (2) the emergence of new ground glass opacities on chest CT; and (3) the detection of *Pneumocystis* via conventional staining or positive *Pneumocystis* PCR on LRT specimens. Two separate physicians (L.Y.Y. and C.Y.) reviewed the medical records and assessed the diagnosis of PJP. Disagreements were resolved by a third physician (P.J.M.).

Data collection

Medical records were retrospectively reviewed to collect various clinical and laboratory data. These included demographic features, underlying disease, severity of disease upon ICU admission (evaluated using the Acute

Physiology and Chronic Health Evaluation [APACHE] II, PaO_2/FiO_2 ratio and the use of mechanical ventilation), microbiological findings related to *Pneumocystis* on LRT specimens, the highest serum results of BDG and lactate dehydrogenase within three days of the respiratory sample collection date, absolute total and $CD4^+$ lymphocyte counts, and ICU and in-hospital outcomes.

Information regarding potential confounders associated with positive serum BDG results was collected. These included recent intravenous immunoglobulin (IVIG) therapy, other blood product transfusions, antimicrobial therapy, continuous renal replacement therapy (CRRT) within 3 days before BDG testing, concurrent other IFIs caused by BDG-producing organisms within one week before or after serum BDG testing, and bacteremia within 2 days before or after serum BDG testing.

Detection of *Pneumocystis jirovecii*

The measurement of serum BDG levels was conducted using the Fungus (1–3)-β-D Dextran Test Kit (Charles River Zhang Jiang, China A & C Biological LTD.), with a linear detection range of 10–500 pg/mL and a correlation coefficient of $|r| > 0.990$, as determined from a calibration curve with standard solutions. The maximum detection limit was 10,000 pg/mL, and BDG levels below 10 pg/mL (the minimum detection limit) were set at 10pg/mL. Samples exceeding the specified range were diluted using the kit's provided dilution vial (0.9 mL diluent) and reassayed accordance with the instructions provided in the kit. A serum BDG level of ≥150 pg/mL was considered positive according to the manufacturer's defined threshold.

For the detection of *Pneumocystis*, methods included direct microscopy using GMS staining and qPCR.The qPCR assay in this study was an in-house PCR, targeting the mitochondrial large subunit (mtLSU) rRNA gene of *P. j* [13]. Primers and probes targeting the human albumin gene were used as internal controls. The result was considered positive when the cycle threshold (Ct) value for the mtLSU gene was ≤37 and negative when Ct was >37.

Statistical analysis

The data are presented as the frequency and percentage for categorical variables and as the mean±standard deviation (SD) or median with interquartile range (IQR) for continuous variables. Differences in continuous data were analysed using the exact Mann–Whitney *U* test or the Kruskal–Wallis test, while Fisher's exact test was used for frequency data.

Logistic regression analysis with a backwards variable selection procedure was performed to identify factors associated with serum BDG positivity. Variables with a *P* value<0.2 in the univariate analysis or with clinical significance were included in the multivariable model. The factors causing BDG positivity, apart from PJP, are denoted as confounders of BDG positivity. Patients were stratified into two groups based on the presence of these confounders: the group with confounders and the group without confounders. The diagnostic performance of BDG for PJP, in addition to the determination of optimal cut-off levels, was separately evaluated in these two groups and in the entire study population.

Receiver operating characteristic (ROC) curves were generated to diagnose PJP, and the areas under the ROC curves (AUC_{ROC}) were compared using the nonparametric technique described by Delong et al. The optimal BDG cut-off value for diagnosing PJP was identified using the maximum Youden's index (J=Sensitivity+Specificity −1). The sensitivity, specificity, PPV, and NPV were calculated for both the manufacturer-recommended cut-off and the optimal cut-off.

P values≤0.05 were considered to indicate statistical significance. All the statistical analyses and graphical representations were generated using SPSS Statistics (version 29.0, IBM Corp., Armonk, NY, US) and GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA).

Results

Study population

Between January 1, 2015, and June 30, 2021, a total of 639 non-HIV immunocompromised patients were admitted to the medical ICU due to suspected pneumonia. Among them, 412 underwent real-time PCR and microscopy with GMS staining on LRT specimens and concomitant serum BDG testing. Of the LRT samples, 96% were BALF, while only 4% were tracheal aspirates. After excluding 12 patients who had received more than one week of anti-PJP treatment before serum BDG testing, 400 patients were included in the final analysis (Fig. 1). The average age of the patients in this study was 58 (IQR 43–65) years, and 56.3% were female. Upon ICU admission, the mean APACHE II score was 21, and 84% of patients required invasive mechanical ventilation. The most prevalent underlying immunocompromised condition was SAIID (80.1%), followed by hematological malignancies (11.3%) and solid malignancies receiving chemotherapy (6.0%). Complicated interstitial lung disease was found in 165 patients (41.3%). More than half of the patients received corticosteroids. Only 20 patients (5%) received PJP prophylaxis with trimethoprim-sulfamethoxazole.

Among the 400 patients included in this study, 167(41.7%) was diagnosed with PJP, including 41 with both positive GMS staining and positive *P.j* PCR and 126 with positive *P.j* PCR alone. There were no statistically significant differences in age or sex between the groups with and without PJP. The PaO_2/FiO_2 ratio and MV requirement were similar in both groups. Patients with PJP had higher serum BDG and LDH levels and lower

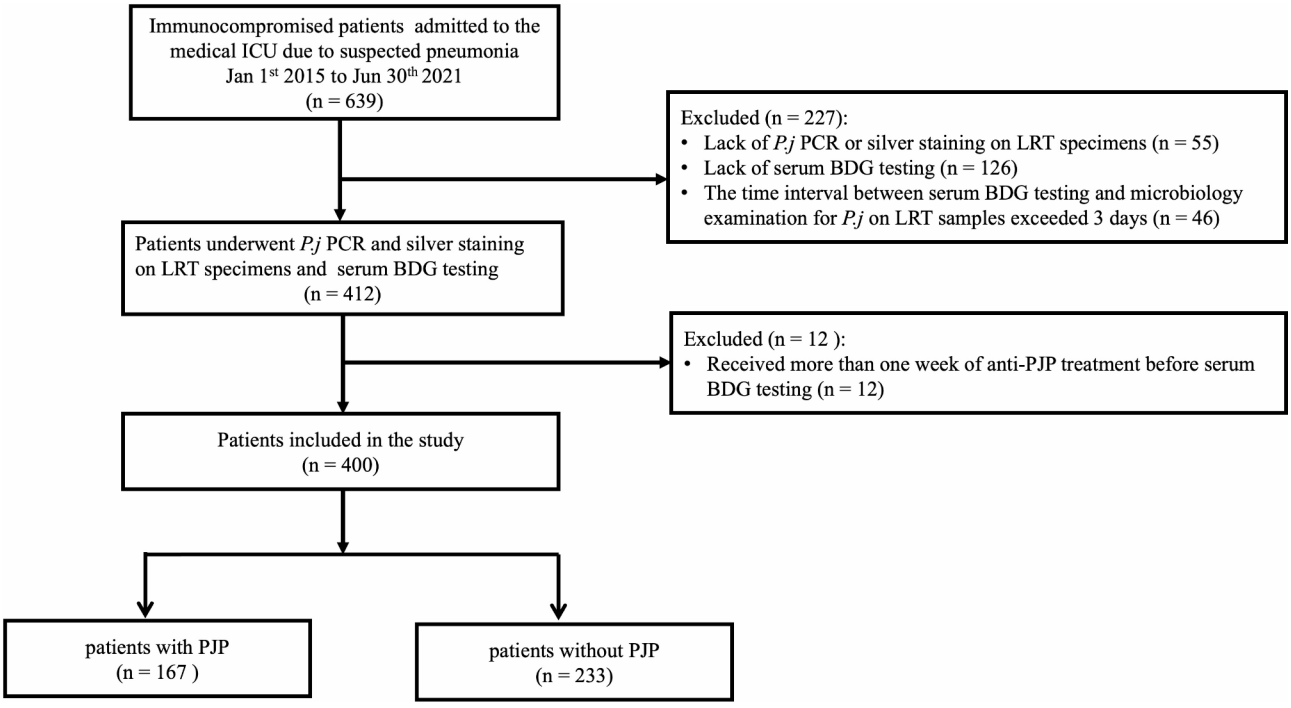

Fig. 1 Flowchart of the patients included in this study. BDG, (1–3)- β-D-glucan; PCR, polymerase chain reaction; PJP, *Pneumocysitis jirovecii* pneumonia; *P.j*, *Pneumocysitis jirovecii*

CD4+cell counts than patients without PJP (Table 1). ICU and hospital mortality rates were significantly greater in the PJP group. (67.7% vs. 52.8%, $p=0.009$; 68.9% vs. 54.5%, $p=0.012$, respectively).

Serum BDG assay results

The median serum level of BDG in the overall patients was 230 pg/ml (IQR, 54–767), with 233 patients (58.3%) exhibiting a positive BDG based on the manufacturer's cut-off (150 pg/ml). In patients with PJP, the median serum BDG level was significantly greater at 820 pg/ml (IQR, 401–1412) than that in non-PJP patients, for which the median was 72 pg/ml (IQR 28–221) ($p<0.001$). The rates of serum BDG positivity in PJPand non-PJP patients were 94% and 32.6%, respectively. There was no significant difference in serum BDG between patients with microscopic evidence of PJP and those without microscopic evidence (820 pg/ml vs. 827 pg/ml, $p=0.717$).

Confounders of positive serum BDG

Patients with a positive BDG were more likely than were those with a negative serum BDG to have PJP, other IFIs, bacterial pneumonia, or gram-negative bacteremia and to have recently received IVIG infusions (Additional file 1: Table S1). No significant associations were found between a positive BDG and other reported confounders, including blood product transfusions, specific antibiotics, or hemodialysis therapy. According to the multivariate logistic regression analysis, in addition to PJP, three confounders were shown to be associated with a positive BDG result: recent IVIG infusion (within 3 days before serum BDG testing) (odds ratio [OR] 9.24, 95% CI 4.09–20.88; $p<0.001$), other IFIs (within a one-week window of serum BDG testing) (OR 4.46, 95% CI 2.10–9.49; $p<0.001$), and concurrent gram-negative bacteremia (within a 2-day window of serum BDG testing) (OR 29.02, 95% CI 9.03–93.23; $p<0.001$) (Table 2).

All patients were categorized into two groups based on the presence of confounders: the group with confounders and the group without confounders. Among the entire population, more than one-third ($n=133$) were categorized into the confounders group. The incidence of PJP was not significantly different between the two groups (44.5% in the group without confoundersvs. 36.0% in the group withconfounders). However, the serum BDG was significantly higher in the group with confounders than in the group without confounders (370 pg/ml vs. 189 pg/ml, $p=0.002$), as was the BDG positive rate (67.7% vs. 53.6%, $p=0.007$).

Diagnostic performance of BDG for the diagnosis of PJP

Analysis of the ROC curve for serum BDG in the diagnosis of PJP showed a promising AUC of 0.90 (95% CI: 0.87–0.93) in the overall population. Comparable performance was observed in the subgroup analyses, such as SAIIDs patients (AUC 0.89, 95% CI: 0.86–0.93) or those on mechanical ventilation (AUC 0.90, 95% CI: 0.87–0.93). However, the presence of confounders affected

Table 1 Demographic and clinical characteristics of study patients with and without PJP

Variable	Total (*n* = 400)	Patients with PJP (*n* = 167)	Patients without PJP (*n* = 233)	*P* value
Age (years)	58 (43,65)	58 (41,65)	58 (45, 65)	0.529
Female sex	212 (53.0)	94 (56.3)	118 (50.6)	0.265
Complicated interstitial lung disease	165 (41.3)	57 (34.0)	108 (64.4)	0.014
Immunocompromised conditions				
SAIID	321 (80.1)	149 (89.2)	172 (73.8)	< 0.001
Hematological malignancy	45 (11.3)	8 (4.8)	37 (15.9)	0.001
Solid tumor with chemotherapy	24 (6.0)	5 (3.0)	19 (8.2)	0.032
HSCT/ SOT	10 (2.5)	5 (3.0)	5 (2.0)	0.592
Corticosteroid exposure*	223 (55.8)	129 (77.2)	94 (40.3)	< 0.001
Concomitant infections				
Bacterial pneumonia	123 (30.8)	59 (35.3)	64 (27.5)	0.093
Virus pneumonia	94 (23.5)	54 (32.3)	40 (17.2)	0.210
Other IFIs**	81 (20.3)	36 (21.6)	45 (19.3)	0.582
Gram negative bacteremia***	36 (9)	9 (5.4)	27 (11.6)	0.033
Gram positive bacteremia***	13 (3.3)	5 (3)	8 (3.4)	0.807
Disease severityat time of PJP diagnosis				
APACHE II score	21 (17, 25)	19 (16, 23)	22 (18, 26)	< 0.001
PaO_2/FiO_2 (mmHg)	100 (75, 141)	100 (81,141)	100 (70, 141)	0.531
Invasive mechanical ventilation	336 (84)	141 (84.4)	195 (83.7)	0.842
Septic shock	130 (32.5)	41 (24.6)	89 (38.2)	0.004
Medical treatment (within 3 days before serum BDG testing)				
CRRT	65 (16.3)	16 (9.6)	49 (21.0)	0.002
IVIG infusion	56 (14)	18 (10.8)	38 (16.3)	0.116
Other blood products infusion‡	87 (21.8)	24 (14.4)	63 (27.0)	0.002
Laboratory dataat time of PJP diagnosis				
Positive GMS staining	41 (10.3)	41 (24.6)	0 (0)	< 0.001
Positive *Pneumocystis* PCR	187 (46.8)	167 (100.0)	20 (8.6)	< 0.001
Absolute neutrophil count, 10^3 cells/μL	7.2 (4.3, 10.9)	6.6 (4.3, 10.2)	7.7 (4.3, 12.2)	0.126
Absolute lymphocyte count, 10^3 cells/μL	0.42 (0.22, 0.65)	0.37 (0.22, 0.55)	0.47 (0.23, 0.75)	0.003
$CD4^+$ T lymphocyte count, cells /μL	115 (56, 225)	97 (48, 185)	143 (58, 250)	0.005
Serum BDG positivity ‡	233 (58.3)	157 (94.0)	76 (32.6)	< 0.001
Serum BDG (pg/ml)	230 (54,767)	820 (401,1412)	72 (28, 221)	< 0.001
Serum LDH (IU/L)	532 (333, 802)	674 (492, 883)	427 (264, 650)	< 0.001
Outcomes				
ICU mortality	236 (59.0)	113 (67.7)	123 (52.8)	0.009
Hospital mortality	242 (60.5)	115 (68.9)	127 (54.5)	0.012
ICU LOS	11.0 (7.0, 20.2)	12.0 (7.0, 20.7)	11.0 (7.0, 20.0)	0.604
Hospital LOS	23.5 (12.0, 38.7))	23.0 (10.0, 35.0)	24.0 (12.8, 42.0)	0.088

Data are presented as no. (%) or median (interquartile range) unless otherwise indicated

Abbreviations: APACHE, acute physiology and chronic health evaluation; BDG, (1,3)-β-D-glucan; CRRT, continuous renal replacement therapy; GMS, Gomori methenamine silver; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit; IFIs, invasive fungal infections; IVIG, intravenous immunoglobulin; LDH, lactate dehydrogenase; LOS, length of stay; PCR, polymerase chain reaction; PJP, *Pneumocysitis jirovecii* pneumonia; SAIID, systemic autoimmune or inflammatory diseases; SOT, solid organ transplantation

*Corticosteroid exposure was defined as corticosteroids at a therapeutic dose of ≥ 0.3 mg/kg of prednisone equivalent for ≥ two weeks in the past three months

**Microbiologically proven IFI caused by BDG-producing organisms other than PJP within a one-week window of serum BDG testing; Other IFIs in this study included invasive pulmonary aspergillosis (*n* = 64), invasive candidiasis (*n* = 10), invasive pulmonary mucormycosis (*n* = 3), invasive fusariosis (*n* = 2), disseminated cryptococcosis (*n* = 2)

***Bacteremia within a 2-day window of serum BDG testing. bacteria species of gram-negative bacteremia included *Pseudomonas aeruginosa* (*n* = 14), *Acinetobacter baumannii* (*n* = 8), *Klebsiella pneumoniae* (*n* = 9), Escherichia coli (*n* = 4), and *Enterobacter cloacae* (*n* = 1). Bacteria species of gram-positive bacteremia included *Staphylococcus aureus* (*n* = 4), *Staphylococcus epidermidis* (*n* = 3), *Streptococcus* (*n* = 2), *Enterococcus faecalis* (*n* = 2), and *Corynebacterium striatum* (*n* = 2)

†Other blood products included packed red blood cells, undiluted platelets, fresh frozen, coagulation factors, and human albumin, excluding IVIG

‡Serum BDG positivity was defined as a serum BDG value equal or above the manufacturer cut-off (150 pg/ml)

Table 2 Multivariable analysis of factors associated with serum BDG positivity*

Variables	Odds Ratio	95% CI	*P* value
PJP	75.76	34.56–166.05	< 0.001
Other IFIs†	4.46	2.10–9.49	< 0.001
Gram negative bacteremia‡	29.02	9.03–93.23	< 0.001
IVIG therapy§	9.24	4.09–20.88	< 0.001

Variables in the logistic regression model with backward LR included presence of PJP, other invasive fungal infections, concomitant bacterial pneumonia, Gram negative bacteremia, recent IVIG therapy, Blood products transfusion, recent CRRT, Use of meropenem/imipenem; Hosmer Lemeshow Goodness-of-fit for this model: $P=0.268$

Abbreviations: BDG, (1,3)-β-D-glucan; CI, confidential interval; CRRT, continuous renal replacement therapy; IFIs, invasive fungal infections; IVIG, intravenous immunoglobulin; PJP, *Pneumocysitis jirovecii* pneumonia

*Serum BDG positivity was defined as a serum BDG value equal or above the manufacturer cut-off (150 pg/ml)

†IFI caused by BDG-producing organisms except PJP within a one-week window of serum BDG testing

‡Bacteremia within a 2-day window of serum BDG testing

§IVIG therapy within 3 days before serum BDG testing

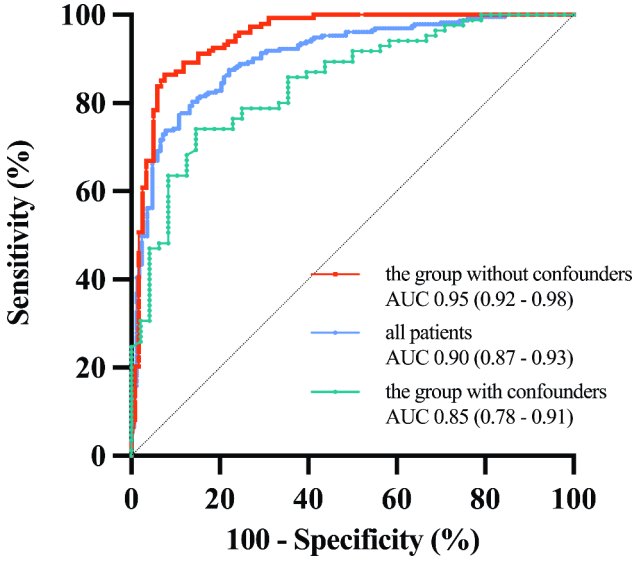

Fig. 2 ROC curve of BDG for PJP in all patients and subgroups with and without confounders. AUC, area under curve; BDG, (1–3)-β-D-glucan; PJP, *Pneumocysitis jirovecii* pneumonia; ROC, receiver operating characteristic

the performance of the BDG assay. The AUC_{ROC} was significantly greater in the group without confounders compared to the group with confounders (0.95, [95% CI: 0.93–0.98] vs. 0.85 [95% CI: 0.78–0.91], $p=0.005$) (Fig. 2).

The ROC analysis revealed that the optimal cut-off values for BDG in the diagnosis of PJP were 202 pg/ml (non-confounder group), 390 pg/ml (confounder group), and 252 pg/ml (overall population). The sensitivity, specificity, PPV, and NPV were calculated at these cut-off values and at the manufacturer's cut-off of 150 pg/ml. Using the manufacturer's cut-off of 150 pg/ml, while maintaining a constant sensitivity of 94% across groups, significant differences were found in terms of specificity: 78% in the non-confounder group, 47% in the confounder group, and 67% in the overall population. Despite the high incidence of PJP (41.4%) in this study, the PPV was suboptimal, ranging from 50 to 78% across different groups (Table 3). However, upon adopting the specific optimal cut-offs for each group, there was a significant increase in specificity, particularly in the confounder group (0.47 [95% CI: 0.37–0.58] at 150 pg/ml vs. 0.74 [95% CI: 0.63–0.82] at 390 pg/ml). Although the improvements in specificity and positive predictive value were accompanied by slight decreases in sensitivity and negative predictive value, the latter still remained within acceptable and reasonable ranges.

Discussion

We conducted a retrospective study to assess the performance of the serum BDG assay for the diagnosis of PJP among non-HIV immunocompromised patients admitted to the ICU while also exploring optimized cut-off values to increase its accuracy. Our results demonstrated that serum BDG testing exhibited satisfactory sensitivity and an NPV above 90% but inadequate specificity and a PPV below 70% at the manufacturer's recommended cut-off. By adopting an optimized cut-off higher than the manufacturer's recommendation, the specificity and PPV could be enhanced. Additionally, the diagnostic performance of BDG in the ICU setting could be further

Table 3 Serum BDG's diagnostic performance for PJP in all patients and subgroups with and without confounders

Group	Cut-off type	Cut-off (pg/mL)	Sensitivity	Specificity	PPV	NPV
All patients	Manufacturer	≥ 150	94 (89, 97)	67 (61, 73)	67 (61, 73)	94 (89, 97)
(*n* = 400)	Optimal†	≥ 252	87 (81, 91)	79 (74, 84)	75 (68, 81)	89 (84, 93)
Patients without confounders‡	Manufacturer	≥ 150	94 (88, 97)	78 (71, 84)	78 (71, 84)	94 (89, 97)
(*n* = 267)	Optimal	≥ 202	87 (79, 92)	88 (82, 92)	85 (77, 91)	89 (83, 93)
Patients with confounders	Manufacturer	≥ 150	94 (82, 98)	47 (37, 58)	50 (40, 60)	93 (80, 98)
(*n* = 133)	Optimal	≥ 390	85 (72, 93)	74 (63, 82)	65 (52, 76)	90 (80, 95)

Results of sensitivity, specificity, PPV, and NPV are expressed as percentages (95% confidence interval)

Abbreviations: BDG, (1–3)- β-D-glucan; PJP, *Pneumocysitis jirovecii* pneumonia; PPV, positive predictive value; NPV, negative predictive value

†Optimal cutoff was defined as the cutoff with the highest Youden index provided that both sensitivity and specificity values are at least ≥ 50%

‡Confounders included other invasive fungal infections caused by BDG-producing organisms except PJP within a one-week window of serum BDG testing, IVIG therapy within 3 days before serum BDG testing, and gram-negative bacteremia within a 2-day window of serum BDG testing

improved by using different cut-off values for patients with and without confounders associated with an elevated BDG level.

The diagnostic performance of serum BDG for invasive fungal infections varies significantly across different patient populations, notably reflecting decreased accuracy in ICU patients [14]. Studies on the performance of BDG for diagnosing PJP in the ICU population are limited, with the majority of those focusing on critically ill patients with hematologic malignancies [15, 16]. While these studies reported high sensitivities ranging from 85 to 100%, they consistently revealed lower specificities (54–75%) and strikingly low PPVs (<40%). Our study, comprising a more diverse spectrum of critically ill patients with immunocompromised conditions, yielded similar results, reinforcing the critical limitation of serum BDG in the diagnosis of PJP in ICU patients with a broader clinical context. Our findings were in accordance with recent research involving a sizable cohort of 600 ICU patients, which similarly demonstrated that BDG testing alone had a suboptimal PPV of 39% in diagnosing PJP [12]. The implications of such limited PPVs are substantial, potentially leading to unnecessary administration of anti-PJP therapy with trimethoprim in approximately one-third of patients. Moreover, false positives may prematurely lead to diagnostic closure, impeding accurate identification of the causative pathogen and subsequent delays in initiating appropriate treatment. These critical limitations, especially in the ICU population, significantly constrain the feasible diagnostic application of serum BDG for PJP.

The poor specificity and PPV of BDG for diagnosing PJP identified in our study and other studies may be partially explained by the inappropriate manufacturer' cut-off value for positivity. The initial establishment and validation of the serum BDG cut-off did not include PJP patients [17]. Previous studies have suggested that manufacturer-defined BDG thresholds are more suitable for ruling out rather than diagnosing PJP, regardless of HIV or non-HIV status [18, 19]. Patients with PJP commonly exhibit a marked increase in serum BDG, with some exceeding the upper limit of detection, suggesting the potential for higher positive thresholds of BDG for the diagnosis of PJP [20]. Several studies have demonstrated that higher cut-offs improved the specificity and PPV for diagnosing PJP without compromising sensitivity, even for critically ill patients [15, 21–24]. For instance, in a study of 50 non-HIV immunocompromised patients with acute respiratory distress syndrome, employing a cut-off value three times higher than the manufacturer's recommendation increased the specificity from 54 to 84% [15]. Similarly, our study revealed improvements in specificity and PPV without significant compromises on sensitivity or NPV by using a higher serum BDG cut-off value of 252 pg/ml, determined by the ROC curve, instead of the manufacturer-recommended cut-off of 150 pg/ml.

Another potential reason for the low specificity and PPV of BDG for the diagnosis of PJP among critically ill patients is the presence of numerous infectious and non-infectious factors in the ICU setting, apart from PJP, that lead to BDG positivity. The mean BDG levels were found to be significantly higher among patients with ICU admission [25]. In a multicenter retrospective study involving 737 ICU patients with hematological malignancies, a positive BDG was observed in 35% of patients without invasive fungal infections [16]. Similarly, Digby et al. observed elevated serum BDG levels in patients with confirmed infection regardless of microbial etiology [26]. Hence, identifying factors related to serum BDG positivity in the ICU is crucial. According to our multivariable analysis, other IFIs, IVIG infusions, and gram-negative bacteremia were significantly associated with serum BDG positivity, even after adjusting for the presence of PJP. While the correlation between BDG positivity and bacteremia has not been as extensively documented as that between IVIG infusion or other invasive fungal infections, it has nevertheless been referenced in the literature [27–29]. This phenomenon may be attributed to false positives resulting from the cross-reactivity of antigens from specific bacterial strains with the BDG assay, or the capacity of certain bacteria to produce BDG [27, 28]. Nevertheless, this finding remains somewhat controversial [30]. Our study revealed that only Gram-negative bacteremia was associated with positive BDG results, whereas Gram-positive bacteremia was not. Similarly, the majority of previously reported bacteremia with elevated serum BDG were caused by gram-negative bacteria [27–29]. BDG levels were significantly higher in patients with Gram-negative bacteremia in comparison to those with bacteremia due to Gram-positive bacteria [27]. These findings are not yet fully understood. Further research is needed to elucidate this phenomenon. Notably, in our study, factors such as blood product transfusions other than IVIG, certain antibiotic treatments, or hemodialysis, which were previously reported to potentially cause positive serum BDG, showed no significant association with BDG positivity. Although we identified only three confounders, fewer than the reported factors, nearly one-third of patients in our study had at least one confounder. As shown in Table 3, the specificity and PPV of BDG in patients with confounders were approximately 50%.

According to the latest updated EORTC/MSG diagnostic criteria for PJP, BDG is considered one of the mycological diagnostic criteria only after excluding confounders causing positive BDG [8]. However, as observed in our study and others, confounders are commonly observed among immunocompromised patients admitted to the

ICU [16]. This finding implies that a considerable portion of this population may be unable to utilize serum BDG for the diagnosis of PJP, thereby undermining the advantage of serum BDG in diagnosing PJP. To address this issue, the present study stratified patients based on the presence or absence of confounders and established specific serum BDG cut-offs accordingly. Utilizing a higher BDG cut-off value of 390 pg/ml for patients with confounders notably increased the diagnostic specificity from 47 to 74%, albeit with a slight decrease in sensitivity.

This study has several limitations. First, the generalizability of our results might be affected by the fact that the BDG detection kit we used was not one of the commonly used commercial kits in previous studies [11, 31]. Discrepancies in substrate, detection method, and cut-off value of positivity across various commercial BDG kits may affect the diagnostic performance of detection [32, 33]. Second, the majority of PJP patients in this study did not undergo two consecutive BDG at the time of PJP, which could increase the risk of PJP overdiagnosis due to false positives resulting from testing errors. When using serum BDG testing to aid the diagnosis of PJP in an ICU setting, combining it with other diagnostic methods such as PCR and clinical manifestations is crucial. Lastly, the study is based on data collected from a single center, where the spectrum of immunocompromised diseases primarily consisted of SAIID accounting for 80% of the patients. Further validation of these findings is warranted in a more diverse population in the ICU setting.

Conclusions

In conclusion, customizing a serum BDG cut-off specifically for PJP diagnosis and adjusting it based on the presence of confounding factors can improve the specificity and PPV while preserving the essential sensitivity and NPV in non-HIV immunocompromised ICU patients. Our study explored the practical application of serum BDG for the diagnosis of PJP, offering valuable insights for further exploration of its significance and utility in this particular population.

Abbreviations

APACHE	Acute Physiology and Chronic Health Evaluation
AUC	Area under curve
BDG	(1,3)-β-D-glucan
CRRT	Continuous renal replacement therapy
EORTC/MSG	European Organization for Research and Treatment of Cancer/Mycoses Study Group
GMS	Gomori methenamine silver
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IFIs	Invasive fungal infections
IQR	Interquartile range
IVIG	Intravenous immunoglobulin
LRT	Lower respiratory tract
NPV	Negative predictive value
OR	Odds ratio
Pj	Pneumocystis jirovecii
PJP	Pneumocystis jirovecii pneumonia
PCR	Polymerase chain reaction
PPV	Positive predictive value
ROC	Receiver operating characteristic
SD	Standard deviation
SAIID	Systemic autoimmune or inflammatory diseas

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions

J-M P and BD contributed to study concept and design; Y-Y L, YC, SL, Q-W Y, and R D assisted in the acquisition and interpretation of clinical data; Y-Y L and YC performed the statistical analyses; Y-Y L and J-M P drafted the manuscript; BD contributed to critical revision of the manuscript; J-M P and BD supervised the study; All authors reviewed the manuscript.

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Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethics statement

The Institutional Review Board of the Peking Union Medical College Hospital approved the study under number K23C3112. Given that the study was noninterventional and retrospective in nature with anonymized data, written informed consent had been waived.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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