

Usefulness of 1,3 Beta-D-Glucan Detection in non-HIV Immunocompromised Mechanical Ventilated Critically Ill Patients with ARDS and Suspected *Pneumocystis jirovecii* Pneumonia

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Received: 18 September 2016 / Accepted: 26 March 2017 / Published online: 4 April 2017
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Abstract

Introduction *Pneumocystis jirovecii* pneumonia (PCP) is a major cause of disease in immunocompromised individuals. Diagnosis is typically obtained by microscopy and/or PCR. For ambiguous PCR results, we evaluated the new biomarker 1,3-Beta-D-Glucan (BDG).

Methods BDG serum levels were assessed and correlated to PCR results in immunosuppressed patients with ARDS.

Results 11 (22%) out of 50 patients had suspected PCP. APACHE II (26 vs. 24; $p < 0.002$), SOFA score (16 vs. 14; $p < 0.010$) and mortality rate (34 vs. 69% $p < 0.004$; 34 vs. 80% $p < 0.003$) were significantly altered in patients with positive (pPCR) and slightly positive (spPCR) PCJ PCR as compared to patients

with no-PCP (nPCP). BDG levels were significantly lower in patients with nPCP (86; 30–315 pg/ml) than in patients with pPCR (589; 356–1000 pg/ml; $p < 0.001$) and spPCR (398; 297–516 pg/ml; $p < 0.004$) referring to the cutoff in this study for PCP of 275 pg/ml. An overall sensitivity (S) of 92% (95% CI 86–96%) and specificity (SP) of 84% (95% CI 79–85%) for PCP were found for the BDG Fungitell assay. In detail, S of 98% (95% CI 94–100%) and SP of 86% (95% CI 82–92%) for pPCP and S of 98% (95% CI 96–100%) and SP of 88% (95% CI 86–96%) for spPCO were found.

Conclusion Serum BDG levels were strongly elevated in PCP, and the negative predictive value is high. BDG could be used as a preliminary test for patients with suspected PCP, especially in patients with slightly positive PCR results.

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Keywords *Pneumocystis jirovecii* pneumonia · 1,3-Beta-D-Glucan · Critically ill · ARDS

Introduction

Immunocompromised patients, particularly critically ill, are at increased risk for *Pneumocystis jirovecii* pneumonia (PCP). PCP still remains a serious and potentially life threatening infection linked with a high morbidity and mortality [1, 2]. Incidence of PCP is increasing due to wide spread use of immunosuppressants [1].

In immunosuppressed critically ill patients, PCP presents in nearly 10% as acute respiratory distress syndrome (ARDS) [3]. Without appropriate antibiotic therapy, the mortality from PCP in non-HIV-infected patients rises up to 90% [1, 3].

However, early identification of PCP is still challenging, especially in critically ill. Not only the patients' complexity, moreover, accompanying signs and symptoms are in most cases nonspecific and may not be present until the disease is advanced or disseminated [4, 5].

Diagnosis of PCP is typically obtained by microscopically staining and polymerase chain reaction (PCR) of bronchoalveolar lavage (BAL) fluids which is the gold standard [4, 5]. However, as reported in former studies lack of sensitivity of these techniques in non-HIV than in HIV infected patients may be a problem in early identification [4]. Moreover, ambiguous results regarding PCP in critically ill patients with ARDS may be fatal not only because of increasing morbidity but also considering side effects of therapy with an inappropriate indication.

The noninvasive serum biomarker 1,3-Beta-D-Glucan (BDG), a typical cell wall component of most pathogenic fungi, including *P. jirovecii*, could be a potentially useful marker in early detection of PCP [6, 7]. The so-called Fungitell BDG assay (Associates of Cape Cod, East Falmouth, MA) is approved by the FDA for the diagnosis of invasive fungal diseases [8, 9]. Up to now, data about the performance characteristics of the Fungitell BDG test for the diagnosis of PCP have been scarce, especially in critically ill patients [9, 10]. Therefore, we examined in this observational study the use of the 1,3 Beta-D-Glucan Fungitell assay for early detection of PCP and potentially discrimination in not clearly stated diagnostic results in immunocompromised critically ill patients with ARDS.

Materials and Methods

Setting and Patients

This observational study was conducted at the medical ICU of the University Hospital Technische Universität München, Germany. All immunocompromised patients (18 years of age or older) without HIV with ARDS and suspected PCP between December 2014 and December 2015 were included. All patients with

respiratory failure received bronchoalveolar lavage (BAL) and computed tomography of the chest.

This study was reviewed and approved by the ethics committee of our university hospital, and all data were processed anonymously. Need for informed consent was waived for this analysis.

Definitions and Microbiological Testings

ARDS was defined following the ARDS Definition Task Force conducted Berlin definition [11]. For definition of fungal disease, we used the criteria proposed by the EORTC/MSG Consensus Group [12]. Sepsis was defined following the International Guidelines for Management of Severe Sepsis and Septic Shock from 2012 [13].

Standard Microbiological Testing

BAL Fungal Testing

Microbiological specimens were tested at the Institute of Microbiology, by direct examination, culture and with an automated microbiology growth and detection system.

1,3-Beta-D-Glucan

BDG was detected with the Fungitell assay as recommended by the manufacturer (Associates of Cape Cod). BDG levels of ≥ 80 pg/ml were considered positive results. BDG levels of >275 pg/ml were considered as positive in patients with PCP.

PCJ Testing

PCJ diagnostic was performed in all patients by microscopy with direct fluorescent antibody staining and real-time polymerase chain reaction (PCR) assay targeting mitochondrial rRNA from *P. jirovecii* [14]. PCR was set as positive over 10^4 copies/ml (pPCP), slightly positive between 10^3 and 10^4 copies/ml (spPCP) and negative below 10^3 copies/ml (nPCP) in BAL fluid.

Statistics

We used IBM SPSS Statistics 23 (SPSS inc., Chicago, IL, USA) for all statistical analyses in this study. A

p value below a significance level of 5% ($p < 0.05$) indicates statistical significance. The diagnostic performance of BDG Fungitell assay test was evaluated as sensitivity and specificity, with 95% confidence intervals (CI). The optimal BDG cutoff was determined with the maximum Youden index.

Results

Patients' Characteristics

We analyzed the medical records of all patients with ARDS and suspected PCP that were treated at the ICU between December 2014 and December 2015. Out of these 97 patients, we identified 50 (51%) immunocompromised patients without HIV. The demographic and clinical characteristics of the patients are presented in Table 1.

A high overall APACHE II and SOFA score could be observed in this study population which expresses the severity of the underlying diseases. APACHE II and SOFA score were significantly higher in patients with positive and slightly positive PCJ PCR as compared to patients with no-PCP (26 vs. 24; $p < 0.002$ and 16 vs. 14; $p < 0.010$).

All patients were admitted because of ARDS and/or sepsis. 40% needed renal replacement therapy. Thus, an overall mean ICU stay of 20 days and mortality rate of 66% could be observed (see Table 1).

Comparing patients with no and with positive and slightly positive PCJ PCR, some significant differences could be observed: Positive and slightly positive PCJ PCR were significantly more frequently observed in patients with hematological malignancies (40 vs. 50% $p < 0.044$; 40 vs. 64% $p < 0.035$) and after chemotherapy of solid tumors (34 vs. 5%; $p < 0.035$). Moreover, neutropenia was significantly more often in patients with positive and slightly positive PCJ PCR (17 vs. 41% $p < 0.042$; 17 vs. 40% $p < 0.045$).

The Horowitz Index (paO₂/FiO₂) as expression of ARDS severity was significantly lower in patients with positive and with slightly positive PCJ PCR, which means worse ARDS, as compared to patients with no-PCP (97 vs. 79 $p < 0.002$; 97 vs. 71 $p < 0.001$).

Mortality rate was significantly higher in patients with positive and slightly positive PCJ PCR as compared to patients with no-PCP (34 vs. 69% $p < 0.004$; 34 vs. 80% $p < 0.003$).

1,3-Beta-D-Glucan Results and Microbiological Findings

The results of 1,3-Beta-D-Glucan (BDG) levels are presented in Table 2. All PCP patients presented elevated BDG concentrations.

The BDG cutoff for invasive fungal diseases was set beyond 80 pg/m as recommended by the manufacturer. For others such as PCP, a clearly defined cutoff is not available; therefore, we performed a ROC analysis on our data. Following these data, the optimal BDG cutoff for the diagnosis of PCP, determined with the maximum Youden index, would be >90 pg/ml.

However, to optimize specificity and sensitivity we tripled the BDG cutoff for PCP up to 275 pg/ml.

The BDG levels measured in patients with no-PCP ranged from 30 to 1000 pg/ml, with a mean concentration of 186 pg/ml. After exclusion of two patients with invasive aspergillosis (IA), the BDG levels ranged from 30 to 315 pg/ml with a mean concentration of 86 pg/ml which was only slightly elevated beyond the overall cutoff of 80 pg/ml and below the cutoff for PCP of 275 pg/ml.

In patients with positive and slightly positive PCJ PCR, significantly higher BDG mean levels of 589 pg/ml (range 356–1000 pg/ml; $p < 0.001$) and 398 pg/ml (range 297–516 pg/ml; $p < 0.004$) could be observed as compared to patients without PCP. Even after exclusion of one patient with IA in each group, the BDG concentrations were significantly beyond the cutoff of 275 pg/ml (mean 563 pg/ml, range 356–893, $p < 0.001$ and 344 pg/ml; range 297–489 pg/ml, $p < 0.001$) compared to the PCP negative group. Microscopic staining was in two patients positive (4%, BDG level 893 and 489 pg/ml, respectively).

Leukocyte count, C-reactive protein, procalcitonin levels and especially LDH were not statistical significant different between patients with elevated BDG levels and positive/slightly positive PCJ PCR ($p = 0.138$; $p = 0.433$; $p = 0.323$; $p = 0.123$ and $p = 0.244$; $p = 0.267$; $p = 0.344$; $p = 0.455$) compared to PCP negative patients.

The fungal findings of *Candida spp.* in BAL were interpreted as colonization in most cases.

Four patients with elevated BDG levels had findings of *Aspergillus spp.* in BAL and standard microbiological testing (see Table 2). Following the EORTC/MSG Consensus Group, these cases were interpreted as *probable* invasive fungal disease. These

Table 1 Presents the basic parameters

	All patients (<i>n</i> = 50)	Patients with suspected PCP: PCR/microscopy negative (<i>n</i> = 39)	Patients with suspected PCP: positive PCR (<i>n</i> = 6)	<i>p</i> value	Patients with suspected PCP: slightly positive PCR; (<i>n</i> = 5)	<i>p</i> value
Age	59; 18–84; 13	58; 18–78; 12	58; 40–71; 15	<i>p</i> = 0.467	69; 56–84; 11	<i>p</i> = 0.105
Male gender (%)	58	56	83	<i>p</i> < 0.030	40	<i>p</i> = 0.167
APACHE II	25; 18–30; 3	24; 18–30; 3	26; 22–28; 2	<i>p</i> < 0.002	26; 22–28; 3	<i>p</i> < 0.002
SOFA score	15; 10–18; 2	14; 10–18; 2	16; 14–18; 1	<i>p</i> < 0.010	16; 14–17; 1	<i>p</i> < 0.010
Host factors (%)						
Hematological malignancies	64	40	50	<i>p</i> < 0.044	64	<i>p</i> < 0.035
After chemotherapy of solid tumors	8	5	34	<i>p</i> < 0.035		
Solid organ transplantation	12	13	16	<i>p</i> = 0.465	20	<i>p</i> < 0.035
Autoimmune disorders treated with immunosuppressive agents	16	18			40	<i>p</i> = 0.055
Neutropenia (%)	36	17	41	<i>p</i> < 0.042	40	<i>p</i> < 0.045
Norepinephrine dosage (ug/h)	893; 0–2000; 527	885; 0–2000; 497	750; 400–1800; 546	<i>p</i> = 0.133	1120; 200–1800; 769	<i>p</i> = 0.090
Horowitz index	92; 45–225; 42	97; 45–225; 45	79; 56–113; 23	<i>p</i> < 0.002	71; 55–114; 24	<i>p</i> < 0.001
ICU stay (<i>d</i>)	20; 5–95; 16	20; 5–95; 17	21; 5–47; 16	<i>p</i> = 0.466	19; 10–28; 7	<i>p</i> = 0.535
Mortality rate (%)	66	34	69	<i>p</i> < 0.004	80	<i>p</i> < 0.003

Acute Physiology And Chronic Health Evaluation (APACHE), Sequential Organ Failure Assessment score (SOFA). Data are presented as mean ± SD, and statistical significant parameters are highlighted

Table 2 Demonstrates the 1,3-Beta-D-Glucan levels, standard infection parameters and possible influencing factors

	All patients (<i>n</i> = 50)	Patients with suspected PCP: PCR/microscopy negative (<i>n</i> = 39)	Patients with suspected PCP: positive PCR (<i>n</i> = 6)	Patients with suspected PCP: slightly positive PCR; (<i>n</i> = 5)	<i>p</i> value	<i>p</i> value
1,3-Beta-D-Glucan levels pg/ml						
All	232; 30–1000; 278	186; 30–1000; 246	589; 356–1000; 97	398; 297–516; 113	<i>p</i> < 0.001	<i>p</i> < 0.004
Exclusion of patients with invasive mycosis		86; 30–315; 82	563; 356–893; 102	344; 297–489; 92	<i>p</i> < 0.001	<i>p</i> < 0.001
In patients with positive staining		0/39	893	489		
Microscopic staining positive (<i>n</i>)			1/6	1/5		
1,3-Beta-D-Glucan						
Sensitivity	92%		98%	98%		
Specificity	84%		86%	88%		
Negative predictive value		96%				
LDH U/l	582; 169–2240; 394	557; 169–2240; 414	658; 263–1125; 337	684; 378–1178; 330	<i>p</i> = 0.138	<i>p</i> = 0.244
Leukocytes G/l	10.8; 0.1–50.8; 11.9	11.2; 0.1–50.8; 13	11.9; 3.4–22.9; 7.3	5.8; 0.2–16.5; 6.7	<i>p</i> = 0.433	<i>p</i> = 0.267
C-reactive protein mg/dl	21.5; 1.1–46.4; 13.1	22; 2.4–46.4; 13.1	13.6; 1.1–31.7; 12.1	27.3; 15.5–45.6; 11.6	<i>p</i> = 0.323	<i>p</i> = 0.344
Procalcitonin ng/ml	13.3; 0.1–124.1; 25.5	14.5; 0.1–124.1; 26.1	2.4; 0.1–2.4; 0.3	19.1; 0.2–78.8; 33.6	<i>p</i> = 0.123	<i>p</i> = 0.455
Used antibiotics (<i>n</i>)						
Meropenem		16	4	3		
Piperacillin/azobactam		14	2	2		
Ceftazidime		8	2	1		
Linezolid		14	4	3		
Vancomycin		8	1	1		
Trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxes (<i>n</i>)		18	2	2		
Invasive aspergillosis (<i>n</i>)		2	1	1		

Data are presented as mean ± SD. Statistical significant parameters are highlighted

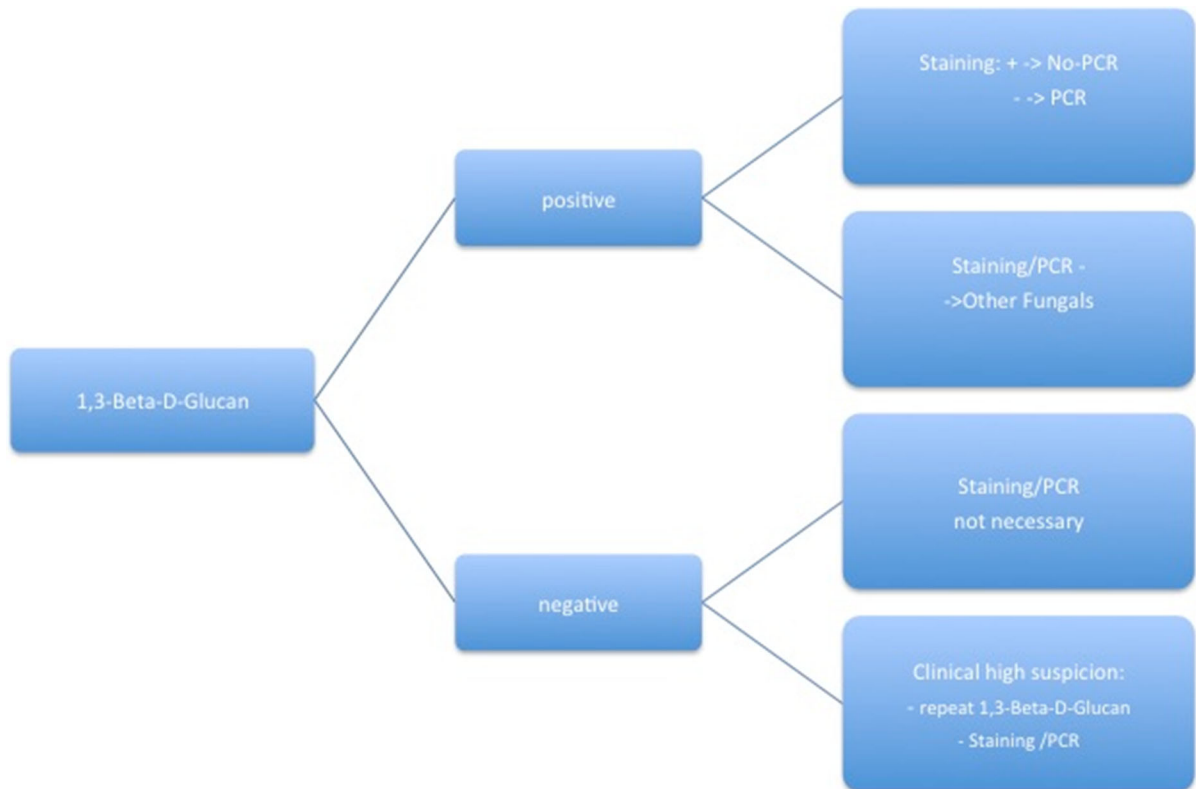


Fig. 1 Diagnostic approach using 1,3-Beta-D-Glucan in suspected PCP

patients were excluded, and the BDG levels were adjusted.

Trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxes and the other used antibiotics are listed in Table 2.

An overall sensitivity of 92% (95% CI 86–96%) and specificity of 84% (95% CI 79–85%) for PCP were found for the BDG Fungitell assay. In detail, a sensitivity of 98% (95% CI 94–100%) and specificity of 86% (95% CI 82–92%) for PCJ positive PCR and a sensitivity of 98% (95% CI 96–100%) and specificity of 88% (95% CI 86–96%) PCJ slightly positive PCR was found. The negative predictive value was 96%.

Discussion

In our observational study, we evaluated the usefulness of 1,3 Beta-D-Glucan (BDG) detection in non-HIV immunocompromised critically ill patients with ARDS and suspected *P. jirovecii* pneumonia (PCP).

Our results presented a highly reliable diagnostic value for the BDG Fungitell assay with an overall sensitivity of 92% and a specificity of 84%.

The measurement of BDG levels is based on the *Limulus* test. The activity of this reaction can be measured with the use of colorimetric or turbidimetric methods, in which optical density is converted to beta-D-glucan concentrations [8, 9, 15].

According to the interpretation criteria of the manufacturer, BDG concentrations higher than 80 pg/ml are considered to be a positive result [8]. However, this cutoff value is used for all findings and not specific for PCP [16–18]. Our results confirm that elevated BDG levels are detected in sera from patients with PCP and also illustrate that generally very high levels are measured.

Therefore, we set a high cutoff of 275 pg/ml in our study, especially to discriminate between slightly positive PCJ PCR results. Thus, we found for positive PCJ PCR a sensitivity of 98% and specificity of 86% and for slightly positive PCJ PCR a sensitivity of 98%

and specificity of 88%. Our data are too limited to validate a cutoff value but suggest that a higher cutoff value for the diagnosis of PCP than for the diagnosis of invasive aspergillosis or candidiasis could be used safely. BDG levels alone cannot prove the existence of PCP, and they must be interpreted considering clinical findings. However, with a negative predictive value of 96%, a BDG level <80 pg/ml almost rules out PCP.

The development of PCR techniques for the diagnosis of PCP improved the reproducibility and the sensitivity of the laboratory diagnosis of PCP, but differentiation between colonization and infection by these techniques remains challenging [4, 19].

However, differentiation is vital especially in critically ill patients with ARDS, because early detection and effective therapy is the cornerstone in reducing mortality in PCP.

As presented in our study, high BDG values can confirm a positive PCJ PCR. However, much more important is detection of a high BDG value in patients with slightly positive PCJ PCR to confirm suspected PCP even though microscopic staining was positive in only 4%. Thus, BDG measurement can reduce time delay and early therapy of PCP.

As reported in our study, patients with ARDS and with suspected PCP are severely ill. Moreover, this can also be observed regarding the mortality rates which are significantly increased in patients with positive PCJ PCR but are highest in the group with slightly positive PCJ PCR (34 vs. 69% $p < 0.004$; 34 vs. 80% $p < 0.003$). This underlines the necessity of definitely diagnosis.

A limitation of the BDG test is its panfungal character [8, 20]. As reported in this study, four patients were detected with invasive aspergillosis. However, as a standard procedure in critically ill patients, different diagnostic approaches such as galactomannan detection in serum and BAL fluid samples and culture of respiratory samples are used to different microbiological and fungal findings [20, 21].

Beyond these possible disruptive factors, the BDG assay can be safely used for detecting PCP early in the course of infection, prior to the onset of overt clinical findings as reported in this study. Therefore, following our results we described a possible diagnostic approach using 1,3-Beta-D-Glucan in managing suspected PCP as presented in Fig. 1.

Conclusion

To conclude, using the BDG assay appeared to shorten the time interval between suspected infection and established diagnosis, when compared with waiting for clinical signs and symptoms of disease. However, this strategy has not been validated in critically ill patients, but as presented in our study combining PCR results with BDG levels could be used to initiate early therapy and aware suspicion for PCJ pneumonia.

Authors' contribution Tobias Lahmer (TL), Clarissa Prazeres da Costa (DC), Jürgen Held (JH), Sebastian Rasch (SR), Ursula Ehmer (UE) and Roland M Schmid (RMS), Wolfgang Huber (WH) were involved in study design. Tobias Lahmer (TL), Sebastian Rasch (SR) and Ursula Ehmer (UE) collected the data. Tobias Lahmer (TL), Clarissa Prazeres da Costa (DC), Jürgen Held (JH), Sebastian Rasch (SR), Ursula Ehmer (UE) and Roland M Schmid (RMS), Wolfgang Huber (WH) analyzed the data. Tobias Lahmer (TL), Clarissa Prazeres da Costa (DC), Jürgen Held (JH), Sebastian Rasch (SR), Ursula Ehmer (UE) and Roland M Schmid (RMS), Wolfgang Huber (WH) wrote the paper

Compliance with ethical standards

Conflict of interest None of the authors has any potential financial or non-financial conflicts of interest related to this manuscript.

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