Audit Report

Use of β-D-glucan in diagnosis of suspected *Pneumocystis jirovecii* pneumonia in adults with HIV infection

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Abstract

Objectives: An elevated serum (1-3)-β-D-glucan (BDG) concentration has high sensitivity for a diagnosis of *Pneumocystis* Pneumonia (PCP) in people with HIV (PWH). At the current manufacturer-recommended positive threshold of 80 pg/mL (Fungitell), specificity for PCP is variable and other diagnostic tests are required. We evaluated the utility of serum BDG for diagnosis of suspected PCP in PWH at three inner-London hospitals to determine BDG concentrations for diagnosis and exclusion of PCP.

Methods: From clinical case records we abstracted demographic and clinical information and categorised patients as having confirmed or probable PCP, or an alternative diagnosis. We calculated sensitivity, specificity, and positive predictive value (PPV) of serum BDG concentrations >400pg/mL and negative predictive value (NPV) of BDG <80pg/mL.

Results: 76 patients were included; 29 had laboratory-confirmed PCP, 17 had probable PCP and 30 had an alternative diagnosis. Serum BDG >400 pg/mL had a sensitivity of 83%, specificity of 97% and PPV 97% for diagnosis of PCP; BDG <80 pg/mL had 100% NPV for exclusion of PCP.

Conclusions: In PWH with suspected PCP, BDG <80pg/mL excludes a diagnosis of PCP whereas BDG concentrations >400pg/mL effectively confirm the diagnosis. Values 80 - 400 pg/mL should prompt additional diagnostic tests.

Introduction

Clinical and radiological features of *Pneumocystis* Pneumonia (PCP) are non-specific; a confirmed diagnosis requires visualisation or molecular detection of the pathogen in bronchoalveolar lavage fluid (BALF) or induced sputum.[1][2][3] Previous studies in people with HIV (PWH) showed elevated serum (1-3)-β-D-glucan (BDG) concentrations have high sensitivity for diagnosis of PCP.[4][5][6] Commercial BDG assays are widely available, so an algorithm using BDG alongside clinical probability of PCP has been proposed for diagnosis of PCP, potentially avoiding invasive investigations.[5]

BDG has variable specificity for PCP using the manufacturer's (Fungitell: Associates of Cape Cod) threshold of 80 pg/mL. Serum BDG can be elevated in other fungal infections as this cell wall antigen is shared by many fungi. Using a higher threshold for a positive BDG result may improve specificity for PCP. We examined this in PWH presenting with pneumonitis to establish a BDG threshold that provided acceptable predictive value for diagnosis, or exclusion, of PCP.

Methods

We retrospectively identified case records of 76 consecutive PWH admitted to King's College, Royal Free London, and University College London Hospitals, between January 2018 and June 2020. Inclusion required presence of respiratory symptoms, radiological pneumonitis (diffuse interstitial or alveolar infiltrates), and an available BDG result. We collected demographic and clinical information, results of microbiological tests, treatments, discharge diagnoses, and clinical outcomes up to three months post-BDG measurement. Participants were categorised by aetiology of pneumonitis: PCP (laboratory-confirmed or probable), or not PCP. Laboratory-confirmed PCP was defined by visualisation of *P. jirovecii* in BALF using Grocott-Gomori stain and/or detection of *P. jirovecii* DNA at $>10^6$ copies/mL . Probable PCP was defined in individuals who did not undergo bronchoscopy by typical clinical and radiological presentation, clinical response to specific anti-*Pneumocystis* treatment and no alternative diagnosis identified. Patients without PCP were defined by a

microbiologically-confirmed alternative diagnosis (in BALF, naso-pharyngeal aspirate [NPA], or blood) and/or by negative Grocott-Gomori staining, and/or *P. jirovecii* DNA being undetectable or detectable at low levels (<10^4 copies/mL), in BALF. *PCP qPCR thresholds were at locally verified levels (unpublished) using an assay targeting the Pneumocystis mitochondrial large subunit mRNA gene.*

At all three sites measurement of serum BDG used the Fungitell assay (positive >80 pg/mL).[7] We determined sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of BDG (above or below 80 and 400 pg/mL (five-times diagnostic threshold) for diagnosis of PCP.[8]

Results

Demographic, clinical and laboratory characteristics are shown in Table 1. Patients with PCP were predominantly male, median age 48 years, severely immunodeficient and with high HIV viral loads. About half were diagnosed with HIV during admission with PCP while 41% had HIV diagnosed many years previously; of these most had defaulted care and were not taking antiretroviral therapy (ART). Of 29 with laboratory-confirmed PCP, diagnosis was made by PCR in 18, PCR and Grocott-Gomori in four, and by Grocott-Gomori in seven. PCP treatment was administered for 21 days and two-thirds received adjunctive corticosteroids. Those without PCP had other infections or non-infectious diagnoses, were younger, more often of black ethnicity, and were less severely immunosuppressed when compared to those with PCP (Table 1).

Serum BDG levels were higher among those with laboratory-confirmed and probable PCP than in those without PCP (median >500 [IQR 435.5 to >500]) and >500 [>500 to >500] vs. 60 [14 to 148] pg/mL, $p<0.05$; Kruskal Wallis test) (Table 1). Serum BDG >400 pg/mL had a sensitivity of 83% and high specificity (97%) and PPV (97%) for PCP, whereas BDG >80 pg/mL had 100% sensitivity but poor specificity (53%) and BDG <80 pg/mL had high NPV (100%). Serum BDG concentrations between 80-400 pg/mL had poor discriminatory value for PCP (PPV 38%; NPV 62%).

Discussion

In PLH presenting with clinically-suspected PCP, serum BDG <80 pg/mL had high NPV (100%) and thus excludes PCP, replicating findings from previous studies.[4][5][9][10] Several studies propose using higher BDG cut-off values to provide better specificity.[9][11][12][13] Indeed, increasing the BDG positive threshold to >400 pg/mL led to much better specificity (97%) with only minimal reduction in sensitivity (83%). BDG results in the intermediate range of 80-400 pg/mL discriminate poorly between those with and without PCP and additional diagnostic tests remain important to exclude or confirm PCP.

In only one patient (CD4 count 40, not taking ART, who did not undergo BAL), serum BDG was >400 pg/mL and RSV was detected in an NPA. Clinically, there was no evidence of candidiasis, empirical anti-fungals were not given: recovery occurred without treatment for PCP. This patient may have had dual pathology with RSV and PCP with clinical improvement related to ART initiation and co-trimoxazole prophylaxis, may have been colonised with *P. jirovecii*, or had a false-positive BDG result.

In summary, our results indicate that the use of dual thresholds of 400 and 80 pg/mL identifies patients in whom PCP is either highly likely or highly unlikely, respectively, thus potentially avoiding unnecessary investigations to confirm PCP or unnecessary treatments directed at PCP.

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Figure 1. β-D-glucan levels among 76 HIV-infected patients, with laboratory-confirmed PCP, probable PCP, and those who did not have PCP. Horizonal bars represent medians.

Table 1. Demographic, clinical, and laboratory characteristics of 76 adult HIV-infected adults presenting with a pneumonitis.

Characteristic	Measure	Laboratory confirmed PCP (n=29)	Probable PCP (n=17)	Not PCP* (n=30)
Age, years	Median (IQR)	48 (35-57)	49 (38-52)	42 (37-51)
Gender, male	n (%)	21 (73)	13 (76)	18 (60)
Ethnicity				
Black	n (%)	10 (35)	6 (35)	17 (57)
White/"other"	n (%)	19 (65)	11 (65)	13 (43)
Time since HIV diagnosis				
>5 years	n (%)	11 (38)	9 (53)	20 (67)
<1year	n (%)	3 (10)	1 (6)	6 (20)
Diagnosed concurrently	n (%)	15 (52)	7 (41)	4 (13)
ART status				
Naive	n (%)	20/28 (71)	9/14 (64)	14/29 (48)
Taking ART at presentation	n (%)	1 (3)	0	7 (23)
CD4 count, cells/mm^3	Median (IQR)	19 (10-33)	19 (10-56)	49 (19-133)
HIV viral load, log_{10} copies/mL	Median (IQR)	5.8 (5.3-6.2)	6.0 (5.1-6.5)	4.9 (4.6-5.5)
Radiographic features (CXR/CT)				
Diffuse interstitial infiltrates	n (%)	19 (65)	10 (59)	22 (73)
Diffuse "Ground Glass Opacities"	n (%)	25 (86)	15 (88)	15 (50)
PCP treatment				
PCP treatment given	n (%)	29 (100)	17 (100)	5/29 (17)**
Duration of PCP treatment, days	Median (IQR)	21 (21-21)	21 (21-21)	3 (2-5)**
Adjunctive corticosteroids	n (%)	16 (55)	15 (88)	6/29 (21)**
Primary PCP prophylaxis	n (%)	NA	NA	26/29 (90)
Outcome at 3 months				
Alive	n (%)	24 (83)	15 (88)	25 (83)
Lost to follow up	n (%)	1 (3)	1 (6)	3 (10)
Died	n (%)	4 (14)	1 (6)	2 (7)
β-D-glucan, pg/mL	Median (IQR)	>500 (435->500)	>500 (>500->500)	60 (14-148)
<80	n (%)	0	0	16 (53)
80-400	n (%)	6 (21)	2 (12)	13 (43)
>400	n (%)	23 (79)	15 (88)	1 (3)

Key: *Diagnoses included: other infections (bacterial [12], mycobacterial [7], viral [5] or fungal (aspergillus [1]); and non-infectious diagnoses (pulmonary Kaposi sarcoma, metastatic cancer of unknown primary, pulmonary oedema, alveolar haemorrhage, immune reconstitution inflammatory syndrome). **PCP treatment (+/- adjunctive corticosteroids) commenced at presentation and discontinued when an alternative diagnosis was made.