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Serum and bal beta-D-glucan for the diagnosis of *Pneumocystis* pneumonia in HIV positive patients

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Background/purpose: The diagnosis of patients with pulmonary infiltrates and human immunodeficiency virus (HIV) infection remains a challenge. In current clinical practice the gold standard for *Pneumocystis jirovecii* pneumonia (PCP) diagnosis remains the identification of the organism in bronco alveolar lavage (BAL) using microscopy (e.g., silver stain). (1->3)-β -d-glucan (BG) is a polysaccharide that is present within the cell wall of *Pneumocystis* and other fungi.
Methods: We analyzed serum and BAL lavage fluid from a cohort of 119 patients that did have HIV, a diagnosis of pneumonia and underwent bronchoscopy (FOB) for diagnosis of PCP.
Results: The discriminative power of serum BG for the diagnosis of PCP in this group of patients was very high. Using a cutoff of 300 pg/mL, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 91%, 92%, 89% and 93% respectively. A model for ROC with just serum BG ($N = 108$) had an AUC of 0.95. Serum procalcitonin (PCT) and BAL BG were not as accurate for the diagnosis of PCP. For BAL BG using a cutoff of 783 pg/mL, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 72%, 79%, 72% and 79% respectively. The differences between the medians for serum PCT between the group with a without PCP did not reach statistical significance ($p = 0.6137$).
Conclusion: The measurement of serum BG should be incorporated in the diagnostic work up of HIV positive patients with dyspnea and infiltrates on chest X X-ray. Our study confirms the diagnostic value of serum BG previously reported by others but we add a cutoff value that we believe is more accurate for patients with AIDS and suspicion of PCP.

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Introduction

The diagnosis of patients with pulmonary infiltrates and human immunodeficiency virus (HIV) infection remains a frequent challenge [1]. Very often the only way to distinguish between *Pneumocystis jirovecii* pneumonia (PCP), community-acquired pneumonia (CAP) and other entities is by fiberoptic bronchoscopy (FOB), an invasive procedure used to collect microbiologic samples. Many times patients with PCP lack a productive cough and so sputum cannot be obtained [2]. Sputum samples have a much lower sensitivity for detection of PCP than bronchoalveolar lavage (BAL) samples even with adequate induction. A meta-analysis [3] found an overall sensitivity of 55.5% and specificity of 98.6% for sputum induction; there was a difference in sensitivity when immunofluorescence was used compared to cytochemical stains (67.1 versus 43.1%). In current clinical practice the gold standard for PCP diagnosis remains the identification of the organism in BAL using microscopy (e.g., silver stain) [4,5]. More recently, polymerase chain reaction (PCR) has been used for diagnosis and quantification of fungal load but may be limited by the detection of colonization [1].

In recent years a promising serologic test for the detection of invasive fungal infections has been established. Serum measurement of (1->3)-β-D-glucan (BG) is based on the level of this polysaccharide that is present within the cell wall of *Pneumocystis* and other fungi [6].

The first description of elevated BG, both in serum and BAL, of HIV positive patients with PCP was in 1996 [7]. Since that time several studies have been reported. Using plasma samples from a previous study, Sax et al. [8] found in 252 patients with HIV, that BG had a sensitivity of 92% and a specificity of 65% for the diagnosis of PCP, using a cutoff value of 80 pg/mL. In another study comparing 28 serum samples of patients with HIV or hematological malignancy vs. 28 control patients, the sensitivity and specificity of BG for PCP were 100% and 96.4%, respectively [9]. A prospective study from Japan in immunocompromised patients [10], using 40 BAL and 107 sputum samples of patients with clinical suspicion of PCP, found a sensitivity and specificity of 100% and 80% using a cutoff of 15 pg/mL of BG for the diagnosis of definitive PCP (positive silver staining). The values in probable PCP (negative silver staining but clinical presentation compatible) were 76.2% and 73.3% at a cutoff value of 6 pg/mL. In the Japanese study the kit used for BG quantification was different from the kit currently employed in the US. They did use the BG test WACO that has a recommended cutoff value of 11 pg/mL and uses different reagents for quantification, we did use for our study the Fungitell® assay, from Associates of Cape Cod, Inc. that has a recommended cutoff value of 80 pg/mL. A small prospective study from the Netherlands in HIV-negative patients [11] also showed excellent diagnostic accuracy of serum BG levels for the diagnosis of PCP. Using the same samples of Sax et al. [8], but restricting the study to patients with respiratory symptoms, Wood et al. [12] were able to achieve a positive predictive value of 96.3% using a cutoff of 80 pg/mL for the diagnosis of PCP. A recent study from Portugal by Esteves et al. [13] employed serum BG and serum lactate dehydrogenase for the diagnosis of PCP in 100 HIV positive patients and 50 healthy blood donors. They propose a cutoff of 400 pg/mL for serum BG as optimal.

Several meta-analysis for the use of serum BG for the diagnosis of invasive fungal infections have been published. A recent one from He [14] included 4214 patients but did focus mainly on invasive infections by Candida and Aspergillus. More recently Karageorgopoulos et al. [15] published an excellent meta-analysis about the accuracy of BG for the diagnosis of PCP. In their analysis they did include 14 studies, with 357 patients that had PCP and 1723 controls. Their definition of PCP did include the detection of the organism in BAL but also in sputum or by PCR. Most of those patients were HIV-negative and did have another cause for immunosupresion. Their overall sensitivity and specificity was 94.7% and 86.3%. They suggest that a negative serum BG could reasonably exclude PCP in patients with low or moderate pretest probability for the disease.

BG was measured in BAL and serum in 109 immunocompromised patients with possible fungal infections in a recent study from the Mayo Clinic [16]. Of 8 patients that were diagnosed with PCP, 7 had positive serum and BAL levels for BG. In those patients with PCP the median values for BG in serum and BAL were 406 pg/mL and 500 pg/mL, respectively. When BG has been measured in BAL [17,18] the data appear to indicate that it does not add to the diagnostic accuracy of serum levels alone. Of note, both studies involved ventilator-associated pneumonia patients, not HIV patients with acute pulmonary infiltrates. A more recent paper [19] studying the utility of BAL BG testing in invasive fungal infections did conclude that this measurement has poor specificity and reproducibility. Of note the measurement of BG in BAL is not validated for the Fungitell® assay. The lack of standardization in the BAL technique could be in part a reason for this poor reproducibility.

Another serologic marker, procalcitonin (PCT), has been shown to be quite valuable and more specific than previous markers for bacterial infections [20]. It is the peptide precursor of the hormone calcitonin and is ubiquitously produced in response to endotoxin and bacterial mediators. PCT levels strongly correlate with the extent and severity of illness in several types of bacterial infections [21]. More specifically, the use of procalcitonin in bacterial pneumonia has proven to help with antibiotic guidance and improve outcomes in several randomized controlled trials [22,23].

PCT levels were assessed in a 2006 study from South Africa [24] involving 266 patients admitted with a clinical diagnosis of CAP. In 169 of those a microbiological diagnosis was made; 44 patients were found to have tuberculosis, 31 PCP, and 35 bacterial pneumonia. The mean PCT levels for tuberculosis, PCP and bacterial pneumonia were 4.164 ng/mL (95% CI 1.749—6.579), 1.138 ng/mL (95% CI .543—1.734) and 19.479 ng/mL (95% CI 8.021—30.938), respectively. Moreover, a well-designed prospective study from 2007 [25] included 107 consecutive patients with various reasons for their immune-compromise state. In that study, serum PCT had an area under the-curve of .746 (95% CI .602—.889) for the prediction of bacterial infection, defined as positive bacteriologic results in the BAL. In