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Test Performance of Blood Beta-Glucan for *Pneumocystis Jirovecii* Pneumonia in Patients with AIDS and Respiratory Symptoms

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Abstract

Objective—To define the test characteristics of plasma beta-glucan for diagnosis of *Pneumocystis jirovecii* pneumonia in AIDS patients with respiratory symptoms.

Design—Analysis of baseline blood samples in a randomized strategy study of patients with acute opportunistic infections, limited to participants with respiratory symptoms.

Methods—Participants in the 282-person ACTG A5164 trial had baseline plasma samples assayed for beta-glucan testing. As part of A5164, two study investigators independently adjudicated the diagnosis of PCP. Respiratory symptoms were identified by investigators from a list of all signs and symptoms with an onset or resolution in the 21 days prior to or 14 days following study entry. Beta-glucan was defined as positive if ≥ 80 pg/mL and negative if <80 pg/mL.

Results—Of 252 study participants with a beta-glucan result, 159 had at least one respiratory symptom, 139 of whom had a diagnosis of PCP. The sensitivity of beta-glucan for PCP in participants with respiratory symptoms was 92.8% (95% CI: 87.2%–96.5%), and specificity 75.0% (50.9%–91.3%). Among 134 individuals with positive beta-glucan and respiratory symptoms, 129 had PCP, for a positive predictive value of 96.3% (91.5%–98.8%). Fifteen of 25 patients with a normal beta-glucan did not have PCP, for a negative predictive value of 60% (38.7%–78.9%).

Conclusion—Elevated plasma beta-glucan has a high predictive value for diagnosis of PCP in AIDS patients with respiratory symptoms. We propose an algorithm for the use of beta-glucan as a diagnostic tool based on the pretest probability of PCP in such patients.

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Keywords

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Introduction

Definitive diagnosis of *Pneumocystis jirovecii* pneumonia (PCP) can be difficult due to inability to culture the organism and need to visualize the organism on microscopic stains of respiratory secretions [1,2]. Measurement of blood levels of (1→3)-β-D-glucan (beta-glucan), which is a component of the cell wall of *P. jirovecii* and other fungi, has emerged as a potentially useful diagnostic tool in immunocompromised patients, including those with HIV [3–8].

We previously reported the performance characteristics of plasma beta-glucan in HIV-positive participants with a broad range of opportunistic infections (OIs) [6]. In that study of patients with advanced immunosuppression and diverse OIs, plasma beta-glucan was strongly associated with the diagnosis of PCP. However, in clinical practice, beta-glucan testing would likely have the greatest utility in patients with respiratory symptoms and suspected PCP, not as a screening test in all patients with acute OIs. As a result, here we describe the test characteristics of beta-glucan testing only in study participants who presented with respiratory symptoms.

Methods

The study population was participants in ACTG A5164, a strategy study of early versus deferred antiretroviral therapy in patients with acute AIDS-related OIs. Eligible HIV-related OIs for study enrollment specifically excluded tuberculosis (due to potential drug-drug interactions with ART) and OIs requiring antiretroviral therapy for treatment (e.g. progressive multifocal leukoencephalopathy, AIDS-related dementia, and cryptosporidiosis). Oral and esophageal candidiasis were not eligible OIs for inclusion on their own, but investigators were required to note the diagnosis if present on entry. Study participants were required to be able to take oral medications and to provide written informed consent for participation.

Two study investigators independently adjudicated the diagnosis of PCP after reviewing reports from study sites. Participants could be enrolled with either “confirmed” or “probable” PCP. The study defined confirmed PCP as: 1) a history (within 3 months) of shortness of breath, dyspnea on exertion, cough, or fever; plus 2) histological or cytological evidence of *P. jirovecii* in a bronchoalveolar lavage, lung biopsy, or sputum specimen. Probable PCP was: 1) a history (within 3 months) of shortness of breath, dyspnea on exertion, cough, or fever; plus 2) abnormal chest radiograph or CT scan or hypoxia based on arterial blood gas partial pressure of oxygen less than 80 mm Hg or alveolar-arterial oxygen difference greater than 15 mm Hg on room air; plus 3) specific anti-pneumocystis therapy initiation. The primary analysis showed no significant difference or trend in difference in

beta-glucan results between confirmed versus probable PCP [6], so these two categories were considered together for the present analysis.

Respiratory symptoms were identified by investigators from a list of all signs and symptoms with an onset or resolution between 21 days prior to study entry and 14 days following study entry. Beta-glucan was categorized as positive if ≥ 80 pg/mL or negative if <80 pg/mL. Values of <31 pg/mL were censored at 31 pg/mL and values of >500 pg/mL were censored at 500 pg/mL. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of beta-glucan for diagnosis of PCP among patients with respiratory symptoms were calculated based on the results.

Results

Among the 282 participants in A5164, 252 (89%) had a baseline sample yielding an analyzable beta-glucan result. The participants had advanced HIV-related immunosuppression, with a median CD4 lymphocyte count of 26 cells/ μ L (interquartile range [IQR], 10–53 cells/ μ L) and a plasma HIV RNA level of 5.02 log copies/mL (IQR, 4.69–5.60 log copies/mL). The most common qualifying OI was PCP (69%), followed by cryptococcal meningitis (14%) and bacterial pneumonia (9%). One hundred twelve participants (44%) had concomitant oral or oroesophageal candidiasis with a qualifying OI. Full baseline characteristics of the study population have been previously described [6].

Of 252 study participants with beta-glucan assay results, 159 (63%) reported at least one baseline respiratory symptom; the most common were dyspnea or shortness of breath (52.1%), cough or sputum production (44.4%), and pleuritic chest pain (3.6%). One hundred thirty-nine of 173 PCP cases (80%) had at least one baseline respiratory symptom.

One hundred twenty-nine of the 139 participants with respiratory symptoms and PCP had beta-glucan ≥ 80 pg/mL, for a sensitivity of 92.8% (95% CI: 87.2–96.5%) (Table 1). Among the 20 participants with respiratory symptoms without PCP, 15 had a negative beta-glucan, for a specificity of 75.0% (95% CI: 50.9–91.3%). The 20 participants without PCP had the following diagnoses: 11 had bacterial pneumonia, including one with pneumonia secondary to *Nocardia*; seven had cryptococcal meningitis, including one with concomitant pulmonary cryptococcosis; one had toxoplasmosis; one had “bacterial infection of deep tissue;” and one had disseminated histoplasmosis, including pulmonary disease. The five participants with positive beta-glucan, respiratory symptoms and no PCP included the participant with disseminated histoplasmosis and four with bacterial pneumonia (not *Nocardia*).

Among the 134 study participants with a baseline respiratory symptom and beta-glucan ≥ 80 pg/mL, 129 had PCP, for a PPV of 96.3% (95% CI: 91.5–98.8%). Conversely, among the 25 participants with a beta-glucan result <80 pg/mL, 15 did not have PCP, yielding a NPV of 60% (95% CI: 38.7–78.9%).

The median beta-glucan value among all participants with respiratory symptoms and PCP was 405 pg/mL (IQR, 209–500 pg/mL), and among those without PCP was 40.5 pg/mL (IQR, 31–84.5 pg/mL) (Figure 1). Sixty of 62 participants (97%) with beta-glucan >500 pg/mL and respiratory symptoms had PCP. Thirty-four participants had PCP with no

respiratory symptoms; among these participants, beta-glucan was positive in 31 (91.2%) and negative in three.

Discussion

In this study, we demonstrate that a blood test for beta-glucan has a high PPV (96.3%) for PCP in AIDS patients with respiratory symptoms when 80 pg/mL is used as the cutoff for positive. This is higher than the PPV we previously reported (85%) when examining the performance of beta-glucan in patients presenting with a wider range of OIs [6]. The PPV in the current analysis is higher than in the previously published paper because of the higher pretest probability of PCP in AIDS patients presenting with respiratory symptoms. Additionally, certain fungal infections that may yield a positive beta-glucan (such as histoplasmosis or, less commonly, cryptococcosis) are not primarily respiratory pathogens in patients with AIDS.

An inevitable limitation of the beta-glucan test is that it is not specific to *Pneumocystis jirovecii*, and hence will have a lower PPV than the gold standard for diagnosis, cytologic staining of respiratory secretions. Furthermore, false-positive results can occur with the administration of certain agents that are filtered through cellulose filters, such as albumin or immunoglobulin. False-positive results may also be caused by hemodialysis if cellulose membranes are used, by serosal exposure to cellulose gauze, or possibly by certain antimicrobials [9,10]. Therefore, careful consideration of potential confounding factors is important when ordering the test.

Improved tests for PCP are needed because the diagnosis has many inherent challenges; most importantly, the organism cannot be cultured, so the diagnosis depends on examination of respiratory secretions [1,2]. The sensitivity of induced sputum examinations for PCP is highly variable and depends on patient and laboratory factors [11]. While bronchoscopy and lung biopsy are more sensitive, they are invasive. Other non-invasive markers, such as lactate dehydrogenase, have even lower specificity, and PCR is not readily available [11,12]. As a result, beta-glucan can provide a useful, non-invasive adjunctive marker.

In this study, 97% of participants with beta-glucan >500 pg/mL and respiratory symptoms had PCP. There were two participants with respiratory symptoms, beta-glucan >500, and no diagnosis of PCP. On review, one of these patients was treated with trimethoprim-sulfamethoxazole and prednisone, yet the diagnosis entered in the case report form was bacterial pneumonia. It is possible that this patient actually had PCP and was miscoded. The other patient received piperacillin-tazobactam as part of treatment for bacterial pneumonia; data are conflicting as to whether antimicrobials like piperacillin-tazobactam cause false elevations of beta-glucan, so it is unclear if antibiotic administration led to the elevated result this case [9,13,14]. There was also a patient who had respiratory symptoms and beta-glucan of 411 pg/mL but no diagnosis of PCP; this patient was diagnosed with disseminated histoplasmosis.

Forty-four percent of the study participants had concomitant oral and/or oroesophageal candidiasis (candidiasis alone was not an eligible study criteria, so all participants with

candidiasis also had a concomitant OI, the most common being PCP). As we reported in our original analysis of beta-glucan in A5164, the relationship between presence of candidiasis and elevated beta-glucan was not statistically significant [6]. However, given the high rate of PCP among those with mucosal candidiasis, candidiasis is an important clinical marker of high PCP risk. High rates of PCP in the setting of candidiasis have been noted since early in the AIDS era [15].

We propose the following algorithm for use of blood beta-glucan as a diagnostic tool for PCP based on the pretest probability in an AIDS patient with respiratory symptoms (Figure 2): For a patient with low clinical suspicion for PCP, such as a patient with high CD4 count, acute onset of symptoms, focal or lobar infiltrate on X-ray, or absence of fever, cough or dyspnea, we recommend first treating more likely alternate diagnoses, then checking the beta-glucan if there is lack of clinical improvement. If positive, we would then initiate treatment and confirmatory testing for PCP (induced sputum and, if negative, BAL), particularly if the beta-glucan result were significantly elevated – the median result in our study for patients with positive beta-glucan, respiratory symptoms and PCP was 451 pg/mL.

For a patient with moderate to high pretest probability of PCP, such as a patient with CD4 count <200 cells/mm³, subacute onset of fever, dyspnea, cough, or hypoxia, with diffuse interstitial infiltrates on chest radiograph or a combination of these factors, we recommend initiating empiric PCP treatment and checking beta-glucan as a way of all but confirming the diagnosis. In such patients, a beta-glucan result >500 pg/mL would arguably obviate the need for cytologic confirmation of PCP. An intermediate result in this setting would necessitate confirmatory testing for PCP and a negative result should trigger additional work-up for alternate diagnoses.

An important caveat to any diagnostic strategy for patients with advanced HIV-related immunosuppression is that multiple infections may be present simultaneously. For example, in highly histoplasmosis-endemic areas, a high beta-glucan level could be triggered by PCP, histoplasmosis, or concomitant infection with both pathogens. In this setting, supplementing beta-glucan with urine histoplasmosis antigen testing would help clarify the diagnosis. Furthermore, the beta-glucan test has not been thoroughly evaluated in less common fungal infections, such as coccidioidomycosis, blastomycosis, paracoccidioidomycosis, and penicilliosis, so should be used in conjunction with other diagnostic modalities in patients at risk for those infections. One report does describe a high rate of positivity in coccidioidomycosis, while another found no positivity in blastomycosis, though was limited by small sample size [16,17].

Limitations to our study include the lack of a consistent protocol for PCP diagnosis between study sites, leading to variable strategies (some sites were more likely to confirm the diagnosis than others) and the possible miscoding of one case that in hindsight probably did have PCP. In addition, we do not know how the test would perform in settings where PCP is a less-common opportunistic infection; this limits our ability to predict how the test would perform in a group with lower pretest probability. Another possible limitation is that A5164 excluded patients with tuberculosis; while tuberculosis is not known to raise the beta-glucan

level, this may have excluded some patients with advanced immunosuppression and other concomitant infections, including fungal infections.

An important potential area of future research would be to conduct a similar analysis in resource-limited settings, where availability of definitive PCP diagnostics is limited and often relies on clinical parameters [18]. Challenges to implementing the beta-glucan test in such settings will be the need for refrigeration of samples, equipment (such as a computer and an incubating plate reader), and a trained technician, as well as cost of the test. Currently, the Fungitell kit, as used in this study, permits testing of up to 42 patient samples in duplicate, per kit, and costs approximately \$1,229 (US currency) [Associates of Cape Cod, personal communication].

In summary, we found that in patients with advanced HIV-related immunosuppression and respiratory symptoms, a positive beta-glucan test had a high predictive value for the diagnosis of PCP. In particular, values >500 pg/mL were strongly suggestive of the diagnosis. We believe that, if available, blood beta-glucan testing can provide helpful adjunctive diagnostic information for work-up of respiratory symptoms in this population.

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Figure 1. Distribution of beta-glucan results by PCP status among participants with respiratory symptoms

Distribution of beta-glucan results at baseline in those with and without *Pneumocystis jirovecii* pneumonia (PCP) among A5164 participants with respiratory symptoms. The median value for those with PCP (horizontal line) was 405 pg/mL (interquartile range [IQR], 209–500 pg/mL) and for those without PCP was 40.5 pg/mL (IQR, 31–84.5 pg/mL). Both continuous and categorical beta-glucan levels were significantly different in persons with and without PCP ($P < .001$). Note one participant with no PCP and beta-glucan >500 pg/mL may have had PCP, as treatment for bacterial pneumonia was given with trimethoprim-sulfamethoxazole and prednisone; the second participant with no PCP and beta-glucan >500 pg/mL received piperacillin-tazobactam for bacterial pneumonia. The participant with no PCP and glucan 411 pg/mL had histoplasmosis.

Figure 2. Algorithm for use of beta-glucan as a diagnostic tool for AIDS patients with suspected PCP

Table 1

Beta-Glucan results by PCP among participants with a baseline respiratory symptom

Beta-Glucan (pg/mL)	Positive or Probable PCP	Non-PCP OI Diagnosis	Total	P-Value*
Positive (≥ 80)	129 (93%)	5 (25%)	134 (84%)	<0.001
Negative (<80)	10 (7%)	15 (75%)	25 (16%)	

* Fisher's Exact Test