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β-D-Glucan and S-adenosylmethionine serum levels for the diagnosis of Pneumocystis pneumonia in HIV-negative Patients: A prospective study

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Summary *Objective:* To prospectively assess the diagnostic utility of S-adenosylmethionine (AdoMet) and (1→3)-β-D-glucan (β-D-glucan) serum markers for Pneumocystis pneumonia (PCP) in HIV-negative patients.
Methods: HIV-negative, immunocompromised patients suspected of PCP based on clinical presentation and chest imaging were included. PCP was confirmed or rejected by results of direct microscopy and/or real-time PCR on broncho-alveolar lavage (BAL) fluid. Measurement of serum β-D-glucan and AdoMet was performed on serum samples collected at enrollment and during follow-up. Both serum β-D-glucan and AdoMet were assessed for diagnostic accuracy and correlation with clinical and laboratory parameters.
Results: In 31 patients enrolled (21 PCP-positive, 10 PCP-negative), AdoMet levels did not discriminate between patients with and without PCP. Elevated serum β-D-glucan was a reliable indicator for PCP with a sensitivity of 0.90 and specificity of 0.89 at the 60 pg/ml cut-off. In PCP-positive patients β-D-glucan serum levels decreased during treatment and inversely correlated with Pneumocystis PCR cycle threshold values in BAL fluid.
Conclusions: The level of serum β-D-glucan — but not AdoMet — was diagnostic for PCP within the clinical context and may serve as marker for pulmonary fungal load and treatment monitoring.

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Introduction

Pneumocystis pneumonia (PCP), caused by *Pneumocystis jirovecii* is an important cause of morbidity and mortality in patients with human immunodeficiency virus (HIV) infection and other conditions associated with immunosuppression.[1] The diagnosis of PCP is based on microscopy methods (silver, giemsa and immunoflorescent staining) and real-time PCR performed on broncho-alveolar lavage (BAL) samples obtained from patients with a compatible clinical picture.[2] Microscopy techniques are limited by their sensitivity and time demanding procedures. Currently used real-time PCR methods to detect *P. jirovecii* yield high sensitivity but might lack the required specificity by detecting *P. jirovecii* also in patients who are colonized but do not suffer from PCP.[3–6] Furthermore, the need for both sensitive and specific serum tests for PCP becomes particularly evident when invasive diagnostic procedures cannot be performed due to a patient's clinical condition. Hence, a number of serum markers, including (1→3)-β-D-glucan (β-D-glucan) and S-adenosylmethionine (AdoMet) levels were recently studied for their ability to discriminate between PCP and other pulmonary conditions.[7–10]

The polysaccharide β-D-glucan is one of the major components of the cyst wall of *P. jirovecii,* but is present also in the cell wall of other fungal pathogens e.g. *Aspergillus fumigatus* and *Candida* spp.[11,12] Its potential as a discriminative marker in serum was first proposed after studies in PCP infected rats as well as in human case series.[10,13] Watanabe et al. recently demonstrated the high potential of β-D-glucan as a discriminative marker for PCP in a study with HIV-infected patients.[14] Yet, prospective data about its use for diagnosing PCP in solid organ transplant recipients and patients with other causes of immunodeficiency is limited. Alternatively, being proposed as a useful biochemical marker in 2003, the measurement of AdoMet levels in serum was recently re-introduced as a promising diagnostic test for PCP in patients infected with HIV, yielding a sensitivity and specificity of >90%.[7,15] In the cell's metabolism, AdoMet serves as an essential intermediate substance e.g. for methylation reactions and polyamine synthesis. In contrast to almost all other micro-organisms capable of causing disease in humans, *Pneumocystis* spp. seem to depend on exogenous AdoMet although conflicting data were published.[16,17] Contrary to high intracellular concentrations, extracellular concentrations are low and may be depleted during PCP.[17]

The question has remained whether the observations in HIV-infected individuals with regard to the accuracy of these new serum markers for PCP can be extrapolated to the HIV-negative population. HIV-related PCP and non-HIV-related PCP are known to be different in terms of clinical characteristics.[18,19] Several studies demonstrated that a higher load of *P. jirovecii* is present in the lungs of patients with HIV as compared to patients with PCP due to other underlying disorders.[20,21] Despite the lower amount of antigen, the inflammatory response of the immune system appeared to be more severe in HIV-negative immunocompromised individuals with PCP, which is thought to account for the more severe clinical picture and higher mortality reported in this group.[19,22] In this study we prospectively assessed whether serum AdoMet, its most direct metabolite adenosylhomocysteine (AdoHcy), the AdoMet/AdoHcy ratio and β-D-glucan, would be reliable indicators for the diagnosis of PCP in HIV-negative immunocompromised individuals. In addition, the correlation of serum AdoMet and β-D-glucan levels with real-time PCR results as well as with other biochemical and clinical parameters were evaluated.

Methods

Patients

In this prospective observational study, consecutive HIV-negative, adult immunocompromised patients suspected of having PCP based on presentation and chest imaging were enrolled during admission in the Leiden University Medical Center, a tertiary care and teaching hospital in The Netherlands.Videobronchoscopy was performed and a segment of an involved lung zone was lavaged using 20 ml aliquots. The diagnosis of PCP was confirmed or rejected by results of direct microscopy methods and/or real-time PCR of the dihydropteroate synthetase (DHPS) gene of *P. jirovecii* on the BAL fluid.[23] Patients who were thereafter considered PCP-negative served as a control group. None of the case- or control patients had other proven invasive fungal infections. Demographical characteristics and data about medical history, symptoms at clinical presentation, treatment and disease outcome were extracted from the medical files. Levels of Lactate dehydrogenase (LDH) and leukocyte count in BAL fluid were acquired from the hospitals' electronic database. PCR cycle threshold values (ct-values) were obtained from the database of the Department of Medical Microbiology. The time of diagnosis was defined as the date when microbiological evidence was first obtained, i.e. the date of the BAL procedure. Data were anonymously noted on case record forms (CRFs) and a database was constructed. The study was approved by the institutional review board of the Leiden University Medical Center and all patients provided written informed consent for participation in the study. Because in our hospital performing a bronchoscopy is part of the standard work-up protocol for immunocompromised patients presenting with pneumonia in which atypical or fungal micro-organisms are suspected to be the cause, informed consent for bronchoscopy was obtained seperately by the lung physician as part of clinical routine.

Sample collection and measurement of S-adenosylmethionine and (1→3)-β-D-glucan

Serum samples were prospectively collected around the time of diagnosis and during one or more time points during the first week of follow-up. Per protocol, blood was drawn for study purposes when venipuncture was performed for other clinical reasons. AdoMet and AdoHcy were measured by a method adapted from Gellekink et al.[24,25] In short, after withdrawal and rapid centrifugation of EDTA-blood for determination of AdoMet and AdoHcy, plasma aliquots were stored at −80°C until the time of analysis. AdoMet, AdoHcy and their ratio were determined using liquid chromatography mass spectrometry (LC-MS/MS). After thawing of the non-acidified plasma samples, portions of 10 ul were injected on a 50 × 2.1 mm Atlantis C-18 column (Waters) and eluted in