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Original article

Serum markers as an aid in the diagnosis of pulmonary fungal infections in AIDS patients

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ABSTRACT

Introduction: The etiology of pulmonary infections in HIV patients is determined by several variables including geographic region and availability of antiretroviral therapy.
Materials and methods: A cross-sectional prospective study was conducted from 2012 to 2016 to evaluate the occurrence of pulmonary fungal infection in HIV-patients hospitalized due to pulmonary infections. Patients' serums were tested for (1–3)-β-D-Glugan (BG), galactomannan, and lactate dehydrogenase (LDH). The association among the variables was analyzed by univariate and multivariate regression analysis.
Results: 60 patients were included in the study. The patients were classified in three groups: *Pneumocystis jirovecii* pneumonia (PJP; 19 patients), community-acquired pneumonia (CAP; 18 patients), and other infections (23 patients). The overall mortality was 13.3%. The time since diagnosis of HIV infection was shorter in the PJP group (4.94 years; $p = 0.001$) than for the other two groups of patients. The multivariate analysis showed that higher BG level (mean: 241 pg/mL) and LDH (mean: 762 U/L) were associated with the diagnosis of PJP. PJP was the aids-defining illness in 11 out of 16 newly diagnosed HIV-infected patients.
Conclusion: In the era of antiretroviral therapy, PJP was still the most prevalent pulmonary infection and BG and LDH may be suitable markers to help diagnosing PJP in our HIV population.

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Introduction

The epidemiology of pulmonary infections varies according to geographical regions and to whether the infection occurred before or after active antiretroviral therapy (ART) availability. In some low-income countries, tuberculosis and pneumocystosis are still the most frequent pulmonary complications in HIV/AIDS patients.[1] Conversely, in higher resources settings, the frequency of pulmonary diseases associated with AIDS has decreased due to the benefits of the ART.[2–4] Hospitalization due to chronic obstructive respiratory disease, lung cancer, and bacterial pneumonia has become more frequent than pulmonary infections due to *Pneumocystis jirovecii*, tuberculosis, and cytomegalovirus.[5,6] However, lower respiratory tract infections (LRTI) are still 25-fold more common in the HIV population compared to the general community causing an estimate number of 20–25 episodes per 100 hospitalizations worldwide.[6,7]

Fungal infections in HIV-infected patients are neglected diseases, predominantly in countries with limited resources, and represent a significant cause of pulmonary infections. The burden of HIV-related mycosis worldwide is estimated to account, per year, for more than 950,000 cases of cryptococcosis, 400,000 cases of PJP, and 300,000 of disseminated histoplasmosis.[8]

The majority of the diagnoses of pulmonary diseases are based on clinical symptoms and X-ray findings resulting in patients being treated empirically. Identification of the etiologic agent is a complex task requiring the combination of microbiologic exams, serologic markers, molecular techniques, and invasive procedures, such as lung biopsy and bronchoalveolar lavage (BAL). New molecular techniques have been studied to improve the diagnosis of pulmonary infiltrates in the HIV-infected population. *Loop-mediated isothermal amplification* (LAMP) has been evaluated for the detection of *Pneumocystis* DNA in respiratory specimens and could serve as a diagnostic tool.[9] Serum (1-3)-β-D-Glugan (BG) has been a promising non-culture method for the diagnosis of some fungal infections, including, *Candida* spp., *Fusarium*, and *P. jirovecii*. Recent data have shown that serum BG could be correlated with the diagnosis of PJP[10–12] with a sensitivity range of 90–100% and specificity of 65–100%.[13–15]

In low/middle income countries there is an urgent need for prospective studies to clarify the infectious causes of pulmonary diseases that lead to hospitalizations in AIDS patients. In this scenario, the proper identification of the causative agents will indicate a better therapeutic approach and improve the understanding of the epidemiology of pulmonary affections responsible for hospitalizations in our country.

The aim of this study was to evaluate the occurrence of pulmonary fungal infections and the role of serum markers in HIV-infected patients hospitalized with acute respiratory symptoms, in a tertiary care referral hospital in Campinas, Sao Paulo, Brazil.

Materials and methods

Study population

We conducted a prospective cross-sectional study, from 2012 to 2016, at the Hospital das Clinicas of the University of Campinas (UNICAMP), Sao Paulo, Brazil, which is the reference hospital for more than six million inhabitants.

The Ethical Committee approved this study (No. 8876/2012) and all patients signed the informed consent form. The inclusion criteria included HIV infection, age over 18 years old, and hospitalization due to symptoms of lower respiratory tract infection. Exclusion criteria included pregnant women and patients with nosocomial pulmonary infections. At the time of hospitalization, a physiotherapist (AIPM) performed a pulmonary examination in all patients and collected sputum and oral lavage for the molecular diagnosis of *P. jirovecii*. A radiologist assessed all chest radiographies and tomographies. For the assessment of the pneumonia severity, the patients were classified using CURB-65 score.[16] At the day of admission, the following data were abstracted from the patients' records: age, gender, duration of HIV infection (in years), duration of hospitalization (in days), outcome, previous opportunistic infections, hemoglobin level, hematocrit, total leukocytes, serum creatinine, serum urea, CD4 T cell count, and HIV viral load in the last two months. In addition, serum cryptococcal antigen, serology for paracoccidioidomycosis, blood culture for bacteria and fungi, culture for mycobacteria and fungi in sputum, serum LDH, BG and GM, LAMP of respiratory specimens (sputum, BAL) and oral lavage (OL) for *P. jirovecii* were also tested.

The clinical diagnosis of pneumonia due to *P. jirovecii* was established according to WHO clinical criteria of case definition for HIV-related opportunistic diseases,[17] and a chest X-ray showing bilateral interstitial infiltrates or a chest tomography showing alterations compatible with PJP (bilateral patchy ground grass opacity with a central perihilar predominance). A definitive diagnosis was based on the identification of *P. jirovecii* in cytology or immunofluorescent microscopy of induced sputum or BAL or histology of lung tissue.

Community-acquired pneumonia (CAP) was defined as the presence of cough together with one or more of the symptoms: chest pain, dyspnea, presence of new pulmonary infiltrate on chest radiography.[18] Criteria for diagnosis of CAP and LRTI due to bacteria, fungi other than *P. jirovecii*, or parasites were based on the identification of the etiologic agent in cultures of blood and respiratory secretions.

BG and GM in serum and in BAL specimen

Serum and BAL samples were tested for GM and BG. Concentrations of GM were determined by using the Platelia *Aspergillus* Ag assay (Bio-Rad, Marnes-la-Coquette, France), according to the manufacturer's instructions. An OD ≥ 0.5 was considered as a positive result. BG levels were determined by the Fungitell® assay (Associates of Cape Cod, Inc., Cape Cod, MA, USA), according to the manufacturer's recommendations. BG levels ≥80 pg/mL were considered as positive results.

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