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Galactomannan detection in bronchoalveolar lavage fluid for the diagnosis of invasive aspergillosis in patients with hematological diseases—the role of factors affecting assay performance

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SUMMARY

Background: We evaluated the performance of a galactomannan (GM) assay in bronchoalveolar lavage (BAL) fluid compared to serum samples for the diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematological diseases.
Methods: Two hundred and fifty-five bronchoscopies were performed on 230 patients. Bronchial and alveolar samples from BAL fluid as well as serum samples were analyzed in the GM assay.
Results: Twenty-eight cases of IPA (11%) were diagnosed. The sensitivity, specificity, positive predictive value, and negative predictive value of the GM assay using a cut-off of 0.5 were 57.1%, 99.3%, 94.1%, and 92.5%, respectively, for the alveolar sample; 44.0%, 99.3%, 91.7%, and 91.4%, respectively, for the bronchial sample; and 60.7%, 100%, 100%, and 92.9%, respectively, for serum. The highest sensitivity (78.6%) with good specificity (98.6%) was obtained with a 'triple detection' of GM in bronchial, alveolar, and serum samples. Neutropenia and antifungal therapy for only 24 h increased the sensitivity, while antifungal treatment for $\geq$2 days decreased assay performance. Moreover, a trend towards a higher volume of aspirated fluid in GM-negative BAL ($p = 0.092$) was observed.
Conclusions: In contrast to recently published data, we found only moderate sensitivity, but high specificity and high positive predictive value of the detection of GM in BAL fluid. In addition, neutropenia, antifungal therapy, and BAL standardization affected GM assay performance.

1. Introduction

Invasive aspergillosis (IA) represents the most frequent invasive fungal disease occurring in patients with hematological malignancies, especially those with acute leukemia and after allogeneic hematopoietic stem cell transplantation (HSCT).[1,2] The lungs comprise the primary site of IA in the vast majority of cases, and therefore invasive pulmonary aspergillosis (IPA) represents the most frequently occurring form of this disease.[3] The high morbidity and mortality rates of this infection, as well as difficulty in obtaining an early diagnosis by conventional diagnostic procedures, has led to the development of new non-culture based techniques.[4]

The detection of Aspergillus galactomannan (GM) in serum using the Platelia Aspergillus enzyme immunoassay (EIA) (Bio-Rad, France) has been studied extensively and represents a sensitive, non-culture-based tool for the early diagnosis of IA in patients with hematological malignancies.[5] Moreover, GM can also be detected in body fluids other than serum, since a water-soluble carbohydrate is released during hyphal tip growth.[6] Recently, in vitro studies[7] and animal models[8] have shown that during IPA, GM is released at an earlier time and at a higher concentration in the bronchoalveolar lavage (BAL) fluid than in serum. Therefore, in an attempt to improve the sensitivity of the GM assay in patients with IPA and to shorten the amount of time to final diagnosis, several groups have investigated the utility of GM detection in BAL fluid[9] in non-hematological or mixed populations,[10–19] as well as in patients with hematological malignancies.[10,20–27]

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However, the clinical utility and benefit of the GM EIA assay compared to regular serum GM screening methods remains a matter of debate, primarily due to substantial inconsistencies in the reported sensitivity, which ranges from 57%[24] to 100%,[20] even in samples from patients with hematological malignancies.

The heterogeneity may be related to several factors that can influence the results, including patient characteristics,[28] variability in the definitions of IPA,[29,30] and different cut-off optical density (OD) values of GM used in BAL fluid.[9] Moreover, there are several covariates that may markedly influence the assay performance. First, the administration of mold-active antifungal drugs (including prophylactic treatment) that can influence the hyphal growth and antigen release into body fluids may affect the assay performance.[31,32] Second, neutropenia can lead to higher fungal burden.[32,33] Finally, there is a lack of BAL standardization among studies that have analyzed the presence of GM in BAL fluid, and therefore the role of pre-analytical variables has not been assessed. It is well-known that the return from the first infused aliquot during lavage is enriched for bronchial components, and should be analyzed separately from the returns from subsequent aliquots, which are of alveolar origin.[34,35] In addition, the total volume of BAL fluid varies substantially among studies, and this can influence the fungal antigen concentration, and consequently the sensitivity of the GM assay. To date, no study has analyzed these pre-analytic factors.

The purpose of this study was to evaluate the performance and clinical usefulness of GM detection in BAL fluid compared to serum samples for the diagnosis of IPA in a large cohort of patients with hematological malignancies. Moreover, we compared assay performance using bronchial and alveolar samples from BAL fluid, and analyzed the effect of the total BAL fluid volume on the GM assay. Finally, we investigated the role of neutropenia and mold-active antifungal treatment on the sensitivity of the GM assay in our study population.

2. Methods

2.1. Study population

We retrospectively reviewed the data obtained from patients treated at the Department of Internal Medicine, Hematology and Oncology of Masaryk University and University Hospital Brno, Brno, Czech Republic, from July 2003 to September 2009, who underwent a bronchoscopy with BAL for the evaluation of new pulmonary infiltrates and who had BAL fluid tested for GM. A patient who underwent repeated sampling could be included in the analysis if BAL had been performed on pulmonary infiltrate that was clearly a new presentation. All patients gave informed consent before the procedure and the Institutional Review Board approved the study.

Twenty-six BAL samples obtained from patients who had a clear false-positive result for GM in the serum, which was caused by the administration of GM-positive lots of piperacillin–tazobactam and amoxicillin–clavulanate or infusion of Plasma-Lyte solution (Baxter Healthcare), were excluded from the analysis.

Patients were hospitalized in standard air controlled rooms or in high-efficiency particulate air-filtered single rooms. Patients with neutropenia less than 1.0×10^9/l (48.2% of episodes) received antifungal prophylaxis at the time of the bronchoscopy; however, only 5.9% of the patients received mold-active drugs.

Regular monitoring of serum GM was performed twice a week in all patients with acute leukemia and in patients who received an allogeneic HSCT. For all other patients, the serum GM was tested only in cases of pulmonary infiltrate or febrile neutropenia. If febrile neutropenia did not respond to antibiotic treatment, the patients received empirical antifungal treatment, which predominantly consisted of conventional amphotericin B or echinocandin. This treatment was followed by a routine full diagnostic work-up for invasive fungal disease, including a high-resolution lung computed tomography (CT) and BAL in the case of pulmonary infiltrates.

2.2. Fiberoptic bronchoscopy and sample investigation

The site of the fiberoptic bronchoscopy was guided by a high-resolution lung CT. Eight to 10 sequential, 20-ml aliquots of sterile saline solution were infused into the lower respiratory tract, and each aliquot was immediately aspirated. The return from the first aliquot (bronchial sample) was processed separately from the returns from the subsequent aliquots, which were pooled together and homogenized (alveolar sample). Both portions of BAL fluid were submitted for cytology assessment, direct examination, bacterial, fungal, and mycobacterial culture, and GM detection. Viral and *Pneumocystis jiroveci* screening by PCR was performed on the alveolar samples only.

2.3. GM detection

GM detection was performed for both samples of BAL fluid separately (i.e., bronchial and alveolar samples). Moreover, the Platelia Aspergillus GM EIA (Bio-Rad, France) test was used to detect GM in serum samples taken immediately prior to the bronchoscopy. The BAL specimens were centrifuged at 1000 rpm for 10 min and the supernatant was used for GM detection. The Platelia Aspergillus GM EIA test was performed according to the manufacturer's recommendations for testing serum samples. The assay was performed three times a week in a routine microbiology laboratory. Samples were stored at −20 °C until used for testing. All tests with an OD index $\geq$0.5 were repeated on the same specimen.

2.4. Case definition and statistical analysis

The medical records of all patients were reviewed, and each case of pulmonary infiltrate for which BAL was performed was classified as proven, probable, possible, or no IPA based on the revised European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) case definition.[30] The detection of GM in BAL fluid and serum samples obtained during bronchoscopy was not included as one of the microbiological criteria.

The Mann–Whitney test or Kruskal–Wallis analysis of variance (ANOVA) was used to compare independent subgroups of bronchoscopies in continuous variables, and the Wilcoxon test was used for a pair-wise comparison. A two-sided p-value of <0.05 was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated when bronchoscopies without IPA were considered as being true-negatives and episodes with proven and probable IPA were considered as true-positives. A receiver operating characteristic (ROC) curve and area under the curve (AUC) were used to estimate the discriminatory capability of the GM assay performed in both samples of BAL fluid and of the serum sample obtained during bronchoscopy for IPA detection. For statistical analysis, the software Statistica version 9.0 (StatSoft) and R (R Development Core Team) were used.

3. Results

During the study period, the bronchial and alveolar samples of BAL fluid obtained from 255 bronchoscopies in 230 patients were tested for GM. The baseline characteristics are shown in Table 1. IPA was documented in 105 (41.2%) of the bronchoscopies