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A cross-sectional observational to assess the role of Bronchoalveolar lavage fluid galactomannan in the diagnosis of invasive pulmonary aspergillosis in non-neutropenic patients

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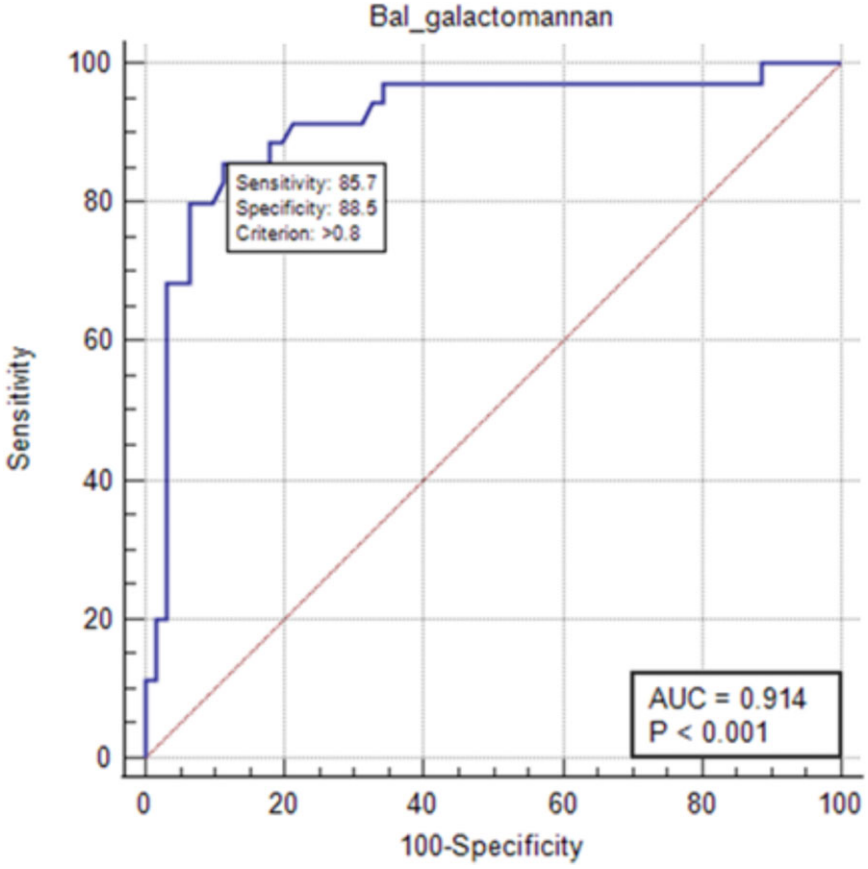
Objective: This study was done to assess the role of Bronchoalveolar lavage fluid (BALF) galactomannan (GM) in the diagnosis of invasive pulmonary aspergillosis (IPA) in non-neutropenic patients and to determine the optimal cut-off value of BALF GM for diagnosing IPA in non-neutropenic hosts.

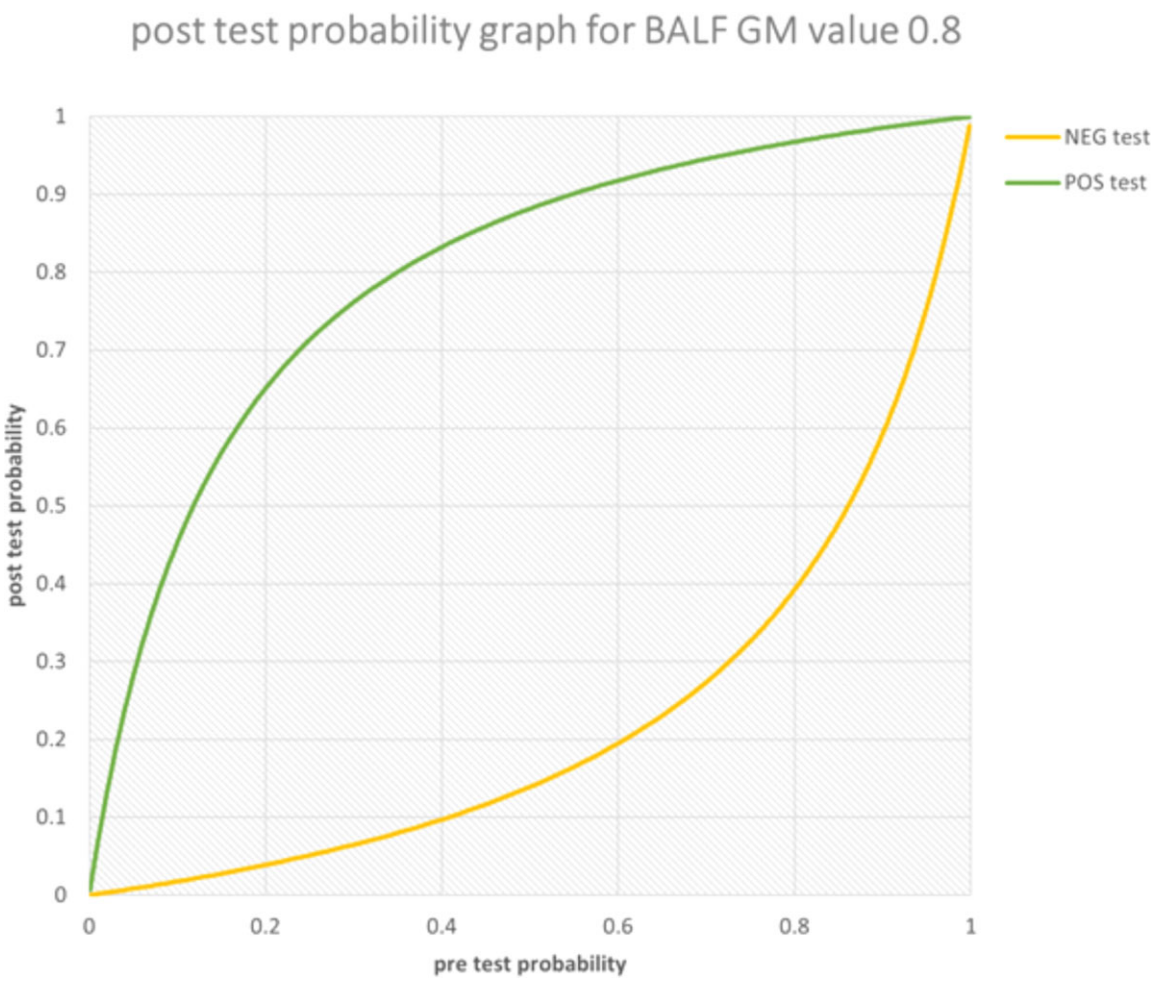
Methods: We conducted a cross-sectional observational study on 96 non-neutropenic patients of age > 18 years with suspected pulmonary infections of fungal etiology. A detailed history of predisposing conditions was obtained and routine laboratory investigations with chest computerized (CT) tomography were performed. Fiberoptic bronchoscopy under local anesthesia and sedation was done using a video bronchoscope. Bronchoalveolar lavage was done from the segment or lobe of interest instilling 60-100 ml of saline withdrawn under low pressure and the sample was tested for a battery of investigations; fungal KOH smear; fungal culture; GM by Platelia Aspergillus enzyme immunoassay; and others required for diagnosis. A protected brush specimen and transbronchial lung biopsy were done wherever feasible. The yield from the BALF GM assay was compared with diagnosis based on definitions given by EORTC/MSG excluding host factors. Patients were classified into three groups namely non-IPA, probable IPA, and proven IPA. For statistical analysis, probable and proven IPA were taken as one group i.e., the IPA group. Mann-Whitney test and Chi-Square test along with Fisher's exact test were performed for inter-group comparison between quantitative and qualitative variables respectively. The receiver operator characteristic curve was used to establish a cut-off point of BALF GM and Serum GM for predicting IPA. Sensitivity, specificity, PPV, and NPV were calculated. The McNemar test was used for the comparison of sensitivity and specificity. Inter-rater kappa agreement was used to find the strength of agreement of BALF GM, BALF culture, and BALF DM.

Results: Out of 96, 1 was diagnosed with proven IPA, 34 were probable IPA cases and 61 were not IPA cases. Chronic kidney disease (CKD) as a risk factor was more common in IPA cases compared to non-IPA cases (25.7% vs. 8.2%, *P*-value .019). Chest CT showed cavity in a significant number of IPA patients compared to non-IPA cases (60% vs. 29.5%, *P*-value .003). BALF direct microscopy, culture, and serum GM had sensitivities < 60% but specificities close to 95%. BALF GM showed promising results with a sensitivity of 88.5% and specificity of 85.7% at cutoff value of 0.8.

See Figures below.

Conclusions: Our study highlights the magnitude of IPA in non-neutropenic hosts with unconventional risk factors like CKD, diabetes, and the need for increased vigilance for diagnosis of IPA in such patients. We suggest a lower cut-off value of BALF GM against 1 as in EORTC/MSG criteria and consider CKD as one of the risk factors for IPA.





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Validation panel for MALDI-TOF identification of fungi

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Sciensano, the Belgian federal scientific institute for public and animal health, houses the BCCM/IHEM Fungi Collection which contains more than 15 000 strains, belonging to over 1 500 different species. The collection is managed according to ISO 9001 standards.

Its purpose is to make fungal strains available for academics, clinicians, industry, and education.

Fungal pathogens are not as often encountered as bacteria in the clinical laboratory. Additionally, laboratories may not have the knowledge or logistics for the long-term preservation of axenic fungal isolates. Without an array of fungal strains with confirmed identity, it is complicated to implement new protocols and equipment when these need to be validated for the identification of fungi.

To short-cut this problem and support laboratories in identifying clinical fungi in routine activities, BCCM/IHEM has developed two validation panels for the identification of fungi via MALDI-TOF mass spectrometry: there is a validation panel with yeasts and a validation panel with filamentous fungi. The selection of strains is based on species that are routinely encountered in a clinical laboratory, and also contains some rarer, but emerging fungal pathogens, like *Trichophyton indotineae* and *Candida auris*. The identity and purity of the strains in these panels have been verified according to ISO 17025 accredited protocols. This allows the laboratory to evaluate in a short term the extraction protocol, the MALDI-TOF machine, and the database of reference mass spectra.

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***Aspergillus fumigatus* complicates one third of the patients with suspected bronchial asthma or pulmonary tuberculosis: Clinical validation of indigenously developed diagnostic kits**

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Objectives: *Aspergillus fumigatus*, an opportunistic fungus, causes complications in about 5%-20% of bronchial asthma and about 26% of pulmonary tuberculosis patients. Detection of *Aspergillus fumigatus* specific IgG and IgE antibodies in the patient serum is an excellent tool to screen for *Aspergillus* sensitization early on to employ anti-fungal drugs in the clinical management to stall the progression of lung fibrosis.

Methods: Novel indigenous AfuPEPLISA assays were developed for the detection of specific IgG and IgE, based on the 12 amino acid long synthetic peptide epitope of Asp f1, an 18 kDa major allergen/antigen. The novel diagnostic kits were manufactured at a licensed GMP facility under a test license. Independent validation of the kits was pursued at PGIMER and VPCI hospitals in suspected bronchial asthma patients ($n = 1307$), and the diagnostic efficiency was compared with currently used ImmunoCAP assay.

Results: The diagnostic specificity and sensitivity were found to be 95.7% and 89.8%, respectively, for IgG; and 94.2% and 70%, respectively for IgE AfuPEPLISA, and were not significantly different from ImmunoCAP assay. Screening of the suspected patients of pulmonary tuberculosis (PTB) at RBIPMT Hospital for the presence of *A. fumigatus* specific IgG and IgE antibodies was pursued using AfuPEPLISA kits. A total of 82 out of 254 suspected PTB patients (32.3%) were seropositive in agreement with the previous reports.

See Figures 1 and 2 below.

Conclusion: The study inferred that indigenously developed AfuPEPLISA kits are an economically viable option to integrate in the clinical management of patients with suspected bronchial asthma or PTB for efficient diagnosis of *Aspergillus* sensitization.