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Original article

Aspergillus galactomannan detection in exhaled breath condensate compared to bronchoalveolar lavage fluid for the diagnosis of invasive aspergillosis in immunocompromised patients

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ABSTRACT

Objectives: Exhaled breath condensate (EBC) is a noninvasive means of sampling the airways that has shown significant promise in the diagnosis of many disorders. There have been no reports of its usefulness in the detection of galactomannan (GM), a component of the cell wall of *Aspergillus*. The suitability of EBC for the detection of GM for the diagnosis of invasive aspergillosis (IA) using the Platelia *Aspergillus* enzyme-linked immunosorbent assay was investigated.
Methods: Prospective, cross-sectional study of lung transplant recipient and haemotologic malignancy patients at a university centre. EBC samples were compared to concomitant bronchoalveolar lavage (BAL) samples among lung transplant recipients and healthy controls. EBC was collected over 10 minutes using a refrigerated condenser according to the European Respiratory Society/American Thoracic Society recommendations, with the BAL performed immediately thereafter.
Results: A total of 476 EBC specimens with 444 matched BAL specimens collected from lung transplant recipients ($n = 197$) or haemotologic malignancy patients ($n = 133$) were examined. Both diluted and untreated EBC optical density (OD) values (0.0830, interquartile range (IQR) 0.0680–0.1040; and 0.1130, IQR 0.0940–0.1383), respectively, from all patients regardless of clinical syndrome were significantly higher than OD values in healthy control EBCs (0.0508, IQR 0.0597–0.0652; $p < 0.0001$). However, the OD index values did not correlate with the diagnosis of IA (44 samples were associated with IA). Furthermore, no significant correlation was found between EBC GM and the matched BAL specimen.
Conclusions: GM is detectable in EBC; however, no correlation between OD index values and IA was noted in lung transplant recipients. **A. Bhimji, Clin Microbiol Infect 2017;▪:1**

Introduction

Invasive aspergillosis (IA) is a major cause of morbidity and mortality in immunocompromised patients, particularly patients with haemotologic malignancy [1,2], stem cell transplant recipients [3,4] or solid organ transplant (SOT) recipients [5]. The 12-month cumulative incidence after transplantation is 3.4% and 3.1% in stem cell and SOT recipients, respectively.

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Despite advances in antifungal therapy, IA results in mortality rates of 25% to 58%, in part as a result of the difficulty in diagnosing IA in its early stages [3,5–9]. Prompt diagnosis of IA improves survival [10]. Diagnosis generally depends on the recovery of *Aspergillus* spp. in culture from respiratory tract samples or the detection of hyphae within biopsy specimens. This approach is limited by the invasiveness of biopsy techniques. Although the complications from bronchoscopy are low (1% in all comers) [11,12], it carries risks, and a number of patients are not considered as a result of low platelet counts and tenuous respiratory status [12].

A surrogate diagnostic marker based on fungal antigen detection, galactomannan (GM), has been developed and incorporated by the 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) for the diagnosis of probable IA [13]. However, it has not been evaluated in exhaled breath condensate (EBC).

EBC is an appealing method to conveniently and noninvasively evaluate a wide range of aerosolized molecules from the respiratory tract. A few studies have correlated levels of various substances in bronchoalveolar lavage (BAL) and EBC [14,15]. The ease of sample collection, combined with the sensitivity of the GM enzyme-linked immunosorbent assay (ELISA), makes EBC a novel and potentially important diagnostic sample type for IA.

The aim of this study was to evaluate the feasibility of utilizing EBC in detecting GM in immunocompromised patients. The evaluation was performed prospectively over a 3-year period. The performance of the GM ELISA assay in diagnosing IA using EBC was assessed using the EORTC/MSG diagnostic criteria. In order to verify the utility of GM detection in EBC for the diagnosis of IA, we compared its detection with that of paired BAL fluid samples.

Materials and methods

Study participants

This was a single-centre prospective study among adult (18–85 years) lung transplant recipients and patients with haemotologic malignancy receiving chemotherapy or undergoing haematopoietic stem cell transplantation at the University Health Network between 2013 and 2015 who underwent bronchoscopy for clinical indications including surveillance or diagnostic evaluation. In addition to immunocompromised individuals, adult healthy volunteers ($n = 43$) were enrolled as a control group in order to determine an optimal cutoff index for GM in EBC specimens. Of 43 controls, 21 (48.84%) were women, and the median age was 31 years. With respect to race, 25 of them (58.14%) were of Asian descent (11 East Asian, 9 South Asian and 5 Middle Eastern), while 14 (32.56%) were white, 2 (4.65%) were African American/African Canadian and 2 (4.65%) were mixed race. They were recruited from within the hospital or university setting, did not have any underlying disease or malignancy, and did not undergo bronchoscopy as part of their participation. EBC and BAL sample collection was conducted with the written informed consent of all study participants, and the study protocol was approved by the local ethics committee (Research Ethics Board of the University Health Network). Relevant patient clinical information was collected. Radiologic findings up to 7 days before and 2 days after the bronchoscopy date were collected.

Definitions

Patients were classified as having fungal colonization, proven, probable, possible or no fungal infection on the basis of the revised EORTC/MSG case definitions [13]. International Society for Heart and Lung Transplantation (ISHLT) guidelines were adapted for lung transplant recipients to define anastomotic bronchial infections or tracheobronchitis [16]. Proven IA was diagnosed if patients had histopathologic evidence of tissue invasion by hyphae with morphology suggestive of *Aspergillus* and the isolation of *Aspergillus* species in respiratory specimen culture. Probable IA required the presence of clinical features for lower respiratory tract fungal disease (dense, well-circumscribed lesions with or without a halo, air-crescent sign or cavity on chest computed tomograph, and mycologic evidence of *Aspergillus* infection by cytology, direct microscopy, culture or positive BAL GM). Serum GM and (1→3)-β-D-glucan testing results were not available for categorization. The same criteria for probable IA were used to classify probable invasive mould infection cases due to non-*Aspergillus* moulds (e.g. *Fusarium* species). Possible invasive fungal infection (IFI) was defined by the presence of clinical criterion but without mycologic support in patients with haemotologic malignancy only. Only patients with proven or probable IA were evaluated as IA cases. Colonization with *Aspergillus* or other non-*Aspergillus* mould was noted when patients had a positive BAL culture for *Aspergillus* or non-*Aspergillus* moulds but when both the bronchoscopy and chest computed tomographic scans were normal. Exploratory analyses of EBC GM positivity with the clinical syndrome of possible IFI and *Aspergillus* colonization were also performed.

EBC and BAL collection

The EBC samples were collected using a commercially available device, the RTube (Respiratory Research, Charlottesville, VA, USA). Subjects were asked to perform normal tidal breathing into the mouthpiece for 10 minutes at an initial condenser temperature of −80°C. EBC was collected on the day of bronchoscopy or up to 48 hours before, then stored for assessment (frozen at −80°C). BALs were collected and processed as previously described by instillation of two 50 mL and one 25 mL aliquots of normal saline solution into separate traps [17]. Residual BAL fluid from the clinical microbiology laboratory was collected and stored without preservation at −80°C.

GM Platelia Aspergillus assay

Both EBC and BAL supernatant samples were analysed for GM by using the direct double-sandwich Platelia *Aspergillus* GM ELISA (Bio-Rad, Hercules, CA, USA) by technicians blinded to the source of the sample and the clinical data. The assay was performed according to the manufacturer's recommendations for testing serum or BAL specimens. EBC samples were tested treated (diluted) and untreated with ethylenediaminetetraacetic acid (EDTA) at 120°C for 6 minutes, followed by centrifugation before testing. Each BAL sample was treated with EDTA as per the manufacturer's recommendations.

BAL specimens were tested in real time as they were received as part of clinical care, and EBC specimens were tested in batches. For validation, each run contained in duplicate one negative control, one positive control containing 10 ng/mL of GM and a standardized serum sample containing 1 ng/mL GM for calibration and conversion of the measured absorbance into indexes. As cleared by the US Food and Drug Administration, a positive BAL specimen was defined as one with an optical density (OD) index of $\geq$0.5 with repeat testing.

The presence or absence of GM in the BAL samples was determined by the calculation of an index for each specimen. The index was the OD value of the specimen divided by the mean OD of the wells containing the cutoff the control sample. In order to determine a level for positivity for EBC GM, the cutoff levels for diluted EBC samples were determined by adding 3 standard deviations to the mean OD values of 40 healthy control samples, after removal of outliers ($n = 4$). The cutoff value for diluted EBC was calculated as

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