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Influence of mould-active antifungal treatment on the performance of the *Aspergillus*-specific bronchoalveolar lavage fluid lateral-flow device test

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ABSTRACT

The effect of mould-active antifungal (AF) therapy/prophylaxis on the performance of the *Aspergillus*-specific lateral-flow device (LFD) test for diagnosing invasive pulmonary aspergillosis (IPA) was evaluated. This was a retrospective analysis of patients diagnosed with probable or proven IPA (according to revised EORTC/MSG criteria) at the Medical University of Graz (Austria) and the University Hospital of Mannheim (Germany) between February 2011 and December 2014. In total, 60 patients with 63 bronchoalveolar lavage fluid (BALF) samples were included in the analysis. Patient charts were reviewed regarding AF treatment at the time of bronchoscopy, and the influence of AFs on the performance of the LFD and BALF galactomannan (GM) ELISA results was calculated. Overall, 54 patients (57 BALF samples) had probable IPA and 6 patients (6 samples) had proven IPA. In 21/63 samples (33%) (from 19 patients), systemic mould-active AFs had been initiated before bronchoscopy. Of 63 BALF samples, 16 (25%) yielded a false-negative LFD result. The sensitivity of the LFD for probable/proven IPA was significantly lower in those receiving mould-active AFs compared with those without (52% vs. 86%; $P = 0.006$). Similar results were found for BALF GM, with sensitivities decreasing under systemic AFs (71% vs. 95%, $P = 0.013$ with the 0.5 ODI cut-off; 52% vs. 81%, $P = 0.036$ with the 1.0 cut-off). These results suggest that the sensitivity of the BALF LFD and BALF GM assays may be reduced in the presence of mould-active AF treatment. Negative results in patients on AFs should therefore be interpreted with caution.

1. Introduction

Invasive pulmonary aspergillosis (IPA) is a fatal disease associated with high mortality rates owing to the progressive nature and its refractoriness to therapy if initiated late in the course of the disease [1,2]. Early mould-active antifungal (AF) treatment is a key factor for survival and therefore AF treatment is frequently initiated upon suspicion and before mycological evidence for IPA is obtained [3]. In addition, mould-active AF prophylaxis is a common

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approach in high-risk patients to reduce the incidence of IPA. Overall, AF prophylaxis is considered efficient and breakthrough fungal infections are rarely observed. However, the occurrence may vary and breakthrough rates as high as 13% have been reported from some centres [4–6].

The European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) defined revised criteria for the diagnosis of invasive fungal infections in 2008 [7]. Diagnosis of proven IPA requires lung tissue examination and histopathological evidence of fungal invasion, but this is often not possible in high-risk patients. Critical conditions such as severe respiratory failure or low platelet count may not allow invasive procedures owing to the high probability of procedure-related complications. Therefore, the majority of non-fatal IPA cases are classified as probable disease, which requires fulfilment of (i) predefined host factors, (ii) clinical criteria and (iii) mycological criteria that include fungal culture [8]. Owing to low sensitivity and a long turnaround time of conventional culture [9], however, more reliable mycological tests are needed to enable early diagnosis in clinical routine.

Determination of galactomannan (GM) antigen in bronchoalveolar lavage fluid (BALF) samples and in serum samples has become a routine method for IPA diagnosis in clinical practice [10,11]. However, GM determination is also subject to important limitations. The positive predictive value of serum GM is limited among patients receiving mould-active AF prophylaxis owing to the low prevalence of IPA in this setting [12–14]. Furthermore, GM determination is limited by varying turnaround times (up to 3 days or more in some centres), false-positive test results, and limited sensitivity in cases of pre-emptive or targeted systemic AF therapy [11,15–18].

The *Aspergillus*-specific lateral-flow device (LFD) test is a novel and rapid single-sample test developed at the University of Exeter (UK). This point-of-care assay uses a monoclonal JF5 antibody to detect an extracellular mannoprotein that is exclusively secreted during active growth from *Aspergillus* spp. [19]. Minimal required training, simple handling by using BALF samples without any pre-treatment, no need for specially equipped laboratories, rapid availability of test results within 15 min and low costs are the major advantages of the LFD [19–23]. Previous studies have indicated that the BALF LFD might be a promising alternative to BALF GM testing [21–27]. Furthermore, it was shown in an animal model of IPA that the performance of the BALF LFD was not strongly influenced by systemic AFs [24].

However, little is known about the influence of systemic AF prophylaxis and therapy on the performance of the LFD in human BALF samples.

The aim of this analysis was therefore to evaluate the effect of mould-active AF treatment on the performance of the *Aspergillus*-specific LFD assay for detection of IPA.

2. Materials and methods

This retrospective analysis of a cohort study was performed at the Medical University Hospital of Graz (Austria) and the University Hospital of Mannheim (Germany). The primary objective was to evaluate the influence of systemic mould-active AF treatment on the performance of the LFD test for diagnosing IPA using BALF samples.

2.1. Participants

The study included a total of 63 BALF samples obtained from 60 patients (59 samples from the Medical University of Graz and 4 samples from Mannheim University Hospital). In part these patients and samples have been reported previously [21,22,25–27].

Patients who were diagnosed with probable or proven IPA between February 2011 and December 2014 and who had complete data on AF treatment for ≥7 days prior to bronchoscopy were included in this retrospective analysis. IPA was classified according to the 2008 EORTC/MSG criteria, with the inclusion of BALF GM optical density index (ODI) >0.5 as the mycological criterion [7]. Sample collection and testing was performed prospectively in both centres, whilst classification of IPA was performed retrospectively. For this analysis, charts of patients who fulfilled probable or proven IPA criteria were re-reviewed regarding mould-active treatment at the time of bronchoscopy (defined as mould-active AF initiated ≥24 h prior to bronchoscopy).

LFD testing was performed prospectively and always on the day of BALF sample collection in the Microbiology Laboratory, Department of Internal Medicine, Medical University of Graz and in the Scientific Laboratory, Department of Haematology and Oncology, Mannheim University Hospital. Testing was performed according to the manufacturer's (OLM Diagnostics, Newcastle upon Tyne, UK) recommendations [19,23]. The interpreters of the LFD test results were blinded to clinical information regarding the patient, ensuring an unbiased interpretation of the test line intensities [ranging from strong positive (+++) to weak positive (+) or negative (−) depending on the antigen contents of BALF samples]. Regardless of the intensity of the test lines, all positive results indicate germination of spores and the existence of hyphae in the lungs and were therefore interpreted as positive.

Conventional culture and direct microscopy were performed routinely and prospectively in all BALF samples included in this analysis. GM determination was performed in accordance with the manufacturer's specifications (Platelia™ *Aspergillus* Ag ELISA; Bio-Rad Laboratories, Munich, Germany).

2.2. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v.22.0 (IBM Corp., Armonk, NY). Sensitivities of the BALF LFD, GM test and conventional culture in patients with and without AF therapy were calculated. Sensitivities were compared using Fisher's exact test and a P-value of <0.05 was considered statistically significant.

This study was conducted in accordance with the Declaration of Helsinki 1996, Good Clinical Practice and applicable local regulatory requirements and law. Data collection and processing were done according to legal regulations and local Ethics Committee requirements. All data presented have been anonymised and are therefore not attributable to individual patients; the ethics committees therefore waived written informed consent of participating patients. The study protocol was approved by the local Ethics Committee of Medical University Graz and was registered at ClinicalTrials.gov (identifier NCT02058316; registered 20 January 2014). Performance evaluation of a medical product was also reported to the Austrian Agency for Health and Food Safety (Protocol no. INS-621000-0478).

3. Results

A total of 63 BALF samples from 60 patients were included in the analysis. All patients had been classified with probable (n = 54 patients with 57 BALF samples) or proven IPA (n = 6 patients with 6 BALF samples). Of the 63 BALF samples, 59 were from Graz and 4 were from Mannheim. The samples included from Mannheim were two each of probable and proven IPA (three underlying haematological malignancy and one primary central nervous system malignancy). In 21/63 samples (33%) (from 19 patients), systemic mould-active AF agents had been initiated before