Journal of Microbiological Methods 140 (2017) 32–39

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Comparative performance of *Aspergillus* galactomannan ELISA and PCR in sputum from patients with ABPA and CPA☆,☆☆

Samuel Fayemiwo[a,b,c,*], Caroline B. Moore[b,c], Philip Foden[d], David W. Denning[c,e], Malcolm D. Richardson[b,c]

[a] Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, Ibadan, Nigeria
[b] Mycology Reference Centre, University Hospital of South Manchester, Manchester Academic Health Sciences Centre, Manchester, UK
[c] Institute of inflammation and Repair, Faculty of Medicine and Health Sciences, University of Manchester, UK
[d] Medical Statistics Department, University Hospital of South Manchester, Manchester, UK
[e] National Aspergillosis Centre, University Hospital of South Manchester, Manchester Academic Health Sciences Centre, University of Manchester, UK

ARTICLE INFO

Keywords:
Sputum
Galactomannan
Aspergillus DNA
Allergic bronchopulmonary aspergillosis
Chronic pulmonary aspergillosis (CPA)

ABSTRACT

Objectives: Galactomannan (GM) and *Aspergillus* DNA detection are useful tools for the diagnosis of invasive pulmonary aspergillosis (IPA), primarily in blood and bronchoscopy samples. This study aimed to evaluate the utility of both markers for detection of *Aspergillus* in sputum from patients with allergic bronchopulmonary aspergillosis (ABPA) and chronic pulmonary aspergillosis (CPA).

Methods: ABPA or CPA demographic patient data were retrieved. This retrospective observational audit included 159 patients with at least one sputum pair. 223 sputum sample pairs were analysed, as well as six control samples for GM only. Real time PCR was performed following sputum DNA extraction using the MycAssay™ Aspergillus kit and cycle thresholds were subtracted from 38 to give positive values (transformed Ct, TCt).

Results: The mean age of the patients was 61.81 years (SD: ± 11.06; range 29–100). One hundred and twenty-six (79.2%) had CPA. Cultures were positive for fungi in 13.1% of the samples, and *A. fumigatus* was the commonest (11.9%) fungus isolated. Receiver operating characteristic (ROC curve) analysis of sputum GM comparing TCt of > 0.0, and > 2.0 to derive GMI cut-off values showed a cut-off of 6.5. About 50% of sputa with strongly positive PCR values had GM values > 6.5. Two of six (33%) control samples had GM indices > 6.5.

Conclusion: It is not clear that GM determinations in sputum are useful for diagnosis of either CPA or ABPA, or following therapy.

1. Introduction

Aspergillus species may cause life-threatening respiratory infections including invasive pulmonary aspergillosis, and allergic and chronic lung disease (Hope et al., 2005). A small number of species of *Aspergillus* are responsible for pulmonary aspergillosis including allergic bronchopulmonary aspergillosis (ABPA) and chronic pulmonary aspergillosis (CPA) (Hope et al., 2005; Denning et al., 2011). While ABPA is a non-invasive form of *Aspergillus* lung disease with hypersensitivity manifestations in patients who have pre-existing atopy, asthma or cystic fibrosis (Kousha et al., 2011), CPA manifests in patients who have impaired immunity and chronic obstructive pulmonary diseases (COPD) (Kousha et al., 2011). Globally, about 6.8% of asthmatic patients and those with corticosteroid-complicated asthma develop ABPA (Agarwal et al., 2013). Among cystic fibrosis patients, the prevalence of ABPA was found to be 2–15% (Denning et al., 2003a), increasing to 18% among children (Sharma et al., 2014). The global burden of CPA complicating ABPA has been estimated to be approximately 400,000 patients (Denning et al., 2013a). Most subjects with a simple aspergilloma are asymptomatic except for haemoptysis which occurs in about 50–90% of cases (Patterson and Strek, 2014). Confirmation of diagnosis of these infections requires assessment of clinical symptoms, positive *Aspergillus* spp. culture from respiratory secretions, histological demonstration of fungal invasion, detection of *Aspergillus* IgG antibody

☆ **Authors' contributions:** DWD and MDR supervised the operational aspect of the study that was conducted by SAF; SAF, CBM supervised the data entry; SAF, CBM, PF and DWD undertook the analysis of data; SAF, DWD and MDR wrote the manuscript which was reviewed by all authors.

☆☆ This article contributes new data on the utility of *Aspergillus* galactomannan (GM) and *Aspergillus* DNA detection in sputum from patients with allergic bronchopulmonary aspergillosis (ABPA) and chronic pulmonary aspergillosis (CPA).

* Corresponding author at: Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, University College Hospital, Ibadan, Nigeria.
E-mail address: safayemiwo@comui.edu.ng (S. Fayemiwo).

http://dx.doi.org/10.1016/j.mimet.2017.06.016
Received 20 March 2017; Received in revised form 17 June 2017; Accepted 18 June 2017
Available online 21 June 2017

and other immunological tests as well as review of radiological appearances (Denning et al., 2003a; Zmeili and Soubani, 2007; Saraceno et al., 1997; Agarwal et al., 2015).

Most diagnostic tests for the detection of *Aspergillus* and other fungi utilise serum and bronchoalveolar lavage (BAL) fluid for the confirmation of pulmonary aspergillosis and acute invasive aspergillosis (Agarwal et al., 2015). BAL requires bronchoscopy that is invasive and may be difficult to obtain in critically ill patients (Denning et al., 2011). However, sputum that is either expectorated or induced is non-invasive and its diagnostic significance in term of volume yield, sensitivity and specificity cannot be overemphasized (Fraczek et al., 2014; Schelenz et al., 2015; Kimura et al., 2008).

The detection of galactomannan (GM), a polysaccharide that is released during *Aspergillus* growth, is a useful and reliable non-invasive diagnostic test for screening and management of aspergillosis and is often more sensitive than culture (Schelenz et al., 2015; Fisher et al., 2013). The level of galactomannan in body fluids such as serum and BAL is positively associated with the fungal burden of *Aspergillus* (Fisher et al., 2013). Two studies have shown evaluated GM detection in sputum, one in patients with invasive aspergillosis (Kimura et al., 2009), one in cystic fibrosis (Baxter et al., n.d.). The optimal cut-off for the galactomannan index (GMI), as determined by constructing receiver operating characteristic (ROC) curve, and for the diagnosis of invasive pulmonary aspergillosis had been set at equal or > 0.5 for serum and 1.0 for BAL respectively (Schelenz et al., 2015; Kimura et al., 2009; Khorvash et al., 2014; Morrissey et al., 2013; He et al., 2011; Richardson and Warnock, 2012). There have been considerable variations in the sensitivity and specificity of GMI detections in body fluids (Fisher et al., 2013). In BAL fluid, the sensitivity ranges between 29 and 100% (Hope et al., 2005), whilst it ranges between 17 and 100% in serum (Schelenz et al., 2015). There is considerable controversy surrounding the optimal cut-off for respiratory secretion GMI values. A cut-off value of 1.2 in sputum was proposed for *Aspergillus* GM in IPA among haematological patients with sensitivity and specificity set at 100 and 62.2% respectively (Kimura et al., 2008; Kimura et al., 2009).

Real-Time PCR (RT-PCR) has now become an important diagnostic procedure for the diagnosis of aspergillosis (Fraczek et al., 2014). Detection of *Aspergillus* DNA has been applied to most body fluids including blood, BAL and sputum specimens using commercially available kits, for example MycAssay® *Aspergillus* (Baxter et al., 2013) (Lab21, Cambridge, UK). Both GM and Aspergillus PCR have shown higher sensitivity than culture for the detection of *Aspergillus* species in sputum in CF patients (Baxter et al., 2013; Baxter et al., 2011) and PCR in non-CF patients (Denning et al., 2011; Langridge et al., 2016). A cycle threshold (Ct) value < 36.0 is regarded as positive for *Aspergillus* DNA in RT-PCR in respiratory fluids (Tuon, 2007). However, there have been many inconsistencies in the reported RT-PCR Ct and GM index values for positive detection of *Aspergillus* (Kimura et al., 2009).

The survival of patients with aspergillosis is highly dependent on the ability to make an early and accurate diagnosis. Since the isolation of organisms is rarely successful, this study was designed to determine the GM index and PCR Ct values to evaluate the cut-off values for positive sputum detection of *Aspergillus* in patients with ABPA and CPA.

2. Materials and methods

2.1. Study design

The audit was a retrospective observational study involving routinely collected sputum samples from patients with ABPA and CPA, whether they have been treated or not. Baseline data for patients with a diagnosis of ABPA or CPA, as previously described (Denning et al., 2011; Denning et al., 2003b), were collected and included age, gender, sputum microscopy, fungal culture and current antifungal treatment. The audit was registered with the University Hospital of South Manchester audit department.

2.2. Patient selection

Patients with ABPA or CPA who had submitted sputum samples for microscopy, fungal culture, Aspergillus RT-PCR and detection of GM antigen by ELISA within a two weeks interval were identified. Approximately 60 ABPA and CPA patients attend the National Aspergillosis Centre (NAC) clinics each week, and some do not produce sputum. The pairs of values of results for GM and PCR were recorded for each patient. Unselected sputa submitted by general practitioners for routine bacterial culture were used as controls for GM only.

2.3. Laboratory procedures

The sputum samples were divided into aliquots for the routine direct microscopy, culture, galactomannan testing and RT-PCR. Consistency of sputum specimen was also graded as watery, salivary, mucoid, mucopurulent, and purulent and blood stained.

2.3.1. Microscopy

Sputum samples were digested with dithiothreitol (Sputasol®) (Stokell et al., 2014) and then examined under bright-field and fluorescent microscopy with calcofluor white. Aliquots of digested sputum were cultured on Sabouraud dextrose agar plates (SAB) and incubated for a total of 7 days at 30 °C and 37 °C (Denning et al., 2013b). Fungal identification was determined by conventional mycological procedures, and by DNA sequencing when required (Denning et al., 2011; Denning et al., 2003a).

2.3.2. Galactomannan assay

GM levels were determined using a commercially available kit (Platelia™ Aspergillus, BIO-RAD, France). The kit has been validated only for serum and bronchoalveolar lavage (BAL). A BAL sample result that was greater than a 1.0 index value was considered to be positive while 0.5 was the validated index value for serum. However, the performance for sputum has not been validated. The results interpreted according to the manufacturer recommendations, are usually expressed as the ratio of optical densities (OD) of either serum or BAL samples to the mean cut-off of the threshold control OD.

2.3.3. Aspergillus PCR assay

Aspergillus spp. DNA was detected using the commercially available real-time PCR diagnostic assay MycAssay™ Aspergillus (Lab21, Cambridge, UK) kit. The control reagent was set at a cycle threshold (Ct) of 38.0. Interpretations of the Ct value according to the manufacturer guidelines indicate that a Ct value of 36.0 or below is positive while a Ct of 38.0 or greater is regarded as negative. However, based on local experience with this assay an additional interpretation is that a Ct value between 36 and 38 has been assigned as a weak positive which could represent aspergillosis or colonization. In the interpretation of the Ct values, all Ct values were subtracted from 38 to provide a positive and increasing value ('Transformed Ct '(TCt)) correlating with the strength of signal and *Aspergillus* load. A TCt of 0.0 is equivalent to Ct of 38.0 or above.

2.4. Statistical analysis

The data generated were sorted and coded serially. The analysis was carried out using SPSS version 17.0. Data was cleaned after entry by removing duplicated data that were misclassified as disease-type. Summary statistics for both categorical and continuous variables were estimated. Patients were selected based on their diagnosis of ABPA or CPA. Transformed Ct values were set at either > 0.0 or > 2.0 denoting the positivity of *Aspergillus* DNA detection while GM index cut-off values were set at 0.5–6.5. The sensitivity, specificity, positive predictive and negative predictive values were calculated for each possibility of the cut-off values. The Youden J-statistic (Youden Index (YI)), which is