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A prospective study of fungal biomarkers to improve management of invasive fungal diseases in a mixed specialty critical care unit

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ABSTRACT

Purpose: The diagnosis of invasive fungal diseases (IFD) in critical care patients (CrCP) is difficult. The study investigated the performance of a set of biomarkers for diagnosis of IFD in a mixed specialty critical care unit (CrCU).
Methods: A prospective observational study in patients receiving critical care for ≥7 days was performed. Serum samples were tested for the presence of: (1-3) - β-D-glucan (BDG), galactomannan (GM), and *Aspergillus fumigatus* DNA. GM antigen detection was also performed on bronchoalveolar lavage (BAL) samples. The patients were classified using published definitions for IFD and a diagnostic algorithm for invasive pulmonary aspergillosis. Performance parameters of the assays were determined.
Results: In patients with proven and probable IFD, the sensitivity, specificity, PPV and NPV of a single positive BDG were 63%, 83%, 65% and 83% respectively. Specificity increased to 86% with 2 consecutive positive results. The mean BDG value of patients with proven and probable IFD was significantly higher compared to those with fungal colonization and no IFD (p value < 0.0001).
Conclusion: New diagnostic criteria which incorporate these biomarkers, in particular BDG, and host factors unique to critical care patients should enhance diagnosis of IFD and positively impact antifungal stewardship programs.

1. Introduction

Globally, invasive fungal diseases (IFD) are recognised as a major and frequent complication during treatment of critical care patients (CrCP). *Candida* spp. are the most common fungal pathogens although recently *Aspergillus* spp. have emerged as important pathogens in this cohort [1-7]. The main challenge is making a timely diagnosis of IFD since delayed initiation of appropriate antifungal therapy has been shown to increase morbidity and mortality [8-10].

Candida species have been reported as the 5th leading cause of nosocomial bloodstream infections (BSIs) in CrCP [11]. However, given the poor sensitivity of blood cultures, the true incidence of candidemia may be greater [12]. In addition, intra-abdominal candidiasis (IAC) may be an under-diagnosed clinical syndrome due to the non-specificity of clinical signs and the difficulty of interpretation of culture results from intra-abdominal samples where cultured *Candida* spp. could be interpreted as colonizers of the intestinal tract [13].

Abbreviations: IFD, invasive fungal disease; CrCP, critical care patients; CrCU, critical care unit; BDG, 1-3-beta-D-glucan; GM, galactomanan; DNA, deoxyribonucleic acid; qPCR, real time polymerase chain reaction; BAL, bronchoalveolar lavage; NPV, negative predictive value; PPV, positive predictive value; BSI, bloodstream infection; IAC, intra-abdominal candidiasis; APACHE, acute physiologic assessment and chronic health evaluation; SOFA, sequential organ failure assessment; ANC, actual neutrophil count; IA, invasive aspergillosis; IC, invasive candidiasis; IPA, invasive pulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; BM, biomarker; EORTC/MSG, European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Disease Mycoses Study Group; GICU, general intensive care unit; HDU, high dependency unit; MV, mechanical ventilation; HD, haemodialysis; GI, gastro-intestinal; BSA, broad spectrum antimicrobial; TPN, total parenteral nutrition; CSF, cerebrospinal fluid; ODI, optical density index; ITS, internal transcribed spacer; BSC, biological safety cabinet; EAPCRI, European *Aspergillus* PCR initiative; Ct, cycle threshold; ICo, internal control; ROC, receiver operator curve; AUC, area under the curve; CABG, coronary artery bypass graft; EVAR, endovascular aneurysm repair; HAP, hospital-acquired pneumonia; AFT, antifungal therapy; HSCT, haematopoietic stem cell transplant; GVHD, graft versus host disease; CA, cancer.

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Invasive aspergillosis (IA), most commonly caused by *A. fumigatus*, has been increasing in incidence in CrCP who do not have the traditional host factors for this disease [2,4,6,14,15]. Like invasive candidiasis (IC), the clinical presentation and radiological findings of IA in non-neutropenic patients are non-specific, therefore a high index of suspicion is necessary when a patient is not responding to broad-spectrum antibacterial agents. Blot et al. [16] have proposed a diagnostic algorithm for invasive pulmonary aspergillosis (IPA) in CrCP where patients were classified as proven, putative IPA (which from hereon will be referred to as probable IPA) or *Aspergillus* colonization. This algorithm requires isolation of *Aspergillus* species from a respiratory specimen when not all patients with IPA will satisfy this mycological criterion. Importantly, the algorithm excludes fungal biomarkers such as galactomannan (GM) or (1-3)-β-d-glucan (BDG). Of particular interest is the increased recognition that patients with COPD, most of whom are receiving corticosteroids, are at risk for IPA during critical care [6,17,18]. Diagnosis of IPA in this cohort is also challenging. To address this, Bulpa et al. have proposed definitions for diagnosis of IPA in COPD patients but these require further clinical validation [17].

Given the difficulty with diagnosis of IFD and the low sensitivity of fungal cultures, other approaches have been investigated. Two key advances are fungal antigen detection and molecular assays to detect fungal DNA in clinical samples. Several studies have investigated the potential usefulness of BDG and GM antigen in the diagnosis of IFD both in neutropenic and non-neutropenic patients [19-22]. These biomarkers (BM) have been included as mycological criteria in the European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG) revised definitions of IFD [23]. Detection of fungal DNA was not included as this was stated to require further standardisation and validation although in the recently proposed revisions to these guidelines, PCR for the detection of *Aspergillus* and *Pneumocystis jirovecii* are likely to be included (PL White, Personal Communication). The potential usefulness of these biomarkers for early diagnosis of IFD and their impact on the management of non-neutropenic critical care patients needs further evaluation.

To address these deficiencies, a prospective observational study was performed to document the incidence of IFD in our CrCP, to enumerate the host factors associated with an increased risk for acquiring IFD and to determine the performance of BDG, GM and our in-house *Aspergillus fumigatus* specific real time polymerase chain reaction (qPCR) assay for diagnosis of IFD in CrCP. Detection of *Candida* DNA was not included because we did not have an in-house assay for this fungal pathogen.

2. Materials and methods

This study was conducted at St. James's Hospital (SJH) Dublin Ireland, a 1010 bed tertiary referral centre. Our 23 bed critical care unit (CrCU), which includes the general intensive care (GICU) and high dependency (HDU) units, is a mixed specialty unit.

2.1. Ethics statement

Prior approval for this study was obtained from the St. James's Hospital Research Ethics Committee (Reference 2012/39/01).

2.2. Patients

All patients who were admitted to our CrCU for 7 days or longer and who were expected to require at least a further week's stay on the unit were eligible for inclusion in the study. Patients, or more commonly next of kin, were approached for their consent and an information leaflet with the details of the study was provided. Patients or patients' next of kin who refused to consent/assent for the study and patients known to have infections with hepatitis B virus, hepatitis C virus or HIV were excluded from the study.

2.3. Clinical data

The following data were collected: patients' demographics including age, sex, underlying diagnoses, Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) admission scores, reason for admission, host factors for IFD which included neutropenia (Actual neutrophil count, ANC < 0.5×10^9/L), haematological malignancies, bone marrow or haematopoietic stem cell transplant, solid tumours, solid organ transplant, receipt of T-cell immune-suppressants, mechanical ventilation (MV) for ≥3 days, haemodialysis/haemofiltration (HD) ≥ 3 days, gastrointestinal (GI) surgery, broad spectrum antimicrobial (BSA) therapy, presence of indwelling medical devices, corticosteroid therapy (prednisolone equivalent of 0.3 mg/kg/day for ≥3 weeks), total parenteral nutrition (TPN) ≥ 3 days, other co-morbidities such as history of COPD, diabetes and liver cirrhosis, pertinent laboratory and radiological results from the day of admission to the unit and the outcome of critical care admission.

2.4. Definitions

The patients were categorised either as having no evidence of IFD, fungal colonization, possible, probable or proven IFD. Patients with *Candida* infection were categorised using the following definitions: patients with proven IC had clinical sepsis where *Candida* sp. was isolated from normally sterile sites e.g. blood, CSF, tissue, or other body fluid obtained at the time of drainage such as peritoneal fluid, pleural fluid, synovial fluid, or an ophthamologic examination consistent with endophthalmitis, or histologically documented candidiasis [23,24]; probable IC where patients had signs and symptoms of clinical sepsis, ≥3 host risk factors and isolation of *Candida* sp. from ≥2 non-sterile sites such as superficial swabs, sputum, urine and fluid from indwelling drains; possible IC where patients had signs and symptoms of clinical sepsis, ≥3 host factors and isolation of *Candida* spp. from 1 non sterile site; fungal colonization applied to patients who may or may not have had host risk factors for *Candida* infection, without signs of sepsis, with *Candida* recovered from one or more non-sterile sites [25]; patients with no evidence of IC had no clinical signs and no microbiological evidence of *Candida* infection. When applicable, we used the EORTC/MSG revised definitions for IFD [23] and the clinical algorithm proposed by Blot et al. [16] to differentiate *Aspergillus* colonization from probable and proven IPA. All the imaging results were reviewed by a Radiology Specialist who was blinded to the patients' clinical diagnoses and laboratory results. IFD was categorised as either unit-acquired if patients were diagnosed to have probable or proven IFD >48 h after admission or non-unit-acquired if the diagnosis was made within 48 h of admission.

As this was an observational study and the biomarker tests were performed in batches, the results were not available to the intensivists for clinical decision making. To evaluate the diagnostic performance of these BMs, the results were excluded as mycological criteria during the evaluation and categorisation of the study population. Additionally, there was no protocol followed for sampling of sterile and non-sterile sites for culture or for initiation of antifungal therapy (AFT). These decisions were left to the discretion of the clinical team/s based on the patients' clinical status and the reported results of laboratory and imaging investigations.

After evaluation of all data following completion of the study, the patients were reviewed and re-categorised with the inclusion of the BM results using the EORTC/MSG definitions for IFD [23] and the diagnostic algorithm for IPA by Blot et al. [16] if applicable.

2.5. Clinical samples

Ten milliliters of blood were collected in a BD Vacutainer® without anticoagulant from the study participants twice weekly. Blood samples were allowed to clot and serum was extracted after centrifugation at