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ORIGINAL ARTICLE

Threshold of galactomannan antigenemia positivity for early diagnosis of invasive aspergillosis in neutropenic children

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Purpose: Invasive aspergillosis (IA) is an important cause of morbidity and mortality in immunocompromised patients. Pediatric data on the accuracy and optimal cutoff of galactomannan antigen detection to diagnose IA is sparse and controversial. We evaluated the utility and optimal serum galactomannan assay (GA) cutoff in children.
Methods: Children with febrile neutropenia due to malignancy, hematopoietic stem cell transplant, aplastic anemia, or congenital neutropenia, were prospectively included from 2007 to 2011. All new episodes of febrile neutropenia were recorded. In case of a previous diagnosis of IA, subsequent episodes were excluded. One to four GA were tested by enzyme immunoassay during each episode. Bronchoalveolar lavage and other relevant samples for mycological diagnosis, and computed tomography of chest/sinus were performed wherever appropriate. IA was classified as "proven", "probable", and "possible" as per the 2008 European Organisation for Research and Treatment of Cancer and Mycoses Study Group Guidelines. The optimal cutoff value was determined using receiver operating characteristic curves in episode-wise analysis.
Results: There were 145 patients with 211 febrile episodes included: hematopoietic stem cell transplant ($n = 15$), oncological ($n = 113$), and hematological disorders ($n = 17$). Forty-five children (31.0%) developed IA (5 proven, 15 probable, and 25 possible). Cutoff value of single GA ≥ 0.7 for proven/probable/possible IA offered the best combination of sensitivity (82.2%)/

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specificity (82.5%), and 94.4% negative predictive value. Two consecutive positive GA $\geq$ 0.7 had a sensitivity/specificity of 75.0%/91.0%. Index GA $\geq$ 1.9 was associated with significantly higher mortality in children with IA and overall.
Conclusion: Serum GA is sensitive to diagnose IA in pediatric patients with excellent negative predictive value at an optimal cutoff of $\geq$0.7. Considering two consecutive values $\geq$0.7 increases specificity to 91.0%.

Introduction

Invasive fungal infections (IFIs) are a major cause of mortality and morbidity in neutropenic patients after chemotherapy for hematological malignancies or hematopoetic stem cell transplant (HSCT).[1] Opportunistic yeasts and molds are the most prevalent pathogens associated with IFI.[2] The incidence of invasive aspergillosis (IA) has increased in recent years, particularly among patients receiving new intensive chemotherapy regimens for malignancies or undergoing allogeneic HSCT.[1,3] Despite therapeutic advances, IA is associated with high morbidity and mortality, reaching 30—70% in HSCT recipients.[4] Prompt institution of antifungal therapy is necessary to decrease mortality, making early diagnosis a critical factor to improve outcomes in patients with IA. However, early diagnosis of IA remains difficult, because clinical and radiological signs are nonspecific, whereas microbiological culture techniques have a low sensitivity and require expertise for species identification.[1] Although histological diagnosis by tissue biopsy is the gold standard for IA,[5] it is invasive and may lead to life-threatening complications, especially among patients with coagulopathy and thrombocytopenia.

Detection of circulating *Aspergillus* antigens in the serum has proven to be a powerful tool for non-invasive early detection of IA.[3,6,7] Galactomannan, a heat stable heteropolysaccharide cell-wall component of *Aspergillus* species, is released into biological fluids during fungal growth in the tissues.[8] Quantitative enzyme immunoassay (EIA) for serum galactomannan detection is highly sensitive and superior to polymerase chain reaction (PCR)-based assays.[9,10] However, the ideal threshold for positive galactomannan assay (GA) remains controversial,[11] and may differ between adults and children, as the latter display higher false positivity rates. The cutoff optical density (OD) was initially set at 1.5 and applied in Europe, then lowered to 0.5 in the USA to allow earlier detection performance for adult hematology patients.[12] Pediatric studies are sparse and have used various thresholds.

In an effort to standardize the definitions of IFI, an international consensus of the Invasive Fungal Infections Cooperative Group of the European Organisation for Research and Treatment of Cancer (EORTC) and Mycoses Study Group (MSG) proposed three levels of probability of IA: "proven", "probable", and "possible", based on host factors, and microbiological and clinical criteria.[13] GA was included as mycological criterion in the revised definitions of IA by the EORTC/MSG, which recommends adopting the threshold values set by manufacturers.[14] However, a systematic review showed variable accuracy of GA for surveillance and detection of IA.[3]

Thus we conducted a prospective study to assess the utility of GA for diagnosing IA in pediatric patients with febrile neutropenia and to determine the optimal GA cutoff value.

Methods

Patients with febrile neutropenia younger than 18 years admitted in the Hematology Oncology Unit, Department of Pediatrics Sir Ganga Ram Hospital, Rajender Nagar, New Delhi, India, were included prospectively. Causes of neutropenia included chemotherapy for malignancy, allogenic or autologous HSCT recipients, aplastic anemia, and congenital immunodeficiency. Febrile neutropenia was defined as either a single oral temperature above 38.5°C or an oral temperature of 38°C for >1 hour in a child with an absolute neutrophil count below 1.0×10^9/L. More than one treatment episode was included per patient, but once proven or probable IA was diagnosed, these patients were no longer eligible for inclusion during subsequent neutropenic episodes.

Demographic and clinical data were recorded. Patients were tested for the presence of galactomannan antigen if they met at least one of the following criteria: persisting neutropenic fever despite administration of 5 days of broad-spectrum antibiotics, unexplained fever relapsing after 48 hours of deffervescence while still neutropenic and on antibiotics, clinical signs or symptoms suggestive of IFI (lower respiratory tract infection; nasal eschar, maxillary tenderness, nodular, and/or necrotic skin lesions), appearance of new pulmonary infiltrate while receiving broad-spectrum antibiotics or steroids, isolation of molds, or demonstration of hyphae in respiratory secretions. All HSCT recipients received antifungal prophylaxis with fluconazole (6 mg/kg/day) from the start of pretransplant conditioning. Positive GA during surveillance of HSCT patients resulted in a preclinical start of empirical therapy with either amphotericin B or voriconazole. In case of clinical suspicion of IFI, empirical antifungal therapy was started after sampling for GA.

Blood samples (2 mL) were collected in dry tubes for GA. Paired sera at a minimum of 1 day-interval and follow-up samples to assess response to antifungal therapy were evaluated, if possible. High-resolution computed tomography (CT) of the chest and sinus was done in patients with clinical signs or symptoms suggestive of pulmonary or sinus IFI, and in case of positive GA. Other relevant samples were