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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

taken from the study population wherever appropriate [bronchoalveolar lavage (BAL), endotracheal aspirate, and biopsy specimens] and were examined for the presence of fungi by microscopy and culture as previously described.[15] IA was classified as "proven", "probable", and "possible" as per EORTC/MSG 2008 Guidelines,[12] excluding galactomannan antigen as a mycological criterion.

Serum samples were stored at +2–8°C, for testing within 24 hours, or kept frozen at −20°C otherwise, for a maximum of 4 days. Twice weekly testing was done. Quantitative determination of serum *Aspergillus* galactomannan antigen by one-stage sandwich immunoenzymatic technique was done using the Platelia Aspergillus EIA kit (Bio-Rad, Marnes-la-Coquette, France) as per the manufacturer's instructions. Positive, negative, and threshold control samples provided by the manufacturer were included in each assay. GA was initially considered positive above an index cutoff OD of ≥1 and borderline positive at 0.5–1. An experienced laboratory technician trained in EIA conducted the test and the optical density measurement, which was then interpreted by two mycologists, unaware of the patients' clinical details.

The outcome of all febrile neutropenic episodes was assessed by clinical response, follow-up GA on antifungal therapy and 30-day all cause mortality. Statistical analysis was done on SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Chi-square test was applied to compare categorical variables. Two-sided $p < 0.05$ was considered as significant. Receiver operating characteristic curves were used to determine the optimal cutoff value in episode-wise analysis, using the maximum index value obtained during each episode. The diagnostic value of galactomannan index was assessed using the area under the curve (95% confidence interval), along with pooled sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) calculated for proven/probable/possible IA versus no IA using 2 × 2 contingency tables with single index value and two sequential index values above various threshold OD (0.5, 1, 1.5, and optimal threshold value) for GA positivity. Comparison of non-normally distributed variables between the two groups was done using Mann–Whitney *U* test.

Results

Patients

There were 145 patients included prospectively over 5 years (from January 2007 to December 2011) during 211 episodes of febrile neutropenia (Table 1 and Fig. 1). There were 101 boys and 44 girls, aged from 3 months to 18 years (median 5 years). Thirty-six patients had high-risk neutropenia after HSCT ($n = 15$), intensive chemotherapy for relapsed, refractory, or high-risk acute lymphoblastic leukemia ($n = 18$), and antithymoglobulin therapy ($n = 3$), whereas 109 patients had low-risk neutropenia. Proven, probable, possible IA, and no evidence of IA were seen in five children, 15 children, 25 children, and 100 children, respectively. Forty-five patients with IA were no longer included during subsequent episodes of febrile neutropenia. IA was pulmonary in 39 patients, sinonasal in seven individuals, intestinal in one individual, and pericardial and hepatic in one patient. The diagnosis of IA was based on a combination of positive culture or smear in 20/50 (40.0%) children tested, suggestive CT imaging in 39/73 (53.4%), and biopsy specimens positive in 5/8 (62.5%). BAL or endotracheal aspirate was positive in 14 (33.3%) out of 42 patients. Fungal mold isolates in 16 patients were *Aspergillus flavus* ($n = 10$), *Aspergillus fumigatus* ($n = 4$), *Fusarium* sp. ($n = 2$), *Mucor* sp. ($n = 2$), and *Conidiobolus* sp. ($n = 1$). Positive smear and negative culture were mostly observed in children receiving antifungal therapy.

Galactomannan antigenemia

One to four GA were tested during each febrile episode. Eleven patients for which only one GA was tested were excluded from analysis of two consecutive GA. Of 405 serum samples, 134 (33.3%) were above the recommended positivity cutoff (≥0.5). Positive GA was observed from 34 days before to 27 days after CT scan suggestive of IA (median, 3 days) and from 37 days before to 4 days after positive BAL (median, 3 days). The receiver operating characteristic curves drawn to assess the value of a single GA and of two consecutive GA in pooled proven/probable/possible IA showed that a cutoff value of GA ≥ 0.7 offered the best compromise between increased specificity and lower sensitivity (Fig. 2).

The GA positivity rate was highest in probable and proven IA as compared to possible IA ($p < 0.001$, Table 1). Out of five proven IA, a false negative result was concurrent to prior administration of amphotericin B, leading to poorer performance of GA in the proven IA group than the probable IA group. Thus sensitivity of a single GA ≥ 0.7 and of two consecutive GA ≥ 0.7 was higher in cases with probable IA than in proven IA and possible IA, whereas specificity was identical in all three categories. Single GA and two serial GA ≥ 0.7 had a NPV of 94.5% and 92.8% respectively. The PPVs of single and serial GA ≥ 0.7 were 56.1% and 70.2%, respectively.

During all 211 episodes, 28/45 (62.2%) children with clinical symptoms suggestive of IA developed proven, probable, or possible IA, versus 2/19 (10.5%) episodes of HSCT surveillance and 15/147 (10.2%) with pyrexia of unknown origin (PUO, $p < 0.001$). GA positive yield was significantly higher among children with clinical suspicion of IA than those with PUO or on routine HSCT surveillance of GA ($p < 0.001$). GA was positive for 1–34 days (median, 6 days) prior to the detection of radiological signs in 17/39 (43.6%) patients with chest CT scan suggestive of IA.

False positive single GA ≥ 0.7 ($n = 29$, 13.7%) was seen in association with bacterial septic shock ($n = 5$), *Mucor* sp. induced intestinal perforation ($n = 2$), and one case each of *Candida* sepsis, gastroenteritis, mucositis, gut graft-versus-host disease grade IV, piperacillin–tazobactam administration, whereas there was no identifiable cause in 17 cases. Eleven children had only one GA tested, and either chest CT not suggestive of IFI ($n = 3$) or BAL negative ($n = 2$) or neither performed ($n = 6$). Follow-up of these children, who did not receive long-term antifungal therapy, confirmed the absence of IFI. Seven children had only one positive GA, the subsequent ones being <0.7.

Table 1 Characteristics of 145 children with febrile neutropenia

	Total	Proven IA	Probable IA	Possible IA	No IA
Patients	***n* = 145**	***n* = 5**	***n* = 15**	***n* = 25**	***n* = 100**
Median age, y	5	9	7	8	5
(range)	(0.25–19)	(3–11)	(0.5–19)	(2–14)	(0.25–16)
Diagnosis					
HSCT					
Allogenic	9	—	2	—	7
Autologous	6	—		—	6
ALL	92	3	7	19	63
AML	14	—	2	3	9
NHL	5	—	2	—	3
Bone marrow failure					
Acquired	14	2	1	3	8
Inherited	2	—	1	—	1
SCN	1	—	—	—	1
Solid tumor	2	—	—	—	2
Episodes	***n* = 211**	***n* = 5**	***n* = 15**	***n* = 25**	***n* = 166**
Median index GA	0.1 (0–5.5)	1.9 (0.1–2.4)	2.8 (1.5–5.5)	1.5 (0–4.3)	0.1 (0–4.7)
Single GA					
≥0.5	72 (84.4/79.5)	4 (80/79.5)	15 (100/79.5)	19 (76/79.5)	34
≥0.7	66 (82.2/82.5)	4 (80/82.5)	15 (100/82.5)	18 (72/82.5)	29
≥1.0	65 (80.0/82.5)	4 (80/82.5)	15 (100/82.5)	17 (68/82.5)	29
≥1.5	56 (75.6/86.7)	3 (60/86.7)	15 (100/86.7)	16 (64/86.7)	22
2 consecutive GA	*n* = 200	*n* = 5	*n* = 14	*n* = 24	*n* = 157
≥0.5	48 (74.4/89.8)	3 (60/89.8)	13 (92.9/89.8)	16 (66.7/89.8)	16
≥0.7	47 (75.0/91.0)	3 (60/91.0)	14 (93.3/91.0)	16 (66.7/91.0)	14
≥1.0	43 (69.8/91.7)	3 (60/91.7)	12 (85.7/91.7)	15 (62.5/91.7)	13
≥1.5	30 (53.5/95.5)	3 (60/95.5)	11 (78.6/95.5)	9 (37.5/95.5)	7
Clinical groups	*n* = 211	*n* = 5	*n* = 15	*n* = 25	*n* = 166
PUO	147	1	5	9	132
HSCT surveillance	19	4	2	—	17
Clinical suspicion of IA	45		8	16	17
Aspergillus sp. in mycological samples	*n* = 52	*n* = 5	*n* = 15	*n* = 15	*n* = 17
Culture + smear positive	15	4	9	—	—
Smear positive	7	1	6	—	—

Data are presented as *n*, *n* (range), or % (sensitivity/specificity).
ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; GA = galactomannan assay; HSCT = hematopoietic stem cell transplantation; IA = invasive aspergillosis; NHL = non-Hodgkin lymphoma; PUO = pyrexia of unknown origin; SCN = severe congenital neutropenia.

When two consecutive GA ≥ 0.7 were considered for positive assay, only 11/200 children (5.5%) had false positive results. Of these, neither chest CT nor BAL was performed in 3 children. In the rest, chest CT ($n = 7$) and BAL ($n = 2$) were negative. One of those children with at least two consecutive GA ≥ 0.7 had sinusal mucosal thickening on CT scan and no evidence of pulmonary IA during the first episode, and was classified as having no IA; he developed possible pulmonary and sinusal IA 10 months later.

We observed 31.6% false positive among HSCT recipients when single GA ≥ 0.7 was used as diagnostic criterion, as compared to 13.6% in PUO and 6.7% in children with clinical suspicion of IA ($p = 0.03$). However, the use of two serial GA ≥ 0.7 lowered false positive rate to 5.5%: 11.1% among HSCT recipients, 6.5% in children with PUO and none in children with clinical suspicion of IA.

False negative single GA (<0.7) was seen in four children on antifungal therapy (amphotericin B or voriconazole, 1 proven IA, and 3 possible IA) and four children without identifiable cause (all possible IA).

Outcome

Response to antifungal therapy was favorable in 31 children with IA, and GA became <0.7 in 2–54 days (median 8 days) in those with positive galactomannan antigenemia. The episode outcomes of 45 patients with IA were survival in 32 cases, death in 11 (24.4%) cases, discharge on request and loss to follow-up in two cases (Table 2). Causes of death included invasive fungal infection *per se* in five patients, sepsis in five patients and hemophagocytic

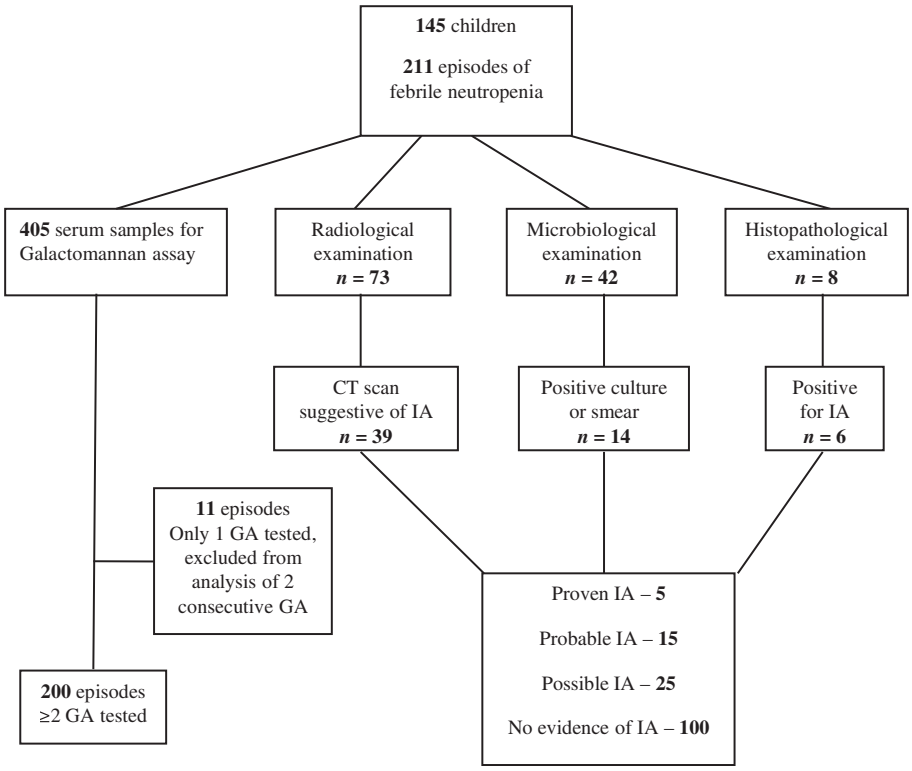

Figure 1. Flow diagram of 145 patients included in the study and investigations to diagnose invasive aspergillosis (IA). GA = galactomannan assay.

lymphohistiocytosis with acute hepatic failure in one patient. Nine (9.0%) children without evidence of IA died (6 of sepsis, 3 of hemorrhage). Three children discharged on request and lost to follow-up were excluded from mortality data. Mortality was significantly higher in children with IA (proven/probable/possible) than in those with no evidence of IA. One out of two HSCT recipients with probable IA died. *Aspergillus* coinfection with *Mucor* sp. and with *Conidiobolus* sp. plus *Fusarium* sp. was seen in two proven IA, and none survived the episode. Episode-wise mortality rate was 40% in proven IA, 50% in probable IA, and 8.3% in possible IA. However, because the probable IA group included only children with positive cytology or fungal culture, 18 children with possible IA were later included in the probable IA group as per the revised definitions of IA, using GA $\geq$ 0.7 as an indirect mycological criteria, for correct estimation of mortality in probable IA.[14] Thus the true mortality rate was 29.0% (9/31) in probable IA and 0% (0/7) in possible IA.

The highest GA index value per episode was compared between patients with favorable and unfavorable outcome. Median GA (interquartile range) was significantly higher in patients with 30-day mortality than in survivors [1.87 (0.11–2.69) vs. 0.09 (0.11–2.69), $p < 0.001$]. An index value $\geq$ 1.9 ($n = 31$) was significantly associated with episode mortality in children with IA (42.9% vs. 9.1%, $p = 0.016$) and in the whole cohort (33.3% vs. 5.6%, $p < 0.001$). Septic shock and multiorgan failure was seen more often in children with GA $\geq$ 1.9 (16.1% vs. 3.3%, $p = 0.012$). A high proportion of patients whose serial GA index values did not decrease below the threshold of 0.7 did not survived the febrile neutropenia episode (41.7% vs. 8.3%, $p = 0.059$). Children who died had higher GA index values in the 2nd week and 3rd week of pre-emptive antifungal therapy than those who survived. Conversely, favorable response to antifungal therapy was associated with return to a negative GA within 2–54 days (median, 8 days).

Discussion

In our children with febrile neutropenia due to HSCT, hematological, or oncological disorders, serum EIA galactomannan detection was found to be highly sensitive and specific for the early detection of IA. Our data suggest that, in the pediatric age group, a higher positivity threshold should be chosen than the 0.5 threshold proposed by the manufacturer. However, the positive predictive value of a single test was <60% and 70% for two serial GA.

Previous combined adult and pediatric studies showed that GA specificity is significantly lower in children as in adults.[16] The first European study, including 347 children and 450 bone marrow transplant patients, considered two consecutive sera samples $\geq$1.5 as criterion for positive Galactomannan antigen assay, with sensitivity and specificity of 90.6% and 94%, respectively.[17] Two recent studies were exclusively done in pediatric patients. A single assay threshold positivity of $\geq$0.5 yielded high sensitivity, specificity, positive, and negative predictive values (90%, 92%, 81.8% and 96%) in 62 febrile neutropenic children with either leukemia or pancytopenia.[18] In that population, accuracy and reliability of GA and nested PCR were comparable. Sensitivity of 91.3% and 81.7% specificity were

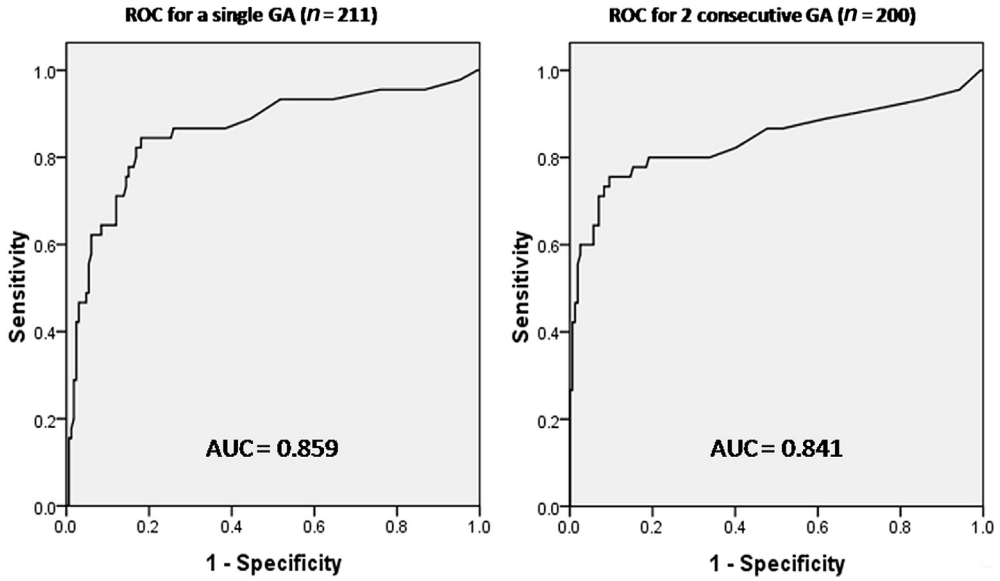

Figure 2. Receiver operating characteristic curves for galactomannan assays (GA) in proven/probable/possible invasive aspergillosis versus no invasive aspergillosis. AUC 95% confidence interval for a single GA = 0.788—0.930; for two consecutive GA = 0.756—0.927.

reported in 99 children with cancer and HSCT recipients, using two GA ≥ 0.5 on two occasions as criteria for positivity.[19]

The 2002 EORTC/MSG classification recommended using two positive results as diagnostic criterion.[13] The 2008 revised classification no longer makes two positive samples mandatory.[14] In our series, the criterion of two consecutive values ≥0.7 yielded lower sensitivity, as repeat GA 2—4 days after initiation of antimold antifungal therapy was followed by a decrease of GA below the threshold positivity

Table 2 Thirty-day all-cause mortality in 145 children and in 211 febrile neutropenia episodes

	Total	Died	Survived	*p*	Lost to follow-up
Patients	***n* = 145**	***n* = 2**	***n* = 122**		***n* = 3**
Group					
Proven IA	5	2 (40.0)	3 (60.0)	<0.001	0
Probable IA	15	7 (46.7)	7 (46.7)		1 (6.7)
Possible IA	25	2 (8.0)	22 (88.0)		1 (4.0)
No IA	100	9 (9.0)	90 (90.0)		1 (1.0)
Single highest GA					
≥0.7	62	14 (22.6)	46 (74.2)	0.007	2 (3.2)
<0.7	83	6 (7.2)	76 (91.6)		1 (1.2)
≥1.9	32	11 (34.4)	19 (59.4)	<0.001	2 (1.1)
<1.9	113	9 (8.0)	103 (91.2)		1 (3.2)
Episodes	***n* = 211**	***n* = 20**	***n* = 188**	***p***	***n* = 3**
Group					
Proven IA	5	2 (40.0)	3 (60.0)	<0.001	0
Probable IA	15	7 (46.7)	7 (46.7)		1 (6.7)
Possible IA	25	2 (8.0)	22 (88.0)		1 (4.0)
No IA	166	9 (5.4)	156 (94.0)		1 (0.6)
Median index GA	0.1	1.9	0.1	<0.001	1.3
(IQR)	(0.0—1.5)	(0.1—2.7)	(0.0—1.1)		(0.7—1.8)
Single highest GA					
≥0.7	66	14 (21.2)	50 (75.8)	<0.001	2 (3.0)
<0.7	145	6 (4.1)	138 (95.2)		1 (0.7)
≥1.9	31	10 (32.3)	20 (64.5)	<0.001	2 (1.1)
<1.9	180	10 (5.6)	168 (93.3)		1 (3.2)

Data are presented as *n* (%) or *n* (IQR).
GA = galactomannan assay; IA = invasive aspergillosis; IQR = interquartile range.

cutoff. However, specificity increased substantially, particularly among HSCT recipients. Various authors have shown the benefit of lowering the assay cutoff value from 1.5 to 0.7 or even 0.5 in nonallo-HSCT adults.[16] However, most false-positive results occur in children and in allogeneic HSCT patients, with false positive rates of 75% in pediatric HSCT recipients.[16,20,21] Although our data include only 19 episodes of HCST surveillance, false positivity rate was only 5% using either 1.5 as single GA positivity cutoff or two serial GA $\geq$ 0.7. Thus GA monitoring appears to be a good surveillance option for HSCT recipients on prophylactic fluconazole, so as to administer early preemptive antimold therapy.

False positive GA may be caused by the passage of galactomannan of food origin through the intestinal mucosa, as well as antibiotics such as piperacillin–tazobactam.[22,23] Out of 29 false positive GA $\geq$ 0.7, we identified intestinal mucosa damage (e.g. intestinal perforation, gastroenteritis, mucositis, and severe gut graft vs. host disease) as possible causes of false positivity in five children, and piperacillin–tazobactam in one child. In such a clinical scenario, repeating GA after a few days would reduce the false positivity rate, thus increasing specificity.

Despite using preemptive antifungal therapy with amphotericin B or voriconazole, we report high mortality in proven (40%) and probable IA (29%), similar to previous pediatric report.[19,24] Mortality increased to 100% when the coinfection of *Aspergillus* with *Mucor* sp. and the coinfection with *Conidiobolus* sp. plus *Fusarium* sp. were seen (2 cases of proven IA). The initial higher mortality figure in probable IA was inflated by considering as possible IA 18 children without direct mycological evidence of IA, but with positive indirect mycological criterion. However, a large number of children with possible IA benefited from preemptive antifungal therapy. We found that high GA index value ($\geq$ 1.9) was associated with septic shock and with significant mortality in our pediatric population. Higher mortality was also seen wherever positive GA index did not decrease below threshold positivity. The use of high GA index value as a potential poor prognostic marker may be prospectively assessed in larger studies.

Serum galactomannan antigen monitoring is accurate and reliable to diagnose invasive aspergillosis in febrile neutropenic children, with an optimal positivity cutoff of $\geq$0.7. Its specificity increases to 91% when two consecutive values $\geq$0.7 are considered. A negative GA practically rules out IA. A GA value $\geq$ 1.9 is associated with higher risk of fatal outcome. Prompt return to a negative GA may be a good indicator of clinical response to antifungal therapy and predictor of favorable outcome.

Conflicts of interest

All authors declare no conflicts of interest.

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