International Journal of Infectious Diseases 54 (2017) 8–12

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Utility of the serum galactomannan assay for the diagnosis of invasive aspergillosis in children with acute lymphoblastic leukemia

Gulhadiye Avcu [a], Deniz Yilmaz Karapinar [b,*], Ayse Burcu Akinci [b], Zuhal Onder Sivis [b], Akkiz Sahin [b], Zumrut Sahbudak Bal [a], Suleyha Hilmioglu Polat [c], Dilek Yesim Metin [c], Fadil Vardar [a], Yesim Aydinok [b]

[a] Department of Pediatrics, Division of Pediatric Infectious Diseases, Faculty of Medicine, Ege University, Izmir, Turkey
[b] Department of Pediatrics, Division of Pediatric Hematology, Faculty of Medicine, Ege University, 35100 Bornova, Izmir, Turkey
[c] Department of Medical Microbiology/Mycology, Faculty of Medicine, Ege University, Izmir, Turkey

ARTICLE INFO

Article history:
Available online

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Galactomannan
Aspergillosis
True-positive
False-positive
Children
Acute lymphoblastic leukemia

SUMMARY

Objectives: Invasive aspergillosis (IA) is an important cause of mortality and morbidity in children with hematological malignancies. The monitoring of serum galactomannan (GM) antigen is considered useful in the diagnosis of IA . The aim of this study was to determine the utility of serum GM monitoring in the early diagnosis of IA and the role of positive antigenemia in the management of children with acute lymphoblastic leukemia (ALL).

Methods: The cases of 141 children who were being treated for ALL in the Division of Pediatric Hematology of the Medical School of Ege University between January 2006 and February 2015 were reviewed retrospectively. Cases of proven and probable IA were defined according to the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria.

Results: The incidence of proven and probable IA was 3.5% (5/141). The incidence of positive GM antigenemia among 3264 serum samples was 5.5% (n = 179). Of the cases detected, 21.7% were true-positive, 52.1% were false-positive, and the remaining 26.1% were classified as 'undetermined.' An increase in the incidence of true-positive tests and induction of antifungal therapy was determined through multiple consecutive positive tests.

Conclusions: GM may be detected in the serum before the clinical signs of IA appear, but its sensitivity and specificity are variable. False-positivity is a significant disadvantage, and consecutive positive GM must be taken into account in the case of clinical and imaging findings that are relevant to IA.

1. Introduction

Invasive fungal infections (IFIs), especially those due to *Aspergillus spp*, are increasing in children with hematological malignancies, and despite the introduction of new antifungal agents, this increase is associated with high morbidity and mortality rates.[1] The early diagnosis and treatment of invasive aspergillosis (IA) is critical to improving patient outcomes. The mortality from IA is in the range of 40–90%, and this is affected by the timing of initiation of therapy.[2,3] A mortality rate of 90% has been reported for patients with pulmonary aspergillosis first treated with antifungal agents more than 10 days after the onset of pneumonia .[4]

Difficulties in the early diagnosis of IA result in delays in the initiation of antifungal therapy. Due to the non-specific clinical symptoms, low sensitivity, long wait for results, difficulty in obtaining positive blood cultures, and lack of appropriate conditions (uncorrected thrombocytopenia, coagulopathies, etc.) for procedures like biopsy for tissue culture or bronchoscopy (bronchoalveolar lavage (BAL)), the early diagnosis of IA in pediatric hematological malignancies is very difficult. Computed tomography (CT) images may show lesions that are compatible with IA (nodular or cavitary lesions, halo sign), but the incidence of these findings is low in pediatric patients. Thus, the use of non-culture methods such as the galactomannan (GM) assay, 1–3-beta-D-glucan test, and fungal DNA via PCR-based assays has become more important in the early diagnosis of IA.[5]

* Corresponding author. Tel.: +90 2323901113.
E-mail address: dyilmazk@yahoo.com (D.Y. Karapinar).

http://dx.doi.org/10.1016/j.ijid.2016.10.027

GM is a component (polysaccharide) of the *Aspergillus spp* cell wall. It is released during cell growth and can be measured in serum using an enzyme immunoassay (EIA). GM testing allows the early diagnosis of aspergillosis and the prompt initiation of antifungal therapy,[6] which are criteria for the treatment of IFI defined by the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG).[7] Many studies on adult populations have evaluated the utility, sensitivity, and specificity of GM for the early diagnosis of IA, but data on serum GM testing in children are limited. In addition, most pediatric studies that have been conducted have focused on patients diagnosed with high-risk hematological/oncological malignancies or acute myeloid leukemia (AML), or patients undergoing hematopoietic stem cell transplantation (HSCT).

The aim of this retrospective study was to investigate the effect of a positive serum Aspergillus GM antigen assay on the management of IA. It was also sought to determine its utility for the diagnosis of IA in children with acute lymphoblastic leukemia (ALL).

2. Methods

Data on pediatric patients with ALL during the period 2006–2015 were analyzed retrospectively. GM testing was performed twice a week during the period of neutropenia for all ALL patients. The demographic and clinical characteristics of the patients were evaluated for IFI. The total number of GM serum tests performed, number of positive and negative test results, and number of consecutive positive tests were analyzed. The effect of positive test results on the management of patients and how they affected clinician decision-making, imaging rates, and therapeutic strategies were investigated. Antifungal therapies given for IA despite negative test results were also recorded. Patients with at least one positive GM assay were investigated in detail for the presence of clinical findings (prolonged or unexplained fever, sinus tenderness, lower respiratory tract infection, cough, sputum, respiratory distress, hepatosplenomegaly), laboratory findings (neutropenia, microbiological results, direct examination for hyphae or cultures), and imaging findings (new pulmonary infiltrates while receiving broad-spectrum antibiotics, consolidation, 'halo' sign, 'air crescent' sign, cavitation) suggestive of IFI.

The diagnosis of possible, probable, and proven IFI was determined using the EORTC/MSG criteria.[7] Thus, IA was considered 'proven' in the presence of positive microbiology (a positive microscopy, culture) or histopathology from a sterile site. 'Probable' IA was defined by the presence of typical clinical and/or radiological findings with mycological criteria. The mycological criteria include microscopy, culture (sputum or BAL), and positive GM antigenemia (two or more consecutive positive serum results of $\geq$0.5, or a positive BAL GM result of $\geq$0.5). IA was considered 'possible' in cases with the appropriate host factors and with sufficient clinical evidence consistent with IFI but for which there was no mycological support.

2.1. Positive galactomannan antigenemia

GM test results with an optical density index (ODI) of $\geq$0.5 are considered positive at the study hospital. Thus, episodes of positive GM antigenemia were defined as at least one ODI of $\geq$0.5 in this study. Consecutive positivity was defined as two or more consecutive test results of $\geq$0.5. Test results were evaluated in terms of true-positives and false-positives. True-positive antigenemia was defined as a positive GM test with the diagnosis of proven or probable IA. GM antigenemia was considered to be false-positive in the absence of the criteria suggestive of proven or probable IA. These criteria are explained as follows: no specific radiographic abnormalities on CT, or no specific clinical symptoms so CT was not performed. GM antigenemia was considered to be 'unclear' if non-specific abnormalities were determined on CT during antifungal therapy.

2.2. Impact on management

A positive GM was considered to provide a clinical effect in the following cases: if a CT scan was performed after the positive test result and/or if a positive test result led clinicians to start, add, or change antifungal therapy.

2.3. Statistical analysis

The data analysis was performed using IBM SPSS Statistics version 20.0 software (IBM Corp., Armonk, NY, USA). Dependence with each of the variables was analyzed by Chi-square test.

3. Results

3.1. Patient characteristics

A total of 141 patients were included in the study. Sixty-one were female and 80 were male. The median age was 55 months (range 3–208 months). One hundred and twenty-three of the patients (87.2%) had pre-B-cell ALL, 15 (10.6%) had T-cell ALL, and three (2.1%) had biphenotypic ALL. Fifty-six of the patients (39.7%) were classified as standard risk, 38 (26.9%) as medium risk, and 47 (33.3%) as high risk. Furthermore, t(9;22) translocation was positive in 6.3% of ALL cases. A poor prednisolone response at day 8 was seen in 43 patients (30.5%) (peripheral blood smear revealed $>1000/mm^3$ lymphoblasts). Bone marrow examination revealed <25% lymphoblasts in 113 (80.1%) and <5% lymphoblasts in 35 (24.8%) of the patients at day 15. Under 5% lymphoblasts in bone marrow at day 33 was found in 128 of the patients (90.7%), and remission was achieved. Relapse occurred in eight patients (5.6%) (the characteristics of the patients with ALL are summarized in Tables 1 and 2).

3.2. GM results

A total of 3264 serum samples from the 141 patients were analyzed. The median number of GM tests performed for each patient was 55 (range 0–84). One hundred and seventy-nine (5.5%) serum samples from 76 patients with an ODI of $\geq$0.5 were considered to represent positive GM antigenemia. The number of negative tests was 3085 (94.5%) from 65 patients.

Table 1
Characteristics of the patients with acute lymphoblastic leukemia

	Patients (N = 141), n (%)
Age	
Median	55 months
Range	3–208 months
Sex	
Male	80 (56.8%)
Female	61 (43.3)
Type of ALL	
Pre-B-cell ALL	123 (87.2%)
T-cell ALL	15 (10.6%)
Biphenotypic	3 (2.1%)
Risk	
Standard risk	56 (39.7%)
Medium risk	38 (26.9%)
High risk	47 (33.3)

ALL, acute lymphoblastic leukemia.

Table 2
Comparison of the characteristics of patients with positive and negative galactomannan antigenemia

	GM-positive ($n=76$), n (%)	GM-negative ($n=65$), n (%)	p-Value
Risk classification			NS
High risk	30 (39.4%)	17 (26.1%)	
Medium risk	12 (15.7%)	26 (40%)	
Standard risk	34 (44.7%)	22 (33.9%)	
Initial WBC count			NS
$<1.5\times10^9$/l	15 (19.7%)	12 (18.4%)	
$>20.0\times10^9$/l	23 (30.2%)	32 (49.2%)	
$>100.0\times10^9$/l	9 (11.8%)	9 (13.9)	
Peripheral blood smear at day 8, lymphoblasts			0.011
$<1.0\times10^9$/l			
$>1.0\times10^9$/l	58 (76.3%)	55 (84.6%)	
	18 (23.7%)	10 (15.4%)	
Bone marrow examination at day 15, lymphoblasts			0.008
<5%			
<25%	15 (19.7%)	20 (30.7%)	
>25%	59 (77.6%)	54 (83%)	
	17 (22.3%)	11 (16.9%)	
Bone marrow examination at day 33, lymphoblasts			0.007
<5%			
>5%	67 (88.1%)	61 (93.8%)	
	9 (11.9%)	4 (6.2%)	
t(9;22) positive	6 (7.8%)	3 (4.6%)	NS
Relapse	6 (7.8%)	2 (3%)	NS
HSCT	8 (10.5%)	4 (6.1%)	0.013

GM, galactomannan; NS, non-significant; WBC, white blood cell; HSCT, hematopoietic stem cell transplantation.

3.3. Positive GM results

The data of 76 patients who had at least one positive GM with an ODI of ≥0.5 were analyzed in detail. Positive serum GM results were observed only once in 22 patients (28.9%), twice in 16 patients (21.1%), and three times in 15 patients (19.7%). Twenty-three patients (30.3%) had consecutive positive GM antigenemia; 16 (21.1%) had at least one consecutive positivity, while seven (9.2%) had two or more consecutive positive results.

Among the 23 patients with at least one consecutive positive GM antigenemia, 21.7% were true-positive. The true-positive rate was 12.5% when there was one consecutive positive and 42.9% when there were two or more consecutive positives. An increase in the probability of detection of true positivity was found in the case of multiple consecutive positives ($p = 0.001$).

The false-positive rate was 52.1%. Six of the patients (26.1%) were classified as 'undetermined'; patients who had non-specific radiographic abnormalities on CT scans and/or a diagnosis of IA could not be excluded because of ongoing antifungal therapy with anti-mold agents.

3.4. Clinical and radiological findings

Febrile neutropenia was treated empirically with broad-spectrum antibiotics (piperacillin–tazobactam or meropenem with amikacin); in the case of hemodynamic instability, a glycopeptide was added empirically. If the fever persisted more than 72–96 h, the patient was evaluated for IFI. A thorax CT was performed in addition to sinus CT if there was a clinical suspicion of fungal sinusitis, and an antifungal agent was initiated empirically.

In the GM-positive group, clinical findings were not consistent with fungal pneumonia in 69 patients (90.8%), while seven of the patients (9.2%) were considered to have clinical signs suggestive of fungal pneumonia.

With positive GM antigenemia, the clinicians performed thorax/sinus CT for 32 patients (42.1%), while CT was not performed for 44 patients (57.9%) because of a positive GM assay (in these 44 patients, CT had already been performed recently).

Thorax CT revealed findings compatible with IA in 12 (15.8%) of the 76 patients with positive GM antigenemia. There were no significant findings consistent with IA on CT scans in 64 patients (84.2%).

A statistically significant relationship between the compliance of clinical signs and CT findings in the diagnosis of fungal pneumonia was observed ($p < 0.010$). CT findings were compatible with IA in 12 patients, while they were incompatible with IA in 64 patients. Clinical symptoms of seven patients were consistent with IA, while they were found to be incompatible in 69 cases. In addition, 95.3% of the patients with normal CT findings had no clinical symptoms compatible with IA; in contrast, only 4.7% of them had specific clinical symptoms of IA.

There was a statistically significant relationship between CT findings and true-positive GM antigenemia for IA ($p < 0.001$). CT findings were found to be consistent with IA in all five patients who had true-positive GM antigenemia.

The patients with CT findings of IA were those for whom clinicians decided to perform CT because of the positive GM antigenemia. There was a statistically significant relationship between the CT decision due to GM positivity and CT findings suggestive of IA ($p < 0.001$). This study revealed that clinicians performed CT without waiting for the test results for all febrile neutropenia episodes with prolonged fever.

3.5. Antifungal therapy

When a positive serum GM test was detected, 35 (46.1%) of the patients were already on antifungal therapy: 15 (19.7%) were receiving caspofungin, 11 (14.5%) were receiving liposomal amphotericin B, six (7.8%) were receiving voriconazole, and three (3.9%) were receiving combination therapy. The detection of positive GM antigenemia led physicians to initiate antifungal therapy in six patients (7.9%), to add a second antifungal agent in another six patients (7.9%), and to change antifungal therapy in only one patient (1.3%). No changes were made in the treatment of 63 patients (82.9%) with positive serum GM antigenemia. In addition, clinicians started antifungal therapy in 56 of 65 patients despite a negative serum GM test result.

An increase in the number of positive GM tests was found to increase the rate of initiation of antifungal therapy. There was a statistically significant correlation between these two variables ($p = 0.004$). The ratio of starting antifungal therapy was 13.6% in the case of one GM positivity detected, while it increased to 28.6% with two or more consecutive positive tests.

3.6. Effect on management

A clinical effect was observed in 33 patients (43.4%). Positive GM antigenemia resulted in a CT scan in 21 of these patients (27.6%). CT and changes in the treatment were performed for 10 patients (13.2%); only a change in the treatment was made in one of the patients (1.3%).

4. Discussion

The aim of this retrospective study was to evaluate the results of GM testing in children with ALL and the effect of a positive serum GM antigenemia result on the management of the condition. GM testing was performed twice a week during the period of neutropenia. The results showed that the number of serum GM tests performed per patient was very high, while consecutive positive results were detected in only a few patients . It was observed that some patients who did not have a positive test had more than 50 serum samples analyzed for GM antigenemia . Despite so many tests being performed, a clinical effect due to positive GM antigenemia was found in less than half of the patients (43.4%). It was found that clinicians performed CT in all patients for whom there was a clinical suspicion of IA before the test results were returned, and clinical findings were more important than GM positivity for patient management. Because it takes time to obtain GM results (average of 2 days for a single result, 5 days for consecutive results), clinicians tend to perform CT scans for patients with significant clinical signs or administer antifungal therapy without previous CT. This explains why clinicians did not perform a CT scan for 57.9% of the patients, and there was no change in the treatment of 82.9% of the patients resulting from a positive GM antigenemia.

GM may be detected in the serum before clinical signs of IA appear, but its sensitivity and specificity are variable. The sensitivity of GM testing varies in the range of 30–100%, while specificity has been reported to be >75%.[8] Serial GM testing is recommended both for early diagnosis and the follow-up of patients with IA as a prognostic marker to evaluate the course of the disease and the response to antifungal treatment.[9] Although the GM assay is frequently used to help clinicians in the early diagnosis of IA, a disadvantage of GM testing is the likelihood of false-positive results. Factors that are known to cause false-positive results are as follows: cross-reactivity of the GM assay with a number of filamentous fungi including the species *Fusarium*, *Penicillium*, *Cladosporium*, *Histoplasma*, *Blastomyces*, *Paracoccidioides*, *Cryptococcus*, *Nigrospora*, *Paecilomyces*, *Trichothecium*, *Lichtheimia ramosa*, and *Geotrichum*; treatment with piperacillin–tazobactam, amoxicillin–clavulanate, or cyclophosphamide; the presence of GM in some food, drink, enteral nutrition products, and intravenous solutions (Plasma-Lyte). In patients undergoing cytotoxic chemotherapy or who have graft-versus-host disease (GVHD), gastrointestinal translocation of fungal GM from food may explain false-positive GM antigenemia.[10] In neonates and infants, immaturity of the intestinal mucosa may lead to the translocation of lipoglycans of Bifidobacterium, resulting in false-positive GM antigenemia.[11,12] Greater false-positive results are reported within the first 2 to 4 weeks of cytotoxic chemotherapy in hematological malignancy and HSCT patients.[13]

False-positive GM antigenemia was higher than expected (52.1%) in this study. This may be due to several factors: (1) serum GM tests were studied twice a week, but the index values were not always reported. The laboratory reported results only as 'positive' or 'negative' (GM with an ODI of ≥0.5 was reported as positive). Consecutive positive tests with an ODI of ≥0.5 were deemed to represent positive GM antigenemia, while a single result with an ODI of ≥0.7 could not be determined and accepted as positive GM antigenemia. Some of the single positive results may have been ≥0.7 and should have been in the true-positive group. (2) All of the patients were treated with piperacillin–tazobactam for febrile neutropenia. (3) Foods, drinks, or enteral nutrition products and gastrointestinal mucosal damage due to cytotoxic chemotherapy may have contributed to false-positive GM antigenemia.

It is important to know the false-positivity rate of the GM assay, especially in serum samples, because of the difficulties with other diagnostic methods, such as BAL or tissue biopsy. This is also important to note because GM is used as a microbiological criterion of probable IA according to the EORTC/MSG consensus group. Higher false-positivity rates in pediatric cancer patients than in adult patients have been reported in the literature.[14] One combined adult–pediatric study showed false-positive antigenemia in 10.1% of pediatric patients and only 2.5% of adult patients, while another study found false-positive results in 44% of pediatric patients and only 0.9% of adult patients.[15] Nevertheless, several pediatric studies have shown that the false-positive rate for GM testing is lower than estimated. Hayden et al. reported 12.8% false-positive GM results in 56 pediatric oncology patients from 990 serum samples using an EIA GM index value of ≥0.5 for positive GM antigenemia.[15] The false-positive rate of GM in 70 patients undergoing allogeneic HSCT or treatment for acute GVHD after allogeneic HSCT was reported to be 12.7% by Steinbach et al., including only one patient with proven or probable IA.[13] Lower false-positive rates of GM in pediatric patients were also reported in patients with AML or allogeneic HSCT, including a few patients with proven/probable IA.[16,17] In a study of pediatric hematology patients (leukemia, solid tumor, HSCT) comparing serum and urine samples in terms of GM positivity, the false-positive rate of serum samples was 5.2%, while that of urine samples was 20.2%.[18] Various clinical and laboratory studies have shown that the simultaneous use of antifungal agents reduces serum GM levels.[19,20] The impact of mold-active antifungal prophylaxis and empirical or pre-emptive therapy in patients who are considered to be at the highest risk of developing IA can explain the low rates of proven/probable IA and the predominance of negative GM results. Similar to the present research, a study on adults showed that among the positive GM results of patients undergoing allogeneic HSCT, 65.5% were defined as false-positive, 15.5% as true-positive, and 19% as inconclusive.[21] High false-positive results were considered to be mainly related to gastrointestinal mucosal damage.

Other than the groups of patients mentioned above (AML, HSCT, recurrent leukemia), data on patients with low-risk hematological diseases, which were considered in the present study, are limited. Although the retrospective nature and lack of data relating to false-negative patients are potential limitations, this study may provide guidance on how serum GM should be monitored in pediatric patients with ALL. False-positivity is a significant disadvantage. In light of the latest data, consecutive positive GM results must be taken into account when clinical and imaging findings are relevant to IA. Further validation of the serum GM assay for the early diagnosis of IA in both low- and high-risk patients is necessary and will be better demonstrated in prospective studies on pediatric patients.

Conflict of interest: None.

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