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

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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]

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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.