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Using the galactomannan antigen assay in the diagnosis of invasive aspergillosis after hematopoietic stem cell transplantation

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Abstract

Invasive aspergillosis (IA) is the most common life-threatening infections after hematopoietic stem cell transplant (HSCT). The serum galactomannan (GM) is recognized as an indirect mycological criteria for an early diagnosis of IA. Starting January 2011, we implementing in Fundeni Clinical Institute, Bucharest, for the first time in Romania, the detection of GM antigen (*Platelia Aspergillus* EIA, Bio-Rad). In 2011, patients undergoing HSCT were screened with the galactomannan ELISA; we performed a retrospective chart review of 162 SCT patients who underwent galactomannan testing. Thirteen of the patients (8.02%) had at least one positive galactomannan ELISA, and four had multiple positive tests. When calculated in reference to a proved or probable diagnosis of aspergillosis, the galactomannan ELISA had a sensitivity of 0.857 and a specificity of 0.913. The positive predictive value was 0.46, and the negative predictive value was 0.993. The *Platelia Aspergillus* galactomannan antigenemia assay may assist physicians in making an early diagnosis of IA, in correlation with clinical and radiological criteria. The test has a high sensitivity and specificity and a very good negative predictive value. We found the screening of GM ELISA to be a highly specific diagnostic tool in detecting IA manifested in patients undergoing HSCT.

Keywords: galactomannan, invasive aspergillosis, hematopoietic stem cell transplant.

Introduction

The frequency of invasive fungal infections has risen dramatically in recent years [1, 2]. Opportunistic invasive fungal infections (IFIs) are a major cause of morbidity and mortality in immunocompromised patients. The majority of IFI occur in patients with hematological malignancies (HM), particularly in patients with acute myeloid leukemia (AML) and those who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) [3–5].

The dramatically increase of IFD during the last decades is attributed to host defense impairment due to intensive cytotoxic chemotherapies, hematopoietic stem cell transplantation, ablative radiation therapy, use of corticosteroids, cyclosporine and new immunosuppressive agents [6–10].

Aspergillus spp. infections remain the most common cause of death in HM; the overall incidence of *Aspergillus* spp. ranges from 0.3% to 12% depending on the underlying hematological condition. Patients undergoing allo-HSCT appear to have two periods for the peak occurrence of invasive aspergillosis (IA). The first period is before engraftment, during the neutropenic state. The second period occurs later during the post-engraftment period, when patients are on immunosuppressive therapy or suffer for GVHD [10].

Aspergillus species are ubiquitous moulds to which humans are commonly exposed. Of approximately 180 species, it is estimated that 34 are medically significant [11]. Most persons who are exposed to the fungus remain asymptomatic. Patients who are immunocompromised, however, are susceptible to more severe invasive disease, usually marked by an acute progressive infection, often resulting in death. The prognosis of IA is grim, with a case mortality rate of 58% [12].

Although newer, less toxic antifungal agents have been developed, successful management of IA is contingent on early detection, which, unfortunately, can be difficult.

The gold standard for the diagnosis of IA is tissue biopsy demonstrating invasion on histopathological examination and identification of the organism in culture. However, obtaining tissue specimens for the diagnosis of IA is often difficult because patients in whom IA is suspected often have medical conditions, such as thrombocytopenia that preclude biopsy.

A recent diagnostic modality for IA is the galactomannan (GM) assay. GM is part of the outer layer of the

Aspergillus cell wall, and is released during growth of the fungus at the tips of hyphae [13].

GM is a cell wall component of many fungi, including *Aspergillus*, *Penicillium*, *Paecilomyces*, and *Geotrichum* species. The antigen can be detected using a commercially available sandwich ELISA (*Platelia Aspergillus*, BioRad, France) (PA-ELISA), which employs a monoclonal antibody (EB-A2) that binds galactofuran epitope of the GM antigen [14].

Patients and Methods

In 2011, all patients with HSCT at the Fundeni Clinical Institute, Bucharest, were screened with serum GM ELISAs while hospitalized. We performed all assays on peripheral blood serum, as per standard protocols.

Methodology

The assay is a sandwich enzyme immunoassay using rat monoclonal antibodies to *Aspergillus fumigatus*. This antibody was produced by immunizing rats with a mycelial extracts of *A. fumigatus*, and it recognizes a galactomannan epitope that contains β(1→5)-linked galactofuranose. The antibody reacts with several *Aspergillus* species, including *A. fumigatus*, *A. flavus*, *A. niger*, *A. versicolor*, and *A. terres* [14].

Testing procedure

The test serum is first boiled for 3 minutes in the presence of 4% ethylenediaminetetraacetic acid (EDTA) to dissociate immune complexes and destroy interfering substances. The resultant coagulum is centrifuged at 10 000×g for 10 minutes, and the supernatant is removed and may be stored at 2–8^{0}C for up to 72 hours before testing. *Aspergillus* grows well in contaminated serum stored at 2–8^{0}C, highlighting the importance of careful specimen processing and storage.

Testing is performed by adding a peroxidase-linked detector antibody followed by 50 µL of the test specimen into the pre-coated microplate wells, then incubating at 37^{0}C for 90 minutes. Next, a tetramethyl-benzidine (TMB) chromogene substrate is added, and the plate is incubated in dark for 30 minutes at 30^{0}C; if antigen is present, then a blue color appears. The enzyme reaction is stopped by adding sulfuric acid stopping solution, which changes the color to yellow. The microplate wells are aspirated and washed between steps and read in a microplate reader at both 450 nm and 620/630 nm wavelengths after the last step.

Calculation of results

A positive control well, negative control well, and two cut-off control wells are included for quality control and calculation of antigen results, reported as an Index. The results are determined by comparison with the cut-off control. The optical density (OD) of the test specimen is divided by the mean OD of the cut-off control, and results with an index value of 0.5 or higher are considered positive [15–18].

A positive GM test result was defined as two consecutive tests with an optical density index of ≥0.5 or a single test with an optical density index of ≥0.8. The date of the first positive GM test result was considered the date of diagnosis of IA, in high-risk patients with radiological signs of IA.

Patients were retrospectively evaluated from January 1 to December 31, 2011. Data from all clinical data for diagnosing fungal infections, including microbiology cultures, cytology reports from bronchoalveolar lavage procedures, biopsy findings, and CT scans, were reviewed. The probability of having an invasive fungal infection at any time after HSCT was determined by EORTC/MSG criteria [19]. We calculated the sensitivity, specificity, negative and positive predictive values of the GM ELISA, in regards to probable or proven IA infection.

Results

GM ELISA was tested 230 times among the 162 patients with hematopoietic stem cell transplants. After a complete retrospective review of each patient's clinical course, 102 of the 162 patients had no clinical, radiographic, or microbiological criteria for IA. Of the remaining 60 patients, one had proven IA by biopsy (one patient with pulmonary aspergillosis by open lung biopsy). Six patients had a probable diagnosis of IA, and 53 had a possible diagnosis if IA. Thirteen of the 162 (8.02%) patients had at least one positive GM ELISA test. GM assays were positive in six of the seven proven or probable IA patients. We identified seven false positive GM assays, in patients with at least one positive GM assay and no proven or probable IA. Four of these false positive results were considered to be due to the use of antibiotics. One single patient was diagnosed with AI and had a negative GM results, probably due to the previous exposure of antimould prophylaxis.

Invasive aspergillosis

As shows in Table 1, the galactomannan ELISA had a sensitivity of 0.857 and a specificity of 0.913. The positive predictive value was 0.46, and the negative predictive value was 0.993.

Table 1 – ***Diagnosis validity of the GM ELISA test***

		+	-
Galactomannan ELISA	**+**	6	7
	-	1	148

Sensitivity: 6/7 (0.857); Specificity: 148/162 (0.913); PPV: 6/13 (0.46); NPV: 148/149 (0.993).

A total of 148 adult and 14 pediatric patients had at least two GM ELISA tested while undergoing HSCT in 2011. These patients ranged in age from one to 68.1 years (mean 32.4 years). Ninety-six of the recipients were male (59.9%). With one exception, all the tested patients received peripheral hematopoietic stem cells. The source of hematopoietic stem cell grafts varied; 136 received sibling donor stem cells, 15 received unrelated donor stem cells, 11 received autologous stem cells. Three adult patients had a positive sputum direct microscopic exam for *Aspergillus* (Figure 1).

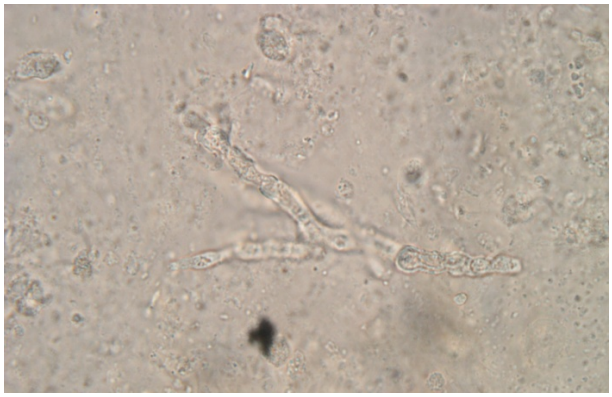

Figure 1 – *Sputum direct microscopic exam: branched septatae hyphae.*

Discussion

Minimally invasive tests for the diagnosis of aspergillosis pose several difficulties, as the coagulopathy caused by pancytopenia, in association with HSCT, can lead to unspecific biopsies with low sensitivity thresholds.

In our experience, serum GM ELISA as a screening test also provides sufficient sensitivity and specificity in detecting IA after HSCT. The test has also a very good negative predictive value -0.993. Our data may be limited by the fact that only one of 162 patients had a proved diagnosis of IA, but due to the clinical situation of the patients, more invasive diagnosis procedures were inappropriate. However, our retrospective classification of proven or probable IA in 8.02% of patients is consistent with the incidence reported in other HSCT population studies [20].

Within the past decade detection of the *Aspergillus* antigen galactomannan has become an important and reliable tool for the early diagnosis of invasive aspergillosis. The test has a high sensitivity and specificity, but it not replaces careful microbiological and clinical evaluation. There is now standardized a word-wide cut-off 0.5 and both false negative and false positive reactivity is encountered.

There are many false-positive test results reported with the GM assay. Because produces GM, it is not surprising that a number of β-lactam antibiotics, including *Penicillium*, piperacillin/tazobactam, amoxicillin/clavulanate, ampicillin have yielded positive *Platelia* EIA results. False positive results may occur more frequently in children and it was suggested that GM present in milk or protein-rich nutrients is the cause of false-positive results in children. The false-positive rate was reported to be high in up to 83% of newborn babies [13]. Other factors related with false positive results are: infection with organisms that share cross-reacting antigens with *Aspergillus*, reduced renal clearance, patients undergoing liver transplantation for autoimmune liver disease, patients undergoing cyclo-phosphamide treatment and patients undergoing lung transplantation for cystic fibrosis and chronic obstructive pulmonary disease.

The main reasons for a false-negative result are exposure to antifungal agents and high cut-off values, but there are also others reasons for false negative tests: inappropriate diagnostic criteria for IA, inadequate frequency of galactomannan testing, patients with non- or minimally invasive manifestation of aspergillosis, low volume of sampling or long-term storage of samples.

Antigen has been detected in body fluids other than serum: broncho-alveolar lavage fluid (BAL) or central nervous system (CNS). Although these specimens are not standardized yet, they may be superior to serum for testing in certain circumstances.

It is recognized now that GM detection facilitates early diagnosis of IA and can precede CT findings by one week or more. Twice-weekly antigenemia monitoring is very useful for detecting IA in high risk patients, inclusive allogeneic stem cell recipients, but testing GM antigenemia can be use also for monitoring the effectiveness of the therapy.

Conclusions

A positive serum GM for a probable diagnosis of invasive fungal infection may be appropriate and more clinically useful and allow initiation of preemptive antifungal therapy. It also may allow earlier initiation of effective treatment, which is especially important in very-high-risk populations like HSCT recipients.

Our findings indicate that screening with *Platelia Aspergillus* galactomannan antigenemia assay may assist physicians in making an early diagnosis of IA, in correlation with clinical and radiological criteria and represent a highly specific diagnostic tool for detecting invasive aspergillosis in patients undergoing HSCT.

Acknowledgments

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