1. Wheat3003 (truncated)

Review article

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# Rapid diagnosis of invasive aspergillosis by antigen detection

**Key words:**
aspergillosis; diagnosis; immunoassay; PCR; antigen detection

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**Abstract:** Aspergillosis is a serious and often fatal infection in the bone marrow or organ transplant patient, for which improved methods of diagnosis are desperately needed. Currently, the diagnosis is most often made based on clinical findings and radiographic findings, which are nonspecific, and toxic therapies are initiated empirically, often without ever establishing the diagnosis. Without a definitive diagnosis, physicians often withhold or reduce the doses of the antifungal agent when toxicity develops or the patient improves, permitting progression of disease in those with invasive aspergillosis. The Platelia *Aspergillus* galactomannan antigenemia assay may assist physicians in making these decisions. With a sensitivity of 81% and a specificity of 89% in the studies leading to its FDA clearance, physicians still must be aware of the potential for false-positive and false-negative results; the test does not replace careful microbiological and clinical evaluation. This report will review the relevant literature and provide guidelines for use of the test in patient management.

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Aspergillosis continues to be a serious and common opportunistic infection in immunocompromised subjects. Invasive aspergillosis (IA) occurs in 5–20% of individuals who undergo allogeneic stem cell transplantation, while a lower proportion occurs in solid organ allograft recipients (1–4). Furthermore, mortality remains high, above 50% in most studies (1). While the effect of the serious underlying disease has a profound impact on outcomes, delayed diagnosis contributes to mortality.

Diagnosis of IA may be difficult (5). Although air-crescent and halo signs seen on radiographs or computed tomography (CT) scans suggest IA (6), they are neither specific nor sensitive, and often are not correctly identified (7, 8). Often, definitive diagnosis by biopsy is not feasible because of coagulation abnormalities. Bronchoscopy to obtain specimens for cytology or culture may be possible in such patients, but the sensitivity for diagnosis is only about 25% (9). More often, antifungal therapy is initiated empirically, and the diagnosis is not proven.

Today, empiric therapies may include voriconazole (8) or caspofungin (10, 11), which, while active against *Aspergillus*, may not be effective

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against other molds, an emerging problem following bone marrow (12) or solid organ transplantation (13). The availability of rapid, specific, and non-invasive diagnostic tests could be highly useful in such cases, permitting earlier initiation of effective treatment. Furthermore, negative tests for aspergillosis may alert the clinician to modify empiric therapy to include agents that are active against other molds, or to pursue additional diagnostic procedures.

Antigen detection for diagnosis of IA was first reported in the late 1970s, and was made a reality by the production of monoclonal antibodies (14) and creation of a standardized and reproducible assay in the early 1990s (15). Available in Europe for over 5 years, the Platelia® *Aspergillus* antigen immunoassay, produced by Bio-Rad Laboratories (Hercules, CA, USA), was cleared by the Food and Drug Administration (FDA) for diagnostic use in the US in May 2003. This review will focus on the clinical uses of the new *Aspergillus* antigen assay, as well as its limitations.

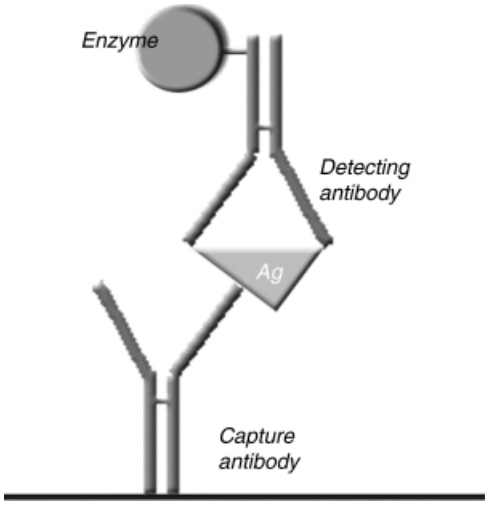


***Fig. 1.*** Schematic describing the steps of the assay. The test uses microplates that are pre-coated with the captured antibody. Next, the detector antibody and test serum are added in that order, and the plates are incubated at 37°C for 90 min. The plates are aspirated and rinsed, and then TMB chromogen substrate is added, and the plate is incubated in the dark for 30 min at 30°C. After the reaction is stopped with $H_2SO_4$, wells that contain antigen become yellow, and the color is recorded using a microplate reader.

## 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 extract of *A. fumigatus*, and it recognizes a galactomannan epitope that contains β (1→5)-linked galactofuranose (14). The antibody reacts with several *Aspergillus* species, including *A. fumigatus, A. flavus, A. niger, A. versicolor,* and *A. terres* (14); and with exoantigens from several other molds: *Penicillium digitatum, Trichophyton rubrum* and *interdigitalis, Botrytis tulipae, Wellemia sebi,* and *Cladosporium cladosporioides* (14); *Cladosporium herbarum, Acremonium spp, Alternaria alternata, Fusarium oxysporum, Wangiella dermatitidis*, and *Rhodotorula rubra* (16); *Paecillomyces variotii* and *Penicillium chrysogenum* (17).

### Testing procedure

The test serum is first boiled for 3 min 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 min, and the supernatant is removed and may be stored at 2–8°C for up to 72 h before testing. *Aspergillus* grows well in contaminated serum stored at 2–8°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°C for 90 min (Fig. 1). Next, a tetramethylbenzidine (TMB) chromogen substrate is added, and the plate is incubated in the dark for 30 min at 30°C; if antigen is present, then a blue color appears. The enzyme reaction is stopped by adding $H_2SO_4$ 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 and 620/630 nm wavelengths after the last step.

### Calculation of result

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 to be positive. In some reports, several specimens containing known amounts of galactomannan were included to permit more exact quantitation. However, the assay is not linear at ODs above 2.0 and may not accurately reflect galactomannan concentration in such specimens.

## Sensitivity

The sensitivity of the Bio-Rad Platelia® *Aspergillus* EIA was 80.7% in the studies used for FDA approval in the US. In other studies, the sensitivity has ranged from less than 50% to over 90%. A few large recent

Rebound antigenemia after treatment was stopped suggests relapsing infection and the need for resumption of therapy.

## Comparison of *Aspergillus* EIA and PCR

While this is an area of intense investigation that dates back to the early 1990s, a clinically applicable and reliable method has yet to be identified and the findings are inconsistent between studies. Focusing on studies comparing polymerase-chain reaction (PCR) to the Platelia® *Aspergillus* EIA, Kami et al. (34) reported a sensitivity of 79% by PCR compared with 58% by EIA. Specificity was 92% by PCR and 97% by EIA (34). Bretagne et al. (26) noted a lower sensitivity for PCR than antigenemia, 50% vs. 77.8%, respectively. In another report using a rat model of IA, antigenemia testing was superior to PCR (55). Kawamura et al. (29), however, noted lower sensitivity for antigenemia than PCR.

PCR methods for the detection of *Aspergillus* DNA in specimens other than blood have also been described. *Aspergillus* DNA has been detected in BAL specimens, but comparison with antigenemia testing remains incomplete (47, 56–58). Kami et al. (53) reported the detection of *Aspergillus* DNA in the CSF of 5 patients with CNS aspergillosis, 4 of whom also had elevated levels of antigen by EIA. Spiess et al. (59) evaluated a real-time PCR assay on BAL and blood from patients with IA and controls, noting it to be specific but less sensitive than a previously described nested PCR. However, they did not compare PCR with antigen detection (59). Sanguinetti et al. (35) recently compared real-time PCR and nested PCR to galactomannan antigen detection in BAL from patients with hematologic disorders. Using EORTC criteria, the sensitivity of galactomannan detection was 100%, compared with 90% for both real-time and nested PCR (35). The results were negative in control patients.

Research is required to define the most appropriate PCR method before molecular diagnostics can be accurately compared with antigenemia testing.

## Proposed uses and limitations

To provide accurate results, the laboratory must be proficient in performance of the assay; to use them appropriately in patient management, the clinician must be aware of the assay's limitations. The indications and guidelines for use of antigenemia testing are summarized in Table 6. The most important point is that a negative test cannot rule out the diagnosis of IA, and a positive test alone is not diagnostic of the infection. Accordingly, the tests must be used in conjunction with and not in place of other diagnostic procedures and expert clinical judgment. Also of note is the importance of confirming positive results before accepting them as diagnostic of IA. The cost of frequent antigenemia testing is a cause for concern. Will the benefits of early diagnosis and appropriate therapy justify these added costs of care (36)? Prospective studies are needed to assess the optimal, cost-effective use of the test.

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2. Moriss2008 (truncated)

Title: **Using the galactomannan assay in the diagnosis of invasive aspergillosis**
Author(s): Robert Wood-Morris and Glenn Wortmann
Source: ***The AIDS Reader.*** 18.6 (June 2008): p318.
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Aspergillus species are ubiquitous molds to which humans are commonly exposed. Of approximately 180 species, it is estimated that 34 are medically significant. (1) Most persons who come in contact with the fungus remain asymptomatic. However, some experience mild morbidity demonstrated by recurrent sinusitis, asthma exacerbations, or allergic bronchopulmonary aspergillosis.

Patients who are immunocompromised, however, are susceptible to more severe invasive disease, usually marked by an acute progressive infection, often resulting in death. A survey of 89 physicians whose experience with a combined 595 patients with proven or probable invasive aspergillosis (IA) showed that 32% of patients had undergone bone marrow transplantation, 29% had a hematological malignancy, 9% had undergone solid organ transplant or had another condition requiring immunosuppressive therapy, 9% had pulmonary disease, and 8% had AIDS. (2)

The prognosis of IA is grim, with a case mortality rate of 58%. (3) Although newer, less toxic antifungal agents have been developed, successful management of IA is contingent on early detection, which, unfortunately, can be difficult.

## DIAGNOSIS OF IA

IA is usually suspected when signs of infection refractory to broad-spectrum antibiotics develop in an immunocompromised patient. The gold standard for the diagnosis of IA is tissue biopsy demonstrating invasion on histopathological examination and identification of the organism in culture.

The finding of acute-angle branching, septated, non-pigmented hyphae on histopathological examination is not specific for IA because other molds, including Fusarium, Paecilomyces, and Pseudallescheria boydii, can have a similar appearance. For unclear reasons, growth of Aspergillus in culture occurs in only 30% to 50% of histopathologically suggestive cases. (4) 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.

## GALACTOMANNAN ANTIGEN TESTS

A recent diagnostic modality for IA is the galactomannan (GM) assay. GM is a cell wall component of many fungi, including Aspergillus, Penicillium, Paecilomyces, and Geotrichum species. An enzyme-linked immunosorbent assay-based kit is commercially available as the Platelia Aspergillus EIA (Bio-Rad Laboratories, Redmond, Wash) and was cleared by the FDA for diagnostic use in May 2003. GM antigen positivity is among the microbiological criteria proposed by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group for the diagnosis of IA.

The Platelia EIA is an immunoenzymatic sandwich microplate assay that uses monoclonal antibodies that bind to side-chain residues of the GM molecule. (5) A sandwich enzyme immunoassay format is used for detection. The performance of the assay varies according to the threshold value used.

When the Platelia EIA became available in Europe a decade ago, the manufacturer recommended an optical density (OD) index (also called a GM index) of 1.5 as the cutoff between positive and negative results. Lowering the threshold to between 0.7 and 1.5 in an effort to improve sensitivity was validated, and an analysis of 986 serum samples determined that a cutoff of 0.5 increased sensitivity with minimal loss of specificity. (6,7)

In the United States, the suggested OD index threshold is 0.5; in Europe, a cutoff of 0.7 is commonly used. (8) The overall reported sensitivity of the Platelia EIA ranges from 30% to 90%, with a reported specificity of greater than 93%.9,10

Causes of false-positive GM assay results

False-positive test results with the GM assay have been reported by a number of investigators (Table). Because Penicillium produces GM, it is not surprising that a number of [beta]-lactam antibiotics, including piperacillin/tazobactam, amoxicillin/clavulanate, ampicillin, and phenoxymethylpenicillin, have yielded positive Platelia EIA results. (11,12)

Other reported causes of false-positive results include various foods, Geotrichum capitatum (a rare cause of invasive disease), Bifidobacterium species (frequent GI colonizers), and an electrolyte solution (containing sodium gluconate produced by Aspergillus niger fermentation) used for bronchoalveolar lavage (BAL). (13-16)

GM testing on nonserum specimens

The GM assay has been validated only for serum samples. However, investigators have reported results of testing other body fluids, including BAL fluid, cerebrospinal fluid (CSF), and urine.

Because 70% of cases of IA involve the lungs, testing of BAL fluid specimens for GM seems intuitive. Use of the Platelia EIA when testing BAL fluid in 49 cases of proven or probable IA and 47 control patients revealed a sensitivity of 76% (61% to 87%) and a specificity of 94% (84% to 99%). (17) A similar study demonstrated a sensitivity of 100% when testing BAL fluid samples from 20 patients with proven or probable pulmonary IA. (18)

Data on the use of the GM assay for testing of CSF are sparse. One study reported that CSF GM values in 5 patients with probable CNS IA were significantly higher than GM values in 16 control patients. (19)

As with CSF sampling, a paucity of reports are available on the use of the GM assay for testing urine. In one study that used the Platelia EIA, only 2 of 6 patients with confirmed IA tested positive for GM in their urine. (20)

Use of serial GM testing

The use of sequential serum GM assays as a strategy to improve sensitivity and specificity has been proposed.

This approach was evaluated in a prospective study involving 88 neutropenic patients who had received chemotherapy for leukemia or myelodysplastic syndrome or myeloablative allogeneic hematopoietic stem cell transplant. (21) Surveillance tests using Platelia EIA were performed 3 times a week, and treatment with amphotericin B was initiated only if 2 or more GM assays had positive results or chest CT findings suggested invasive fungal infection (supported by a culture or microscopic examination finding of molds). Application of these guidelines led to a 78% reduction in use of antifungals compared with use of antifungals for neutropenic fever when treatment decisions were based on standard guidelines.

Another study evaluated serial GM testing in 74 allogeneic stem cell transplant recipients who had serum GM assays performed twice weekly and who underwent chest CT if a fever of unknown focus persisted for more than 72 hours. (22) Nine patients had proven or possible IA. The GM test demonstrated a sensitivity of 100% and specificity of 93%, and the sensitivity of chest CT was comparable (100% had abnormal findings). Only 2 of the 9 patients died as a result of IA, leading to the conclusion that Aspergillus GM surveillance and early chest CT should be considered to detect IA in its initial stages.

## CONCLUSION

The timely diagnosis of IA is problematic, and its delayed diagnosis is associated with high mortality rates. The GM assay offers the potential of securing a diagnosis through relatively noninvasive means. Clinicians should consider using this test when they suspect IA in immunocompromised patients. The test may prove especially helpful in patients in whom invasive tissue biopsy is contraindicated. There are published shortcomings of the GM test, and questions remain about GM detection in tissue and fluid other than serum and whether surveillance sampling in at-risk populations can both decrease use of empiric antifungal therapy and improve survival.

Key words: Aspergillus * Aspergillosis * Galactomannan * Platelia enzyme immunoassay

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3. Foy2007 (truncated)

# Galactomannan Antigen Enzyme-Linked Immunosorbent Assay for Diagnosis of Invasive Aspergillosis after Hematopoietic Stem Cell Transplantation

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## ABSTRACT

**Invasive aspergillosis is difficult to diagnose in patients undergoing hematopoietic stem cell transplantation (HSCT). In 2003, a serum enzyme-linked immunosorbent assay (ELISA) test for the detection of galactomannan (a glycoprotein found on the *Aspergillus* cell wall) became available in the United States. In 2004, patients undergoing HSCT were screened biweekly with the galactomannan ELISA at our institution. We performed a retrospective chart review of 121 SCT patients who underwent galactomannan testing. Thirteen of the patients (10.7%) had at least 1 positive galactomannan ELISA, and 4 had multiple positive tests. When calculated in reference to a proved or probable diagnosis of aspergillosis, the galactomannan ELISA had a sensitivity of 0.50 and a specificity of 0.94. The positive predictive value was 0.46, and the negative predictive value was 0.94. Galactomannan ELISA had fewer false-positive and false-negative results in pediatric patients than in adult patients. In 4 of the 12 cases of invasive aspergillosis, the galactomannan ELISA was positive before other microbiologic evidence of aspergillosis was available. In these cases, a positive ELISA predated culture and cytologic evidence of invasive aspergillosis by a mean of 5 days (range, 1-8 days). Our findings indicate that a biweekly serum galactomannan ELISA is a highly specific diagnostic tool for detecting invasive aspergillosis in patients undergoing HSCT. When used regularly, the ELISA may allow for earlier diagnosis of invasive aspergillosis in some patients. The test is troubled by a low sensitivity and high frequency of false-negative tests.**



## KEY WORDS

*Aspergillus* galactomannan antigen • Enzyme-linked immunosorbent assay • Hematopoietic stem cell transplantation • Invasive aspergillosis • Pulmonary nodules

## INTRODUCTION

Invasive aspergillosis (IA) is a frequent and devastating complication in patients who have undergone hematopoietic stem cell transplantation (HSCT). The diagnosis of IA remains a clinical challenge. Blood, sputum, and bronchoalveolar lavage cultures have low specificity and sensitivity in detecting IA [1,2]. Radiographic findings on computed tomography (CT) of the chest are also frequently nonspecific [3,4].

In May 2003, the US Food and Drug Administration (FDA) approved a serum enzyme-linked immunosorbent assay (ELISA) test to detect the aspergillus cell wall glycoprotein known as galactomannan (GM). Similar antigen ELISAs for GM have been available in Europe since 1996 [5]. In 2001, the European Organization for the Research and Treatment of Cancer (EORTC)/National Institute of Allergy and Infectious Diseases (NIAID) consortium included a positive serum GM ELISA as part of its definition of clinical opportunistic fungal infection in HSCT and cancer patients [6]. Initial reports suggested specificity ranging from 81% to 94% and sensitivity ranging from 64% to 96% [7-9]. More recent observations suggest a high rate of false-positive tests associated with beta-lactam antibiotics, particularly piperacillin-tazobactam [10-16].

The aims of the present study were to better define the positive and negative predictive value of qualitative GM ELISA and to assess whether screening could lead to earlier diagnosis of IA in HSCT patients.

## PATIENTS AND METHODS

In 2004, all patients undergoing HSCT at the University of Minnesota Medical Center were screened with biweekly serum GM ELISAs while hospitalized. Additional testing of GM on HSCT patients outside the hospital was done as determined by clinical providers. Assays were performed on peripheral blood serum in accordance with the manufacturer's specifications (Bio-Rad, Redmond, WA). The tests were performed on Tuesdays and Fridays in a clinical hospital laboratory. Control samples were included in each assay batch to validate performance of the ELISA.

GM ELISA was reported as either positive or negative, with a cutoff of 0.5 ng/mL. Patients were retrospectively evaluated through June 30, 2005, to allow a minimum of 6 months to a maximum of 18 months follow-up. Prospectively collected data from the University of Minnesota Blood and Marrow Transplant Database, as well as 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/NIAID criteria at the end of the follow-up period. A patient was classified as proved IA if he or she had a sterilely obtained specimen that was culture-positive for aspergillus; as probable IA if he or she had microbiologic evidence of aspergillus infection from other specimens, including sputum, bronchoalveolar lavage, and skin biopsy; as possible IA if he or she had CT evidence of pulmonary infiltrates in the setting of high clinical suspicion for fungal infection (neutropenia, fever, or graft-versus-host disease [GVHD]); and as no risk of IA if he or she failed to meet other criteria. These probabilities were determined both with and without GM testing in each patient.

Review of GM-positive cases allowed for determination of true and false-positive GM assays. Sensitivity, specificity, and positive and negative predictive values of the GM ELISA were calculated based on the clinical diagnosis of probable or proven IA infection. Secondary analysis of adult and pediatric populations was obtained with a cutoff of age 18 years at the time of transplantation. In addition, the lead time from positive GM assay to proven or probable invasive fungal infection was calculated.

A total of 71 adult and 50 pediatric patients had at least 2 GM ELISAs tested while undergoing HSCT in 2004. These patients ranged in age from 0.4 to 68.1 years (mean, 29.5 years) at the time of transplantation. Median age for the tested patients was 25.3 years; 69 of the recipients were male (57%). The source of hematopoietic stem cell grafts varied; 52 patients received 1 or more units of umbilical cord blood, 39 received sibling donor stem cells, 15 received unrelated donor stem cells, 11 received autologous stem cells, and 1 received allogeneic natural killer cells. Three patients underwent 2 consecutive (tandem) courses of myeloablative therapy (including total body irradiation) with autologous peripheral blood HSCT.

## RESULTS

GM ELISA was tested 1523 times among the 121 patients, for an average of 12.6 tests per patient (range, 2-53 tests). After a complete retrospective review of each patient's clinical course, 28 of the 121 patients had no clinical, radiographic, or microbiological criteria for IA. Of the remaining 93 patients, 2 had proven IA by biopsy (1 patient with pulmonary aspergillosis by open lung biopsy and 1 patient with cerebral aspergillosis by brain biopsy). Ten patients had a probable diagnosis of IA, and 81 had a possible diagnosis if IA.

Thirteen of the 121 (10.7%) patients had at least 1 positive GM ELISA test. GM assays were positive in 6 of the 12 proven or probable IA patients. Seven patients who had at least 1 positive GM assay were determined not to have proven or probable IA (false-positive GM assays): of these, 5 had possible IA, and 2 had no IA. Four of the 12 patients with proven or probable IA met the additional EORTC/NIAID criteria for invasive fungal infection by having 2 positive serum GMs.

The use of screening GM changed EORTC/NIAID classification from possible IA to probable IA in 2 patients. Clinical diagnosis of IA including and excluding GM testing is outlined in Table 1. As shown in Figure 1, the GM ELISA had a specificity of 0.94, sensitivity of 0.46, positive predictive value of 0.46, and negative predictive value of 0.94.

**Table 1.** *Clinical Certainty of IA Infection with and without the Use of the GM ELISA*

| EORTC/NIAID Classification of Fungal Infection | Without Use of GM ELISA, n (%) | With Use of GM ELISA, n (%) |
|---|---|---|
| None | 28 (23%) | 28 (23%) |
| Possible | 81 (67%) | 79 (65%) |
| Probable | 10 (8%) | 12 (10%) |
| Proven | 2 (2%) | 2 (2%) |

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REVIEW

# Current therapeutic approaches to fungal infections in immunocompromised hematological patients

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SUMMARY

Invasive fungal infections are significant causes of morbidity and mortality in patients with hematological malignancies. Patients with acute myeloid leukemia and those who have undergone allogeneic hematopoietic stem cell transplantation are at especially high risk. Various fungal agents are responsible for this complication, but *Aspergillus* spp. and *Candida* spp. are the most frequently isolated micro-organisms; less commonly, infections could be caused by *Zygomycetes* or other rare molds or yeasts.

Several new systemically-administered antifungal agents have been approved for clinical use since 2001; these agents include liposomal amphotericin B, voriconazole, caspofungin, and posaconazole, and they represent a major advance in antifungal therapy and have improved the prognosis of patients with hematological malignancies.

This review focuses on therapeutic aspects of the management of fungal infections in hematological patients.



## Introduction

The proportion of patients with malignancies who develop invasive fungal infections (IFI) has increased dramatically worldwide over the past few decades. The majority of these infections 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).[1–3]

Because of difficulties in early and accurate diagnosis, systemic antifungals have been used for universal prophylaxis and/or on an empirical basis in high-risk populations; this use results in increased costs, toxicity, and concerns about emergent drug resistance. In the past, health providers largely relied on amphotericin B deoxycholate (d-AMB) for the target treatment of proven IFI, but recently, this treatment has been replaced with lipid formulations, new triazoles, and a novel class of echinocandins. The availability of new treatments offers an opportunity to revise traditional approaches to antifungal therapy and to perhaps improve outcomes.

## Epidemiology and risk factors

Historically, systemic *Candida* infections have been primarily responsible for IFI in HM. However, autopsy and epidemiologic studies have confirmed that over the last ten years mold infections have increased.[1–6] This change in epidemiologic trends of IFI may be related to a real increase in the incidence of molds or to the introduction of more accurate diagnostic procedures. Furthermore, particularly in HSCT patients, the widespread use of fluconazole prophylaxis could also play a role; fluconazole targets many *Candida* spp., but not *Aspergillus* spp. or other molds.[4]

*Aspergillus* spp. infections remain the most common cause of death in HM; the overall incidence of *Aspergillus* spp. ranges from 0.3% to 12.8%, depending on the underlying hematological condition.[1,3,6–9] Patients undergoing allo-HSCT appear to have two timeframes 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 graft-versus-host disease (GVHD).[5]

Yeast infections are less common than mold infections, and *Candida* is still the predominant yeast pathogen. Recently, the emergence of other opportunistic mold pathogens (e.g., *Fusarium* spp. and Zygomycetes) has been reported, while infections due to other fungal pathogens remain rare.[10–14]

In general, the patient's immune status, degree of organ damage (i.e., mucositis or GVHD), microbial exposure (i.e., colonization,

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**Table 6**
Characteristics of different approaches in the therapy of invasive fungal infections in patients with hematological malignancies and literature suggestions.

| | Description | Pro | Con | Suggested antifungal agent |
|---|---|---|---|---|
| Prophylaxis | Applicable to uninfected patients who are at risk for IFI | • Possible reduction of the incidence of IFIs<br>• Possible reduction of fungal infection-related mortality | • Potential onset of resistance<br>• Drug toxicity<br>• Drug interactions<br>• High costs | • Posaconazole<br>• Fluconazole (in allo-HSCT) |
| Empirical approach | Early treatment of occult fungal infection, when patients have clinical signs and symptoms of infection but no clearly identifiable pathogen or radiological signs | • Suggested by many international guidelines<br>• Early therapy for patients with an undetected but possible IFI, because survival seems to be affected by the early initiation of treatment | • High risk of overtreatment with potentially toxic and/or expensive drugs<br>• It is a less targeted antifungal treatment compared with diagnostic-driven strategies | • L-AmB Caspofungin |
| Pre-emptive approach | Administered in neutropenic patients with persistent fever who show image-documented pneumonia, acute sinusitis, or a positive galactomannan test | • Early detection of asymptomatic infections thanks to the intensive use of screening markers<br>• Reduction in the use of antifungal drugs compared with an empirical approach | • Need for an early and intensive diagnostic work-up<br>• Availability of serial microbiologic and radiologic tests required<br>• Expensiveness of diagnostic work-up | • Not specific indication |
| Target therapy | Administered in patients with a clear evidence of fungal infection | • Possibility to administer an antifungal treatment really effective against the pathogen<br>• Reduction in the use of antifungal drugs compared with an empirical approach | • The diagnosis can be late<br>• Frequent inapplicability of histological exams in HM (due to thrombocytopenia, hemodynamical instability, performance status, etc.) | • Voriconazole for aspergillosis<br>• Echinocandins for candidemia<br>• L-AmB for zygomycosis<br>• Voriconazole for scedosporiosis |

tality rate of this infection, a combined approach using azoles, AMB formulations, rh-GF, and GTX should be considered.

The majority of reported *Trichosporon* spp. and *Geotrichum capitatum* infections occurred in patients with HM, particularly acute leukemia.[13] The optimal therapy for trichosporonosis has yet to be identified; however, *in vitro* experiences provide encouraging evidence of the potential role of the new triazoles and of voriconazole in particular.

*Practice points*

- Effective treatment options are available for the majority of mold and yeast infections.
- Echinocandins represent the drugs with a higher efficacy against *Candida* in non-neutropenic patients. In spite of little experience in HM, they could be considered a good choice in these patients also.
- At present, voriconazole has been recognized as the gold standard among anti-*Aspergillus* treatments.
- Zygomycosis must be considered a rare, not emerging fungal infection. Treatment with L-AMB or posaconazole improves the prognosis.

## Conclusions

Over the last ten years, new antifungal agents have become available for the treatment of IFIs. These drugs, along with better supportive care and more effective diagnostic tools, have significantly reduced the mortality rate linked to fungal complications. The differences among the various therapeutic approaches and the best antifungal agent suggested for each strategy have been summarized in Table 6.

IFIs are still a significant cause of morbidity and mortality, and future efforts should focus on further improvements in diagnostic techniques, which would allow for the timely application of antifungal therapy and would reduce the use of treatments in inappropriate settings.

## Conflict of Interest

L.P. has received research grants and honoraries as speaker and consultant from Mercks, Gilead, Pfizer and Schering Plough. L.F., G.V., B.P. and M.C. reported no potential conflicts of interest.

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# Issues with galactomannan testing

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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 galactomannan molecule, that is detected by the commercial sandwich ELISA (Platelia Aspergillus, Biorad), was found not to be a single molecule, but a family of molecules that have the epitope that reacts with the monoclonal antibody. Also the cut off level is now world-wide lowered to 0.5 which will help to further standardize and compare this diagnostic tool. Despite the advantages of galactomannan detection, there are several issues that have impact on its use in clinical practice. Both false negative and false positive reactivity is encountered and although the causes of false reactivity are not fully understood, new insights have become available which help us to optimize the use of the assay. This review discusses present issues with galactomannan testing with a view to future research and management.

**Keywords** Galactomannan, diagnosis, invasive aspergillosis

## Introduction

The detection of circulating galactomannan (GM) has become an important tool in the early diagnosis of invasive aspergillosis (IA). GM is part of the outer layer of the aspergillus cell wall, and is released during growth of the fungus at the tips of the hyphae [1,2]. 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 to the galactofuran epitope of the GM antigen [3,4]. The assay has been extensively studied and is now commonly used to monitor patients at high risk for invasive aspergillosis [2,5–8]. There are several issues that hamper the use of the assay, which will be addressed in this review.

One area of controversy has recently been resolved. The PA-ELISA was originally marketed with a cut-off for positive of 1.5. There was no evidence to support this cut off level and in the past years many researchers have proposed lower cut off levels. The assay was released in the USA with a cut off of 0.5 and the producer of the assay recently decided to lower the cut off to 0.5 in all other countries based on ROC analysis of European data sets of GM monitoring in patients with hematological malignancy [9].

## The GM antigen

Unlike the name suggests, the so-called 'GM antigen' is not a single molecule but a family of molecules which are better called galactofuranose(galf)-antigens. In addition to GM, fungal glycoproteins also react with the EB-A2 antibody, including phospholipase C and phytase, which were shown to have only one terminal galactofuranose unit that was essential for binding with the EB-A2 antibody [10]. These molecules might not only show different PA-ELISA reactivity's but their expression might also be modulated by the fungal environment. A recent study showed that the release of PA-ELISA reactive antigens *in vitro* is influenced by the growth phase of the fungus [11]. Not only are reactive antigens released during early logarithmic growth, mycelial breakdown during the lytical phase results in a further increase of reactive antigens in the culture supernatant. However, the actual galf antigens that circulate in vivo in the

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## Strategy and value of GM monitoring

Regular monitoring of patients with hematological malignancy, who are neutropenic and do not receive mould-active antifungal drugs is the setting in which the PA-ELISA appears to be of most value in the management of high risk patients [7,8,15]. In other patient groups the benefit of this assay and the optimal strategy which incorporates GM detection is less well established [37]. In hematology patients, the most appropriate strategy appears to be a combination of GM monitoring and high resolution CT scan, although some investigators find no additional value of GM-monitoring [38]. This pre-emptive approach was recently found to be feasible in hematology patients [39]. Patients with IA were better identified compared with a historic control group which received empiric antifungal therapy [39]. The number of patients requiring antifungal drugs was also lower in those undergoing the pre-emptive strategy than those receiving empiric antifungal therapy; 17% vs 35%. Only one patient, who died of invasive zygomycosis, was not diagnosed using this approach [39]. Although these results are encouraging, there is at present no study that shows that GM monitoring or the pre-emptive management strategy leads to a survival benefit.

## Conclusions

GM detection remains a useful tool in the diagnosis of IA despite the current drawbacks as discussed above. Future research and improved GM detection techniques, such as described with filtration as part of the pre-treatment procedure, will certainly further contribute to early diagnosis of IA. At present monitoring of circulating GM is the only noninvasive tool with proven usefulness. However, new biological markers like (1→3)-β-D-glucan and fungal DNA might show an additional value. Management strategies should include all possible options for early diagnosis in order to increase the chance of successful treatment of patients with this disease.

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# Current Status of Nonculture Methods for Diagnosis of Invasive Fungal Infections

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## INTRODUCTION

The frequency of invasive fungal infections has risen dramatically in recent years (209, 216). Early and accurate diagnosis of these infections is important for several reasons, including timely institution of antifungal therapy (164) and to decrease the unnecessary use of toxic antifungal agents. In addition, the availability of accurate and timely diagnoses could reduce the use of empirical antifungal therapy, thereby reducing antifungal selection pressure and the emergence of antifungal resistance. Unfortunately, a major obstacle to the successful treatment of invasive fungal infections is the lack of sensitive and specific methods for the early diagnosis of invasive fungal infections. Standard approaches to the laboratory diagnosis of invasive fungal infections include (i) direct microscopic visualization for the presence of organisms in freshly obtained body fluids, (ii) histopathologic demonstration of fungi within tissue sections, and (iii) cultivation of the causative fungus and its subsequent identification. However, these approaches often are not sufficiently sensitive and/or specific to diagnose invasive fungal infections, and they sometimes require invasive procedures to obtain the necessary specimens.

This work reviews recent advances of nonculture methods for the diagnosis of invasive aspergillosis, invasive candidiasis, cryptococcosis, blastomycosis, coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, and penicilliosis. Among the nonculture methods we review, detection of a specific host antibody response is attractive because such tests can be performed rapidly and do not require invasive sampling procedures. However, presence of host antibodies does not always correlate with presence of invasive disease, especially in patients whose abilities to produce specific immunoglobulin responses may be impeded by immunosuppressive drugs and/or serious underlying diseases. Detection of macromolecular microbial antigens generally requires a relatively large microbial burden, which may limit assay sensitivity. Nonetheless, several

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*Infectious Disorders • Research Paper*

# The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study

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Background and Objectives. The aim of this study was to evaluate the incidence and outcome of invasive fungal infections (IFI) in patients with hematologic malignancies.

Design and Methods. This was a retrospective cohort study of patients admitted between 1999 and 2003 to 18 hematology wards in Italy. Each participating center provided information on all patients with newly diagnosed hematologic malignancies admitted during the survery period and on all episodes of IFI experienced by these patients.

Results. The cohort was formed of 11,802 patients with hematologic malignacies: acute leukemia (myeloid 3012, lymphoid 1173), chronic leukemia (myeloid 596, lymphoid 1104), lymphoma (Hodgkin's 844, non-Hodgkin's 3457), or multiple myeloma (1616). There were 538 proven or probable IFI (4.6%); 373 (69%) occurred in patients with acute myeloid leukemia. Over half (346/538) were caused by molds (2.9%), in most cases *Aspergillus spp*. (310/346). The 192 yeast infections (1.6%) included 175 cases of candidemia. Overall and IFI-attributable mortality rates were 2% (209/11802) and 39% (209/538), respectively. The highest IFI-attributable mortality rates were associated with zygomycosis (64%) followed by fusariosis (53%), aspergillosis (42%), and candidemia (33%).

Interpretation and Conclusions. Patients with hematologic malignancies are currently at higher risk of IFI caused by molds than by yeasts, and the incidence of IFI is highest among patients with acute myeloid leukemia. *Aspergillus spp* are still the most common pathogens, followed by *Candida spp*. Other agents are rare. The attributable mortality rate for aspergillosis has dropped from 60-70% to approximately 40%. Candidemia-related mortality remains within the 30-40% range reported in literature although the incidence has decreased.

Key words: fungal infection, aspergillus, Candida, epidemiology, hematologic malignancies.

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The percentage of patients who develop invasive fungal infections (IFI) has increased dramatically in recent decades. Most of these infections occur in patients with hematologic malignancies.[1-3] This increase 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.[3-8] *Candida spp.* have been the main cause of IFI, but recent autopsy and epidemiological findings indicate that an increasing number of infections are being caused by molds.[9-11] Most are attributed to *Aspergillus spp.*, and such infections have become a prime cause of death in patients with hematologic malignancies. During the last 20 years, other opportunistic fungal pathogens, such as *Fusarium spp.* and *Zygomycetes*, have also emorged[12-15] whereas infections caused by other fungi are still rare.[16-19] The true incidence of IFI among patients with hematologic malignancies remains obscure since data in the literature are based largely on reports from single institutions or analyses of selected subgroups of patients (e.g., those with acute leukemia or following stem cell transplantation).[7-8] The aim of the present study was to investigate current incidence and mortality rates for IFI in patients with hematologic malignancies in Italy.

## Design and Methods

This retrospective cohort study was conducted in hematology wards of tertiary care centers or university hospitals located throughout Italy, between January 1999 and December 2003. Enrollment was limited to adult patients (aged over 16 years) with newly diagnosed acute myeloid or lymphoid leukemia (AML and ALL, respectively), chronic myeloid or lymphoid leukemia (CML and CLL, respectively), Hodgkin's or non-Hodgkin's lymphoma (HL and NHL, respectively), or multiple myeloma. Seven centers contributed data only on patients with acute leukemia. Patients with other types of hematologic malignancies (e.g., myelodysplastic syn-

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MAJOR ARTICLE

# Defining Opportunistic Invasive Fungal Infections in Immunocompromised Patients with Cancer and Hematopoietic Stem Cell Transplants: An International Consensus

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**During the past several decades, there has been a steady increase in the frequency of opportunistic invasive fungal infections (IFIs) in immunocompromised patients. However, there is substantial controversy concerning optimal diagnostic criteria for these IFIs. Therefore, members of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group formed a consensus committee to develop standard definitions for IFIs for clinical research. On the basis of a review of literature and an international consensus, a set of research-oriented definitions for the IFIs most often seen and studied in immunocompromised patients with cancer is proposed. Three levels of probability are proposed: "proven," "probable," and "possible." The definitions are intended for use in the context of clinical and/or epidemiological research, not for clinical decision making.**

Opportunistic invasive fungal infections (IFIs) are a major cause of morbidity and mortality in immunocompromised patients. However, there still remains much uncertainty and controversy regarding the best methods for establishing the diagnosis of most IFIs. Practicing physicians approach this uncertainty by treating suspected cases empirically, whereas those who review cases for research purposes tend to accept only cases in which the diagnosis is certain. This disparity of approaches is particularly apparent in the conduct of clinical trials designed to show that a new drug exhibits sufficient efficacy.

These difficulties are not unique to the study of IFIs, and wide practice variations are known to exist in all areas of medicine [1, 2]. The uncertainty in disease definition is thought to be a key contributor to these variations [1]. Strategies to minimize such uncertainties have resulted in movements such as evidence-based medicine [3] and practice guidelines [4]. In studies in which there is no assurance that homogeneous populations are being evaluated, the selection of study subjects may be biased and, therefore, their findings cannot be used to make generalizations about cause, epidemiology, prognosis, treatment, or prevention [5]. Typically, a set of characteristic abnormalities is used for diagnosis of dis-

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