

Detection of Galactomannan Antigenemia by Enzyme Immunoassay for the Diagnosis of Invasive Aspergillosis: Variables That Affect Performance

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Invasive aspergillosis (IA) is a frequent complication of blood or marrow transplantation. Previous studies have reported that the *Aspergillus* galactomannan enzyme immunoassay (GM EIA) may be a useful diagnostic tool for IA, but its sensitivity is variable. We examined the performance of the GM EIA in 986 serum samples from 67 patients. Results demonstrated that decreasing the index cutoff for positivity to 0.5 increased its sensitivity, with minimal loss of specificity. The low cutoff increased the duration of test positivity before diagnosis by clinical means. Sensitivity was highest in patients who did not receive preventative mold-active antifungals (87.5%). A rabbit model demonstrated that the level of circulating antigen correlated with the tissue fungus burden. A quantifiable response to antifungal therapy in clinical samples and the rabbit model supports the development of this assay for early diagnosis and therapeutic monitoring. The 0.5 cutoff may allow for better performance as an early diagnostic test.

Galactomannan (GM), which was originally identified in *Aspergillus* species as a potential diagnostic marker of invasive aspergillosis (IA) by Reiss and Lehman [1], is a polysaccharide cell-wall component that is released by growing hyphae. Several diagnostic tests have since been developed for the detection of GM [2]. The newest of these tests is a double-sandwich EIA that incorporates the β 1–5 galactofuranose-specific EBA2 monoclonal antibody as both the acceptor and detector for GM [3]. The GM EIA (Bio-Rad) has been shown in multiple studies to be a promising diagnostic tool for

IA in neutropenic patients with cancer. However, the reported sensitivity and specificity have been variable—57%–100% and 66%–100%, respectively [4–12]. Previous explanations for these disparate results have included variable criteria to establish diagnosis, variable numbers of serum samples from patients, and biological variables such as the levels of circulating *Aspergillus* antibodies [13].

In patients who undergo allogeneic blood or marrow transplantation (BMT), IA predominantly occurs late, during graft-versus-host disease (GVHD) [14]. Berenguer et al. [15] have suggested that infections that occur during the pre- and postengraftment periods might differ with regard to pathogenesis and disease progression, with high fungal burden and dissemination beyond the lungs occurring primarily in animals and patients with absolute neutropenia. Because the optimal use of this diagnostic test might vary according to treatment and transplant variables, we sought to establish parameters for usage in BMT recipients. By incorporating a detailed clinical review and a rabbit model of IA, we specifically evaluated the effects of antifungal drug administration, neutropenia, and fungal burden.

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SUBJECTS, MATERIALS, AND METHODS

Clinical Study

Patients, samples, and data. Recipients of BMT at the Fred Hutchinson Cancer Research Center (FHCRC) were enrolled prospectively in a blood-collection study. Blood samples were obtained weekly from the time of receipt of conditioning chemotherapy until day 75 after receipt of the stem-cell product. During this interval, blood samples were obtained daily in cases of fever (temperature $>38.0^{\circ}\text{C}$) or after the diagnosis of fungal infection had been established. Serum samples were stored at -70°C until further use. The study was approved by the institutional review board of FHCRC. Human experimentation was performed according to guidelines of the US Department of Health and Human Services. Informed consent was obtained from patients or their legal guardians. Rabbits were cared for according to National Institutes of Health (NIH) guidelines for animal care and in fulfillment of the criteria of the Association for Assessment and Accreditation of Laboratory Animal Care and the National Research Council.

Patients received conditioning chemotherapy, with or without total-body irradiation, and prophylaxis and treatment for GVHD, as described elsewhere [16, 17]. Antifungal prophylaxis included fluconazole (400 mg/day), which was administered for 75 days after BMT [18]. Patients who had persistent fever despite the use of antibiotics received amphotericin B (AmB; 0.5–1.0 mg/kg or equivalent doses of a lipid preparation) until the resolution of fever and neutropenia. Routine procedures were followed to establish the diagnosis of fungal infections. This included weekly chest imaging with x-ray and computed tomography scan and culture of sputum and sterile samples (bronchoalveolar lavage [BAL] and biopsy) when indicated. Therapy after a diagnosis of IA included conventional AmB, lipid formulations of AmB, itraconazole, and investigational azole antifungal compounds.

Patients with and without IA were preliminarily identified by examination of microbiological and histopathologic records. Detailed chart reviews were then performed to determine whether the patients' diagnoses of IA were proven, probable, or possible or whether they had no infection, according to a modification of the criteria developed by consensus of the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [19]. In brief, patients who had clinical signs and symptoms of infection and tissue biopsy that revealed the growth of an organism (and/or positive histopathologic results) were considered to have proven IA. Patients who had clinical signs and symptoms and *Aspergillus* species in BAL culture or positive histopathologic results were considered to have probable IA. Patients who had ≥ 3 clinical signs or symptoms and growth of an organism from nonsterile fluid (i.e., sputum or sinus aspirate) were considered to have possible IA. Patients who had no clinical or radiographic pulmonary find-

ings but who had invasive sinus disease proven by biopsy and culture were considered to have isolated sinus infection. Dissemination to other organs was confirmed by autopsy or biopsy or was considered to be suggestive by clinical findings (skin lesions or radiographic abnormalities). Colonization was defined as the growth of an organism from a nonsterile site in the absence of clinical signs or symptoms. Results of GM EIA were not included among the diagnostic criteria.

Patient demographic and transplant data were collected by query of a computerized database and by chart review. These data included age, underlying disease, type of transplantation, number of days of antifungal use (fluconazole, itraconazole, AmB formulations, or investigational products), and absolute neutrophil counts (ANCs). Neutropenia was defined as an ANC ≤ 1000 cells/ μL . For consistency and objective measurement, we considered the date of clinical signs or symptoms to be the date on which the clinician initiated antifungal therapy. Chart review was performed by clinicians who were blinded to test results.

The availability of frozen serum samples was analyzed to determine the eligibility of case patients and control subjects. Control subjects had to have ≥ 4 serum samples of sufficient volume ($\geq 600 \mu\text{L}$) available for analysis; to be eligible as a case patient, ≥ 2 serum samples had to be obtained sequentially, and ≥ 1 had to be obtained within 1 week before or after the date of diagnosis of IA.

Measurement of GM. Frozen serum samples were re-labeled according to a random number chart. The quantity of GM in each sample was measured using the Platelia *Aspergillus* EIA test kit (Bio-Rad), by an investigator blinded to both the source of the sample and clinical data. Coded serum samples were thawed and analyzed in batches, as directed by the manufacturer [9]. Optical densities were read at 450 and 620 nm. Positive, negative, and threshold control samples provided by the manufacturer were included in each assay. Results were recorded as an index relative to the mean optical density of the threshold controls (GM index = optical density sample/mean optical density of the threshold control samples). Samples that had an index value ≥ 0.5 underwent repeated testing to verify positive results. After all samples had been analyzed, data were combined with the clinical data, which had been collected independently.

Rabbit Model of Pulmonary IA

Healthy female New Zealand White rabbits (Hazleton) were used. Vascular access was established in each rabbit by the surgical placement of a silastic tunneled central venous catheter [20]. Rabbits were killed by the intravenous (iv) administration of sodium pentobarbital (65 mg/kg; Butler) at the end of each experiment. Serum samples were obtained from all rabbits at the initiation of immunosuppression, during the course of pul-

Table 1. Characteristics of patients in the cohort.

Factor	Finding
Age, mean (range), years	40 (5–66)
Stem cell source, no. (%)	
Bone marrow	53 (79)
Peripheral blood stem cells	12 (18)
Cord blood	2 (3)
Underlying disease, ^a no. (%)	
CML	20 (31)
AML	16 (23)
ALL	12 (17)
MDS	11 (16)
Other	8 (13)
Cases (n = 32), no.	
Proven aspergillosis	
Sinus	1
Lung	12
Probable aspergillosis (lung)	11
Possible aspergillosis (lung)	8
Control subjects (n = 35), no.	
No fungal infection, no colonization	19
Candidemia	3
Fusariosis	2
Pseudallescheriosis	1
<i>Aspergillus</i> species colonization	10

^a ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome. Other diagnoses included aplastic anemia (2), chronic lymphocytic leukemia (4), non-Hodgkin lymphoma (1), and paroxysmal nocturnal hemoglobinemia (1).

monary IA, and before death, and GM was quantified as described above.

Pulmonary IA was established by use of a clinical isolate (*Aspergillus fumigatus* NIH strain 4215), as described elsewhere [21]. In brief, *A. fumigatus* conidia were harvested in saline (0.025% Tween 20), and conidial concentrations were adjusted to high (1×10^8) or low (5×10^7) inocula by use of a hemacytometer. Concentrations were confirmed by the culturing of serial dilutions on Sabouraud glucose agar (SGA). Inoculation of the tracheobronchial tree was performed under general anesthesia on day 2. Each rabbit was anesthetized with ketamine (Phoenix Scientific) and xylazine (Bayer), as described elsewhere [21]. A Flagg O straight-blade laryngoscope was inserted in the oral cavity until the vocal cords were visible, and the *A. fumigatus* inoculum was administered intratracheally with a tuberculin syringe attached to a 5 1/4-inch, 16-gauge Teflon catheter.

To simulate the conditions of protracted neutropenia, therapy with cytarabine (Ara-C; Pharmacia-Upjohn) was initiated iv 1 day before the inoculation. Neutropenia (granulocyte level $<100/\mu\text{L}$) was achieved by an initial course of 525 mg Ara-C/m² for 5 consecutive days. A maintenance dose of 484 mg Ara-C/m² was administered for 4 additional days on days 8, 9, 13, and 14. Concomitant thrombocytopenia was 30,000–50,000/ μL . Methylprednisolone (5 mg/kg; Abbott Laboratories) was administered

on days 1 and 2. Ceftazidime, gentamicin, and vancomycin were administered to prevent opportunistic infections, as described elsewhere [21]. Total leukocyte counts and the percentages of granulocytes were monitored twice weekly with a Coulter counter and by peripheral blood smears, respectively.

AmB (Bristol-Myers Squibb) was administered iv (1 mg/kg/day), starting 24 h after the endotracheal inoculation. Antifungal therapy was continued for ≤ 12 days in surviving rabbits. Lung tissue from each rabbit was sampled and cultured by excision. Each specimen was weighed and homogenized with sterile saline, and dilutions of 10^{-1} and 10^{-2} were plated onto SGA. Plates were incubated at 37°C for the first 24 h and then at room temperature for another 24 h. The number of colony-forming units of *A. fumigatus* was counted and recorded for each lobe, and the colony-forming units per gram of tissue were calculated.

Statistical Analyses

The sensitivity and specificity of the GM EIA were calculated according to the proportion of patients with true and false positive and negative tests. To determine the effect of antifungal therapy, analyses were limited to patients who were not receiving mold-active antifungal compounds (itraconazole, AmB formulations, and voriconazole) within 2 weeks after and 1 month before diagnosis. Calculations were also performed according to certainty of infection (proven, probable, or possible).

The sensitivity and specificity were also calculated for all tests in the total population of patients, by use of a variety of cutoffs to determine positivity. In these calculations, sensitivity was defined as the proportion of positive test results among all tests from patients with proven or probable IA, and specificity was defined as the proportion of negative tests among all tests from control subjects. A receiver-operator characteristic (ROC) curve was calculated to illustrate the trade-off in sensitivity versus 1-specificity rates as the cutoff for the test was shifted from high

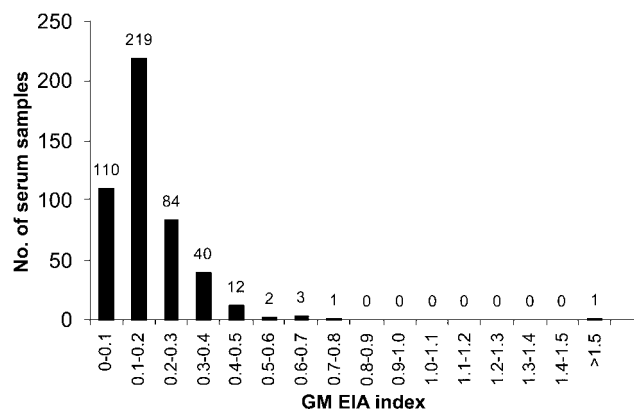


Figure 1. Distribution of galactomannan (GM) EIA index values of serum samples obtained from 35 control subjects. For samples with repeated indices ≥ 1.0 , the first value obtained is shown.

Table 2. Performance of the galactomannan EIA (index cutoff, 1.0).

Diagnosis, by patient group	No. of patients testing positive	Sensitivity, %
Overall cohort		
Proven or probable IA (<i>n</i> = 24)	13	54.2
Proven IA (<i>n</i> = 13)	8	61.5
Probable IA (<i>n</i> = 11)	5	45.5
Receiving antifungal compounds		
Proven IA (<i>n</i> = 5)	1	20
Probable IA (<i>n</i> = 6)	1	16.7
Not receiving antifungal compounds		
Proven IA (<i>n</i> = 8)	7	87.5
Probable IA (<i>n</i> = 5)	4	80

NOTE. IA, invasive aspergillosis.

to low. Specificity was estimated by per-test and per-patient calculations.

To determine the effect of lowering the GM EIA index cutoff to define positivity on the timing relative to clinical diagnoses, the median number of days between positive test results and diagnosis was calculated only among the 11 patients with proven or probable IA who had ≥ 1 positive GM EIA result at all indices (≥ 1.5). Because GM levels decrease with receipt of antifungal drugs, the test may be useful to indicate prognosis and to structure therapy [22]. To explore this potential use of the test, we plotted GM indices longitudinally for all patients with proven IA who had ≥ 2 pre- and postinfection serum samples analyzed and correlated the results with therapy and outcome.

Sensitivity and specificity were determined in rabbits as they were in patients. The relationship between the level of circulating GM and tissue fungal burden was plotted and analyzed by use of Pearson's correlation coefficient. Serum levels of GM in groups of rabbits that received high and low inocula and in those that received antifungal therapy were plotted over time, to further characterize antigen kinetics with different levels of tissue burden.

RESULTS

Clinical study. A total of 1106 serum samples from 79 different BMT recipients were initially identified for analysis. Chart review documented that 35 patients never developed IA, although they may have been colonized with *Aspergillus* species or had disseminated infection with other fungi (table 1). After review of the serum samples available, 12 patients in the cohort were considered not to be evaluable because they did not have samples available within 2 weeks of the date of diagnosis. Final analysis thus included 986 serum samples from 67 patients. Of these, 178 serum samples were analyzed from 13 patients with

proven IA, 201 from 11 patients with probable IA, 135 from 8 patients with possible IA, and 472 from control subjects. Demographic and transplant characteristics of this selected cohort are shown in table 1.

The 472 serum samples from the control subjects are shown in a histogram by index value (figure 1). The median index was 0.14. Only 1 sample from a control subject had a false-positive index with a value >1.0 on repeat testing, and several serum samples from control subjects had GM indices between 0.5 and 1.0 (figure 1).

Sensitivity of the GM EIA according to patient diagnosis is shown in table 2. Using the original manufacturer's definition of "indeterminate to positive" (cutoff ≥ 1.0), the sensitivity of the test in the overall cohort was very low. Because antifungal therapy was given to patients for prophylactic and empirical purposes, we performed the analysis after stratifying patients for receipt of mold-active antifungal therapy (itraconazole or AmB formulations) within 2 weeks before the diagnosis of aspergillosis. Receipt of antifungal compounds decreased the sensitivity of the test (table 2).

To determine whether better performance of the test could be achieved by lowering the index cutoff to define positivity, ROC curves were calculated to illustrate the trade-off in sensitivity versus 1-specificity rates as the cutoff for the test was shifted (figure 2). On this curve, a test that yielded no reliable predictive information would generate a straight diagonal line. Tests with the best performance would have high true-positive values and low false-positive values, even at low cutoffs, yielding an elliptical curve. In the model developed from the GM EIA results, sensitivity increased substantially, with a minimal loss of specificity, after the index cutoff was decreased from 1.0 to 0.5.

To determine the optimal index cutoff, we plotted the sensitivity and specificity of the test in patients who were and were

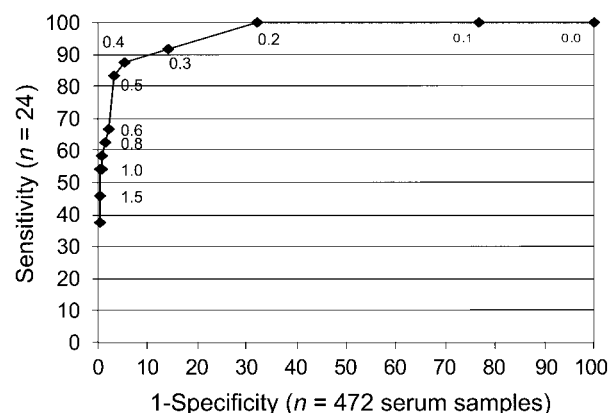


Figure 2. Receiver operator characteristic curve of test results as the galactomannan EIA index cutoff to define positivity is decreased. Indices decrease as the line proceeds to the right (index values are shown for each data point). Sensitivity is plotted per patient, and specificity is plotted per test.

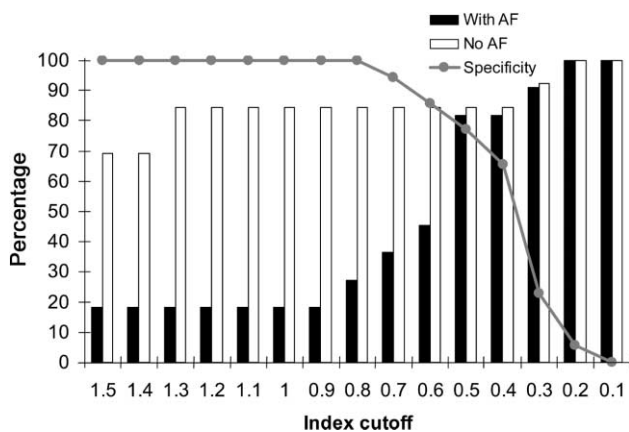


Figure 3. Sensitivity and specificity, from a per-patient analysis, for proven and probable invasive aspergillosis at variable index cutoffs for positivity (x-axis). Overall specificity is shown with the line. Specificity of the test did not differ on the basis of whether the patient was receiving antifungal (AF) compounds.

not receiving mold-active antifungal compounds within the 2-week period before diagnosis (figure 3). In the absence of mold-active antifungal compounds, sensitivity to diagnose proven and probable infection remained high, regardless of the cutoff point for positivity. In patients who were receiving empirical or prophylactic antifungal compounds, a decrease of the cutoff to 0.5 increased the sensitivity to 81.8%, with a decrease in patient specificity from 100% to 77.1%. Specificity of the test did not differ on the basis of receipt of mold-active antifungal drugs (data not shown).

The distribution of test positivity in the control group and in patients with possible IA is shown in table 3. In this table, specificity was calculated by use of a per-test analysis and a per-patient analysis. There were very few false-positive test results overall—18 of 607 serum samples obtained from patients with no IA or possible IA had indices ≥ 0.5 . Accordingly, specificity relative to the total number of tests evaluated was high in all patients. The majority of false-positive results that developed in response to lowering the index occurred in patients who were colonized with *Aspergillus* and who had a diagnosis

of possible IA. False-positive results appeared to be sporadic during the course of BMT and were not associated with the early posttransplant period of mucositis or neutropenia (data not shown).

To evaluate the performance of the test in patients with variable amounts of neutrophils, we analyzed the cohort of 13 patients who had proven infection. Only 3 patients did not develop neutropenia within the 2 weeks before diagnosis, and none had positive GM indices (≥ 0.5). Alternatively, 8 (80%) of 10 patients who had neutropenia within the 2 weeks before diagnosis had positive GM indices ($P = .03$).

The times between test positivity and IA diagnosis according to conventional methods, using different EIA indices used to define positivity, are shown in table 4. Lowering the GM EIA index cutoff increased the time between a positive test result and clinical diagnosis.

Among the small number of patients evaluated, there was an association between the kinetics of positivity after the receipt of antifungal compounds and therapeutic outcome. A decrease in the GM EIA index after the initiation of antifungal therapy was apparent in all 4 patients who survived infection, versus an increase in the GM EIA index in 4 of 5 patients who died from infection. Figure 4 shows serial GM indices in representative patients—1 who died and had IA disseminated to the brain (figure 4A) and another who survived and had disease limited to the lungs (figure 4B). The patient depicted in figure 4A developed high levels of GM while receiving prophylactic fluconazole, but the highest level, which was obtained from the patient in figure 4B, who was receiving AmB empirically, was an index of 0.5. Also, GM levels continued to increase in the patient who ultimately died from infection, whereas they did not increase in the patient who survived.

Rabbit model. Galactomannan was quantified in 10 control (uninfected) rabbits and in 20 rabbits infected with *A. fumigatus* conidia. The overall performance of the test was similar in rabbits and in clinical samples. Of 30 rabbits tested, 1 uninfected rabbit had a false-positive EIA result, 3 infected rabbits had false-negative results, 9 control rabbits had true-negative results, and 17 infected rabbits had true-positive re-

Table 3. Specificity in control subjects and patients with possible invasive aspergillosis (IA), using a galactomannan EIA index cutoff of 0.5.

Diagnosis (no. of samples/no. of patients)	Result/no. of patients		Specificity, %	
	Positive	Negative	Per test	Per patient
No infection or colonization (223/19)	6/5	217/14	97.3	73.7
Colonized with <i>Aspergillus</i> species (189/10)	3/1	186/9	98.4	90
Invasive fusariosis (24/2)	0/0	24/2	100	100
Candidemia (26/3)	1/1	25/2	96	66
Invasive <i>Pseudallescheria boydii</i> infection (10/1)	0/0	10/1	100	100
Possible IA (135/8)	8/4	127/4	94.1	50
Total no. of control subjects (607/43)	18/11	589/32	97	74.4

Table 4. Time between test positivity and clinical diagnosis.

Time, median (range)	GM index cutoff		
	1.5	1.0	0.5
Between test positivity and IA diagnosis	-1 (-41 to 19)	-1 (-41 to 19)	-10 (-59 to 19)
Between test positivity and clinical onset	0 (-21 to 19)	0 (-21 to 19)	-6 (-25 to 19)

NOTE. No. of days before an event (diagnosis or clinical onset) are represented with negative values; no. of days after an event are positive. IA, invasive aspergillosis.

sults, yielding an overall sensitivity of 84% and specificity of 90%. Corresponding positive and negative predictive values were 94% and 75%, respectively. In figure 5, the last GM EIA index obtained before death was plotted against the pulmonary fungal burden. The index values correlated with the pulmonary fungal burden (Pearson's correlation coefficient, 0.7)—rabbits with the highest amount of growth in tissue had the highest circulating GM indices.

To further characterize the plasma kinetics of circulating GM levels, indices were measured longitudinally in rabbits infected with high and low inocula of *A. fumigatus* conidia, as well as in rabbits treated with AmB. The GM index increased in untreated rabbits in a concentration-dependent fashion—rabbits that received higher inocula had higher circulating levels of GM (figure 6). These experiments also assessed the plasma kinetics

of circulating GM levels in rabbits that were treated with AmB after receiving the highest conidial inoculum. After therapy with AmB, circulating levels decreased.

DISCUSSION

The present study presents supportive evidence that the GM EIA has potential utility for screening in immunosuppressed patients, especially in BMT recipients with neutropenia. We combined clinical data and data from a rabbit model to provide novel insights regarding the variables that affect the performance of the test and found that factors that alter fungal burden, such as host immune status and the receipt of antifungal therapy, affected the performance of the assay. This observation has important implications with regard to the interpretation of

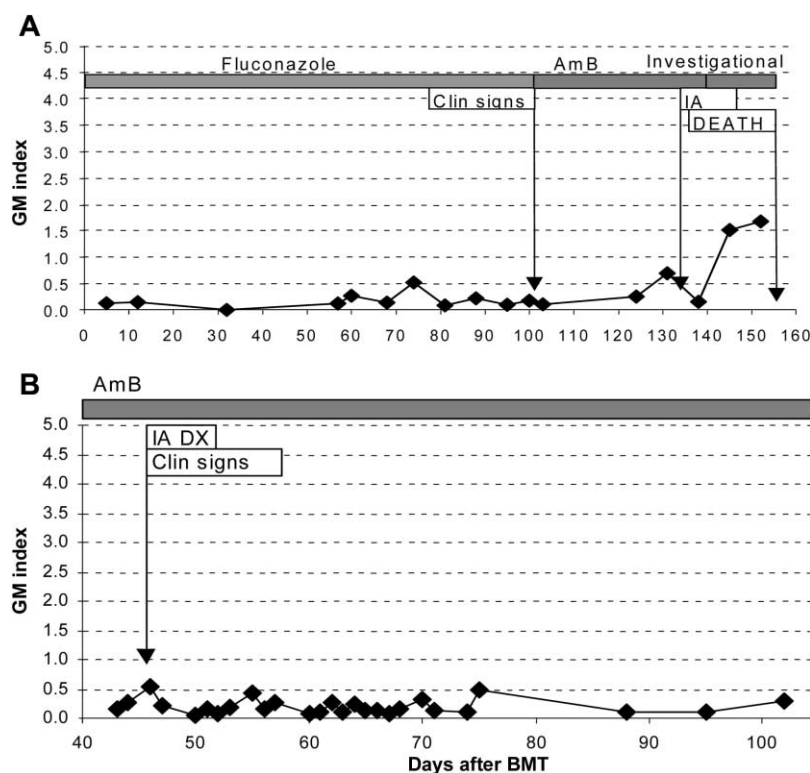


Figure 4. Serial galactomannan (GM) indices in 1 patient who died (A) and another who survived (B) invasive aspergillosis (IA). AmB and lipid AmB, amphotericin B products (formulations not specified); BMT, bone or marrow transplant; clin signs, date of clinical signs or symptoms; IA DX, date of diagnosis of IA; investigational, investigational azole antifungal.

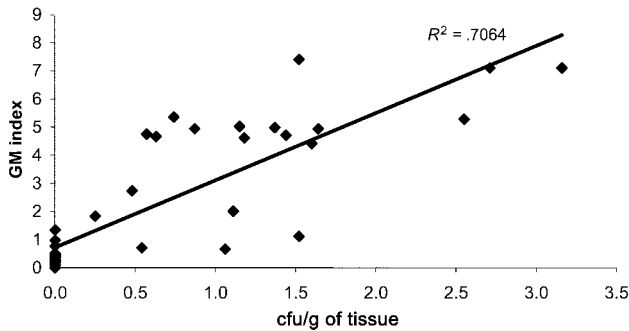


Figure 5. Galactomannan (GM) indices from 20 rabbits infected with 1×10^8 *Aspergillus fumigatus* and 10 uninfected control rabbits. Pulmonary fungal burden, in log colony-forming units/g of tissue (x-axis) was measured by serial dilution and quantitative culture of homogenized lung tissue. The GM EIA index obtained from serum samples the day before death (or killing) of the animal is plotted on the y-axis. A trend line is shown.

clinical data, the design of future studies, and the establishment of parameters for clinical use. Also, results indicate that performance of this GM EIA may be optimized by decreasing the index cutoff to define positivity.

The utility of the GM EIA has been debated because of the wide range of sensitivities previously reported in the literature (38%–100%) and a short interval between the detection of circulating GM and diagnosis by conventional means [4, 12]. Previously, clinical investigators have suggested that reported sensitivities are variable because of different definitions of infection, some of which do not require pathological confirmation [7]. One recent prospective pathology-controlled study, which used an index definition of positivity of ≥ 1.0 , reported a sensitivity of 92.6% and a specificity of 95.4% [7]. In contrast, recent studies have indicated that patients with IA in the setting of different underlying immunologic disorders, such as chronic granulomatous disease and Job's syndrome, may not develop detectable antigenemia [23, 24]. The results of our study suggest that some of the variation in the reported sensitivity may be associated with institutional variations in prophylactic and/or empirical use of antifungal compounds and with analysis between different host populations.

The observation that the administration of antifungal compounds alters circulating GM levels is not unique. Previous studies in the neutropenic rabbit aspergillosis model have demonstrated that therapy with AmB decreases levels of circulating GM, as measured by latex agglutination [21] and EIA [25]. The rabbit studies reported here extend these observations by demonstrating that the level of GM antigenemia varies as a function of pulmonary inoculum and correlates directly with the log tissue burden. Consistent with these findings are the results of clinical reports that have suggested that the course of GM index values closely correlates with the outcome of therapy [8, 26]. The kinetics of GM antigenemia were recently explored in a

review of 37 BMT recipients treated for proven or probable IA [22]. Those investigators found that a 0.5 increase in GM levels above baseline served as a good marker for disease progression and the ultimate failure of therapy. The rabbit data presented in the present study provide an experimental foundation for understanding these observations and indicate that levels of circulating GM correlate with the pulmonary tissue burden of *A. fumigatus*. Larger clinical studies are necessary to define the prognostic utility of the assay for people receiving multiple types of antifungal therapy.

Given the association with fungal burden, it may be possible that the host's immune response can alter the results of the diagnostic test. Verweij et al. [23] suggested that this might occur in the setting of pulmonary "encapsulation" of an *Aspergillus* lesion. Our data suggest that the test's performance might also be altered later after BMT in nonneutropenic but immunosuppressed hosts. This result is similar to that of a recent study that showed that false-negative screening results were more common in patients who developed IA late after BMT [27]. These observations may be explained by a difference in disease progression between hosts with and without neutropenia. In a rabbit model of IA, Berenguer et al. [15] demonstrated that the mechanism of disease progression and fungal burden differed in neutropenic and corticosteroid-treated rabbits. In neutropenic rabbits, pulmonary disease was characterized by intensive hyphal growth and high fungal burden. In rabbits that had been immunosuppressed with corticosteroids and cyclosporine, pulmonary inflammation, with decreased fungal burden, appeared to be the cause of disease progression and death [15]. The results of a subsequent clinical study suggested that analogous disease progression might occur in BMT recipients with and without neutropenia—those without neutropenia developed less extrapulmonary dissemination, more death associated with hypoxia, and pulmonary histopathologic results characterized by diffuse in-

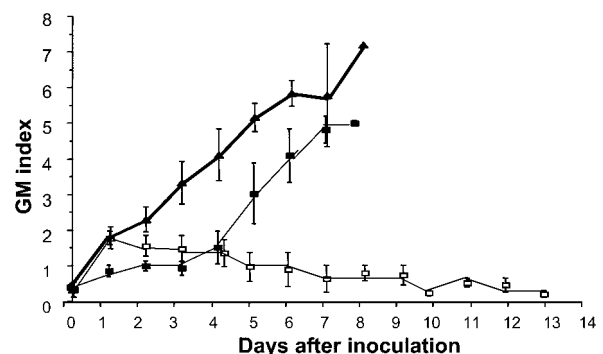


Figure 6. Effect of therapy with amphotericin B. Rabbits were infected with 1×10^8 (black triangles and white squares) and 5×10^7 (black squares) conidia of *Aspergillus fumigatus*. A subset of rabbits was treated at day 1 after inoculation with amphotericin B (0.5 mg/kg/day, white squares) is shown.

flammation [28]. In this setting, the sensitivity of the GM EIA might be compromised. Defining the pathogenesis of IA early and late after BMT and the optimal use of the GM EIA in this setting requires further study.

The mechanism by which circulating levels of GM decrease in the setting of appropriate antifungal therapy and neutrophil response likely involves inhibited hyphal growth. Whether this reflects limited extrapulmonary dissemination and/or decreased fungal burden within the lungs is not known. It is also possible that exposure to AmB might alter the hyphal release of GM (R. Winn, A. Warris, T. Abrahamsen, P. Gaustad, unpublished data).

The specificity of the test was high in the present study compared with previous reports, which have suggested a much higher false-positivity rate, especially in patients who have recently received chemotherapy [9, 11]. Despite sampling non-infected and colonized patients during neutropenia and mucositis, we had few false-positive GM EIA values when we used the cutoff of 0.5. One potential explanation is that false-positive results in previous studies in patients at lower risk reflect partially treated occult infection, whereas the predictive value of invasive disease is higher in more immunosuppressed patients. Although no studies have demonstrated an appreciable effect of freeze-thaw on GM index values, it is possible that the freeze-thaw step in our retrospective study may have decreased the rate of false-positive results by altering levels of a cross-reactive antigen. It is also possible that our population differed from prior cohorts by virtue of other treatment characteristics that may have affected false positivity—for example, the use of potentially contaminated antibiotics such as piperacillin-tazobactam [29]. Finally, our study included 1 child, and children have previously been reported to have a high rate of false-positive results [13].

The European manufacturer of the GM EIA originally suggested that a sample index between 1.0 and 1.5 should be considered within the “gray zone” of positivity. Other investigators have previously suggested that the cutoff for positivity could be safely decreased to >0.7 [13] or >0.8 [30], without a compromise in specificity. Our data support this and suggest that the index to define positivity can be further decreased to 0.5. Using this index to define positivity, the sensitivity and specificity of the test per patient was estimated to be 80%, and the test was positive ~1–2 weeks before clinical onset and diagnosis, respectively. Because the test will be used as an “aid to diagnose IA,” positive results trigger further evaluation for disease (by radiography). In this setting, it seems particularly important to maximize sensitivity for early diagnosis, despite the small decrease in specificity. Accordingly, the US Food and Drug Administration recently cleared the assay using the low cutoff to aid diagnosis in adult BMT recipients with neutropenia. It is possible that the semi-quantitative performance of the GM EIA may be harnessed further to develop personalized treatment strategies for both screen-

ing and therapeutic monitoring; however, larger studies will be needed to define clinical utility and to assess performance in differing patient populations.

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