Aspergillus Galactomannan Detection in the Diagnosis of Invasive Aspergillosis in Cancer Patients

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Purpose: **To assess the *Aspergillus* galactomannan enzyme-linked immunosorbent assay (ELISA) in the diagnosis of invasive aspergillosis (IA) in adult and pediatric oncohematologic patients.**

Patients and Methods: **The study was conducted in four patient groups: those with fever of unknown origin (FUO) during neutropenia, suspected pulmonary infection (PI), or nonpulmonary aspergillosis (NPA) and those undergoing surveillance (S) after hematopoietic stem-cell transplantation (HSCT). IA was classified as definite, probable, or possible, according to European Organization for Research and Treatment of Cancer/Mycosis Study Group definitions.**

Results: **A total of 3,294 serum samples were collected during 797 episodes (FUO, 261; PI, 297; NPA, 28; and surveillance, 211), and 153 episodes of IA were diagnosed (31 definite, 67 probable, and 55 possible). Three episodes were first suspected from galactomannan ELISA; the remaining 150 cases were diagnosed from clinical or radiologic evidence. Sensitivity of the ELISA was 64.5%, 16.4%, and 25.5% in definite, probable, and possible episodes of IA, respectively, and was lower in patients positive for anti-*Aspergillus* antibodies than in antibody-negative patients. Most false-positive results occurred in children and in allogeneic HSCT (allo-HSCT) patients. Overall specificity of the ELISA was 94.8%. It was lower in children compared with adults ($P < .0001$) and in allo-HSCT patients compared with non–allo-HSCT adults ($P = .0002$). Lowering the ELISA cutoff value from 1.500 to 0.700 seemed more relevant for non–allo-HSCT adults (sensitivity, 73.1%, 44.3%, and 44.7% in definite, probable, and possible IA, respectively; specificity, 94%).**

Conclusion: **Galactomannan ELISA seems less sensitive than previously described, and sensitivity can be further reduced by the presence of anti-*Aspergillus* antibodies. A new cutoff value for the ELISA of 0.700 is proposed for non–allo-HSCT adults.**

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THE INCIDENCE OF INVASIVE aspergillosis (IA) has dramatically increased over the last decades. Aspergillosis is now the leading cause of infectious mortality in many hematology and bone marrow transplantation units.[1,2] Prognosis depends mainly on early, effective therapy.[3,4]

Conventional diagnosis of IA is dependent on culture and histopathologic examination of the tissue(s) involved. Microscopy and culture of sputum and bronchoalveolar lavage (BAL) samples are insufficiently sensitive for diagnostic approaches, and biopsies are not always feasible in patients with a severe underlying condition. Thus, in most cases, diagnosis depends on a combination of clinical signs, radiologic abnormalities, and clinical experience.

A serum enzyme-linked immunosorbent assay (ELISA) has recently been developed for the detection of circulating galactomannan (GM), which is a major constituent of *Aspergillus* cell walls. The test is able to detect approximately 1 ng/mL GM, which is 10 times lower than the limit of the latex agglutination test used previously.[5] Initial clinical studies suggest that the ELISA test has a high sensitivity and specificity.[5-9]

We evaluated the GM ELISA in adult and pediatric oncohematologic patients. The evaluation was performed prospectively over a 4-year period in the following groups: (1) neutropenic patients with fever of unknown origin (FUO), persistent despite broad-spectrum antibiotic therapy; (2) transplant recipients undergoing weekly surveillance; (3) patients with suspected pulmonary infection; and (4) patients with extrapulmonary aspergillosis suspected on clinical or radiologic evidence. This long-term surveillance allowed us to collect data on a large number of cases of aspergillosis in patients with various hematologic diseases. The performance of the GM ELISA assay was assessed in IA, using the new European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) definition criteria.[10]

PATIENTS AND METHODS

Patients

GM ELISA became available at the University Hospital of Strasbourg as a routine diagnostic test in January 1997. The study was

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conducted in patients from the pediatric and adult hematology and oncology departments from February 1997 to January 2001.

Definition of Patient Groups

FUO. To be eligible, patients had to be neutropenic (neutrophil count $< 500/\mu L$), with a persistent fever, despite first-line antibiotic therapy. They were to have no microbiologic documentation of the infection, and no other clinical signs suggestive of infection when the first serum sample was collected for antigenemia tests. First-line antibiotic therapy usually included a broad-spectrum beta-lactam and an aminoglycoside.

Serum was collected daily for the detection of antigen, on up to three occasions, when no cause of the fever could be determined. Sampling was stopped when a clinically significant bacterial pathogen, a non-*Aspergillus* fungus, a virus, or a noninfectious cause of the fever was determined, or when the febrile neutropenia resolved.

Suspected pulmonary infection. All patients presenting with clinical signs suggestive of a pulmonary infection had a serum sample collected for antigen testing. Suspicion of an infection was based on symptoms, clinical signs, and the presence of new chest x-ray abnormalities. Patients with pulmonary symptoms clearly related to their underlying disease, or to another noninfectious cause, were not investigated. Antigen tests were carried out as soon as the pulmonary infection was suspected, and continued for 3 consecutive days. Sputum was routinely sampled for microscopy and bacterial and fungal cultures. Computed tomography (CT) scans were obtained whenever feasible, and BAL was performed in the absence of severe hypoxemia when no microbiologic evidence was found in blood or sputum cultures. When a satisfactory non-*Aspergillus* cause of infection was documented, antigen testing was discontinued. When aspergillosis was confirmed or suspected, antigen testing was continued every 7 days until satisfactory response or death.

In all patients dying from pulmonary infections, an autopsy or postmortem pulmonary biopsy was performed, where family consent was available. Autopsy and biopsy samples were collected for microscopy, fungal cultures, and histopathologic evaluation.

Suspected extrapulmonary aspergillosis. Serum samples were taken from all patients with nonpulmonary clinical or radiologic signs consistent with IA for 3 consecutive days, and then every 7 days until resolution of the signs, diagnosis of a non-*Aspergillus* cause of the infection, or death. Diagnostic signs included sinusitis or rhinofacial disease, radiologic evidence of invasive sinusitis, any inflammatory or necrotic skin nodule, and any unexplained lesion on cerebral CT scans.

Surveillance of hematopoietic stem-cell transplant recipients. Patients undergoing autologous hematopoietic stem-cell transplantation (auto-HSCT) or allogeneic hematopoietic stem cell transplantation (allo-HSCT) had weekly serum sampling performed in addition to other routine serologic surveillance. Antigen surveillance was performed during the neutropenic phase for auto-HSCT recipients and for 1 month after hematologic recovery in the absence of graft-versus-host disease (GVHD) in allo-HSCT recipients. In the case of GVHD requiring corticosteroid therapy, antigenemia was assessed every 1 to 2 weeks for up to 4 months after transplantation.

Repeated inclusion. Patients could be included in the study on more than one occasion, although once diagnosed with IA patients were no longer eligible for a subsequent inclusion until complete resolution of the infection. Transplant patients developing a FUO or non–*Aspergillus*-related pneumonia during the surveillance period were included in the FUO or in the suspicion of pulmonary infection group during the episode, and returned to the surveillance group after complete resolution of the episode.

Clinical Definitions

Definition of IA. Diagnosis of IA was classified as definite, probable, or possible on the basis of criteria adapted from the EORTC/MSG definitions.[10] The results of the antigen testing were not included in our classification.

Definite IA was defined by the isolation of *Aspergillus* species from a tissue biopsy specimen or from a normally sterile body fluid (excluding BAL). Probable IA was defined in an immunocompromised patient by the coexistence of clinical and mycologic criteria. Clinical signs were supportive of a diagnosis of probable IA if either one major or two minor criteria were present (as defined by Ascioglu et al[10]). Fungal criteria included (1) positive culture of *Aspergillus* species from sputum, BAL, or sinus aspirate; and (2) the presence of branching septate hyphae in a tissue biopsy, sputum, BAL, sinus aspirate, or any body fluid. Possible invasive pulmonary aspergillosis was considered in immunocompromised patients satisfying the clinical criteria as defined by Ascioglu et al, in the absence of positive fungal criteria or any other explanation for their condition. A diagnosis of possible aspergillosis was never considered before broad-spectrum antibacterial therapy was demonstrated to be ineffective.

*Definition of non-*Aspergillus *pulmonary infections.* Bacterial pneumonia was diagnosed when blood cultures grew a bacterial pathogen (at least two positive cultures were required for coagulase-negative staphylococci) and when no other pathogen was found in respiratory samples. In the absence of concomitant bacteremia, an infection of bacterial origin was presumed when the patient responded to antibacterial therapy alone.

Viral and parasitic lung infections were defined by the isolation of the appropriate pathogen from BAL fluid. *Pneumocystis carinii* pneumonia was diagnosed when the pathogen was detected in BAL fluid, when polymerase chain reaction analysis of the fluid was positive in the absence of other detectable pathogens.

Pulmonary yeast infections were diagnosed when blood cultures grew yeasts and no other pathogen was found in respiratory samples. In the absence of fungemia, the presence of yeasts in respiratory samples without any other pathogen and resolution of the infection after administration of fluconazole led to the diagnosis of presumed yeast pulmonary infection.

The diagnosis of invasive mucormycosis was made on the basis of isolation of broad, nonseptate hyphae or significant positive cultures on the basis of the EORTC/MSG criteria.[10] Diagnosis of other mold infections required a positive culture.

Aspergillus Antigen Testing

The *Aspergillus* GM antigen was detected in serum by direct double-sandwich ELISA (Platelia Aspergillus; Bio-Rad, Marnes La Coquette, France). Samples were processed following the manufacturer's instructions. Serum was heated at 100°C for 3 minutes with a solution of EDTA, to dissociate immune complexes and to precipitate serum proteins that could interfere with the ELISA, and was then centrifuged at 10,000 $\times$ *g* for 10 minutes. Fifty microliters of the supernatant and 50 μL of conjugate (horseradish peroxidase–labeled monoclonal antibody EBA-2) were incubated in the monoclonal antibody EBA-2–coated microplates for 90 minutes at 37°C. After five washes, the plates were incubated (30 minutes in the dark, at room temperature) with a substrate-chromogen solution containing tetramethylbenzidine. The reaction was stopped with 1.5 N sulfuric acid, and

Table 1. Demographics and Number of Antigenemia Tests According to Reason for Antigen Determination

	Reason for Antigenemia Determination			
	Fever of Unknown Origin	Suspected Pulmonary Infection	Extrapulmonary Signs	Surveillance in Transplant Recipients
No. of episodes	261	297	28	211
No. of different patients	220	274	28	206
Age, years				
Median	50	55	54	41
Range	1-85	1-88	22-80	4 months-68 years
Male/female	147/114	202/95	15/13	110/101
No. of antigenemia assays	540	1481	122	1151
No. of antigenemia assays per episode				
Mean	2.1	5.0	4.4	5.5
Range	1-11	1-28	1-19	1-32

plates were read in a Dynatech microplate reader (Dynex Thermobio Analysis, Issy les Moulineaux, France) at 450 nm (reference, 620 nm). For validation, each run contained in duplicate one negative control, one positive control containing 10 ng/mL of GM, and a standardized serum containing 1 ng/mL GM for calibration and conversion of the measured absorbances into indexes. According to the manufacturer's recommendations, sera with an index greater than 1.5 were considered positive. All steps were performed under laminar flow with sterile single-use materials.

Serology

Specific anti-*Aspergillus* antibodies were measured with an in-house ELISA method using an *A fumigatus* antigenic extract. Optical densities were converted to index scores using a control serum, and samples were considered positive for antibodies when the index value ≥ 0.8.

Statistical Analysis

Nongaussian distribution of the antigenemia values was confirmed using the Kolmogorov-Smirnov test. Means were compared using nonparametric tests (Mann-Whitney *U* test to compare two groups and Kruskal-Wallis test with Dunn's posttest to compare three or more groups); $P < .05$ was considered as significant.

RESULTS

Patient Characteristics

A total of 3,294 serum samples were collected during 797 episodes. The median age for the whole study population was 50 years (range, 4 months to 88 years); 48 episodes occurred in children aged younger than 18 years old. Demographic characteristics, median number of antigenemia assays per episode, and underlying diseases, summarized according to the reason for antigenemia determination, are listed in Tables 1 and 2.

In all patient groups, the most frequent underlying diseases were acute leukemia (289 episodes [36.3%]), malig-

Table 2. Underlying Condition in Each of the Patient Groups

	Reason for Antigenemia Determination							
	Fever of Unknown Origin (n = 261)		Suspected Pulmonary Infection (n = 297)		Extrapulmonary Signs (n = 28)		Surveillance in Transplant Recipients (n = 211)	
Underlying Condition	No.	%	No.	%	No.	%	No.	%
Hematologic malignancy								
Acute leukemia	113	43.3	105	35.4	11	39.3	60	28.4
Lymphoma	69	26.4	69	23.2	6	21.4	43	20.4
Chronic myeloid leukemia	6	2.3	15	5.1	1	3.6	14	6.6
Myelodysplastic syndrome	10	3.8	17	5.7	4	14.3	1	0.5
Multiple myeloma	14	5.4	14	4.7	1	3.6	26	12.3
Other	7	2.7	22	7.4	0		2	0.9
Solid tumor								
Digestive tract cancer	7	2.7	16	5.4	0		0	
Urogenital cancer	6	2.3	8	2.7	1	3.6	14	6.6
Breast cancer	9	3.4	3	1.0	0		30	14.2
Lung cancer	0		10	3.4	0		3	1.4
Other	8	3.1	5	1.7	1	3.6	12	5.7
Nonmalignant hematologic diseases	12	4.6	13	4.4	3	10.7	6	2.8
Autologous stem-cell transplantation	48	18.4	40	13.5	1	3.6	135	64.0
Allogeneic stem-cell transplantation	37	14.2	30	10.1	3	10.7	76	36.0

Table 3. Final Diagnosis of the Episodes and Results of Antigen Determination in Each Patient Group

	Reason for Antigenemia Determination				
	Fever of Unknown Origin (n = 261)	Suspected Pulmonary Infection (n = 297)	Extrapulmonary Signs (n = 28)	Surveillance in Transplant Recipients (n = 211)	Total
Final diagnosis					
Absence of aspergillosis	260	149	22	209	640
Non-IA	0	3	1	0	4
Definite IA	0	25	5	1	31
Probable IA	0	67	0	0	67
Possible IA	1	53	0	1	55
Result of antigenemia assay*					
True-positive	1	40	2	2	45
False-positive	13	2	0	18	33
True-negative	247	147	22	191	607
False-negative	0	105	3	0	108
PPV, %	7.1	95.2	100	10.0	57.7
NPV, %	100	58.3	88.0	100	84.9

*Noninvasive cases were not considered (one had positive antigenemia and three were negative).

nant lymphoma (187 episodes [23.5%]), and solid tumor (133 episodes [16.7%]). Nearly half of the episodes occurred in stem-cell transplant recipients: 224 (28.1%) in auto-HSCT recipients and 146 (18.3%) in allo-HSCT recipients. Ten episodes occurred in patients with a history of solid organ transplantation and a posttransplant malignancy.

Antigen Testing

FUO. Of the 540 serum samples assessed, 23 tests (4.3%) from 14 different episodes were positive (Table 3). Only one episode of IA (a possible case in a child) was diagnosed in the FUO host group. This child had a positive GM ELISA. Coughing only developed later during the course of the infection. CT scans showed clusters of micronodules and no mycologic confirmation could be obtained. Four further antigenemia tests were positive.

False-positive results were observed more frequently in children (11 of 25 [44.0%]) than in adults (two of 235 [0.9%]; $P < .0001$). Both adults had only a single positive test, whereas three of the 11 children had repeated positive tests. One patient with a false-positive test had blood cultures positive for *Staphylococcus aureus*, whereas no explanation of the fever could be determined for the remaining 12 patients.

No false-negative results were observed in patients with FUO. Non-*Aspergillus* causes of fever were documented in 121 (50.2%) of the 241 episodes with negative antigenemia. The main causes of fever were bacterial (97 cases) and fungal (19 cases) bloodstream infections. Other identified causes of fever were one case each of esophageal candidiasis, GVHD, oral herpes, lymphoma-related fever, and Epstein-Barr virus infection.

Twenty-two patients with bacteremia and six patients with fungemia developed subsequent pulmonary localization of their infection. None of these patients had mycologic samples positive for *Aspergillus* species, and none had a positive antigen test. Nine of these patients received amphotericin B, but as non-*Aspergillus* pathogens had previously been identified, and the clinical courses of the infections were consistent with those pathogens, diagnoses of possible aspergillosis were not considered. Amphotericin B was given to 28 patients with persistent FUO, but none developed clinical or radiologic signs of infection with *Aspergillus* species, and these cases were not considered as possible aspergillosis.

Suspected pulmonary infections. A total of 1,481 serum samples were investigated from 297 episodes, of which 112 samples were positive using the ELISA test (Table 3). Definite, probable, and possible IA was diagnosed in 25 (8.4%), 67 (22.6%), and 53 (17.8%) episodes, respectively. In addition, three cases (two of tracheobronchitis with negative antigenemia and one of pulmonary aspergilloma with positive antigenemia) were considered as noninvasive. After exclusion of the aspergilloma case, 40 antigenemia tests were true-positives and two (both in children) were false-positives. Pulmonary infection was presumed to be of bacterial origin in both false-positive cases, as they responded to antibiotics before recovery from neutropenia, without the addition of an antifungal agent. One hundred five antigenemia tests were false-negative (Table 3). Among these false-negative cases, eight were definite IA, 56 were probable IA, and 41 were possible IA. Other explanations of the pulmonary disease were mainly documented or presumed bacterial or yeast pneumonia (99 [33.3%]), mucor-

mycosis or other proven filamentous fungi (five [1.7%]), *P carinii* infection (11 [3.7%]), tuberculosis (two [0.7%]), toxoplasmosis (three [1.0%]), viral pneumonia (seven [2.4%]), and noninfectious causes (24 [8.1%]).

Suspected extrapulmonary aspergillosis. Twenty-eight episodes were assessed and 122 serum samples were collected. All episodes occurred in adults. Antigenemia was only positive in two episodes (6.9%), both of which were diagnosed as definite IA (Table 3). A total of five definite cases of IA were documented: two cases of rhinofacial disease with extensive necrosis, one of invasive sinus aspergillosis, one of renal abscess in an AIDS patient, and one of chondrocostal and soft-tissue involvement in a patient with a history of chondrosarcoma treated with radiotherapy. In addition, noninvasive maxillary sinus aspergillosis was diagnosed in one patient with aplastic anemia. Other final diagnoses were cerebral or ocular toxoplasmosis (five cases), primary cerebral non-Hodgkin's lymphoma (three cases), bacterial sinusitis (three cases), Sweet syndrome in febrile leukemic patients (three cases), rhinofacial mucormycosis (two cases), rhinofacial bacterial cellulitis (two cases), necrotic oral or facial herpes (two cases), disseminated fusariosis with necrotic skin lesions (one case), and chronic disseminated candidiasis (one case).

Surveillance in stem-cell transplant recipients. Two hundred six different patients were monitored after a total of 211 transplant procedures (76 allogeneic, 135 autologous). At least one ELISA test was positive in 20 episodes (9.5%) (Table 3).

Two cases of aspergillosis were diagnosed on the basis of antigen surveillance. The first was an adult hospitalized for a second allogeneic transplantation after relapse of acute myeloblastic leukemia. At admission, the initial antigen test gave a value of 0.345, which increased to 0.617 during conditioning chemotherapy and to 6.543 on the day of transplantation. Although amphotericin B therapy was started immediately, the patient developed fever and showed first radiologic abnormalities 2 days later. The patient died rapidly, and the diagnosis of aspergillosis was confirmed at autopsy. The second case was a child treated by intensive chemotherapy followed by an autologous transplantation for Ewing sarcoma. Antigenemia assays were first positive 14 days after transplantation, with an index of 1.978. Two days later, the patient developed fever and cough, and chest CT scans showed a condensation, clusters of micronodules, and pleural effusion. In total, nine antigenemia assays were positive for this patient during the course of the infection. The infection was resolved with amphotericin B followed by itraconazole. As no positive fungal cultures were isolated, the episode was classified as possible aspergillosis.

Nine false-positive results (75.0%) were recorded in the group of 12 children. Seven of these were repeatedly positive (up to 15 times), resulting in unjustified CT scans and therapy with amphotericin B. After 6 months, considering the high rate of false-positive results, the detection of GM as a routine surveillance assay in transplanted children was abandoned.

Nine adults (4.5%) had a false-positive result. Eight of these had a single positive antigenemia test during their surveillance period, and repeat control tests in these patients were negative. The last patient had five consecutive positive tests out of 23 samples collected while receiving itraconazole prophylaxis. Nine further antigen determinations were negative. There were no clinical signs of aspergillosis, and chest and sinus CT scans showed no abnormalities, so diagnosis of IA was considered unlikely.

Global Analysis

Specificity. Overall, specificity of the ELISA test was 94.8% (607 of 640). Specificity was higher in adults (587 of 598 [98.2%]) than in children (20 of 42 [47.6%]) ($P < .0001$). Specificity was lower in adult allo-HSCT recipients (114 of 122 [93.4%]) than in adult auto-HSCT recipients or nontransplant patients (473 of 476 [99.4%]) ($P = .0002$).

Sensitivity. Overall, 153 cases of IA were observed during the study period (definite, 31 cases; probable, 67 cases; and possible, 55 cases). Antigenemia was positive in 45 cases: 14 had only one positive test (mean number of samples, 7.6) and 31 had two or more positive tests (mean number of samples, 9.9). One hundred eight patients had no positive test (mean number of samples collected, 6.7). The sensitivity of the antigen determination was 20 (64.5%) of 31, 11 (16.4%) of 67, and 14 (25.5%) of 55 for the definite, probable, and possible cases, respectively (Table 4).

Serologic tests for antibodies were performed at onset of infection in 150 episodes of IA. Tests were negative in 96 cases (64%) and positive in 54 (36%). Sensitivity to the antigen was lower in patients positive for anti-*Aspergillus* antibodies than in patients with negative serology ($P = .04$) (Table 4).

Positive predictive value. Overall positive predictive value (PPV) was 57.7% (45 of 78). PPV was higher in adult nonallogeneic patients (35 of 38 [92.1%]) than in adult allo-HSCT recipients (six of 14 [42.9%]; $P = .0001$) and in children (four of 26 [15.4%]; $P < .0001$).

Negative predictive value. Overall negative predictive value (NPV) was 84.9% (607 of 715). In adults, NPV was lower in nonallogeneic transplanted patients (473 of 572 [82.7%]) than in allo-HSCT recipients (114 of 121 [94.2%]; $P = .001$). In children, the NPV was 96.7% (20 of 22), not different than in adults ($P = .56$).

Table 4. Sensitivity of *Aspergillus* Galactomannan ELISA in Invasive Aspergillosis According to the Level of Certainty and to the Presence of Anti-*Aspergillus* Antibodies at Onset of Infection, Using the Manufacturer's Recommended Cutoff Value of 1.500

	All Patients		Anti-*Aspergillus* Antibodies*			
			Absent		Present	
	No.	%	No.	%	NO.	%
Definite IA	20/31	64.5	16/21	76.2	4/10	40.0
Probable IA	11/67	16.4	7/38	18.4	4/28	14.3
Possible IA	14/55	25.5	11/37	29.7	2/16	12.5
All episodes	45/153	29.4	34/96	35.4	10/54	18.5†

*Three cases were not tested for antibodies.

†$P = .04$ between cases with and without anti-*Aspergillus* antibodies.

Determination of a new clinically relevant cutoff. As specificity of the GM ELISA was low in children and in adult allo-HSCT recipients and there were few cases of IA in these two subgroups, analysis was focused on adults who had not undergone allo-HSCT. The sensitivity, specificity, PPV, NPV, and clinical efficiency of the assay was evaluated at six selected cutoff points in adults who had not undergone allo-HSCT (Table 5). The lowest selected cutoff was 0.600, a value approximately equal to the mean test score + 2 × SD in noninfected patients. Compared with the recommended cutoff point of 1.500, a new cutoff set at 0.700 gave the best compromise between increased sensitivity (+24%) and reduced specificity (−5.5%). The cutoff point of 0.700 increased the clinical efficiency of the test by 1.0% (Table 5). This new cutoff was approximately equivalent to the mean test score + 3 × SD of the test scores in noninfected adults.

Using the new cutoff point of 0.700, the sensitivity of the assay was calculated in the population of adult patients who had not undergone allo-HSCT, according to both the level of certainty of diagnosis and the presence (or absence) of anti-*Aspergillus* antibodies (Table 6).

DISCUSSION

This study represents a large-scale evaluation of *Aspergillus* GM ELISA in both adult and pediatric cancer patients. The assay was evaluated over a 4-year period in four different patient groups, representative of patients admitted to oncology and hematology departments. A total of 153 cases of IA were diagnosed during the study period, making this the largest GM ELISA study yet reported. There were strong discrepancies between the present study and previous publications, in terms of assay sensitivity.

The first report of GM ELISA suggested a sensitivity of 100% in nine patients.[6] Sensitivity was similar (90% and 100%) in two other small studies of 10 and six cases of invasive disease, respectively.[5,7]

A large study of GM ELISA has been performed in allo-HSCT patients.[9] Nineteen (76%) of the 25 patients with definite aspergillosis and 14 (93%) of the 15 patients with probable aspergillosis had positive antigenemia tests. Similar results were reported by the same authors in a second study, which included children and HSCT patients.[11] Recently, Maertens et al[12] reported a sensitivity of 89.7% in 39 probable and proven cases of IA. In an earlier publication, a similar value (92.3%) was reported in 27 autopsy-proven cases of IA.[13]

The lowest sensitivity of the GM ELISA assay has been reported in allo-HSCT recipients (60%), in liver transplant recipients (56%), and in patients with various other conditions including nonmalignant diseases (52%).[14-16] The re-

Table 5. Impact of Modified ELISA Cutoff Values in Adult Nonallogeneic HSCT Patients

	Cutoff					
	1.500 (%)	1.000 (%)	0.900 (%)	0.800 (%)	0.700 (%)	0.600 (%)
Sensitivity						
Definite IA (n = 26)	57.7	61.5	61.5	65.4	73.1	76.9
Probable IA (n = 61)	16.4	26.2	31.1	36.1	44.3	49.2
Possible IA (n = 47)	21.3	31.9	34.0	36.2	44.7	53.2
All cases (n = 134)	26.1	35.1	38.1	41.8	50.0	56.0
Specificity	99.4	98.5	97.5	95.4	93.9	88.7
PPV	92.1	87.0	81.0	71.8	69.8	58.1
NPV	82.7	84.4	84.8	85.3	87.0	87.7
Clinical efficiency*	83.3	83.8	84.4	83.6	84.3	81.5

*Clinical efficiency: (true-positive + true-negative)/total number of tests.

Table 6. Sensitivity of *Aspergillus* Galactomannan ELISA, According to Level of Certainty of Diagnosis, and to Presence of Anti-*Aspergillus* Antibodies at Onset of Infection in Adult Nonallogeneic HSCT Patients, Using the Newly Defined Cutoff Value of 0.700

	All Patients		Anti-*Aspergillus* Antibodies*			
			Absent		Present	
	No.	%	No.	%	No.	%
Definite IA	19/26	73.1	13/17	76.5	6/9	66.7
Probable IA	27/61	44.3	20/34	58.8	7/26	26.9
Possible IA	21/47	44.7	17/32	53.1	4/14	28.6
All episodes	67/134	50.0	50/83	60.2	17/49	34.7†

*Two cases were not tested for antibodies.

†$P = .007$ between cases with and without anti-*Aspergillus* antibodies.

sults of the present study, in which the overall sensitivity of the GM assay was only 29.4%, differ considerably from these previous reports. Although the mean number of tests per episode was slightly lower in the present study than in other reports, this cannot explain the low sensitivity in probable cases of IA, compared with definite cases, as similar numbers of tests were conducted in these patient groups (mean, 6.8 and 7.5 tests per episode, respectively).

In order to explain such a major discrepancy in sensitivity, it is important to differentiate between cases, according to the level of certainty in their diagnosis. Sensitivity was generally much higher in definite cases than in probable or possible cases of IA. A large proportion of the possible cases, as defined by the EORTC/MSG criteria, are likely to be either nonfungal infections or non-*Aspergillus* fungal pneumonia. In a patient population defined in terms similar to those in the present study, Maertens et al[12] reported a sensitivity of only 7.4%.

The low sensitivity of the assay in patients with probable IA remains a matter of concern. According to the EORTC/MSG criteria, probable cases are defined by either a positive microscopic evaluation or culture of a respiratory tract sample or a positive histopathologic evaluation. It is likely that many of these probable cases are indeed true episodes of IA, although it is impossible to estimate precisely this proportion. Of the 67 probable cases of IA in the present study, 19 were defined by a single positive sputum or tracheal aspiration culture; these patients also had clinical and radiologic signs of pulmonary infection. The fact that only one (5.3%) GM ELISA was positive in these 19 patients suggests that the use of a single positive sputum or tracheal aspiration culture in the diagnosis of IA may not be appropriate. It has been suggested previously that diagnosis on the basis of a single positive culture may lead to an overestimation in the number of cases of IA.[17] In accordance with the EORTC/MSG criteria, we kept these cases in the probable group.

Although disease definition is a crucial issue, there were also several false-negative results in cases of proven IA in the present study. It was clear that the presence of anti-*Aspergillus* antibodies significantly lowers the sensitivity of the GM ELISA test. This has not been described previously for *Aspergillus* antigenemia, although a similar trend has been reported for *Candida* mannan.[18] Few patients with proven candidiasis expressed mannan and antimannan antibodies concomitantly. Furthermore, the appearance of antibodies during the course of the disease was accompanied by a reduction in mannanemia. This is consistent with increased hepatic clearance of *Aspergillus* GM in immunized animals.[19]

The detection of anti-*Aspergillus* antibodies in 36% of patients with possible, probable, or definite IA at the onset of infection is unexpected, as it is widely accepted that severely immunocompromised patients are not able to produce a significant antibody response.[20] Sensitivity of serologic tests in patients with IA is low, ranging from 14% to 36%, depending on the test used.[21] This low sensitivity makes such tests of little use in the diagnosis of IA, except for cases in which the presence of antibodies interferes with the sensitivity of other tests, such as the GM ELISA. In the present study, the sensitivity of the GM ELISA was clearly higher in patients without anti-*Aspergillus* antibodies. As a consequence, in patients with suspected IA, serologic tests should always be performed in conjunction with the antigenemia test, as these can be used to explain false-negative GM ELISA results.

The cutoff value for the GM ELISA kit used in the present study was set by the manufacturer at 1.500, although this has been reduced to 1.000 previously.[12,13] On the basis of the present study, a further reduction, to 0.700 is suggested for adult patients who have not undergone allogeneic transplantation. This proposal is supported by a significant increase in sensitivity (+24%), with only a low decrease in specificity (−5.5%) and an increase in the clinical efficiency of the test. Even with the reduced cutoff score, the PPV for the test remains close to 70% in the whole population, and over 90% in patients with signs of respiratory infection. Considering the high mortality of the

disease, the improved prognosis with early therapy, and the development of better-tolerated anti-*Aspergillus* agents, it is preferable to initiate therapy in patients at high risk for IA, even if their diagnosis is not later confirmed.

For all our calculations, we have accepted a single positive result as a diagnostic criterion. This contrasts with the usual practice observed in other studies and with the recommendations of the EORTC/MSG classification, which suggest that at least two positive results are required to consider the test as a diagnostic criterion.[10] This restriction is probably useful in the two subpopulations with a high false-positive rate (children and allo-HSCT recipients), but does not seem to be necessary for the adult non–allo-HSCT patients. In this latter group, the specificity of the test is high, and asking for two positive results would significantly decrease the sensitivity.

The causes of the numerous false-positive results in the present study, especially in children, remain unclear. We have previously hypothesized that dietary contamination by GM may be a cause of positive antigenemia, and this is supported by the presence of high amounts of GM in the foods (especially cereals and their derivatives) supplied to children with positive tests.[22] Furthermore, GM is heat resistant, and is not eliminated from food by sterilization. Passage of dietary GM into the blood from the intestinal tract could also explain false-positive results in allo-HSCT recipients, as these results occur mainly during the first month after transplantation, when chemotherapy-induced mucosal lesions are maximal.

This explanation may not be the only reason for false-positives, as a false-positive rate of 83% has been reported in premature infants, who are unlikely to have been exposed to foodstuffs contaminated with GM.[16] ELISA cross-reactivity has been observed with other fungi, including *Penicillium* species, *Paecilomyces variotii*, and *Alternaria* species. False-positive reactions have also been seen in bacteremic patients.[23] In the present study, no relationship could be established between false-positive results and non-*Aspergillus* infection.

Analysis of the data by risk factor (reason for undertaking antigenemia testing) indicated that only one case of IA was diagnosed among the 261 episodes of FUO. These results suggest that routine GM ELISA is not useful in patients with febrile neutropenia with no clinical or radiologic signs suggestive of a pulmonary infection.

GM ELISA led to only two diagnoses of IA during the surveillance period for 211 HSCT patients, with each diagnosis preceding the onset of fever by 2 days. This time period between diagnosis and the onset of fever could probably be increased by sampling sera twice weekly, which seems to be a more suitable frequency.[24]

All high-risk patients with a respiratory tract infection or suspected extrapulmonary aspergillosis should be repeatedly tested with GM ELISA, as the PPV of the assay was highest in these patient groups. Positive GM ELISA tests could lead to an earlier appropriate treatment for these patients.

It remains controversial whether IA can be diagnosed by GM ELISA before any clinical or radiologic signs develop. Sulahian et al[9] reported positive antigenemia for more than 30 days before the presentation of clinical or radiologic signs. In contrast, Williamson et al[25] reported a mean delay of 12 days between the first positive test and the onset of fever. In the present study, only two GM ELISA tests were positive before the presentation of the first clinical or radiologic signs of IA. In both cases, detection of GM preceded the clinical signs by only 2 days. Positive GM tests 1 month before the first clinical signs of disease are of questionable reliability, considering the low specificity of the test in allo-HSCT recipients and the often rapid progression of disease in these patients.

Initial enthusiasm for the *Aspergillus* GM test as a diagnostic tool must be tempered, as the sensitivity of the assay is lower than expected. Sensitivity could be improved by lowering the proposed assay cutoff value in adults not undergoing allo-HSCT, with only minor reductions in specificity. The presence of anti-*Aspergillus* antibodies should be assessed, even in cancer patients, as their presence can explain a significant proportion of false-negative reactions.

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