

Use of Circulating Galactomannan Screening for Early Diagnosis of Invasive Aspergillosis in Allogeneic Stem Cell Transplant Recipients

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Screening for galactomannan (GM) has been adopted by many European centers as part of the management plan for allogeneic stem cell transplant recipients. However, the temporal onset of GM antigenemia remains unknown. A series of allogeneic stem cell transplant recipients were monitored prospectively, and the relationship between antigenemia and other diagnostic triggers for initiation of antifungal therapy was analyzed. GM detection had a sensitivity of 94.4% and a specificity of 98.8%. Positive and negative predictive values were 94.4% and 98.8%, respectively. This statistical profile was better than that of other triggers, including unexplained fever, new pulmonary infiltrates, isolation of *Aspergillus* species, and abnormalities seen on computed tomography. Antigenemia preceded diagnosis on the basis of radiologic examination or *Aspergillus* isolation by 8 and 9 days in 80% and 88.8% of patients, respectively. Antigenemia preceded therapy in 83.3% of patients. Detection of GM was especially useful when patients were receiving steroid treatment or when coexisting conditions masked the diagnosis of invasive aspergillosis. Prospective screening for GM allows earlier diagnosis of aspergillosis than do conventional diagnostic criteria.

Allogeneic hematopoietic stem cell transplantation (ASCT) has become a valuable treatment option for an increasing number of patients with malignant and nonmalignant blood disorders [1]. However, this procedure may be complicated by life-threatening conditions, including the development of invasive aspergillosis (IA) [2–4]. The crude mortality rate of this infection is dramatically high and may—at least among allogeneic stem cell transplant recipients—still exceed 80%, despite the introduction of itraconazole and/or the use of lipid-based formulations of amphotericin B [5–7]. This high fatality rate reflects partly the difficulty of establishing a reliable diagnosis at an early stage of the disease; definite diagnosis is not straightforward and usually requires (semi-)invasive procedures that are often precluded by thrombocytopenia or by the critical condition of the patient [8]. Therefore, diagnosis is rarely established during the stages of infection in which the patient has a low fungus burden, when therapy may be most effective. Moreover, autopsy surveys have revealed that a significant portion of cases of IA remain undiagnosed and untreated at death [9,

10]. It has been suggested that survival might be improved by earlier diagnosis followed by prompt treatment [11].

Given the lack of diagnostic certainty, and considering the mortality rate associated with established disease, empirical initiation of antifungal therapy has been advocated, in particular for neutropenic patients with fever who do not respond to broad-spectrum antibiotics [12, 13]. However, this recommendation has never been firmly validated [14, 15] and will, almost inevitably, result in overtreatment, additional toxicity and cost, and alterations of microbial ecology [16–18].

Over the past decade, 2 advances in the diagnosis of IA have been made, especially for diagnosis in patients with hematological disorders [19]. First, the use of high-resolution computed tomographic (HRCT) scanning of the chest may reveal a so-called “halo” sign. This lesion occurs early in the course of IA and corresponds to a central fungal lesion surrounded by a rim of coagulation necrosis and hemorrhage [20]. But, although the presence of this sign is strongly suggestive, it is not pathognomonic for aspergillosis and can also be found in other fungal, as well as bacterial, infections. Second, several European groups have advocated serial screening for galactomannan (GM), a fungal exoantigen released by all pathogenic *Aspergillus* species during growth. Circulating GM can now be detected by a sandwich ELISA that has become commercially available outside the United States [21–23].

However, analyses that have looked at the performance and temporal onset of antigenemia relative to other clinical and microbiological triggers for initiation of antifungal therapy are lacking. The present study complements a study of ours published elsewhere [24] and examines these characteristics, focusing on allogeneic stem cell transplant recipients, who frequently

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suffer from dual infections or concomitant conditions, which makes correct diagnosis even more troublesome.

Patients and Methods

Study population. Between January 1997 and February 2001, 100 consecutive adult patients with hematological disorders underwent myeloablative ASCT at the University Hospital Gasthuisberg in Leuven, Belgium. Patients who received transplants before February 2000 were included in a previous analysis that examined only the statistical profile of the GM assay [24]. Patients <16 years of age, recipients of an autologous transplant, and patients undergoing nonmyeloablative conditioning were excluded. Patient characteristics, including demographic data, underlying disease, donor type, source of stem cells, conditioning regimen, risk stratification, and use of corticosteroids for treatment of graft-versus-host disease (GVHD), are shown in table 1. The mean duration of neutropenia (absolute neutrophil count, <500 neutrophils/ μ L) was 18.7 days (median, 18 days; range, 7–45 days); the mean number of days patients had a neutrophil count <100 neutrophils/ μ L was 14.1 (median, 13.5 days; range, 4–39 days). Patients with acute leukemia (AL) in first remission, chronic myelogenous leukemia (CML) in first chronic phase, myelodysplastic syndrome (MDS) with refractory anemia, and MDS with ringed sideroblasts were considered to be at standard risk. Patients with all other diagnoses and with more-advanced AL, CML, and MDS were considered to be at high risk for transplant-related complications.

The study population was followed up until 1 July 2001 (median follow-up time, 333 days; range, 10–1551 days). The overall mortality rate during the 54-month study period was 59%. Autopsy was performed for 56 (95%) of 59 patients who died during the study period. Eleven patients died within the first month after transplantation, 29 patients died between posttransplantation days 30 and 180, and 19 patients died >6 months after transplantation. The early (within 100 days) transplant-related mortality (all causes of nonrelapse death) was 30%.

Study procedures. All patients were hospitalized in single rooms in a unit supplied with high-efficiency particulate air filters from the beginning of the conditioning regimen until stable engraftment had been achieved. Patients readmitted to the hospital for management of complications could be housed in rooms without air filtration. Antimicrobial prophylaxis during neutropenia consisted of ofloxacin (200 mg every 12 h) and antifungal prophylaxis with either itraconazole capsules (400 mg daily; no dose adaptation according to serum levels) or a combination of aerosolized and low-dose intravenous conventional amphotericin B (0.1 mg/kg). If patients were unable to tolerate oral medication, oral itraconazole could be replaced by intravenous fluconazole (400 mg once daily). Antifungal prophylaxis was continued until the absolute neutrophil count reached at least 500 neutrophils/ μ L for 3 consecutive days. However, prophylaxis was prolonged or reinstated in patients who received steroids for the treatment of GVHD. Health care workers and visitors carried out hand washing procedures before entering the room and wore gloves, masks, and gowns. Most medical procedures were performed in the room; if a procedure had to be done outside the room, gowns and masks were placed on patients before they left the unit.

Table 1. Characteristics of 100 allogeneic stem cell transplant recipients included in a study of the value of galactomannan screening in diagnosis of aspergillosis.

Characteristic	Value
Male sex	67
Age at transplantation	
Mean years (range)	35.6 (17–58)
Age group distribution	
<20 years	12
20–29 years	23
30–39 years	22
40–49 years	31
>50 years	12
Underlying disease	
Acute leukemia	39
Chronic myelogenous leukemia	21
Myelodysplastic syndrome	14
Lymphoma	16
Myeloma	4
Aplastic anemia	4
Other ^a	2
Risk status at transplantation	
Standard risk	45
Poor risk	55
Duration of neutropenia, mean days (range)	18.7 (7–45)
Stem cell source	
Bone marrow	50
Peripheral blood stem cells	50
Conditioning regimen	
Cyclophosphamide/TBI	32
Cyclophosphamide/busulfan	22
Cyclophosphamide/cytarabine/TBI	34
Other	12
Receipt of TBI	75
Donor	
Matched, related donor	74
HLA-mismatched or unrelated donor	26
Receipt of steroids (>20 mg/day of prednisolone equivalent) at stratification	63

NOTE. Data are no. of patients, unless otherwise indicated. TBI, total body irradiation.

^a Thalassemia or polycythemia.

Patients were monitored daily for the development of signs and symptoms of infection. Temperature was measured at least every 6 h. Oropharyngeal washes, as well as stool and urine specimens, were analyzed weekly for the detection of colonization with gram-negative bacteria or fungi. Portable chest x-rays were done weekly in the rooms. Additional x-rays were done at the first day of a febrile episode and when clinically indicated. In cases of persistent fever, chest x-rays were repeated 2–3 times weekly. Antibacterial monotherapy with ceftazidime or meropenem was started at the onset of neutropenic fever (according to published guidelines [25]), after clinical examination was done and samples were obtained for culture of blood, urine, and other specimens. Blood cultures were performed daily until defervescence. In patients who remained febrile after 48–72 h, a glycopeptide antibiotic was added to the treatment regimen. Antifungal therapy was started when fever persisted after 5–7 days of adequate antibiotic therapy or when 1 of these criteria was fulfilled: development of a new infiltrate on chest x-ray in a patient receiving antibacterial therapy; presence of abnormalities on CT with features of invasive mycosis; isolation of molds from any respiratory tract specimen; sudden intracranial event compatible with invasive fungal infection (IFI); and relapse

of neutropenic fever after an afebrile interval of at least 48 h [26]. Patients with preexisting renal impairment (serum creatinine level >2 mg/dL) who received prophylaxis with fluconazole or low-dose amphotericin B could receive itraconazole intravenously on a compassionate-use basis; all others were treated with conventional amphotericin B (0.7–1.0 mg/kg).

In cases of clinical suspicion of IFI, HRCT scans were performed. The HRCT scans were done on a General Electric Hispeed CT/i (General Electric) scanner, using a thin-section technique and a high-speed reconstruction algorithm. Scanning was done during breath holding at full inspiration. Scan collimation was 1 mm, and scan interspacing was 10 mm. Additional expiratory scans (breath hold at full expiration) were performed for most patients, using 20-mm interspacing. Bronchoscopy and guided bronchoalveolar lavage were performed for patients without severe hypoxemia. No endobronchial biopsy samples were obtained during cytopenia. BAL fluid samples were submitted for bacterial, fungal, mycobacterial, and *Legionella* cultures, immunofluorescent staining for *Pneumocystis carinii*, acid-fast staining, silver staining, and viral cultures. Other diagnostic procedures were done as clinically indicated.

Patients who were seropositive for human herpes virus type 1 or varicella-zoster virus received prophylaxis with acyclovir (5 mg/kg 3 times daily). Patients who were seronegative for cytomegalovirus (CMV) received transfusions of blood and/or platelets that were obtained from CMV-seronegative donors. CMV-negative patients who received a graft from CMV-positive donors and CMV-positive recipients of allografts received high doses of prophylactic acyclovir. Ganciclovir was administered preemptively when CMV was detected in blood samples or from at least 2 consecutive urine samples (shell-vial assay). Trimethoprim-sulfamethoxazole or aerosolized pentamidine was given for the prevention of *P. carinii* pneumonia.

Standard GVHD prophylaxis included cyclosporin A and methotrexate; recipients of HLA-mismatched or unrelated donor transplants also received antithymocyte globulin (ATG). Patients with acute GVHD were treated with methylprednisolone at an initial dosage of 2 mg/kg/day. The dose was increased to >5 mg/kg if disease progressed. Nonresponding patients were given a course of ATG. Patients with extensive chronic GVHD were treated on an outpatient basis with steroids and cyclosporin A or tacrolimus.

Unless the family refused, autopsy was pursued for all patients who died during the study period. Tissue specimens were stained with periodic acid-Schiff or Gomori methenamine silver stain for the detection of hyphal elements.

Definitions. Fungal disease was classified as proven, probable, or possible, according to European Organization for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) definitions (ELISA results were not included) [27]. “Proven IA” referred to the histopathologic evidence of tissue invasion by filamentous fungi and positive results of culture for *Aspergillus* species or the isolation of *Aspergillus* species from a sample (excluding BAL fluid and sinus aspirate) from a normally sterile but clinically infected body site obtained by a sterile procedure. Identification of a non-*Aspergillus* mold (e.g., *Fusarium*) in identical settings was considered to be “proven non-*Aspergillus* fungal infection.” Cases in which histopathologic evidence of invasion was present but culture results were negative were classified as “proven fungal infection.” “Probable

pulmonary IA” implied the presence of positive culture results or cytologic evidence for *Aspergillus* species from a lower respiratory tract specimen, in conjunction with 1 major (halo sign or “air crescent” sign on CT scan) or at least 2 minor (symptoms of lower respiratory tract infection, pleural rub, and presence of any new infiltrate in a patient who did not fulfill the major criterion but for whom no alternative diagnosis was available) clinical findings. The fulfillment of a microbiological criterion or 1 major clinical criterion (or 2 minor clinical criteria) in the appropriate host setting defined “possible IFI.” Study patients with neither clinicoradiologic nor microbiological evidence of fungal disease were classified as having “no evidence of fungal infection.” “Colonization” was defined as positive results of culture for molds from a nonsterile site in patients without any other evidence of fungal infection during follow-up. Classification of patients with probable or possible disease could be upgraded after autopsy or surgical procedures.

Clinical samples. Serum samples for the detection of GM were prospectively collected at least twice weekly from admission until death or discharge. In the posttransplantation period, patients were screened at least once weekly as outpatients, until resolution of GVHD and/or cessation of immunosuppressive drugs. Serum samples could be obtained more frequently when the diagnosis of IA became probable or proven. The ELISA (Platelia *Aspergillus*; Bio-Rad Laboratories) was performed according to the manufacturer’s instructions, as described elsewhere [22]. An OD of ≥ 1.0 was considered to be a positive result; however, a result was considered to be true-positive only when 2 consecutive samples for a patient tested positive. Serum samples were stored at -70°C and analyzed weekly by the same technicians. The results of the ELISAs were reported back to the clinicians once weekly, but decisions regarding treatment or preemptive therapy were left to the discretion of the attending physician.

Statistical analysis. Continuous variables were described by use of statistical characteristics (mean, median, and ranges). Discrete variables were described as counts and percentages. To evaluate the value of GM detection as screening test for IA, sensitivity, specificity, and predictive values were calculated from 2×2 tables. Patients without signs indicating IA and those with autopsy-proven absence of IA were considered true control patients; specificity was calculated from this group. Sensitivity was calculated from patients with proven IA. Positive and negative predictive values were estimated from the combination of the 2 groups. The true disease status of patients with probable or possible IA for whom neither biopsy nor autopsy samples were tested could not be accurately validated.

Results

Stratification of patients. On the basis of antemortem findings and following the EORTC/MSG criteria, patients could receive a diagnosis of proven IA ($n = 5$), probable IA ($n = 8$), or possible IFI ($n = 34$). We also identified 6 patients with proven non-*Aspergillus* IFI (2 with mucormycosis, 1 with *Alternaria* infection, and 3 with candidemia). IFI was not evident in the remaining 47 patients. In 47 (88.7%) of 53 patients with proven, probable, and possible infection, 13 cases could be upgraded after postmortem examination, resulting in 18 proven cases of IA (fig-

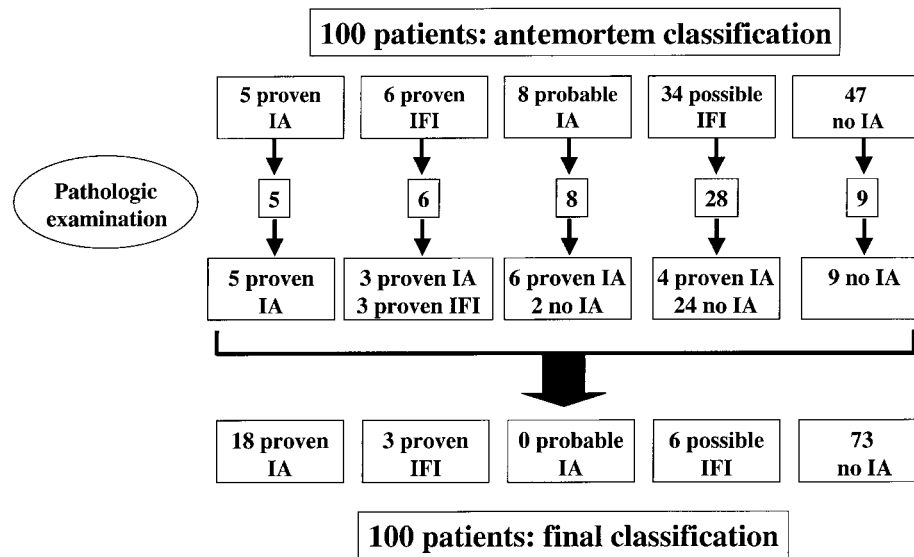


Figure 1. Classification, at study entry (for all patients) and postmortem examination (for 59 patients), of 100 allogeneic stem cell transplant recipients included in a study of the value of galactomannan screening in diagnosis of aspergillosis. IA, invasive aspergillosis; IFI, invasive fungal infection.

ure 1). These additional cases had previously been categorized as probable IA ($n = 6$), possible IFI ($n = 4$), and proven non-*Aspergillus* IFI ($n = 3$; representing dual fungal infections). Alternative diagnoses in the remaining patients initially classified as probable IA or possible IFI included disseminated toxoplasmosis, necrotizing viral pneumonia, *Fusarium* species infection, bacterial pneumonia, marantic endocarditis, malignancy, pulmonary thromboemboli, alveolar proteinosis, diffuse alveolar hemorrhage, and diffuse alveolar damage. Given the absence of autopsy data for 6 patients with possible IFI, the true status of disease in these patients could not be determined. However, open-lung biopsy specimens and brain biopsy specimens from 2 of these patients demonstrated no fungal invasion. Clinoradiologic evidence of fungal infection was absent in 47 patients, of whom 10 eventually died; autopsies were performed in 9 of these cases. Causes of death included hepatic veno-occlusive disease ($n = 2$), malignancy ($n = 5$), CMV pneumonia ($n = 1$), and pulmonary hemorrhage ($n = 1$); no patient showed signs of IFI. After incorporation of these postmortem findings into the analysis, patients were reclassified as follows: 18 patients had proven IA, none had probable IA, 6 had possible IFI, and 76 were without IA. The “no IA” control group also included 3 patients with proven non-*Aspergillus* IFI (*Alternaria* species sinusitis, disseminated *Candida albicans* infection, and *C. glabrata* fungemia).

ELISA screening. Of a total of 2695 serum samples analyzed by ELISA (mean, 26.9 samples/patient; range, 4–81 samples/patient), 240 samples (8.9%) tested positive and 2455 (91%) tested negative. However, positivity was not confirmed by testing of a subsequent consecutive sample for 12 serum samples (5% of all positive results). Two hundred twenty-eight consecutive serum samples (8.5%) in 18 patients tested positive.

When the criterion of 2 consecutive positive results was used, all 5 patients with proven IA (based on antemortem classification) had positive results of ELISA (median, 13 positive serum samples/patient; range, 2–19 positive samples/patient). Six of 8 patients with probable IA were ELISA positive (median, 12 positive serum samples/patient; range, 4–54 positive samples/patient) and 4 of 34 patients with possible fungal infection tested positive (median, 4 positive serum samples/patient; range, 3–16 positive samples/patient). Consecutive positive assays were also found for 3 patients with proven non-*Aspergillus* fungal infection (median, 5 positive serum samples/patient; range, 3–7 positive samples/patient). None of the 47 control patients had 2 consecutive positive ELISA results.

Reanalysis of the data after incorporation of the autopsy findings showed that 17 (94.4%) of 18 patients with proven IA tested positive (median, 10 positive serum samples/patient; range, 2–54 positive samples/patient). Thus, aspergillosis had been detected by ELISA screening before death in 12 of 13 patients whose condition was upgraded to proven IA after autopsy. The remaining seropositive patient who had 3 consecutive positive ELISA results (initially classified as having possible IFI) received a diagnosis of catheter-related *Penicillium* infection (1 false-positive case); testing of open-lung biopsy and autopsy specimens showed no signs of IA. Conversely, an ELISA-negative patient who had been classified as having possible fungal infection proved to be culture and tissue examination positive for *Aspergillus* at autopsy (1 false-negative case). These results are summarized in table 2.

Patients with proven IA. Characteristics of patients with IA are shown in table 3. The median time to onset of aspergillosis after transplantation was 98 days (range, 1–814 days).

Table 2. Results of ELISA screening for galactomannan in 100 allogeneic stem cell transplant recipients.

Data collection point, type of data	Proven IA	Proven non- <i>Aspergillus</i> IFI	Probable IA	Possible IFI	No IA
Antemortem					
No. of patients	5	6	8	34	47
No. of ELISA-positive patients	5	3	6	4	0
ELISA-positive serum samples, median no./patient (range)	13 (2–19)	5 (3–7)	12 (4–54)	4 (3–16)	NA
Combined ante- and postmortem					
No. of patients	18	3	0	6	73
No. of ELISA-positive patients	17	0	0	0	1
ELISA-positive serum samples, median no./patient (range)	10 (2–54)	0	0	0	NA (3)

NOTE. IA, invasive aspergillosis; IFI, invasive fungal infection; NA, not applicable.

Two-thirds of cases were diagnosed after posttransplantation day 50. *Aspergillus fumigatus* was isolated from 17 patients and *Aspergillus versicolor* from 1 patient. Central nervous system involvement was present in 8 patients (44%); this was confirmed by biopsy in 2 patients and by autopsy in 1 patient. In the remainder, brain involvement was presumed on the basis of CT or magnetic resonance imaging findings and *Aspergillus* infection documented at other sites. At diagnosis of IA, 7 patients (39%) were neutropenic, but only 4 (22%) patients received a diagnosis of IA during the pretransplantation neutropenic period. Thirteen patients (72%) with IA received steroids. The all-cause mortality rate was 100% among patients with IA, compared with 50% among control subjects. The median length of survival after the first positive ELISA result was 14 days (range, 2–106 days).

Comparison of GM screening with other diagnostic tools.

The validity of serial screening for GM was compared with other clinical triggers of presumption of IA, such as the presence of fever that does not respond to broad-spectrum antibiotics, the development of new infiltrates on chest x-ray in a patient receiving antibacterial therapy, and the appearance of abnormalities on HRCT that are consistent with infection. Comparison of GM screening with isolation of *Aspergillus* species from BAL fluid and with isolation of *Aspergillus* species from any lower respiratory tract specimen (BAL fluid, bronchial aspirate, or sputum) was also done.

Eleven (61.1%) of 18 patients with proven IA presented with fever refractory to adequate antibacterial therapy, whereas 7 patients (all of whom received steroids) remained persistently afebrile. Twenty-six of 82 control patients also had persistent fever, resulting in a specificity of 68.3%. The positive predictive value of this criterion was only 29.7%, and the negative predictive value was 88.8%. All but 1 patient with IA (94.4%) developed new pulmonary infiltrates visible on conventional chest x-ray. In the control group, new infiltrates were seen in 33 patients, resulting in a specificity of 59.7%. The positive and negative predictive values were 34% and 98%, respectively.

Bronchoscopy with BAL was only performed in patients who fulfilled at least the criterion of having possible fungal infection. *Aspergillus* was isolated from BAL fluid from 9 of 16 patients

with IA who underwent bronchoscopy. BAL fluid yielded *A. fumigatus* in 2 patients who had no evidence of fungal infection on testing of open-lung biopsy and subsequent autopsy specimens. The sensitivity of a positive culture on BAL fluid, calculated using the results of 42 bronchoscopic procedures, was 56.2%; the specificity was 92.3%, the positive predictive value was 81.8%, and the negative predictive value was 77.4%. Isolation of *Aspergillus* species from any lower respiratory tract specimen (including BAL fluid) had a sensitivity of 61.1%, a specificity of 96.3%, a positive predictive value of 78.5%, and a negative predictive value of 91.9% (table 4).

HRCT was performed for 30 patients. Fourteen of 15 patients with IA had abnormalities on CT scan. However, only 5 patients had the characteristic “halo” sign. All of these patients had a neutrophil count <500 neutrophils/mm³ at the time of CT scanning. In the 9 other patients with IA and CT abnormalities, including 7 who were nonneutropenic, ill-defined nodular infiltrates and nonspecific consolidations were observed. In 1 of these patients, follow-up CT scan demonstrated the formation of an “air crescent” lesion. Fourteen of 15 patients without IA also had lesions visible on CT that were compatible with fungal infection, such as ground-glass opacities, ill-defined nodules, and consolidations; however, the typical “halo” sign was observed in 1 patient only. Diagnoses included nocardiosis ($n = 1$), disseminated *Candida* infection ($n = 1$), CMV pneumonia ($n = 1$), adenovirus pneumonia ($n = 1$), disseminated toxoplasmosis ($n = 1$), *P. carinii* pneumonia ($n = 1$), leukemia ($n = 1$), lymphoma ($n = 1$), alveolar hemorrhage ($n = 4$), bronchiolitis obliterans with organizing pneumonia (BOOP; $n = 2$), diffuse alveolar damage ($n = 3$), and microthrombi ($n = 2$); some patients had multiple diagnoses. This resulted in a sensitivity of 93.3%, a very low specificity of 6.6%, and positive and negative predictive values of 50% for CT. However, the presence of a “halo” sign yielded a sensitivity of 33.3%, a specificity of 93.3%, a positive predictive value of 83.3%, and a negative predictive value of 58.3% (table 4).

Seventeen patients with IA had at least 1 positive ELISA result, compared with 12 patients in the control group. The sensitivity and specificity of an unconfirmed positive test result were 94.4% and 85.4%, respectively. The positive predictive

Table 3. Clinical characteristics of 18 patients with proven invasive aspergillosis (IA).

Patient	Fungal disease classification ^a	Sex, age in years	Underlying disease	Type of transplant	ANC, cells/mm ³	Receipt of steroid therapy	Onset, ^b days after transplantation	Survival, ^c days	Treatment ^d
1	Proven (brain)	M, 35	ALL	RD	2139	Yes	108	32	AmB/AmB + 5-FC
2	Proven (brain)	M, 35	AML	RD	700	Yes	65	44	AmB/liposomal Nys
3	Proven (lung)	M, 48	AML	RD	4700	Yes	727	39	Casp
4	Proven (abscess)	F, 26	NHL	MUD	7300	Yes	272	2	Vor/AmB
5	Proven (lung)	M, 27	AML	MUD	200	Yes	551	18	AmB/Vor
6	Probable	F, 51	AML	RD	<500	No	1	85	Itr capsules/AmB/liposomal Nys
7	Probable	M, 33	CML	RD	1056	Yes	122	17	AmB
8	Probable	M, 42	MDS	MUD	0	No	11	106	AmB/Itr oral solution/AmB
9	Probable	F, 34	NHL	MUD	400	Yes	205	17	AmB/Casp
10	Probable	F, 35	ALL	RD	2200	Yes	41	14	Liposomal AmB
11	Probable	F, 48	ALL	RD	1000	Yes	58	8	AmB/liposomal AmB
12	Possible	M, 24	NHL	RD	<100	No	10	10	AmB
13	Possible	M, 19	CML	RD	5000	Yes	88	7	AmB
14	Possible	M, 42	MDS	RD	<500	Yes	132 ^e	NA	AmB
15	Possible	F, 35	AML	RD	16,900	No	29	13	Itr oral solution
16	No IA	M, 38	AML	RD	3100	Yes	814	14	AmB
17	No IA	M, 37	AML	MUD	0	No	5	5	Liposomal AmB
18	No IA	F, 35	CML	MUD	1100	Yes	250	6	Intravenous Itr

NOTE. ALL, acute lymphoblastic leukemia; AmB, amphotericin B; AML, acute myelogenous leukemia; ANC, absolute neutrophil count; Casp, caspofungin; CML, chronic myelogenous leukemia; Itr, itraconazole; MDS, myelodysplastic syndrome; MUD, matched unrelated donor; NA, not applicable; NHL, non-Hodgkin lymphoma; Nys, nystatin; RD, related donor; Vor, voriconazole; 5-FC, 5-fluorocytosine.

^a Stratification was based on antemortem data. The classification system of the European Organization for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group was used [27].

^b Day of first positive ELISA or (patient 11) first isolation of *Aspergillus* from sterile site.

^c Measured from collection of the first of at least 2 consecutive positive samples.

^d Slashes indicate a change in treatment.

^e Day of autopsy; no antemortem diagnosis (negative results of ELISA).

value was 58.6%, and the negative predictive value was 98.6%. Using the criterion of 2 positive ELISA results, specificity and positive predictive value increased to 98.8% and 94.4%, respectively (table 4).

Temporal relationship between GM antigenemia and other triggers. In all 7 patients with proven IA who persistently remained afebrile, a positive antigen test was the first indication of *Aspergillus* infection. Antigen detection preceded ($n = 4$) or coincided with ($n = 2$) the development of fever in 6 of the remaining 11 patients; fever preceded antigenemia in 4 patients by 10, 5, 4, and 3 days, respectively. One febrile patient with aspergillosis remained antigen negative. Antigen detection preceded radiologic changes on conventional chest x-rays for 12 of 15 evaluable patients by a median of 8 days (range, 4–22 days). A positive ELISA result preceded the demonstration of abnormalities on CT scan (typical as well as nonspecific) in 12 of 15 evaluable patients by a median of 6 days (range, 1–12 days). With regard to the isolation of *Aspergillus*, antigenemia preceded the day of collection of the first specimen with a positive culture result in 16 patients by a median of 9 days (range, 2–96 days). One patient had *Aspergillus* isolated from an abscess 59 days before the first detection of GM. Positive ELISA results preceded the initiation of antifungal therapy in 15 patients by a median of 6 days; for 1 patient, initiation of therapy coincided with collection of the first positive sample. In the vast majority of cases (88.8% and 94.4%, respectively), antigenemia preceded a definite diagnosis (based on histopathologic examination and

culture) and death due to or during IA by a median of 14 days (table 5).

Confounding factors. Invasive aspergillosis frequently coincided with other medical conditions that mimicked or masked the clinical diagnosis of aspergillosis. Confounding factors, both infectious and noninfectious, that could also explain the presence of fever and/or the development of pulmonary infiltrates were present at the time that antigen positivity was found in 15 patients. These factors are described in table 6.

Discussion

Although significant center-to-center variation exists, the incidence of IA following ASCT has increased steadily since 1990 and now surpasses the number of invasive *Candida* infections [28, 29]. The high incidence seen in our series has also been reported at other health care centers—both in Europe and in the United States—and can be explained by several findings [29, 30]: First, one-fourth of our patients received a graft from an alternative donor, a group known to be at greater risk of developing IFIs than patients with matched, related donors [3]. Second, established risk factors for early and late IA were present in the majority of our patients, including age >40 years (43%), use of steroids (63%), prolonged pretransplantation neutropenia (mean, 18.7 days), and poor underlying disease status at transplantation (55%) [29]. Third, given the aggressive nature of the reference diagnostic standard, the true incidence remains

Table 4. Comparison of galactomannan antigenemia relative to clinical triggers of invasive aspergillosis (IA) and results of respiratory tract specimen cultures.

Indicator	No. of patients		Value of indicator in diagnosing IA			
	With proven IA (n = 18)	Without IA ^a (n = 82)	Sensitivity	Specificity	PPV	NPV
Persistent fever	11	26	61.1	68.3	29.7	88.8
Infiltrate visible on chest x-ray	17	33	94.4	59.7	34.0	98.0
Positive culture for <i>Aspergillus</i>						
BAL fluid ^b	9	2	56.2	92.3	81.8	77.4
Any specimen	11	3	61.1	96.3	78.5	91.9
Results of CT ^c						
Any abnormality	14	14	93.3	6.6	50.0	50.0
“Halo” sign	5	1	33.3	93.3	83.3	58.3
Positive results of 1 ELISA	17	12	94.4	85.4	58.6	98.6
Positive results of >1 ELISA	17	1	94.4	98.8	94.4	98.8

NOTE. BAL, bronchoalveolar lavage; NPV, negative predictive value; PPV, positive predictive value; CT, computed tomography.

^a Including 6 patients with possible fungal infection (final analysis); no autopsy data were available.

^b Sixteen patients with IA and 26 patients without IA underwent bronchoscopy.

^c A CT scan was done for 15 patients with IA and 15 patients without IA.

undetermined and undoubtedly is underestimated in many epidemiological surveys. However, by maintaining a high rate of autopsy performance (95%), we were able to discriminate between presumed diagnoses of IFI and other etiologies in a series of clinically highly suspected patients, and we confirmed 13 additional cases of IA. Fourth, construction and renovation activities in and around the hospital may have further increased the risk of infection during the study period [31].

As has been found in other reports, the survival rate of patients with IA was dramatically low in the present study, despite the empirical use of amphotericin B and the implementation of investigational antifungal agents [32, 33]. This high case-fatality rate reflects partly the detrimental delay in diagnosis of pulmonary disease, which leads to a high frequency of extrapulmonary dissemination. It is hypothesized that earlier diagnosis, followed by earlier therapy, could reverse the dismal outcome of IA [34]. The present study demonstrates that prospective screening for serum GM by a sandwich ELISA technique makes earlier diagnosis possible and contributes more than other diagnostic criteria to the final diagnosis.

To date, a high index of clinical suspicion for IA, based on knowledge of risk factors and local epidemiology, remains of the utmost importance. Signs of angioinvasion are frequently absent at the early stages of disease, and attempts to make a diagnosis may be thwarted by blunted host inflammatory responses [35]. Therefore, a considerable proportion of invasive fungal infections remain undiagnosed until after death [9, 10, 36]. In the mid-1980s, Gerson et al. [37] developed a discriminant scorecard for the diagnosis of pulmonary aspergillosis in neutropenic patients with leukemia. Many of these criteria still trigger a diagnostic workup in stem cell transplant recipients. Unfortunately, as was also demonstrated in the present study, some of these triggers have low positive predictive value. Persistent fever is a highly imprecise criterion, especially in non-neutropenic ASCT recipients; it may be related to GVHD or

a malignancy, or it may be caused by inadequately treated or undiagnosed nonfungal infections. In addition, fever is frequently absent in patients receiving steroid treatment [38]. Plain chest x-ray lacks specificity, and, although the sensitivity appears to be high, infiltrates only develop at advanced stages of disease or after neutrophil recovery [39, 40]. *Aspergillus* species, although frequently isolated from respiratory tract products, are not always implicated in pathologic processes. Among severely immunocompromised patients, positive predictive values for isolation of *Aspergillus* from respiratory tract specimens range from 70% to 80% (78.5% in our study) [41, 42]. However, the sensitivity is low, especially among patients with focal pulmonary disease; most allogeneic stem cell transplant recipients who present with *Aspergillus* in respiratory specimens have al-

Table 5. Temporal onset of antigenemia in patients with proven invasive aspergillosis (IA).

Time point	No. of evaluable patients	No. (%) of patients with antigen ^a	Days between antigen detection and time point, median (range)
First day of fever	11 ^b	6 (54.5)	3.5 (0–19)
First indication on chest x-ray	15 ^c	12 (80)	8 (4–22)
High-resolution pulmonary CT scan	15 ^d	12 (80)	6 (1–12)
Collection of first sample with positive culture results	18	16 (88.8)	9 (2–96)
Initiation of antifungal therapy	18	16 (88.8)	6 (0–14)
Definite diagnosis of IA	18	16 (88.8)	14 (5–106)
Death	18	17 (94.4)	14 (2–106)

NOTE. CT, computed tomography.

^a At or before time point.

^b Seven patients remained afebrile.

^c One patient had residual lesions from a previous episode of IA, and 2 patients were not evaluable because of preexisting abnormalities visible on chest x-ray (pulmonary non-Hodgkin lymphoma and bronchiolitis obliterans).

^d A high-resolution pulmonary CT scan was not performed for 3 patients receiving mechanical ventilatory support.

Table 6. Alternative explanations of fever and/or pulmonary infiltrates in 18 patients with invasive aspergillosis.

Patient	Concomitant conditions
1	<i>Staphylococcus epidermidis</i> bacteremia and probable toxoplasmosis
2	CMV pneumonia and persistent CMV viremia
3	<i>Streptococcus sanguis</i> bacteremia, CMV viremia, HHV-6 infection, and isolation of <i>Nocardia</i> species from BAL fluid
4	Relapse of pulmonary non-Hodgkin lymphoma and bronchiolitis obliterans with organizing pneumonia
5	Posttransplantation lymphoproliferative disorder
7	Uncontrolled GVHD grade III treated with ATG, alveolar proteinosis, and CMV pneumonia
8	<i>S. epidermidis</i> bacteremia
9	<i>Escherichia coli</i> bacteremia, isolation of <i>Nocardia asteroides</i> from pleural fluid, and CMV viremia
10	CMV pneumonia, prolonged <i>S. epidermidis</i> bacteremia, and TTP
11	<i>Legionella pneumophila</i> pneumonia, <i>Enterococcus faecium</i> bacteremia, and CMV viremia
12	Relapse of anaplastic non-Hodgkin lymphoma
13	<i>Klebsiella pneumoniae</i> bacteremia and pneumonia, <i>Candida</i> peritonitis, uncontrolled GVHD treated with ATG, probable toxoplasmosis, and isolation of HHV-6 from BAL fluid
16	<i>S. epidermidis</i> bacteremia, proven mucormycosis, CMV viremia, and isolation of <i>Mycobacterium scrofulaceum</i> from BAL fluid and sputum
17	<i>Candida tropicalis</i> fungemia, isolation of <i>Cryptococcus</i> and Herpesviridae from BAL, and hepatic veno-occlusive disease
18	Proven mucormycosis and uncontrolled GVHD grade IV

NOTE. ATG, antithymocyte globulin; BAL, bronchoalveolar lavage; CMV, cytomegalovirus; GVHD, graft-versus-host disease; HHV-6, human herpes virus type 6; TTP, thrombotic thrombocytopenic purpura.

ready progressed to frank invasive disease with multifocal or bilateral radiologic abnormalities [41, 42]. Of note, our data on the diagnostic yield of bronchoscopy and BAL (56%) match well with data from a recent EORTC survey (59%) [43] and findings published by Jantunen et al. (57%) [44].

Therapy is often initiated on the basis of these low-predictive value and poorly sensitive criteria. However, not all allogeneic stem cell transplant recipients have the same risk of developing IFI; therefore, such an approach may be associated with significant overtreatment and may result in increased toxicity, induction or selection of drug resistance, and higher medical costs [45]. A higher index of diagnostic certainty would be welcome. The availability of more-sensitive methods for detecting early infection would allow application of more-targeted therapy that is based on a battery of clinical, radiologic, and microbiological data (but not histopathologic proof) and directed toward the highest-risk patients [18].

The early and repeated use of HRCT has been advocated for the diagnosis of pulmonary infections [19]. The “halo” sign appears to be highly indicative of angioinvasive aspergillosis in neutropenic patients and occurs early in the course of disease [20, 46]. As suggested by Caillot et al. [47], an HRCT-based approach improves the diagnosis of probable IA and might have a beneficial impact on survival in febrile neutropenic patients. However, the CT findings of airway aspergillosis—an emerging manifestation—are far less specific; they are similar to the findings seen in patients with bacterial, *Mycoplasma*, or viral bronchiolitis or bronchopneumonia [48]. Moreover, it should be emphasized that the usefulness of HRCT has been validated almost exclusively for neutropenic patients [49]. Our study, as well as others, indicates that similar CT findings lack specificity and predictive value in nonneutropenic stem cell transplant recipients [50]. IA can also manifest as ground-glass opacities or nodular lesions without “halo” sign. In addition, nodular lesions (even when “halo” sign is present) may represent parasitic infection, pulmonary involvement by lymphoma or leukemia, *Nocardia*

infection, obliterative bronchiolitis, and pulmonary thromboemboli [50]. Similarly, diffuse ground-glass opacities can also be seen in association with viral and parasitic infections, BOOP, acute respiratory distress syndrome, and GVHD. Hence, although HRCT may provide more details about the etiology of lung infiltrates in neutropenic patients [49], most stem cell transplant recipients are no longer granulocytopenic when the presumed diagnosis of fungal infection is made. In this case, CT studies can be helpful in findings signs of pulmonary infection and guiding invasive procedures but may not determine the underlying etiology or causative microorganism.

Non-culture-based techniques have focused on the detection of surrogate markers such as fungal DNA [51, 52] and circulating GM, an exoantigen released during invasive disease. A number of techniques, such as EIAs [53], RIAs [54], and latex particle agglutination tests [55], have been evaluated for the detection of GM. However, their routine use has been hampered by poor sensitivity, resulting in the detection of GM only at advanced stages of the disease, when antifungal therapy is no longer useful [56]. Stynen et al. [57] have introduced a sandwich ELISA technique that has a lower threshold of detection of GM than does latex agglutination. We have demonstrated, in the present study, that screening for GM by sandwich ELISA helps to establish an earlier diagnosis of IA after ASCT in adults. The high sensitivity and negative predictive value have been reported elsewhere [21–24, 58]. The positive predictive value was found to be rather low but could be significantly improved by using the criterion of 2 consecutive positive ELISA results, a criterion also used in polymerase chain reaction–based screening [51]. More important, antigenemia preceded clinical diagnosis of IA on the basis of radiologic findings by a median of 8 days and on the basis of a positive *Aspergillus* isolate by a median of 9 days in 80% and 88.8% of cases, respectively. A positive ELISA result preceded the institution of antifungal therapy in 83.3% of patients by a median of 6 days, and the median length of survival after the first positive ELISA result

was 14 days. Whether more-frequent sampling (e.g., 3 times weekly or even daily) or lowering of the threshold for positivity (as recently was suggested by the Strasbourg group for patients at risk of aspergillosis who were not transplant recipients [59]) would further improve the chances of early diagnosis remains to be investigated.

A broad spectrum of coexisting medical conditions, both infectious and noninfectious, that mask the clinical diagnosis of IA was present in our group of patients. In all but 1 of these cases, antigen detection was the first sign of IA. Hence, this test should be performed sequentially as a screening test, rather than as a confirmatory test, when fungal infection is anticipated on clinical grounds. A confirmed positive result (especially an increase in the GM antigen titer) in allogeneic stem cell transplant recipients should encourage clinicians to start anti-*Aspergillus* therapy preemptively, even in the presence of alternative explanations for fever or pulmonary infiltrates [60]. Conversely, if patients repeatedly have negative results of testing, despite fulfilling other criteria for probable or possible fungal infection, then the risk of IA appears to be very low. However, although serologic testing contributes substantially to the diagnosis of IA, it is not a substitute for a thorough diagnostic workup, including CT imaging and bronchoscopy with BAL. In addition, in view of the species-specific nature of the ELISA, the emergence of non-*Aspergillus* mold infections, and the frequent cooccurrence of fungal pathogens [61], making decisions about treatment solely on the basis of negative ELISA results is not advisable, although the chance of incorrectly withholding antifungal therapy is reported to be acceptably low [62]. In addition, a trend toward non-*A. fumigatus* isolates of *Aspergillus* has been observed [29], including species with decreased susceptibility to amphotericin B; therefore, one should try to obtain isolates for species determination whenever feasible. Finally, although the test has been shown to yield positive results before therapy is initiated on the basis of clinical parameters, a randomized trial of antifungal strategy will be needed to investigate whether the incorporation of ELISA will alter the final outcome of IA.

We conclude that prospective screening for circulating GM in allogeneic stem cell transplant recipients allows earlier diagnosis of IA, especially when this method is compared with the use of conventional diagnostic criteria. However, this strategy should be incorporated in a multifaceted and highly sensitive and specific approach that combines serial GM detection and HRCT scanning results. Finally, the presumed beneficial impact on IA-related mortality still needs to be demonstrated in properly designed studies on antifungal strategies.

References

- Gratwohl A, Passweg J, Baldomero H, Hermans J. Blood and marrow transplantation activity in Europe 1997. European Group for Blood and Marrow Transplantation (EGBMT). *Bone Marrow Transplant* **1999**;24:231–45.
- Jantunen E, Ruutu P, Niskanen L, et al. Incidence and risk factors for invasive fungal infections in allogeneic BMT recipients. *Bone Marrow Transplant* **1997**;19:801–8.
- Ninin E, Milpied N, Moreau P, et al. Longitudinal study of bacterial, viral, and fungal infections in adult recipients of bone marrow transplants. *Clin Infect Dis* **2001**;33:41–7.
- Baddley JW, Stroud TP, Salzman D, Pappas PG. Invasive mold infections in allogeneic bone marrow transplant recipients. *Clin Infect Dis* **2001**;32:1319–24.
- Denning DW. Therapeutic outcome in invasive aspergillosis. *Clin Infect Dis* **1996**;23:608–15.
- Patterson TF, Kirkpatrick WR, White M, et al. Invasive aspergillosis: disease spectrum, treatment practices, and outcomes. I3 *Aspergillus* Study Group. *Medicine* **2000**;79:250–60.
- Lin S, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. *Clin Infect Dis* **2001**;32:358–66.
- Rinaldi MG. Problems in the diagnosis of invasive fungal diseases. *Rev Infect Dis* **1991**;13:493–5.
- Barth PJ, Rossberg C, Koch S, Ramaswamy A. Pulmonary aspergillosis in an unselected autopsy series. *Pathol Res Pract* **2000**;196:73–80.
- Groll AH, Shah PM, Mentzel C, Schneider M, Just-Nuebling G, Huebner K. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. *J Infect* **1996**;33:23–32.
- Von Eiff M, Roos N, Schulten R, Hesse M, Zuhlsdorf M, van de Loo J. Pulmonary aspergillosis: early diagnosis improves survival. *Respiration* **1995**;62:341–7.
- Walsh TJ, Finberg RW, Arndt C, et al. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycosis Study Group. *N Engl J Med* **1999**;340:764–71.
- Boogaerts M, Winston D, Bow E, et al. Intravenous and oral itraconazole versus intravenous amphotericin B deoxycholate as empirical antifungal therapy for persistent fever in neutropenic patients with cancer who are receiving broad-spectrum antibacterial therapy: a randomized, controlled trial. *Ann Intern Med* **2001**;135:412–22.
- Pizzo PA, Robichaud KJ, Gill FA, Witebsky FG. Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. *Am J Med* **1982**;72:101–11.
- EORTC International Antimicrobial Therapy Cooperative Group. Empiric antifungal therapy in febrile granulocytopenic patients. *Am J Med* **1989**;86:668–72.
- Wingard JR, Kubilis P, Lee L, et al. Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis. *Clin Infect Dis* **1999**;29:1402–7.
- Dasbach EJ, Davies GM, Teutsch SM. Burden of aspergillosis-related hospitalizations in the United States. *Clin Infect Dis* **2000**;31:1524–8.
- Prentice HG, Kibbler CC, Prentice AG. Towards a targeted, risk-based, antifungal strategy in neutropenic patients. *Br J Haematol* **2000**;110:273–84.
- Denning DW. Early diagnosis of invasive aspergillosis. *Lancet* **2000**;355:423–4.
- Kuhlman JE, Fishman EK, Siegelman SS. Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. *Radiology* **1985**;157:611–4.
- Rohrlich P, Sarfati J, Mariani P, et al. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. *Pediatr Infect Dis J* **1996**;15:232–7.
- Maertens J, Verhaegen J, Demuyneck H, et al. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillosis. *J Clin Microbiol* **1999**;37:3223–8.
- Sulahan A, Boutboul F, Ribaud P, Leblanc T, Lacroix C, Derouin F. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer* **2001**;91:311–8.
- Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening

- for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* **2001**;97:1604–10.
25. Hughes WT, Armstrong D, Bodey GP, et al. 1997 Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. *Clin Infect Dis* **1997**;25:551–73.
 26. Denning DW. Treatment of invasive aspergillosis. *J Infect* **1994**;28(Suppl 1): 25–33.
 27. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer and Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. *Clin Infect Dis* **2002**;34:7–14.
 28. Chandrasekar PH, Alangaden G, Manavathu E. Aspergillus: an increasing problem in tertiary care hospitals [letter]? *Clin Infect Dis* **2000**;30:984–5.
 29. Wald A, Leisenring W, van Burik J, Bowden RA. Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* **1997**;175:1459–66.
 30. Ribaud P, Chastang C, Latgé JP, et al. Survival and prognostic factors of invasive aspergillosis after allogeneic bone marrow transplantation. *Clin Infect Dis* **1999**;28:322–30.
 31. VandenBergh MF, Verweij PE, Voss A. Epidemiology of nosocomial fungal infections: invasive aspergillosis and the environment. *Diagn Microbiol Infect Dis* **1999**;34:221–7.
 32. Ho PL, Yuen KY. Aspergillosis in bone marrow transplant recipients. *Crit Rev Oncol Hematol* **2000**;34:55–69.
 33. Jantunen E, Ruutu P, Piilonen A, Volin L, Parkkali T, Ruutu T. Treatment and outcome of invasive *Aspergillus* infections in allogeneic BMT recipients. *Bone Marrow Transplant* **2000**;26:759–62.
 34. Aisner J, Wiernik PH, Schimpff SC. Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. *Ann Intern Med* **1977**; 86:539–43.
 35. Denning DW. Invasive aspergillosis. *Clin Infect Dis* **1998**;26:781–805.
 36. Vogeser M, Wanders A, Haas A, Ruckdeschel G. A four-year review of fatal aspergillosis. *Eur J Clin Microbiol Infect Dis* **1999**;18:42–5.
 37. Gerson SL, Talbot GH, Hurwitz S, Lusk EJ, Strom BL, Cassileth PA. Discriminant scorecard for diagnosis of invasive pulmonary aspergillosis in patients with acute leukemia. *Am J Med* **1985**;79:57–64.
 38. O'Grady NP, Barie PS, Bartlett JG, et al. Practice guidelines for evaluating new fever in critically ill adult patients. Task Force of the Society of Critical Care Medicine and the Infectious Diseases Society of America. *Clin Infect Dis* **1998**;26:1042–59.
 39. Korones DN, Hussong MR, Gullace MA. Routine chest radiography of children with cancer hospitalized for fever and neutropenia: is it really necessary? *Cancer* **1997**;80:1160–4.
 40. Pasmans HLM, Loosveld OJL, Schouten TC, Thunnissen F, van Engelshoven JM. Invasive aspergillosis in immunocompromised patients: findings on plain films and (HR) CT. *Eur J Radiol* **1992**;14:37–40.
 41. Horvath JA, Dummer S. The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. *Am J Med* **1996**;100:171–8.
 42. Reichenberger F, Habicht J, Matt P, et al. Diagnostic yield of bronchoscopy in histologically proven invasive pulmonary aspergillosis. *Bone Marrow Transplant* **1999**;24:1195–9.
 43. Denning DW, Marinus A, Cohen J, et al. An EORTC multicenter prospective survey of invasive aspergillosis in hematological patients: diagnosis and therapeutic outcome. EORTC Invasive Fungal Infections Cooperative Group. *J Infect* **1998**;37:173–80.
 44. Jantunen E, Piilonen A, Volin L, et al. Diagnostic aspects of invasive *Aspergillus* infections in allogeneic BMT recipients. *Bone Marrow Transplant* **2000**;25:867–71.
 45. de Pauw BE, Meis JF. Progress in fighting systemic fungal infections in haematological neoplasia. *Support Care Cancer* **1998**;6:31–8.
 46. Caillot D, Couaillier JF, Bernard A, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* **2001**;19:253–9.
 47. Caillot D, Casasnovas O, Bernard A, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* **1997**;15:139–47.
 48. Brown MJ, Worthy SA, Flint JDA, Muller NL. Invasive aspergillosis in the immunocompromised host: utility of computed tomography and bronchoalveolar lavage. *Clin Radiol* **1998**;53:255–7.
 49. Heussel CP, Kauczor HU, Heussel GE, et al. Pneumonia in febrile neutropenic patients and bone marrow and blood stem-cell transplant recipients: use of high-resolution computed tomography. *J Clin Oncol* **1999**;17:796–805.
 50. Maschmeyer G. Pneumonia in febrile neutropenic patients: radiologic diagnosis. *Curr Opin Oncol* **2001**;13:229–35.
 51. Hebart H, Löffler J, Meisner C, et al. Early detection of *Aspergillus* infection after allogeneic stem cell transplantation by polymerase chain reaction screening. *J Infect Dis* **2000**;181:1713–9.
 52. Williamson EC, Leeming JP, Palmer HM, et al. Diagnosis of invasive aspergillosis in bone marrow transplant recipients by polymerase chain reaction. *Br J Haematol* **2000**;108:132–9.
 53. Roger TR, Haynes KA, Barnes RA. Value of antigen detection in predicting invasive pulmonary aspergillosis. *Lancet* **1990**;336:1210–3.
 54. Talbot JH, Weiner MH, Gerson SL, Provencher M, Hurwitz S. Serodiagnosis of invasive aspergillosis in patients with hematologic malignancy: validation of the *Aspergillus fumigatus* antigen radioimmunoassay. *J Infect Dis* **1987**;155:12–27.
 55. Hopwood V, Johnson EM, Cornish JM, Foot AB, Evans EG, Warnock DW. Use of the pastorex *Aspergillus* antigen latex agglutination test for the diagnosis of invasive aspergillosis. *J Clin Pathol* **1995**;48:210–3.
 56. De Repentigny L, Kaufman L, Cole GT, Kruse D, Latge JP, Matthew RC. Immunodiagnosis of invasive fungal infections. *J Med Vet Mycol* **1994**; 32:239–52.
 57. Stynen D, Goris A, Sarfati J, Latge JP. A new sensitive sandwich ELISA to detect galactofuran in patients with invasive aspergillosis. *J Clin Microbiol* **1995**;33:497–500.
 58. Verweij PE, Stynen D, Rijs AJMM, de Pauw BE, Hoogkamp-Korstanje JA, Meis JF. Sandwich enzyme-linked immunosorbent assay compared with pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. *J Clin Microbiol* **1995**;33:1912–4.
 59. Letscher-Bru V, Herbrecht R, Oprea C, et al. *Aspergillus* galactomannan ELISA in onco-hematologic patients [abstract J-842]. In: Abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago, 22–25 September 2001). Chicago: American Society for Microbiology, **2001**:386.
 60. Donnelly JP. Infection in the neutropenic and hematopoietic stem cell transplant recipient. *Curr Opin Infect Dis* **2000**;13:337–42.
 61. Maertens J, Demuyck H, Verbeken EK, et al. Mucormycosis in allogeneic bone marrow transplant recipients: report of five cases and review of the role of iron overload in the pathogenesis. *Bone Marrow Transplant* **1999**; 24:307–12.
 62. Severens JL, Donnelly JP, Meis JFGM, De Vries Robbe PF, de Pauw BE, Verweij PE. Two strategies for managing invasive aspergillosis: a decision analysis. *Clin Infect Dis* **1997**;25:1148–54.