

# Galactomannan and Computed Tomography–Based Preemptive Antifungal Therapy in Neutropenic Patients at High Risk for Invasive Fungal Infection: A Prospective Feasibility Study

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(See the editorial commentary by de Pauw on pages 1251–3)

**Background.** Empirical antifungal therapy is the standard treatment for persistent or relapsing antibiotic-resistant neutropenic fever. However, overtreatment resulting in increased toxicity and treatment-related cost is a major shortcoming of such therapy. We assessed the feasibility of a “preemptive” approach based on the incorporation of sensitive, noninvasive diagnostic tests for consecutive high-risk neutropenic patients who had received fluconazole prophylaxis while avoiding empirical therapy.

**Methods.** A total of 136 treatment episodes for persons who were at risk of acquiring invasive fungal infection (IFI) were screened for the presence of galactomannan with an enzyme immunoassay. A diagnostic evaluation, which included thoracic computed tomography scanning (HRCT) and bronchoscopy with lavage, was performed on the basis of well-defined clinical, radiological, and microbiological criteria. Only seropositive patients and patients with a positive microbiological test result plus supportive radiological findings received liposomal amphotericin B.

**Results.** Neutropenic fever developed in 117 episodes, of which at least 41 episodes (35%) satisfied existing criteria for empirical antifungal therapy. However, our protocol-driven preemptive approach reduced the rate of antifungal use for these episodes from 35% to 7.7% (a 78% reduction) and led to the early initiation of antifungal therapy in 10 episodes (7.3%) that were clinically not suspected of being IFI. No undetected cases of invasive aspergillosis were identified; 1 case of zygomycosis was missed. Breakthrough candidemia was diagnosed by conventional culture techniques and was treated successfully. With use of a preemptive approach, the 12-week survival rate for patients with IFI was 63.6% (it was 63.1% for those with invasive aspergillosis).

**Conclusion.** Preemptive therapy based on enzyme immunoassay and HRCT reduced the exposure to expensive and potentially toxic drugs and offered effective antifungal control, but it failed to detect non-*Aspergillus* IFI.

Invasive fungal infections (IFIs) are major causes of morbidity and mortality in neutropenic patients who receive chemotherapy for hematological malignancies or who undergo allogeneic hematopoietic stem cell transplantation. In patients with IFI who have received fluconazole prophylaxis, molds—in particular, *Asper-*

*gillus* species—have become the most likely fungal pathogens [1–3]. Unfortunately, early diagnosis remains a challenge, given the low sensitivity of microbiological culture techniques and the low specificity of standard radiological tools, especially in neutropenic patients [4]. Therefore, infections are often far advanced at the time of diagnostic confirmation, and overall outcome is poor [5, 6].

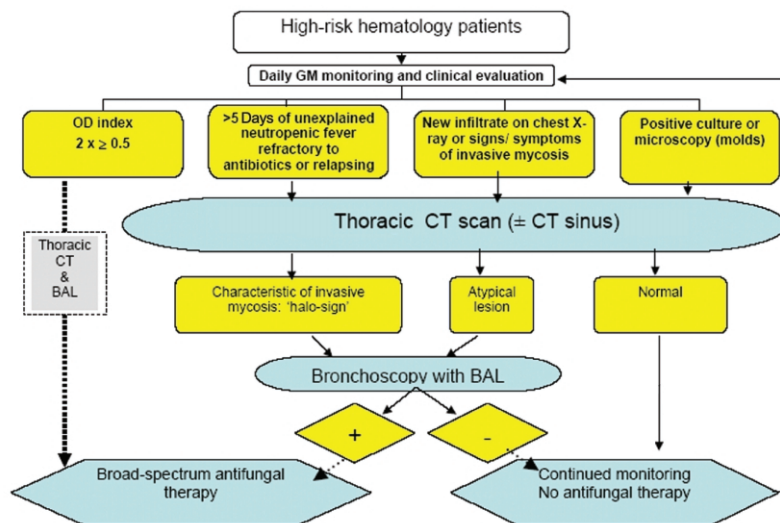
Empirical antifungal therapy is considered standard practice of care in neutropenic patients with fever that persists or recurs while they are receiving broad-spectrum antibiotics and has repeatedly been endorsed by consensus guidelines [7, 8]. The aim is to ensure that patients with possible IFI receive therapy early in the

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**Figure 1.** Diagnostic and treatment algorithm of the study. Thin arrows represent the diagnostic flow chart that was used to assess the presence or absence of fungal disease in high-risk patients. The treatment algorithm is shown by dark dotted arrows: only seropositive patients (i.e., those with 2 consecutive galactomannan (GM) EIA assays with an optical density [OD] of  $\geq 0.5$ ) or patients with positive microbiologic test results (culture or microscopy) plus supportive radiological findings received antifungal treatment. BAL, bronchoalveolar lavage; +, present; –, absent;  $\pm$ , with or without.

course of the disease, because early initiation of therapy seems to improve the survival rate [9–15]. As a result, as many as 40%–50% of the high-risk neutropenic population may receive empirical antifungal therapy, whereas the true incidence of IFI appears to be 10%–15% [16].

Overtreatment, as well as the negative effect of delaying therapy until disease is proven, could be overcome by a preemptive approach. Such a strategy targets the population in which there is sufficient evidence of pathogen invasion but no manifest symptomatic disease. Progress could come from the incorporation of non-culture-based microbiological techniques, including screening for circulating *Aspergillus* galactomannan with an EIA and the early use of high-resolution thoracic CT scanning (HRCT). Both tools have a high diagnostic accuracy in neutropenic adults [17–23]. The aim of our prospective study was to assess the feasibility of a combined EIA/HRCT-based preemptive strategy (while avoiding administration of empirical antifungal therapy) in patients with cancer and prolonged neutropenia.

## MATERIALS AND METHODS

**Study population.** From 1 January 2003 to 31 January 2004, consecutive adult patients (age,  $>16$  years) admitted to the Leukemia and Transplantation Unit of the University Hospital of Leuven (Leuven, Belgium) were included in the study. Patients were eligible for the study if they had received chemotherapy for acute leukemia or myelodysplastic syndrome while having an expected absolute neutrophil count of  $<0.5 \times 10^9$  cells/L for at least 10 days or if they underwent myeloablative

allogeneic hematopoietic stem cell transplantation. Patients who had aplastic anemia or who underwent autologous or nonmyeloablative allogeneic hematopoietic stem cell transplantation were excluded. The study protocol was approved by the institutional review board of the hospital; informed consent was obtained from all patients.

**Study procedures.** Patients were hospitalized in single rooms with high-efficiency particulate air filters from the start of therapy until neutrophil recovery (absolute neutrophil count,  $\geq 0.5 \times 10^9$  cells/L). Antimicrobial prophylaxis consisted of fluconazole (400 mg daily) and levofloxacin (250 mg daily). Patients were surveyed for the development of fever (a temperature of  $>38^\circ\text{C}$  recorded twice or  $>38.5^\circ\text{C}$  recorded once) and for the presence of signs and symptoms of IFI. Surveillance cultures for bacterial and fungal growth from stool and urine samples and oral washes were performed weekly; standard chest radiographs were obtained at hospital admission and once to twice weekly (with a portable machine) thereafter in the room. Neutropenic fever was treated with broad-spectrum antibiotics (i.e., cefepime or meropenem), in accordance with published guidelines [7]. Additional blood cultures, sputum cultures, and cultures of samples from infected sites were performed as clinically indicated. One set of blood cultures was performed daily for patients who received steroids, irrespective of the presence of fever. Vancomycin was added to the treatment regimen for patients who were colonized with methicillin-resistant *Staphylococcus aureus*, who had clinically infected catheter entry-sites, or whose blood cultures yielded gram-positive bacteria. Persistent neutropenic fever was not an indication for the use of

**Table 1. Baseline demographic and clinical characteristics of patients with the study episodes.**

Characteristic	Value
No. of patients	88
Female sex	42 (47.7)
Age	
Median years (range)	44 (16–75)
Age group	
≤40 years	39 (44.3)
40–59 years	36 (40.9)
≥60 years	13 (14.8)
Underlying disease <sup>a</sup>	
Acute myelogenous leukemia	37 (42)
Acute lymphocytic leukemia	17 (19.3)
Myelodysplastic syndrome	3 (3.4)
Relapse of acute leukemia/myelodysplastic syndrome <sup>b</sup>	23 (26.1)
Other <sup>c</sup>	8 (9)
No. of treatment episodes	136
Type of treatment cycle	
Remission-induction	43 (31.6)
Consolidation	38 (27.9)
Reinduction	23 (16.9)
Allogeneic transplantation <sup>d,e</sup>	32 (23.5)
Cytarabine therapy	
Intermediate dose <sup>f</sup>	18 (13.2)
High dose <sup>f</sup>	40 (29.4)
Neutrophil count of <500 cells/ $\mu$ L	134 (98.5)
Duration of neutropenia, days	
Mean $\pm$ SD	21.5 $\pm$ 11.6
Median (range)	19 (4–86)
Neutrophil count of <100 cells/ $\mu$ L	130 (95.6)
Duration of neutropenia, days	
Mean $\pm$ SD	16.6 $\pm$ 9.7
Median (range)	15 (2–65)
Use of corticosteroids <sup>g</sup>	11 (8)
Primary infection with bacteremia <sup>h</sup>	51 (37.5)
Use of broad-spectrum antibiotics	
All	123 (90.4)
Duration, days	
Mean $\pm$ SD	15.9 $\pm$ 9.2
Median (range)	14.5 (4–87)
Use of vancomycin	70 (51.5)
Use of metronidazole	26 (19.1)

(continued)

glycopeptides [24]. Metronidazole was added in cases of necrotizing gingivitis, perianal abscess, or intraabdominal sepsis if it was not treated with meropenem. More than 1 treatment episode could be included per patient, but once IFI was diagnosed, patients were no longer eligible for a subsequent inclusion until complete resolution of the infection.

Findings that triggered a diagnostic evaluation for IFI were as follows: (1) neutropenic fever refractory to 5 days of broad-

**Table 1. (Continued.)**

Characteristic	Value
Samples for EIA	
All	4170
No. of samples per episode	
Mean $\pm$ SD	30.6 $\pm$ 13.7
Median (range)	28 (5–96)

**NOTE.** Data are no. (%) of patients or episodes, unless otherwise indicated.

<sup>a</sup> According to the World Health Organization classification [29].

<sup>b</sup> Includes 9 patients who had relapse after allogeneic transplantation and 7 patients who had relapse after autologous transplantation.

<sup>c</sup> Includes patients undergoing allogeneic hematopoietic stem cell transplantation for relapsed Burkitt lymphoma, relapsed Hodgkin lymphoma, relapsed diffuse large cell non-Hodgkin lymphoma, relapsed follicular non-Hodgkin lymphoma, and refractory post-liver transplantation lymphoma.

<sup>d</sup> Disease phase at transplantation was categorized as early (acute leukemia or poor-risk myelodysplasia in first complete remission, first chronic-phase chronic myelogenous leukemia, or lymphoid malignancy in first remission) in 6 episodes, intermediate (acute leukemia or myelodysplasia in second or higher complete remission, accelerated-phase chronic myeloid leukemia, or lymphoid malignancy in second or higher remission) in 10 episodes, and advanced (refractory or relapsed acute leukemia or myelodysplasia, blast-phase chronic myeloid leukemia, refractory or relapsed lymphoid malignancy, and any relapse after a previous autograft) in 16 episodes.

<sup>e</sup> Donor type was HLA-identical sibling for 11 episodes, HLA-matched unrelated donor for 18 episodes, and mismatched related or unrelated donor for 3 episodes.

<sup>f</sup> Intermediate dose was defined as a total dose per cycle of  $\geq 10$  g/m<sup>2</sup> and <16 g/m<sup>2</sup>; high dose was defined as a total dose of  $\geq 16$  g/m<sup>2</sup> per cycle.

<sup>g</sup> Prednisone (60 mg/m<sup>2</sup>) for 28 days as part of remission-induction therapy for acute lymphocytic leukemia.

<sup>h</sup> Includes 27 cases of gram-positive bacteremia, 12 cases of gram-negative bacteremia, and 12 cases of polymicrobial bacteremia.

spectrum antibiotic treatment or unexplained fever relapsing after at least 48 h of defervescence while the patient was still neutropenic and still receiving antibiotics, (2) clinical signs and/or symptoms suggestive of IFI, (3) appearance of a new pulmonary infiltrate while the patient was receiving treatment with broad-spectrum antibiotics or steroids, (4) isolation of molds or demonstration of hyphae in respiratory specimens, and (5) two consecutive galactomannan EIA assays with an optical density index of  $\geq 0.5$  (figure 1). The diagnostic evaluation included the following: (1) HRCT (with or without sinus CT) within 24 h after request, in accordance with a previously described protocol [25]; and (2) bronchoscopy with bronchoalveolar lavage in patients without severe hypoxemia. Bronchoalveolar lavage samples were submitted for microscopic examination and culture for bacteria, fungi, mycobacteria, and *Legionella* species. Cytomegalovirus, herpes simplex virus, varicella zoster virus, *Toxoplasma gondii*, *Pneumocystis jirovecii*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* were detected by PCR. Endobronchial biopsies were not performed in patients with cytopenia. Other diagnostic procedures could be performed when clinically indicated.

Autopsy was pursued for all fatalities. Specimens were stained with periodic acid-Schiff or Gomori-methenamine silver stain.

**Galactomannan EIA.** Six milliliters of blood was collected daily in tubes that contained EDTA until the resolution of neutropenia or, in cases of invasive aspergillosis, until the end of hospitalization or death. Samples were stored at  $-20^{\circ}\text{C}$  and were analyzed thrice weekly (with the Platelia *Aspergillus* EIA; Bio-Rad Laboratories) by technicians who were unaware of the clinical status of the patient. Processing of the sample and optical density measurements were performed with a semiautomatic analyzer (Behring ELISA processor III; Dade Behring), in accordance with the recommendations of the manufacturer [26]. All reagents were purchased from Bio-Rad Laboratories. Positive, negative, and threshold controls were included in each run. Results were recorded as an index relative to the optical density of the control sample obtained in the same run. An index of  $\geq 0.5$  was considered to be a positive result if it was confirmed with a subsequent sample. After analysis, results were immediately reported back to the clinicians by fax.

**Treatment algorithm.** Only patients with  $\geq 2$  consecutive EIA assays with an index of  $\geq 0.5$  (irrespective of clinical and radiological findings) or with CT findings suggestive of IFI that were supported by a culture or microscopic evaluation positive for molds received liposomal amphotericin B (5 mg/kg iv). In the absence of the aforementioned criteria, suggestive signs and/or symptoms, persistent or relapsing neutropenic fever, and new pulmonary infiltrates were not indications for antifungal therapy. Patients who were refractory to at least 10 days of primary treatment (clinical, radiological, and serological progression) or who were intolerant of liposomal amphotericin B (because of nephrotoxicity or infusion-related side effects) could have their regimen switched to other licensed antifungal agents.

**Analysis.** The primary aim of this nonrandomized pilot study was to evaluate the feasibility of a preemptive approach, as opposed to a fever-driven empirical strategy—that is, how many patients with IFI would receive a misdiagnosis and how many would receive treatment with use of this specific preemptive algorithm, compared with how many would receive treatment with the use of empirical antifungal therapy? Episodes were classified on the basis of the European Organization for the Research and Treatment of Cancer/Mycosis Study Group consensus criteria using an EIA index cutoff of 0.5 [27]. Responses in patients with proven and probable IFI were assessed on the basis of published criteria [28]. Descriptive statistics are reported. Continuous variables are summarized as means  $\pm$  SDs.

## RESULTS

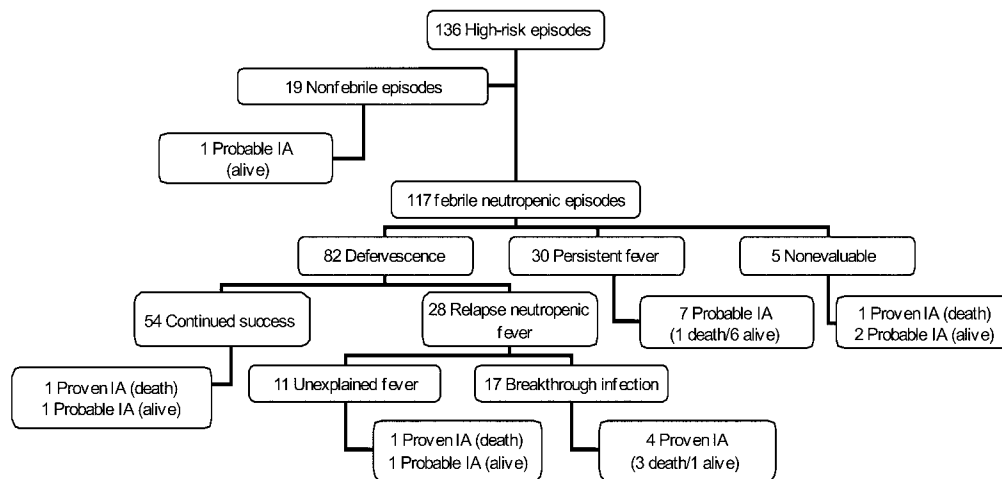
A total of 136 treatment episodes (88 patients) were investigated. Baseline demographic and clinical characteristics are presented in table 1. All episodes involved patients with multiple risk factors for IFI in addition to prolonged neutropenia, such

as use of steroids, older age, prolonged use of broad-spectrum antibiotics, receipt of high-dose cytarabine, and uncontrolled malignancy.

**Classification of study episodes.** Neutropenic fever developed in 117 episodes (86%) (figure 2). After administration of broad-spectrum antibiotics, patients defervesced within 5 days (mean time to defervescence,  $1.84 \pm 1.29$  days) in 82 episodes, whereas fever persisted for  $>5$  days (mean time to defervescence,  $11.7 \pm 4.6$  days; range, 6–26 days) in the remaining 30 evaluable episodes (18 cases of fever of unknown origin, 7 cases of bacteremia with documented microbiological eradication, and 5 cases of clinically documented infection). Overall, 5 episodes were considered to be nonevaluable as a result of confounding causes of fever (i.e., the episode was drug related, disease related, or transfusion related). Time to defervescence for the whole group was  $5.1 \pm 6.1$  days. Antibiotic therapy was also started for 6 of 19 nonfebrile episodes that were due to bacteremia experienced while patients were receiving steroids or due to a pre-existing infection. In 54 successfully treated episodes, patients remained afebrile, whereas a second episode of neutropenic fever occurred in 28 treatment courses. In these latter episodes, the etiology of fever remained unexplained (11 cases) or was attributed to a breakthrough bloodstream infection (17 cases) (fluconazole-resistant *Candida glabrata*, 2 cases; *Staphylococcus* species, 6 cases; *Enterococcus* species, 6 cases; and polymicrobial bacteremia, 3 cases). In retrospect, at least 41 episodes (30%) satisfied criteria for empirical antifungal therapy.

**Preemptive approach.** We analyzed 4170 serum samples (mean,  $30.6 \pm 13.7$  samples per episode; range, 5–96 samples per episode) and identified 19 seropositive episodes (13.9%) (919 samples; mean,  $48.3 \pm 22.8$  samples; range, 19–96 samples). Higher optical density index cutoffs of  $\geq 0.7$ ,  $\geq 1.0$ , or  $\geq 1.5$  identified 16, 14, and 11 of these episodes, respectively. In accordance with European Organization for the Research and Treatment of Cancer/Mycosis Study Group criteria, all seropositive episodes were classified as proven or probable invasive aspergillosis; their distribution, relative to the classification of study episodes, is detailed in figure 2. In the febrile neutropenic group (117 episodes), only 9 episodes were treated preemptively, although at least 41 episodes qualified for empirical antifungal therapy; this represents a reduction in the use of antifungals from an estimated 35% to 7.7% (difference, 27.3%; 95% CI, 9.9%–37.2%). In addition, 10 nonfebrile episodes or febrile episodes with alternative explanations received preemptive therapy on the basis of seropositivity.

Positive galactomannan EIA results were the primary triggers for further evaluation in 16 cases: supportive findings were seen on the first CT (13 episodes) or on a follow-up CT 1 week later (3 episodes). Persistent fever was the primary trigger in 3 remaining episodes; although the initial CT was unrevealing, follow-up CT revealed a classical halo sign. Suggestive clinical



**Figure 2.** Classification of the study episodes and invasive aspergillosis (IA). Although in at least 41 of 117 febrile neutropenic episodes, patients qualified for classical empirical antifungal therapy (30 episodes of persistent fever and 11 episodes of unexplained relapsing fever), in only 9 of these study episodes did patients actually receive antifungals. In addition, 10 episodes not clinically suspected of invasive fungal disease could be classified as invasive aspergillosis (on the basis of European Organization for the Research and Treatment of Cancer/Mycosis Study Group criteria) and were treated with antifungals.

signs and/or symptoms, a new pulmonary infiltrate, or isolation of molds from respiratory specimens were never the trigger for further diagnostic exploration. The temporal relationship between positive EIA results and the presence of suggestive abnormalities on CT could not be investigated, because antigenemia was a trigger for performing CT. Baseline characteristics and outcomes are listed in table 2. Per protocol, all of the patients who experienced these episodes received antifungal therapy. Cases of proven invasive aspergillosis were treated for a median of 34 days (range, 6–75 days); cases of probable invasive aspergillosis were treated for a median of 25 days (range, 11–66 days). All surviving patients received oral maintenance therapy with azoles with activity against molds.

The remaining 117 episodes (3251 samples; mean,  $27.7 \pm 8.8$  samples per episode; range, 5–53 samples per episode) form the seronegative group; 10 samples (0.3%) had optical density indices of  $\geq 0.5$ , but the results were not confirmed with a subsequent sample. Fourteen seronegative episodes (12%) were classified as possible fungal infection; culture and microscopic examination of bronchoalveolar lavage samples from 12 of these cases yielded *Pseudomonas aeruginosa* (2 episodes), methicillin-resistant *S. aureus* (1 episode), or no causative pathogen, including coagulase-negative staphylococci (9 episode). None of these 14 episodes were treated with antifungals. In accordance with European Organization for the Research and Treatment of Cancer/Mycosis Study Group criteria, not a single seronegative episode was diagnosed as proven or probable invasive aspergillosis. However, 2 cases of breakthrough *C. glabrata* fungemia were detected by conventional blood culture techniques and were treated successfully with caspofungin. In addition, a

patient with refractory leukemia who developed a sudden brain hemorrhage (without fever or any other clinical finding suggestive of IFI) proved to have disseminated zygomycosis at autopsy. Thus, 1 (4.5%) of 22 cases of IFI remained undiagnosed antemortem, and the affected patients did not receive adequate therapy.

**Survival and autopsy data.** The overall mortality rate was 18.1% (16 of 88 patients). Seven deaths (41.1%) occurred among patients with positive EIA results. Although autopsy demonstrated hyphal tissue invasion in 6 of 7 patients, invasive aspergillosis was considered the primary cause of death in 2 patients only. For the remaining 4 cases, invasive aspergillosis was a contributing factor, whereas refractory leukemia, disseminated toxoplasmosis, cytomegalovirus disease, and graft failure complicated by bacterial superinfections were the primary causes of death. Interestingly, at autopsy, these latter patients were shown to have organizing necrotic pulmonary nodules without angio-invasion, and all had a favorable serological response after antifungal treatment. None of these patients had signs of extrapulmonary dissemination at autopsy. The only fatal probable case showed a favorable serological response, but the patient died of refractory leukemia; autopsy could not demonstrate fungal involvement. Nine patients (12.7%) from the EIA-negative group died. Autopsy-proven causes of death were attributed to worsening underlying disease or to other nosocomial complications. Except for the case of zygomycosis, no undetected cases of IFI were found. At 3 months, the survival rate was 63.6% for patients with IFI and 63.1% for patients with invasive aspergillosis (it was 76.9% for patients with complete remission of their underlying disease or residual disease

**Table 2. Data for treatment episodes for persons who were at risk of acquiring invasive fungal infection and who had positive EIA results.**

Category, <sup>a</sup> episode	Diagnostic criterion or criteria	BAL finding <sup>b</sup>	CT findings, by scan		No. of samples	No. of EIA-positive serum samples, by OD index cutoff			Radiological response <sup>c</sup>	Serological response	Survival <sup>d</sup>	Response of underlying disease
			At first positive EIA result	Subsequent scan		≥0.5	≥1.0	≥1.5				
Proven IA												
1	Autopsy and culture	NFP	Halo sign	AC sign	71	61	60	58	SD	↓	Death	Ref
2 <sup>e</sup>	Autopsy and culture	NFP	Halo sign	...	24	11	5	3	SD	↓	Death	Ref
3	Autopsy	<i>Aspergillus fumigatus</i>	Halo sign	...	29	14	9	7	Failure <sup>f</sup>	↑	Death	Ref
4	Sterile site culture	<i>A. fumigatus</i>	Pneumothorax	Cavern	33	26	12	7	PR	–	Alive	1st CRem
5 <sup>e</sup>	Autopsy and culture	NFP	Halo sign	AC sign	52	27	21	19	SD	–	Death	Sec GF residual
6	Biopsy	<i>A. fumigatus</i>	Halo sign	AC sign	71	34	14	2	SD	↓	Death	2nd CRem
7	Autopsy and culture	<i>A. fumigatus</i>	–	Nodular lesion	40	8	3	3	Failure <sup>f</sup>	↑	Death	2nd CRem
Probable IA												
8	GM and HRCT	NFP	–	Halo sign	52	33	14	2	CR	–	Alive	2nd CRem
9	GM and HRCT	NFP	Halo sign	...	26	5	2	1	PR	–	Alive	2nd CRem
10	GM and HRCT	NFP	–	Halo sign	52	27	9	1	CR	–	Alive	Ref
11	GM, HRCT, and culture	<i>A. fumigatus</i>	Halo sign	AC sign	96	66	39	27	PR	–	Alive	3rd CRem
12	GM and HRCT	NFP	Halo sign	AC sign	28	2	0	0	PR	–	Alive	Residual
13	GM and HRCT	NFP	Halo sign	AC sign	85	32	7	3	SD	–	Death	Ref
14	GM and HRCT	Not done	Halo sign	...	42	3	0	0	CR	–	Alive	1st CRem
15	GM and HRCT	NFP	Halo sign	...	83	16	0	0	CR	–	Alive	Residual
16	GM and HRCT	Not done	–	...	35	8	0	0	CR	–	Alive	Ref
17	GM, HRCT, and culture	<i>A. fumigatus</i>	Halo sign	AC sign	19	10	4	1	CR	–	Alive	2nd CRem
18	GM and HRCT	NFP	Halo sign	AC sign	46	24	31	19	SD	↓	Alive	3rd CRem
19	GM and HRCT	NFP	Consolidation	AC sign	35	2	1	1	PR	–	Alive	1st CRem

**NOTE.** AC, air crescent; CR, complete response; CRem, complete remission; GM, galactomannan detection; HRCT, high-resolution CT scan; IA, invasive aspergillosis; NFP, no fungal pathogen; OD, optical density; PR, partial response; Ref, refractory disease; residual, residual disease (leukemia) found by immunophenotyping or molecular examination; SD, stable disease; sec GF, secondary graft failure; –, negative (consecutive OD indices of <0.5); ↓, decrease in antigen level; ↑, increase in antigen level.

<sup>a</sup> As defined by European Organization for the Research and Treatment of Cancer/Mycosis Study Group criteria, using a cutoff for galactomannan detection of 2 consecutive serum samples with an OD index of ≥0.5 [23].

<sup>b</sup> Bronchoscopy with lavage was performed for 17 of 19 episodes.

<sup>c</sup> From [28].

<sup>d</sup> Survival status at week 12 after diagnosis.

<sup>e</sup> Patient F/54 and patient M/61 were included twice: once with probable IA, and once with proven IA (relapse during allogeneic transplantation).

<sup>f</sup> Progression of infiltrates on radiological examination.

vs. 30% for patients with refractory disease). Overall, seronegativity was achieved in 68.4% of episodes of invasive aspergillosis (table 2).

## DISCUSSION

Persistent or recurrent unexplained neutropenic fever refractory to antibiotic therapy may represent an IFI and is considered a valid indication for empirical antifungal therapy [8–15]. However, a wide range of biological processes—some infectious, and many noninfectious—can cause fever, including inadequately managed nonfungal infections, drugs, malignancies, graft-versus-host disease, and neutrophil recovery. Conversely, the use of steroids as part of cytoreductive regimens may result in the absence of fever, even in patients with disseminated fungal disease [30]. Thus, we must try to more accurately distinguish between patients truly in need of antifungal therapy and those who do not need antifungals.

This prospective study, which involved adult hematology patients who received fluconazole prophylaxis and who had well-identified risk factors for invasive mold infection, challenges widely accepted empirical concepts by using a strategy based on intensive screening for galactomannan and HRCT. A purely fever-driven approach would presumably have resulted in administration of empirical antifungal therapy for 35% of febrile neutropenic episodes, whereas use of our algorithm resulted in initiation of treatment with antifungals in only 7.7% of these episodes. This finding underscores the potential for overtreatment, toxicity, and increased treatment-related cost associated with recommending early empirical antifungal therapy. This observation also demonstrates the existence of center-related variability regarding the use of empirical antifungal therapy; our 35% rate of empirical antifungal therapy seems on the low side and might suggest that the risk for IFI may be lower than has previously been anticipated, although the IFI rate in this study (22%) was consistent with literature findings [31].

Obviously, the feasibility and safety of targeted strategies depend on the availability of reliable diagnostic tools. Our strategy incorporated both screening for circulating galactomannan and early use of HRCT for the detection of invasive aspergillosis, by far the most common fungal pathogen in neutropenic patients who have received fluconazole prophylaxis. Although reported diagnostic accuracies of galactomannan detection have been disappointing in heterogeneous study populations [32], test performance and reproducibility have been excellent in adult neutropenic patients with a high pretest probability [18–22] and have been sufficiently robust to be used for determination of who should receive preemptive therapy [33]. The excellent negative predictive value of the EIA allowed us to determine whether antifungal therapy can be withheld in patients with refractory neutropenic fever with no other evidence of IFI. Given the nearly 100% fatal outcome of untreated in-

vasive aspergillosis [2], it seems unlikely that we failed to detect patients who were truly infected with *Aspergillus* species, an assumption supported by the lack of undiagnosed cases at autopsy. In addition, positive results were often recorded before the development of fever, which is the trigger for empirical antifungal therapy [20, 25]. Thus, episodes that were not suspected of being invasive aspergillosis (because of absence of fever or presence of confounding factors) were detected early by positive EIA results.

We realize that the exact clinical and pharmaco-economical impact of a preemptive strategy can only be assessed in a randomized, prospective evaluation in which it is compared with an empirical approach. Nevertheless, the results of this pilot study are encouraging and provide rationale for such a challenging study. Achieving seronegativity in almost 70% of neutropenic episodes complicated by invasive aspergillosis is noteworthy, especially because studies indicate that levels of galactomannanemia closely correlate with fungal burden and outcome of therapy [34–36]. When liposomal amphotericin B is used as primary therapy, particularly encouraging is the 76.9% survival rate at 3 months for the subgroup with complete remission or residual disease of their underlying disease. The 30% survival rate for the “refractory disease” subgroup once more underscores the prognostic importance of underlying disease status and immune reconstitution [2].

Three observations regarding the EIA warrant additional discussion. First, several factors that interfere with the performance have been identified, including the prophylactic use of mold-active azoles, receipt of empirical antifungal therapy, and the use of piperacillin-tazobactam [20, 37]. Although none of these factors confounded the performance in this study, they may be present in institutions with different policies. Second, we used 0.5 as an index cutoff for EIA positivity, as was recently accepted by the US Food and Drug Administration [20], but we required at least 2 consecutive positive samples before antifungal therapy was started. Of note, most probable episodes remained seropositive at higher, more conventional cutoffs, and all probable pulmonary cases were supported by suggestive CT findings. Therefore, we hypothesize that, in some cases, the occurrence of high-level antigenemia was prevented by the early institution of antifungal therapy; a similar observation of suppressed expression of antigenemia has been reported in empirically treated patients [20]. Third, the antigen ratio in patients with invasive aspergillosis usually increased from negative (<0.5) to clearly positive (>1.0) in the course of 2 or 3 days; thus, twice- or thrice-weekly sampling seems to be frequent enough and would substantially reduce the cost of our preemptive strategy [21].

The specificity for *Aspergillus* species in our algorithm may keep clinicians from shifting emphasis to preemptive therapy [38]. However, the 2 cases of breakthrough fungemia were

detected by conventional methods, even before the triggering criterion of unexplained relapsing fever was reached. Unfortunately, 1 fatal case of zygomycosis remained untreated; but, given the absence of fever, even an empirical approach would not have rescued this particular patient. Consequently, we feel that a preemptive strategy that targets invasive aspergillosis is feasible and safe and obviates the need for empirical antifungal therapy in high-risk neutropenic patients, provided that adequate *Candida* prophylaxis is given. Of note, the algorithm cannot be extended to nonneutropenic risk populations of persons who often display a different fungal epidemiology—in particular, solid-organ transplant recipients and allogeneic hematopoietic stem cell transplant recipients treated for graft-versus-host disease [39, 40]. For these latter categories, the use of panfungal assays (such as PCR and  $\beta$ -glucan detection) may be more appropriate [41, 42].

In summary, abandoning empirical antifungal therapy for a preemptive approach spared patients from exposure to expensive and potentially toxic drugs. Alternatively, the treatment algorithm offered effective antifungal control for patients with invasive aspergillosis, even for those not qualifying for empirical antifungal therapy, but it failed to identify non-*Aspergillus* infections. A randomized trial comparing outcome and cost-effectiveness of fever-driven empirical therapy versus an EIA- or CT-driven preemptive approach may be in order.

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