MAJOR ARTICLE

Serum Galactomannan Versus a Combination of Galactomannan and Polymerase Chain Reaction–Based *Aspergillus* DNA Detection for Early Therapy of Invasive Aspergillosis in High-Risk Hematological Patients: A Randomized Controlled Trial

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Background. The benefit of the combination of serum galactomannan (GM) assay and polymerase chain reaction (PCR)–based detection of serum *Aspergillus* DNA for the early diagnosis and therapy of invasive aspergillosis (IA) in high-risk hematological patients remains unclear.

Methods. We performed an open-label, controlled, parallel-group randomized trial in 13 Spanish centers. Adult patients with acute myeloid leukemia and myelodysplastic syndrome on induction therapy or allogeneic hematopoietic stem cell transplant recipients were randomized (1:1 ratio) to 1 of 2 arms: "GM-PCR group" (the results of serial serum GM and PCR assays were provided to treating physicians) and "GM group" (only the results of serum GM were informed). Positivity in either assay prompted thoracic computed tomography scan and initiation of antifungal therapy. No antimold prophylaxis was permitted.

Results. Overall, 219 patients underwent randomization (105 in the GM-PCR group and 114 in the GM group). The cumulative incidence of "proven" or "probable" IA (primary study outcome) was lower in the GM-PCR group (4.2% vs 13.1%; odds ratio, 0.29 [95% confidence interval, .09–.91]). The median interval from the start of monitoring to the diagnosis of IA was lower in the GM-PCR group (13 vs 20 days; $P = .022$), as well as the use of empirical antifungal therapy (16.7% vs 29.0%; $P = .038$). Patients in the GM-PCR group had higher proven or probable IA–free survival ($P = .027$).

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Conclusions. A combined monitoring strategy based on serum GM and *Aspergillus* DNA was associated with an earlier diagnosis and a lower incidence of IA in high-risk hematological patients.

Clinical Trials Registration. NCT01742026.

Keywords. invasive aspergillosis; PCR; galactomannan; monitoring; clinical trial.

Invasive aspergillosis (IA) is the most common invasive fungal disease among patients with hematological malignancies and recipients of allogeneic hematopoietic stem cell transplant (allo-HSCT) [1–5]. Although the administration of mold-active antifungal prophylaxis and the prompt initiation of empirical therapy are effective strategies, such approaches may lead to drug-to-drug interactions, side effects, and overuse of antifungals [6].

The management of the high-risk hematological patients has evolved significantly with the advent of new diagnostic methods [7–9]. An increasing number of studies have assessed the role of detecting *Aspergillus* DNA in clinical samples by polymerase chain reaction (PCR) [10–16], overall suggesting that this method might be more accurate for the diagnosis of IA than conventional culture or serum galactomannan (GM) assay [17, 18].

Although the combination of serum GM and PCR assays has shown to perform better in terms of sensitivity and negative predictive values [11, 14, 19], the potential advantage of a diagnostic-driven strategy incorporating both biomarkers remains to be demonstrated. We hypothesized that the serial detection of *Aspergillus* DNA in combination with serum GM quantification might lead to an earlier guided antifungal therapy and, ultimately, decrease the odds of developing invasive disease. The present trial was aimed at comparing the performance of a combined GM and PCR assay–based vs a GM assay–based monitoring strategy for the early diagnosis and therapy of IA in high-risk patients.

METHODS

Study Population and Setting

This open-label, parallel-group, randomized trial (ClinicalTrials.gov identifier NCT01742026) was performed between February 2011 and September 2012 in 13 centers throughout Spain. Patients (≥18 years) with acute myeloid leukemia (AML) or high-risk myelodysplastic syndrome (MDS) undergoing remission induction therapy for newly diagnosed or relapsed or refractory disease and patients undergoing allo-HSCT were potentially eligible. Exclusion criteria included the diagnosis of IA within 6 months prior to or at enrollment, the receipt of antifungal therapy with anti-*Aspergillus* activity within 30 days prior to or at enrollment, and a history of hypersensitivity to azoles. Fluconazole was the only antifungal prophylaxis permitted, and no antimold prophylaxis (ie, extended-spectrum triazoles, echinocandins, or polyenes) was initiated during the study period. Patients could be enrolled only once in the study. The local ethics review committees approved the study protocol, and written informed consent was obtained from each patient.

Study Design and Intervention

Patients were randomized at the time of initiating chemotherapy or on day 1 posttransplant in a 1:1 ratio to 1 of 2 arms: GM-PCR group (experimental) or GM group (control). In an attempt to ensure roughly equal numbers in both arms, an independent statistician performed the procedure by using a computer-generated schedule of randomly permuted blocks. Once the site investigator had obtained the patient's consent, phone confirmation of the allocated group was provided. Monitoring was started from the initiation of the remission induction regimen in AML/MDS patients or from day 1 in allo-HSCT recipients. Serum GM and *Aspergillus* species quantitative real-time PCR (rtPCR) assays were performed twice weekly (on Mondays and Wednesdays) until neutrophil recovery reached $\geq 0.5 \times 10^3$ cells/mm^3 for AML/MDS patients, or until days 180 or 100 for allo-HSCT recipients with or without graft-vs-host disease (GVHD), respectively. The results of both GM and rtPCR assays were provided to the attending physicians of patients allocated into the GM-PCR group; in the GM group, only the results of the GM assay were provided. In both groups, these results were available within 24–48 hours from sampling. When the result of at least 1 of the tests informed to the clinicians turned to be positive (ie, serum GM and/or rtPCR in the GM-PCR group and solely the former in the GM group), ordering a thoracic high-resolution computed tomography (HRCT) scan was mandatory and an antifungal agent with activity against *Aspergillus* species had to be initiated, even if the HRCT scan revealed no radiological signs suggestive of IA according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [20] (Figure 1). Of note, the rtPCR results generated from the GM group had no influence on the clinical management of these patients or on the decision of whether to order an imaging study. Febrile neutropenia episodes were managed with broad-spectrum antibiotics according to the local practice at each center. Attending physicians were allowed to order additional HRCT scan examinations whenever they deemed clinically necessary (even in the absence of any positive monitoring result). Empirical antifungal therapy had to be prescribed after 72 hours of the onset of refractory febrile neutropenia, even if no evidence of IA was present, and

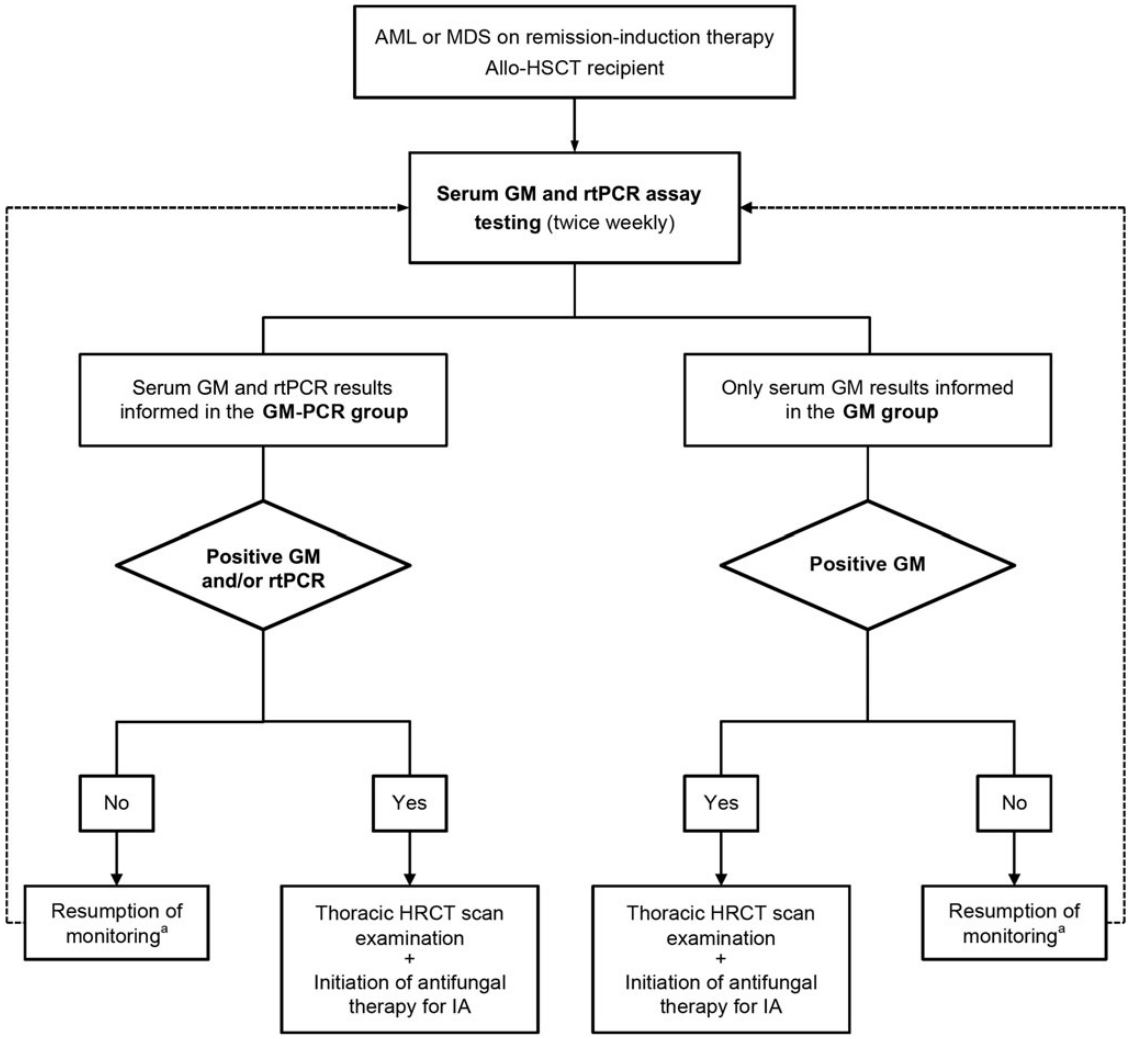

Figure 1. Diagnostic and therapeutic algorithms applied in the study groups. A positive result in any test intended to be provided to the attending physicians (ie, serum galactomannan [GM] and/or real-time polymerase chain reaction [PCR] assays in patients allocated to the GM-PCR group and only the former in those allocated to the GM group) prompted ordering a thoracic high-resolution computed tomography (HRCT) scan and initiating antifungal therapy for invasive aspergillosis (IA). [a]Until neutrophil recovery reached $\geq 0.5 \times 10^3$ cells/mm^3 for acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS) patients, and until days 180 or 100 after transplant for allogeneic hematopoietic stem cell transplant (Allo-HSCT) recipients with or without graft-vs-host disease, respectively. Abbreviation: rtPCR, real-time polymerase chain reaction.

subsequently, the patient's withdrawal from the study was mandatory. Nevertheless, the overall duration of the empirical course of therapy was included within the analyses of antifungal consumption (secondary outcome). Diagnosis of "proven" or "probable" IA was also a criterion for patient's withdrawal. Due to its design, the intervention could not be blinded to patients, attending physicians, or investigators. However, laboratory technicians who performed the monitoring assays were blinded to the intervention and all the study outcomes were reviewed by an independent adjudication committee (further details available as Supplementary Material).

Study Outcomes and Follow-up

The primary outcome was the cumulative incidence of proven or probable IA according to the EORTC/MSG criteria [20]. Secondary outcomes included all-cause and proven or probable IA-attributable mortality; proven or probable IA-free survival; cumulative incidence of any EORTC/MSG category of IA (possible, probable, or proven); and antifungal consumption.

The follow-up period extended from the date of first monitoring until 30 days after the resolution of neutropenia for AML/MDS patients, or until days 210 or 130 days for allo-HSCT recipients with or without GVHD, respectively.

Serum GM and rtPCR Assays

The serum GM assay was performed in the clinical laboratories of each center using the Platelia *Aspergillus* GM enzyme immunoassay kit (Bio-Rad, Madrid, Spain), and was considered as positive in presence of ≥2 consecutive samples with optical density (OD) indexes between 0.5 and 0.7 or a single sample with an OD index ≥0.7. The detection of *Aspergillus* DNA in serum was performed by means of a quantitative rtPCR marked with molecular beacons that detects in a multiplex format all *Aspergillus* species belonging to *A. fumigatus*, *A. terreus*, and *A. flavus* complexes/sections, as previously described [21–23] and in keeping with the requirements drawn by the European *Aspergillus* PCR Initiative [24] (further details available as Supplementary Material). To increase the predictive positive value, an rtPCR assay result was considered positive in presence of ≥2 consecutive positive samples [25]. In case of a first single positive result, no earlier confirmatory assay was ordered, but subsequent sampling was repeated according to the abovementioned schedule.

Study Definitions

IA was classified as proven, probable, or possible according to the EORTC/MSG criteria [20]. Definitions for early (diagnostic-driven), targeted, and empirical therapy are shown in Table 1. Other definitions used in the study were based on accepted criteria [26, 27] and are available in the Supplementary Material.

Table 1. Definitions Used in the Study for the Different Treatment Modalities

	Indication	
	GM-PCR Group	GM Group
Early (diagnostic-driven) therapy	Positive GM assay with either a normal thoracic HRCT scan or any new infiltrate not fulfilling the EORTC/MSG criteria Positive rtPCR with simultaneously negative GM assay, regardless of the findings of the thoracic HRCT scan	Positive GM assay with either a normal thoracic HRCT scan or any new infiltrate not fulfilling the EORTC/MSG criteria
Targeted therapy	Diagnosis of "proven" IA (positive culture or tissue specimen from a sterile site, with or without clinical [radiological] evidence) Diagnosis of "probable" or "possible" IA (radiological findings fulfilling the EORTC/MSG criteria with or without mycological evidence, respectively)	
Empirical therapy	Refractory febrile neutropenia in the absence of mycological, histological, or clinical (radiological) evidence of IA	

Abbreviations: EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; GM, galactomannan; HRCT, high-resolution computed tomography; IA, invasive aspergillosis; PCR, polymerase chain reaction; rtPCR, real-time polymerase chain reaction.

Statistical Analysis

Details on sample size calculation are provided as Supplementary Material [5, 21, 28]. Study outcomes were primarily analyzed according to per-protocol (PP) principle. In addition, intention-to-treat (ITT) analyses were also performed to examine the robustness of the efficacy results. Qualitative variables were expressed as absolute and relative frequencies. Quantitative data were shown as the mean ± standard deviation or the median with range or interquartile range (IQR). Categorical variables were compared using the χ^2 test or Fisher exact test, whereas Student *t* test or Mann–Whitney test were applied for continuous variables. Outcomes were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Survival curves were generated according to the Kaplan–Meier method and compared between groups by the log-rank test. All tests were 2-tailed and *P* values <.05 were deemed significant. Statistical analysis was performed using SPSS software, version 15.0 (SPSS Inc, Chicago, Illinois).

RESULTS

Patient Disposition and Baseline Characteristics

Overall, 224 patients were initially screened for eligibility, 219 of which (97.8%) were enrolled and underwent randomization (ITT population). Four patients did not receive the allocated intervention and 12 patients were further excluded due to major protocol violations, resulting in 96 and 107 patients analyzed in the GM-PCR and GM groups, respectively (PP population) (patient flow diagram available in Supplementary Figure 1). Both arms were overall well balanced, although the duration of neutropenia within the subset of AML patients was longer in the GM-PCR group (*P* = .006; Table 2).

Positivity Rates for GM and rtPCR Assays

The intensity of monitoring was similar in both arms (median of 8 monitoring points per patient). Throughout this period, 23.9% of patients in the GM-PCR group had ≥1 positive rtPCR result compared to 14.0% in the GM group (*P* = .070). Of 19 patients who tested positive at least once in the 2 assays (8 in the GM-PCR group and 11 in the GM group), the positivity of the rtPCR preceded that of the GM assay in 8 cases (42.1%) by a median interval of 5.5 days (IQR, 2.5–12.25 days). Conversely, the GM assay was the first to turn positive in 7 cases (36.8%) by a median interval of 6 days (IQR, 2–7 days). Patients in the GM-PCR group were more likely to have a thoracic HRCT scan examination, in most cases ordered due to a positive monitoring test (Table 3). A detailed description of the reasons for ordering such imaging tests and their contribution to diagnosis is available as Supplementary Figure 2.

Occurrence of Proven or Probable IA (Primary Outcome)

The cumulative incidence of the primary study outcome was significantly lower in the GM-PCR group (4.2% [4/96] vs

Table 2. Baseline and Clinical Characteristics and Follow-up Periods for Both Study Groups (Per-Protocol Population)

Characteristic	GM-PCR Group (n = 96)	GM Group (n = 107)	*P* Value
Age, y, mean ± SD	51 ± 14.6	50 ± 15.3	.458
Male sex	48 (50.0)	49 (45.8)	.549
Underlying hematological condition			
AML	51 (53.1)	55 (51.4)	.889
Disease status at recruitment[a]			
New diagnosis	27 (56.2)	30 (58.8)	.796
Complete remission	17 (35.4)	15 (29.4)	.523
Relapsed or refractory disease	4 (8.3)	6 (11.8)	.410
MDS	4 (4.2)	0 (0.0)	.048
Allo-HSCT	41 (42.7)	52 (48.6)	.481
Conditioning regimen[b]			.264
Reduced-intensity	35 (85.4)	38 (76.0)	
Myeloablative	6 (14.6)	12 (24.0)	
Transplant type[c]			
HLA-identical sibling	15 (41.7)	27 (58.7)	.126
HLA-matched unrelated	11 (30.6)	11 (23.9)	.500
HLA-mismatched	10 (27.8)	8 (17.4)	.259
Graft source[d]			
Peripheral blood	32 (78.0)	37 (78.7)	.939
Bone marrow	4 (9.8)	6 (12.8)	.460
Cord blood	5 (12.2)	4 (8.5)	.413
GVHD prophylaxis			
Antithymocyte globulin	10 (23.8)	10 (19.6)	.624
Cyclosporine A plus methotrexate	18 (42.9)	22 (43.1)	.978
Tacrolimus plus methotrexate	7 (16.7)	6 (11.8)	.497
Tacrolimus plus sirolimus	10 (23.8)	10 (19.6)	.624
Other	3 (3.1)	2 (1.9)	.458
Antifungal prophylaxis			.817
Fluconazole	87 (89.7)	94 (88.7)	
None	10 (10.3)	12 (11.3)	
Quinolone prophylaxis	59 (60.8)	66 (62.9)	.766
Acyclovir prophylaxis	51 (52.6)	65 (61.9)	.180
GVHD	11 (11.5)	17 (15.9)	.418
Acute			
Grades 0–II	7 (53.8)	6 (42.9)	.568
Grades III–IV	3 (23.1)	6 (42.9)	.249
Chronic	3 (23.1)	2 (14.3)	.462
Duration of neutropenia (overall), d, median (IQR)	29 (19–30)	29 (14–30)	.253
Duration of neutropenia (AML patients), d, median (IQR)	30 (22–30)	22 (14–30)	.006
Febrile neutropenia	80 (83.3)	91 (85.0)	.887
Duration of febrile neutropenia, d, median (IQR)	7 (3–16)	9 (3–17)	.535
Piperacillin-tazobactam use	23 (24.0)	29 (27.1)	.608
Follow-up, d, median (IQR)	58.5 (19–115.8)	47 (16–93)	.583

Data are presented as No. (%) unless otherwise specified.

Abbreviations: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; GM, galactomannan; GVHD, graft-vs-host disease; HLA, human leukocyte antigen; IQR, interquartile range; MDS, myelodysplastic syndrome; PCR, polymerase chain reaction; SD, standard deviation.

[a] Data not available for 7 patients.

[b] Data not available for 2 patients.

[c] Data not available for 11 patients.

[d] Data not available for 5 patients.

Table 3. Diagnostic Strategies and Positivity Rates for Serum Galactomannan and Polymerase Chain Reaction Assays in the Per-Protocol Population

Diagnostic Strategy	GM-PCR Group (n = 96)	GM Group (n = 107)	*P* Value
No. of monitoring points (GM and rtPCR) per patient, median (range)	8 (6–15)	8 (5–21)	.533
In AML/MDS patients	7 (5–8.8)	6 (4–8)	.430
In allo-HSCT recipients	20.5 (13–17)	23 (14.5–26.5)	.250
rtPCR assay positivity	23 (23.9)	15 (14.0)	.070
Time to first positive PCR assay, d, median (IQR)	12 (9–20)	18 (9–30)	.425
Positivity only in rtPCR assay[a]	15/23 (65.2)	4/15 (26.7)	
GM assay positivity	8 (8.3)	17 (15.9)	.102
Time to first positive GM assay, d, median (IQR)	16 (13–33)	18 (10–41)	.807
Positivity only in GM assay[b]	0/8 (0.0)	6/17 (35.3)	
Positivity in both assays	8 (8.3)	11 (10.3)	.815
First positive rtPCR preceding first positive GM	5/8 (62.5)	3/11 (27.3)	.181
Interval, d, median (IQR)	6 (3.5–11.5)	4 (. . .)[c]	
First positive GM preceding first positive rtPCR	2/8 (25.0)	5/11 (45.4)	.633
Interval, d, median (IQR)	7 (. . .)[c]	4 (1.5–7)	
Thoracic HRCT scan examination	42 (43.8)	36 (33.6)	.139
Ordered due to a positive monitoring test (GM and/or rtPCR)	24/42 (57.1)	20/36 (55.6)	.887
Other reasons for ordering	18/42 (42.9)	16/36 (44.4)	

Data are presented as No. (%) unless otherwise specified.

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; GM, galactomannan; HRCT, high-resolution computed tomography; IQR, interquartile range; MDS, myelodysplastic syndrome; PCR, polymerase chain reaction; rtPCR, real-time polymerase chain reaction.

[a] GM assays were persistently negative throughout the monitoring period.

[b] rtPCR assays were persistently negative throughout the monitoring period.

[c] IQR not calculable.

13.1% [14/107]; OR, 0.29 [95% CI, .09–.91]; $P = .028$). The absolute risk reduction was 8.9% (95% CI, 1.0–17.0), with a number necessary to treat (NNT) of 11 (95% CI, 6–102). This difference was also confirmed in the ITT population (3.8% [4/105] vs 12.3% [14/114]; OR, 0.29 [95% CI, .09–.89]; $P = .027$;

Table 4. Cumulative Incidence of Invasive Aspergillosis at the End of Follow-up According to Study Group in the Per-Protocol Population

Outcome	GM-PCR Group (n = 96)	GM Group (n = 107)	*P* Value
Proven or probable IA (primary outcome)	4 (4.2)	14 (13.1)	.028
Time to diagnosis, d, median (IQR)	13 (10–26)	20 (14–43)	.022
Any EORTC/MSG diagnostic category of IA (secondary study outcome)	13 (13.5)	17 (15.9)	.695
Possible IA	9 (9.4)	3 (2.8)	.071
Probable IA	4 (4.2)	12 (11.2)	.072
Proven IA	0 (0.0)	2 (1.9)	.499

Data are presented as No. (%) unless otherwise specified.

Abbreviations: EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; GM, galactomannan; IA, invasive aspergillosis; IQR, interquartile range; PCR, polymerase chain reaction.

NNT = 12). In addition, the median time interval to diagnosis was shorter in the GM-PCR group (13 [IQR, 10–26] days vs 20 days [IQR, 14–43]; $P = .022$) (Table 4).

A positive rtPCR assay result preceded or coincided with that of the GM assay in 50.0% (9/18) of patients with proven or probable IA, whereas detection of serum GM preceded rtPCR in 27.8% (5/18) (Table 5). Of note, the rtPCR monitoring remained negative in 4 patients eventually diagnosed with probable IA within the GM group (details are provided in Supplementary Table 1.

Secondary Study Outcomes

Proven or probable IA-free survival was significantly longer in the GM-PCR group (log-rank test $P = .027$; Figure 2). In contrast, we found no differences in the cumulative incidence of IA of any EORTC/MSG diagnostic category (13.5% [13/96] vs 15.9% [17/107]; OR, 0.83 [95% CI, .38–1.81]; $P = .695$; Table 4).

Table 5. rtPCR and Galactomannan Assay Results in Patients Diagnosed With Proven or Probable Invasive Aspergillosis in the Per-Protocol Population

Diagnostic Group	GM-PCR Group, No. (n = 4)	GM Group, No. (n = 14)
Positive rtPCR preceding positive GM	3	3
Simultaneously positive rtPCR and GM	0	3
Positive GM preceding positive rtPCR	1	4
Positive GM, rtPCR always negative	0	4

Abbreviations: GM, galactomannan; PCR, polymerase chain reaction; rtPCR, real-time polymerase chain reaction.

Forty-seven patients received empirical antifungal therapy after a median interval of 13 days (IQR, 8–28 days). Patients in the GM-PCR group were less likely to receive either empirical ($P = .038$) or targeted therapy ($P = .015$) as compared to the GM group. Early therapy was more common in the GM-PCR group ($P < .0001$), with a positive rtPCR assay triggering the initiation of such an approach in most cases (18/19 [94.7%]). There were no differences in the overall duration of antifungal therapy (Table 6).

All-cause mortality did not significantly differ between groups (13.5% [13/96] in the GM-PCR group vs 15.9% [17/107] in the GM group; OR, 0.83 [95% CI, .38–1.81]; $P = .695$). No differences were found either for proven or probable IA-attributable mortality (Table 6). These findings remained unchanged when the analysis was restricted to the ITT population or stratified by underlying condition (data not shown).

DISCUSSION

Previous experiences indicate that *Aspergillus* DNAemia may precede the release of fungal GM into the bloodstream [19, 21], which suggests that DNA detection can offer advantages in the early diagnosis and preemptive therapy of IA. In accordance with this rationale, the application of the combined approach based on the detection of both serum GM and *Aspergillus* DNAemia in high-risk patients not receiving antimold prophylaxis resulted in a relative risk reduction of 68.1% in the primary study outcome (proven or probable IA).

It should be noted that, similarly to previous trials on high-risk patients [29–31], only probable and proven forms of IA were considered as primary outcomes. Because the sensitivity of the PCR method was presumed to be higher than that of the GM assay, we expected a priori that the number of HRCT scans triggered by a positive result would be increased in the GM-PCR group. Thus, the chance to find a radiological image suggestive of IA in the absence of validated mycological criteria —it should be noted that the detection of *Aspergillus* DNA by PCR has not been recognized so far as a diagnostic criterion by the EORTC/MSG [20]— could be increased among these patients and, in turn, their odds of being diagnosed with possible IA, skewing the results against the intervention arm.

By applying the combined strategy, we were able to reduce by 7 days the median interval elapsed from the start of monitoring to the diagnosis of IA. In preliminary studies, it has been shown that PCR positivity is able to anticipate that of GM assay by 2–3 weeks [11, 12, 19]. Differences in kinetics of both biomarkers may underlie these observations [32]. Surprisingly, the PCR monitoring was persistently negative in 4 patients who eventually developed IA in the control group. All of them received the

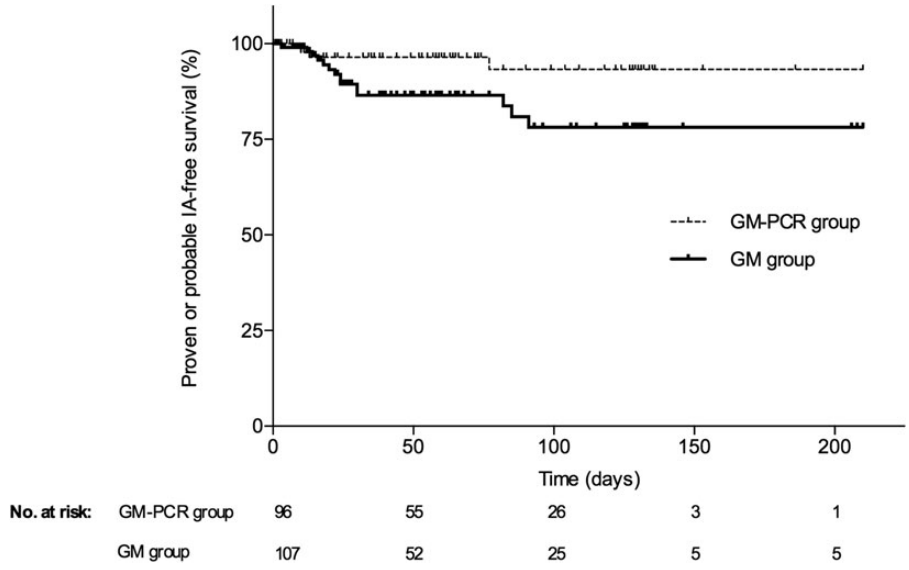

Figure 2. Proven or probable invasive aspergillosis–free survival (Kaplan–Meier curves) in both study groups (per-protocol population) (log-rank test $P = .027$). Abbreviations: GM, galactomannan; IA, invasive aspergillosis; PCR, polymerase chain reaction.

diagnosis of probable disease as per EORTC/MSG criteria based on the presence of host factors, radiological findings, and serum GM positivity. We hypothesize that the high rate of false-positive results with the GM assay [8, 9, 28] and the lack of reliability of our PCR technique to detect species belonging to complexes or sections other than *A. fumigatus*, *A. terreus*, and *A. flavus* might explain this discrepancy, although the possibility of false-negative results in the latter test should also be recognized. The potential advantage in terms of sensitivity from using other samples (ie, bronchoalveolar lavage) for rtPCR monitoring remains to be explored.

The combined GM and PCR-based strategy led to a reduction in the use of empirical and targeted antifungal therapy as compared to the conventional approach. On the other hand, early (diagnostic-driven) therapy was more common in the GM-PCR group, mainly due to the earliness of diagnosis exhibited by the rtPCR assay. Such finding might be interpreted as suggesting that the early detection of *Aspergillus* DNA and therapy ultimately prevented the development of fully established angioinvasive forms. In keeping with this, only 17.4% of patients in the GM-PCR group with a positive rtPCR were finally diagnosed with probable or proven IA compared with 82.3% of those in the control group with a positive GM assay. Overall, these results could indicate that the monitoring for both serum GM and *Aspergillus* DNAemia might provide a better guidance for antifungal treatment by identifying earlier subclinical stages of the infection.

Previous studies have shown that the implementation of PCR-based monitoring may reduce the use of empirical antifungal therapy [10, 33]. Morrissey et al recently demonstrated that a biomarker-based diagnostic strategy (serum PCR and GM assays) in high-risk hematological patients leads to a reduction in the use of empirical therapy compared to the standard approach based on culture and/or histology [14]. Our trial provides further insight by means of a head-to-head comparison of the utility of both biomarkers in the clinical decision-making process. Had the results for the rtPCR assay been available for clinicians in the GM group, 12.9% [4/31] of patients who received empirical therapy would have benefited from avoiding unnecessary treatment or from advancing its initiation.

Only 5 of the deaths observed in this trial were attributable to IA, resulting in an overall case-fatality rate (27.8%) similar to that in recent studies [2, 4, 5, 14, 34]. Although earlier diagnosis and initiation of therapy might have resulted in a better outcome [35] —as suggested by the nonsignificant trend observed toward a decreased IA-related mortality in the GM-PCR group—it should be stressed that the sample size of our trial was not powered to detect differences in any of the secondary outcomes.

This study has several limitations. Most notably, intergroup differences in the PCR positivity rates could suggest that both arms were not entirely comparable in their baseline risk of IA. In accordance, within the subset of AML patients, the neutropenia was more prolonged in those allocated to the GM-PCR group, whereas the presence of other disparities that remained undetected due to inadequate statistical power cannot be excluded. Nevertheless, such an imbalance should have biased the results against the intervention arm. Conversely, the number of patients with a positive GM assay was slightly lower in the GM-PCR group, although this finding may be explained by the effect of the earlier administration of antifungal therapy once

Table 6. Use of Antifungal Therapy (Other Than Fluconazole Prophylaxis) and Overall and Invasive Aspergillosis–Attributable Mortality in the Per-Protocol Population

Outcome	GM-PCR Group (n = 96)	GM Group (n = 107)	*P* Value
Overall use of antifungal therapy	46 (47.9)	57 (53.3)	.446
Empirical therapy	16 (16.7)	31 (29.0)	.038
Early ("diagnostic-driven") therapy	19 (19.8)[a]	3 (2.8)	.000
Targeted therapy	5 (5.2)[b]	17 (15.9)	.015
Other indications[c]	6 (6.2)	6 (5.6)	.846
Duration of antifungal therapy, d, mean ± SD	12.7 ± 24.2	11.8 ± 24.5	.730
Empirical therapy	2.8 ± 9.0	3.5 ± 8.1	.114
Early therapy	5.3 ± 17.0	0.6 ± 4.6	.000
Targeted therapy	3.5 ± 15.8	6.5 ± 23.7	.019
All-cause mortality at the end of follow-up	13 (13.5)	17 (15.9)	.695
30-day mortality	6 (6.3)	5 (4.7)	.759
180-day mortality	13 (13.5)	14 (13.1)	1.000
Proven or probable IA-attributable mortality	1 (1.0)[d]	4 (3.7)	.247
Proven IA-attributable mortality	0 (0.0)	1 (0.9)	. . .
Probable IA-attributable mortality	1 (1.0)	3 (2.8)	. . .

Data are presented as No. (%) unless otherwise specified.

Abbreviations: EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; GM, galactomannan; HRCT, high-resolution computed tomography; IA, invasive aspergillosis; PCR, polymerase chain reaction; rtPCR, real-time polymerase chain reaction; SD, standard deviation.

[a] Includes 8 patients with positive rtPCR assay, abnormal findings on the HRCT scan (although not fulfilling the EORTC/MSG criteria) and negative GM assay; 10 patients with positive rtPCR assay, normal HRCT scan and negative GM assay; and 1 patient with positive GM assay and normal HRCT scan.

[b] Includes 4 patients with probable IA and 1 patient with possible IA (ie, presence of radiological findings suggestive of IA according to the EORTC/MSG criteria, but negative rtPCR and GM assays).

[c] Includes 8 patients who received therapy for an invasive fungal disease other than IA, and 4 in which the fluconazole prophylaxis was substituted with an antimold agent after inclusion.

[d] One additional patient died 12 days after being diagnosed with possible IA on the basis of a positive rtPCR assay associated with a highly suggestive HRCT scan examination.

Aspergillus DNAemia was detected, which could have prevented fungal antigen from being released into the bloodstream during the angioinvasion process. Although we jointly analyzed AML patients and allo-HSCT recipients, the pathogenesis of IA in both types of host is not entirely comparable. Our trial was insufficiently powered to detect potential differences in mortality or subgroup analyses. Finally, the unblinded design raises the potential for bias, the direction of which is not straightforward. Indeed, some clinicians could have been prone to rely on the high sensitivity of the PCR and, therefore, to perform closer surveillance in patients with a positive result, whereas others might have felt more comfortable with the conventional, long-standing GM-based strategy and have underestimated to some extent the positive predictive value of the PCR assay.

In conclusion, this is the first trial to directly compare the serial detection of serum GM and *Aspergillus* DNA with the conventional approach solely based on the GM assay in high-risk hematological patients. Through the earlier diagnosis and initiation of antifungal therapy, the tested strategy was able to significantly decrease the incidence of proven or probable IA. Our results suggest that the PCR should be used to complement, rather than replace, the serum GM assay as the basis of a diagnostic-driven strategy for preventing IA. Further studies are needed to evaluate whether this intervention would offer additional advantages over the increasing use of mold-active antifungal prophylaxis.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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