MAJOR ARTICLE

Earlier Response Assessment in Invasive Aspergillosis Based on the Kinetics of Serum *Aspergillus* Galactomannan: Proposal for a New Definition

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Summary. We show that serum galactomannan–based response criteria for invasive aspergillosis compare favorably to current definitions, allow earlier response assessment, and rely on a simple, reproducible, objective, and *Aspergillus*-specific test and should serve as the primary endpoint in trials of invasive aspergillosis.

Background. Current criteria for assessing treatment response of invasive aspergillosis (IA) rely on nonspecific subjective parameters. We hypothesized that an *Aspergillus*-specific response definition based on the kinetics of serum *Aspergillus* galactomannan index (GMI) would provide earlier and more objective response assessment.

Methods. We compared the 6-week European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) response criteria with GMI-based response among 115 cancer patients with IA. Success according to GMI required survival with repeatedly negative GMI for ≥2 weeks. Time to response and agreement between the 2 definitions were the study endpoints.

Results. Success according to EORTC/MSG and GMI criteria was observed in 73 patients (63%) and 83 patients (72%), respectively. The GMI-based response was determined at a median of 21 days after treatment initiation (range, 15–41 days), 3 weeks before the EORTC/MSG time point, in 72 (87%) of 83 responders. Agreement between definitions was shown in all 32 nonresponders and in 73 of the 83 responders (91% overall), with an excellent κ correlation coefficient of 0.819. Among 10 patients with discordant response (EORTC/MSG failure, GMI success), 1 is alive without IA 3 years after diagnosis; for the other, aspergillosis could not be detected at autopsy. The presence of other life-threatening complications in the remaining 8 patients indicates that IA had resolved.

Conclusions. The *Aspergillus*-specific GMI–based criteria compare favorably to current response definitions for IA and significantly shorten time to response assessment. These criteria rely on a simple, reproducible, objective, and *Aspergillus*-specific test and should serve as the primary endpoint in trials of IA.

Despite recent advances in the management of invasive aspergillosis (IA), this infection remains a leading cause of morbidity and mortality in immunocompromised patients [1]. Better therapeutic strategies are clearly needed and are best tested by means of a randomized controlled trial in which outcome can be rapidly assessed on the basis of *Aspergillus*-specific objective criteria. Rapid assessment of outcome increases trial efficiency and may allow quicker access to potentially life-saving therapies for patients with IA [2]. The European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) recently published the consensus definitions of outcome of IA, which rely on clinical and radiologic findings; negative microbiologic markers, including culture results; and survival at specific time points [3]. In the revised definitions, outcome is assessed at 6 weeks, compared with the previously used 12-week endpoint [4].

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The EORTC/MSG outcome definitions represent an important advance in the field but are somewhat limited by their reliance on subjective criteria (eg, improvement or resolution of clinical and radiologic findings attributable to IA); tests that are not typically available (eg, repeated cultures and/or histopathology); and survival, which is frequently related to the underlying disease, particularly later during the course of IA [1]. These limitations are exemplified by the results of 2 randomized controlled trials of treatment of IA in which 12-week survival rates were surprisingly higher than the rates of aspergillosis response [4, 5]. This suggests the need for alternative more specific methods, such as the application of rapid validated surrogate endpoints, provided that these endpoints can be rapidly achieved. The serum *Aspergillus* galactomannan index (GMI) is an objective marker that fulfills all criteria as a surrogate endpoint for IA [2]; indeed, GMI values correlate with fungal burden in animal models [6] and a strong association between serum GMI values and clinical outcome has been shown by us and others [7–9]. In addition, GMI values typically become positive before overt manifestations of IA become detectable [10].

The purpose of this study was to identify a response definition for IA that is *Aspergillus* specific and allows an earlier assessment than the 6-week EORTC/MSG endpoint. In particular, we evaluated the response rate and time to response of IA among 115 cancer patients with IA using 2 methods: the kinetics of serial serum GMI tests and the EORTC/MSG response criteria [3].

METHODS

Data from a prospective cohort of immunocompromised patients with hematological cancer treated at University of Arkansas for Medical Sciences from January 2003 through January 2010 were reviewed. Patients were managed according to predefined standards of care; serum GMI testing was obtained serially (typically 3 times a week) after the start of antineoplastic therapy [8] and performed according to the manufacturer's instructions (Platelia Aspergillus EIA; Bio-Rad). Fluconazole was given as primary antifungal prophylaxis. Empirical antifungal therapy was not part of the standard of care. The study was approved by the institutional review board. Cases of IA were defined prospectively by 1 of the authors (E. A.) and reviewed by 2 other authors (S. A. N. and M. N.).

Cases were classified as proven or probable IA according to the EORTC/MSG definitions [11]. A third category, "probable IA without prespecified radiologic findings" [12], required the same host and mycological (including positive GMI test results) and clinical criteria per EORTC/MSG definitions but allowed less defined radiologic findings (ie, dense, well-circumscribed lesions, air-crescent sign, or cavity). Cases of possible IA were excluded.

Two response criteria for IA were compared: the revised EORTC/MSG criteria [3], which we defined as the standard; and one based on the kinetics of GMI (*Aspergillus*-specific GMI–based criteria). The study endpoints were the degree of agreement between and the time to outcome evaluation of the 2 definitions. In brief, the EORTC/MSG criteria define success as survival at 6 weeks plus resolution of all attributable symptoms and signs of disease (complete response) or improvement in attributable symptoms and signs of disease and attributable radiological abnormalities (>25% reduction compared with baseline) plus clearance of cultures or reduction of fungal burden, as assessed by a quantitative and validated laboratory marker (partial response); whereas failure included stable disease (6-week survival and minor or no improvement in fungal disease), progressive infection (as determined by a composite of clinical, radiological, and mycological criteria), or death regardless of attribution to IA.

The *Aspergillus*-specific GMI–based response defined success as survival and repeatedly negative serum GMI for ≥2 weeks after the first negative GMI in the absence of new extrapulmonary lesions of IA (eg, a skin lesion culture-positive and/or with hyphal tissue invasion consistent with IA), whereas failure referred to persistently positive serum GMI. Death during the 14-day period was considered failure, unless autopsy examination failed to reveal IA.

Serological relapse was defined if a patient classified as successful by GMI criteria presented any positive GMI test during the 6-week period, provided that no new immunosuppressive therapy for the underlying disease was administered during this period. Neutropenia was defined as an absolute neutrophil count (ANC) $<500/mm^3$ and was considered severe when the ANC was $<100/mm^3$. Bone marrow recovery referred to 3 consecutive ANCs $>500/mm^3$. Chemotherapy regimens that did not result in neutropenia were referred to as nonmyelosuppressive. The date of diagnosis of IA was defined as the date of the first positive GMI. A positive GMI test result was defined as 1 sample with an optical density index ≥0.5. If a patient developed >1 episode of IA, only the first episode was considered in the present study.

The κ correlation coefficient (KCC) was used to determine the correlation between the GMI-based criteria and the EORTC/MSG response criteria. Correlation is considered excellent when $\kappa \geq 0.75$ [13]. Survival was constructed using the Kaplan-Meier method, taking into account the date of diagnosis of IA and the date of death or last follow-up, censored at day 90 after the diagnosis of IA. All analyses were performed using SPSS, version 15.0 for Windows (SPSS). A P value of $<.05$ was considered to reveal a statistically significant difference.

RESULTS

A total of 125 consecutive patients received a diagnosis of IA during the study period. Their characteristics were previously

published [12]. Ten patients were excluded: 4 because their baseline GMI values were not positive and 6 because their response status at 6 weeks could not be assessed. The characteristics of the remaining 115 patients are shown in Table 1. Most patients (90%) had multiple myeloma, and 55 had undergone hematopoietic stem cell transplantation. IA was documented at a median of 27 months following the diagnosis of the underlying cancer (range, 0–186 months) and at a median of 13 days after the last cycle of chemotherapy; 105 (91%) had received corticosteroids within 60 days from the diagnosis of IA, at a cumulative dose of 1080 mg (prednisone equivalent), and 100 patients (87%) were neutropenic (median of 11 days) when IA was diagnosed.

Invasive aspergillosis was classified as proven (5 patients [4%]), probable (73 patients [64%]), or probable without prespecified radiologic findings (37 patients [32%]). The infection involved the lungs only (84 patients), lungs and sinuses (23 patients), or sinuses alone (8 patients). The median number of positive GMI test results was 6 (range, 1–69), with a median value at diagnosis of 1.1 (range, 0.5–7.4) and a median peak of 2.5 (range, 0.6–9.6).

Table 1. Characteristics of 115 Cancer Patients With Invasive Aspergillosis (IA)

Characteristic	Patients
Sex, male:female	68:47
Age, median years (range)	60 (26–81)
Multiple myeloma as underlying disease, no. (%)[a]	104 (90)
Antineoplastic treatment	
Chemotherapy[b]	60 (52)
HCT[c]	55 (48)
Prior HCT, median no. (range)	1 (0–4)
Receipt of corticosteroids during the 60 days preceding IA diagnosis, no. (%)	105 (91)
Neutropenia at diagnosis of IA, no. (%)[d]	100 (87)
Antifungal therapy, no. (%)	
Voriconazole	66 (57)
Liposomal amphotericin B	28 (24)
Other[e]	14 (13)
None	7 (6)
Time from diagnosis of IA to antifungal therapy, median days (range)	3 (0–28)
Duration of antifungal therapy, median days (range)	23 (1–82)

Abbreviation: HCT, hematopoietic stem cell transplantation.

[a] Other underlying diseases: acute myeloid leukemia (4 patients), non-Hodgkin lymphoma (3 patients), solid tumor (2 patients), chronic lymphocytic leukemia (1 patient), and myelofibrosis (1 patient).

[b] There were 50 myelosuppressive and 10 nonmyelosuppressive regimens; 50 autologous and 5 allogeneic HCT.

[c] Seven patients did not receive treatment because of early death (2) or spontaneous response after bone marrow recovery (5).

[d] Neutropenia was defined as absolute neutrophil count $<500/mm^3$.

[e] Other treatments included combinations of various agents: voriconazole, liposomal amphotericin B, micafungin, or anidulafungin.

Seven patients did not receive *Aspergillus*-active antifungal therapy because of death at diagnosis of IA (1 patient) or response with bone marrow recovery (6 patients). Among the 108 patients who received treatment, voriconazole was used in 66 cases (57%) and liposomal amphotericin B in 28 cases (24%). The median duration of treatment was 23 days (range, 1–82 days); 19 patients (18% of those treated) received treatment for $\leq$7 days, whereas 37 patients (34%) were treated for $\leq$14 days. The Kaplan–Meier estimate of survival at 6 weeks was 69%, with 79 patients alive at this time point.

Success according to EORTC/MSG and GMI criteria was observed in 73 patients (63%) and 83 patients (72%), respectively (Table 2). Normalization of serum GMI occurred in 91 patients (79%), at a median of 8 days (range, 1–71 days) after the first positive GMI result, and 7 days (range, 0–69 days) after treatment initiation. Eighty-two (90%) of these 91 patients had repeatedly negative GMI test results for $\geq$14 days and were classified as success according to the *Aspergillus*-specific GMI–based criteria. One additional patient died 3 days after GMI normalization, but because autopsy examination failed to reveal IA, the patient was classified as success, giving a total of 83 responders per GMI-based criteria. In 72 (87%) of the 83 responders, time to response per *Aspergillus*-specific GMI–based criteria was 21 days (range, 15–41 days) after treatment initiation, 3 weeks earlier than the EORTC/MSG assessment time point (Table 2).

Serological relapse was observed in only 1 of the 83 patients classified as success according to GMI criteria. This patient was classified as success 21 days after treatment initiation but experienced a relapse 25 days later (after 6 weeks). At the 6-week time point, the patient was classified as success (partial response) according to the EORTC/MSG criteria. A total of 7 of 25 GMI

Table 2. Outcome of 115 Patients With Invasive Aspergillosis According to European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) and Serum *Aspergillus* Galactomannan Index (GMI)–Based Response Criteria

	Success after start of antifungal therapy				
Criteria	Week 3	Week 4	Week 5	Week 6	After week 6[a]
EORTC/MSG, no. (%) of patients	NA	NA	NA	73 (63)	73 (63)
GMI-based, no. (%) of patients	44 (38)	65 (56)	72 (63)	76 (66)	83 (72)
Agreement, %	…	…	…	85	91[b]

Abbreviations: NA, not applicable.

[a] In 7 patients, the GMI normalized after the 6-week time point.

[b] Agreement between the 2 response definitions was noted in all 32 nonresponders (100%) and in 73 (87%) of the 83 responders, giving an overall agreement of 91%.

Table 3. Characteristics of the 10 Patients With Invasive Pulmonary Aspergillosis (IA) Who Had a Discordant Response (Failure According to European Organization for Research and Treatment of Cancer/Mycosis Study Group [EORTC/MSG] Definition, Success According to *Aspergillus*-Specific Galactomannan Index [GMI] Definition)

Patient	EORTC/MSG response	Time to GMI normalization, days	No. of GMI tests with negative results[a]	Lung infiltrates	Concomitant conditions	Survival after IA diagnosis	Survival after negative GMI test result
1	Failure, stable response	57	2	Stable	None	Alive	3 years
2	Failure, death	12	3	Worse	No IA at autopsy	15 days	3 days
3	Failure, death	20	18	Worse	PE, CMV disease	37 days	17 days
4	Failure, death	7	36	New infiltrates	*Pseudomonas aeruginosa* pneumonia and bacteremia	42 days	35 days
5	Failure, death	4	18	Worse	CMV colitis, VRE sepsis, cardiogenic shock	22 days	15 days
6	Failure, death	17	15	Not tested	*P. aeruginosa* pneumonia, VRE sepsis	33 days	16 days
7	Failure, death	9	30	Improved	PJP, CMV colitis	39 days	30 days
8	Failure, death	13	20	Worse	*Klebsiella pneumoniae* pneumonia	32 days	19 days
9	Failure, death	5	26	Worse	Accidental extubation, VRE sepsis	31 days	26 days
10	Failure, death	4	15	Not tested	Disseminated adenoviral infection, *Clostridium difficile* colitis,VRE sepsis	19 days	15 days

Abbreviations: CMV, cytomegalovirus; PE, pulmonary embolism; PJP, *Pneumocystis jirovecii* pneumonia; VRE, vancomycin-resistant enterococci.

[a] Number of tests between first negative test result and death or last follow-up.

tests performed during this follow-up period had positive results. Chest computed tomographic (CT) scan performed at relapse revealed a cavitary lesion at the site of a previously identified nodule. The patient received voriconazole and cefepime for a concomitant bacterial infection and died 1 month after serological relapse.

Among the 32 patients classified as failure according to GMI-based criteria, 24 died before GMI normalization, at a median of 12 days after diagnosis (range, 2–96 days). For 3 of these 24 patients, an autopsy was performed, which revealed IA. The remaining 8 patients died after GMI normalization but before the predefined 14-day period and hence were classified as failure.

Agreement between the 2 response definitions was present for all 32 patients whose treatment failed (100%) and for 73 (87%) of the 83 patients whose IA responded to treatment, giving an overall agreement of 91%. The sensitivity of the GMI-based criteria was 100%, and the specificity was 76%. A high KCC of 0.819 ($P < .001$) was shown between the GMI-based and the EORTC/MSG response criteria.

Among the 10 discordant cases (9%) (failure according to the EORTC/MSG criteria but success according to the GMI criteria), 9 patients died with uncontrollable cancer and 1 was classified as failure (stable disease per EORTC/MSG). This patient remains alive 3 years later and without relapse of IA. An autopsy examination performed on 1 of the 9 patients who died failed to identify IA. Notably, concomitant serious pulmonary conditions were present among the 6 patients with persistent or worsening lung infiltrates prior to death, including pulmonary embolism, cardiogenic shock, and pneumonia due to bacteria, cytomegalovirus, and adenovirus (Table 3).

DISCUSSION

To our knowledge, this is the first study to evaluate objective and *Aspergillus*-specific criteria to determine the quality of and time to response of IA and to compare these criteria with the current standard for response assessment. More importantly, these findings were examined in a high-risk homogeneous patient population (90% with myeloma) cared for at a single institution, undergoing standardized antineoplastic therapies and infectious disease management, including diagnostic workup and therapy.

Our results indicate that the GMI-based response criteria compare favorably to the EORTC/MSG definition, as shown by the excellent KCC. In addition, the GMI-based criteria allow a much earlier assessment of response in the vast majority of patients and segregate patients into rapid and slow responders. In addition, the characteristics of the 10 discordant cases (success according to GMI and failure according to EORTC/MSG criteria) suggest that they indeed responded to treatment, as discussed earlier, and that the GMI-based criteria may be more precise than the current response definition.

Our findings are supported by several experimental models [6, 11, 14–16], clinical reports [7–9, 17, 18], and autopsy studies of IA [19], all of which revealed a 95%–100% correlation between GMI-determined infection burden and outcome. That relapse was not observed among the 44 responders who were not treated or received ≤14 days of antifungal therapy is further validation of the good performance of the GMI-based response criteria.

The EORTC/MSG response criteria are currently the accepted standard for assessing IA response in clinical trials. However, these criteria rely on "attributable" clinical and radiologic findings. For example, success requires resolution of (or improvement in) all attributable symptoms and signs of disease. This requirement complicates response assessment, because the clinical manifestations of IA are nonspecific, and persistence or recurrence of such symptoms as fever and cough may be due to several other causes [20, 21]. Likewise, positive fungal culture results may represent colonization; repeated sampling is not always feasible; false-negative results are common; and concordance between culture results and survival has not been shown [2, 3].

The other major component of the EROTC/MSG response criteria is radiologic findings. However, radiologic images are not specific for aspergillosis [22, 23] and may even be misleading, because lesions may increase in patients whose condition is responding to treatment, as a result of an inflammatory immune reconstitution syndrome (IRIS) [21].

In contrast, *Aspergillus*-specific GMI serodiagnosis fulfills all criteria as a surrogate endpoint for outcome evaluation [2], including (1) biological plausibility—galactomannan is released by *Aspergillus* species during growth [24], and GMI values correlate with fungal burden in experimental models [11, 25–28]; (2) prediction of outcome—a strong concordance exists between serum GMI test result and outcome [7–9, 17, 18, 25, 29]; (3) excellent attributes—the GMI test is standardized, reproducible, easy to perform, noninvasive, relatively inexpensive, quantitative, and its results rapidly available [2]; and (4) applicability—results are applicable to infections caused by various *Aspergillus* species and at various anatomical sites [2].

In the present study, all patients with persistently positive GMI test results (ie, classification as failure according to the *Aspergillus*-specific GMI–based criteria) were also considered nonresponders according to the EORTC/MSG criteria, albeit it at a much later time point. This is important, because an appropriate and earlier classification of failure may lead to timely changes in treatment strategies. This early classification of response would probably not be possible on the basis of clinical and radiologic criteria only, because worsening clinical and radiologic findings may represent IRIS [21].

Our study shares the limitations of all retrospective studies and is hampered by a low rate of autopsy examination, a trend noted worldwide [30]. In addition, some CT scans were not performed precisely at 6 weeks, hampering a precise assessment of the EORTC/MSG response criteria. Finally, our results should be confirmed in other clinical settings (other underlying diseases, allogeneic transplantation, and so forth).

Our findings that GMI kinetics may be used as endpoint for clinical trials have implications for trial methodology in IA. Determining the sample size for clinical trials using the EORTC/MSG response criteria relies on the difference in proportions of response at a given time point (eg, 70% vs 50% response rate at 6 weeks). This typically requires a large sample size [31]. Relying on serial GMI testing provides an objective and *Aspergillus*-specific endpoint [2], offers the advantage of a smaller sample size for clinical trials (because of the large number of serial GMI endpoints), [31] and further enables us to determine the exact time to response. Although 2 regimens may have similar response rates, one may achieve response faster and would therefore be the treatment of choice, particularly in cancer patients in whom delay of antineoplastic therapy is detrimental. Because of the smaller sample size required with a GMI-based strategy, trials of IA therapies can be conducted more efficiently. In turn, this provides patients with earlier access to potentially life-saving therapies. A randomized controlled trial of combination antifungal therapy in IA using time to normalization of GMI as endpoint is currently underway. The clinical implications of our findings include the opportunity to change therapy in a timely fashion if response is deemed unlikely on the basis of GMI kinetics and to individualize the duration of antifungal therapy.

In conclusion, we show that the *Aspergillus*-specific GMI–based response criteria compare favorably to those of the EORTC/MSG, provide a much earlier time to response assessment, and rely on a quantifiable test that is simple to perform, widely available, reproducible, objective, and *Aspergillus* specific. Therefore, we recommend the adoption of this response definition as a primary endpoint in clinical trials of IA.

Notes

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