Invasive Aspergillosis in Allogeneic Stem Cell Transplant Recipients: Increasing Antigenemia Is Associated with Progressive Disease

François Boutboul,1 Corinne Alberti,2 Thierry Leblanc,3 Annie Sulahian,1 Eliane Gluckman,4 Francis Derouin,1 and Patricia Ribaud4

1Laboratoire de Parasitologie-Mycologie, 2Département de Biostatistique Médicale, 3Service d'Hématologie Pédiatrique, and 4Service de Greffe de Moelle, Hôpital Saint-Louis, Paris, France

The kinetics of serum Aspergillus galactomannan, as determined by enzyme-linked immunosorbent assay, was examined in 37 allogeneic stem cell transplant (SCT) recipients treated for invasive aspergillosis (IA). Fifty-eight periods of response (“response episodes”) were evaluated. There were 42 response episodes that were considered “treatment failures” and 16 that were considered “good” (that is, complete or partial) responses. At baseline (the first day of each new response episode), the patients who experienced treatment failure and those who had good responses did not differ significantly with regard to median galactomannan index (GMI) value. Thereafter, GMI values significantly increased in the treatment failure group, whereas no significant changes were observed in the good response group (P = .002). An increase in the GMI value of 1.0 over the baseline value during the first week of observation was predictive of treatment failure with a sensitivity of 44%, a specificity of 87%, and a positive predictive value of 94%. We conclude that serial determination of serum GMI values is a useful tool for assessing prognosis of IA in allogeneic SCT recipients during treatment.

Invasive aspergillosis (IA) has become one of the leading infectious complications among allogeneic stem cell transplant (SCT) recipients, with a mortality rate of ≧80% [1, 2]. This dreadful prognosis is related to the severe and protracted immunosuppression experienced by these patients, as well as to the frequent delay in diagnosis and insufficient treatment efficiency and/or limiting toxicity of antifungal agents. Recently, the detection, by ELISA, of circulating Aspergillus galactomannan (GM) in samples of serum or other body fluids has received considerable attention as a valuable method for diagnosis of IA that can be used early in the course of infection [3–5]. The usefulness of GM monitoring for assessment of therapeutic response has been established in experimental models of IA [6–9], but its usefulness for humans is still debated. To complement a study of ours published elsewhere [3] about the determination of GM levels for the diagnosis of IA in SCT recipients, we observed the kinetics of GM serum levels in these patients and examined the relationship to outcome.

PATIENTS, MATERIALS, AND METHODS

Study population. We reviewed the medical records for all allogeneic SCT recipients who developed IA during the period from January 1995 through September 1998. All patients had undergone transplantation in the Bone Marrow Transplant Unit at Hôpital Saint-Louis (Paris, France).

Diagnoses of IA were reviewed by the Hospital Aspergillus Study Group. Diagnoses were made on the basis of the findings of clinical charts, imaging studies (including CT scans), and mycological criteria. Patients
with a definite diagnosis of pulmonary IA were those for whom fungal hyphae were observed directly in bronchial alveolar lavage specimens or in 2 consecutive sputum samples and/or in culture of these specimens, in conjunction with findings on a lung CT scan suggestive of IA (i.e., halo sign or air crescent). The diagnosis was considered “probable” if it was based only on findings of a CT scan suggestive of IA. A “definite sinus infection” was defined by a needle aspiration sample or biopsy specimen that was positive for Aspergillus species, in addition to consistent CT findings. A “probable sinus or cerebral infection” was defined by CT findings consistent with this diagnosis in the setting of another site positive for Aspergillus species. All other sites of infection were confirmed by examination of biopsy specimens. Cultures positive for Aspergillus species were not required to define a case when examination of smear or biopsy tissue specimens revealed fungal hyphae [2].

During the study period, IA was diagnosed in 43 patients. Thirty-seven patients met the criteria for confirmed (22 patients) or probable (15 patients) IA and had ≥2 serial determinations of serum GM levels. These patients constituted the study population. There were 23 male and 14 female patients. Median patient age was 26 years (range, 8–54 years). Eighteen patients had received a genoidentical graft and 19 had received an unrelated graft. Aspergillus fumigatus was isolated in 19 patients, Aspergillus flavus was isolated in 2, and Aspergillus niger was isolated in 1. The sites of infection were lung (n = 32), brain (n = 18), skin (n = 5), digestive tract (n = 2), sinus (n = 2), kidney (n = 1), heart (n = 1), and choroid (n = 1). None of the patients had brain lesion(s) without also having another site of infection, and 22 patients had ≥2 sites of infection. The crude mortality rate was 89% (33 patients died). The median duration of survival was 23 days (25th–75th percentiles, 9–92 days) after IA diagnosis and 105 days (25th–75th percentiles, 66–271 days) after transplantation, as determined by a Kaplan-Meier estimate. IA was the main cause of death for 18 patients (54.5%), and it was an associated cause of death for 12 additional patients (36.4%). An autopsy was performed for 3 patients.

Most patients received treatment with several sequential courses of antifungal agents. Patients were switched from one treatment to another for maintenance or because of either treatment failure or toxicity. In this series of 37 patients, 27 patients received amphotericin B deoxycholate (median duration of treatment, 3 days), 28 received a lipid formulation of amphotericin B (median duration, 17 days), 14 received itraconazole (median duration, 43 days), and 4 received voriconazole (median duration, 52 days). Twelve patients underwent adjunctive surgery (11 thoracic and 1 neurosurgical); resection of the lesion(s) was complete for 6 of these patients and incomplete for 6.

Definition of responses and of response episodes. Response to treatment was assessed clinically and by imaging. Responses were classified as “complete response” (i.e., disappearance of all lesions visible on a CT scan), “partial response” (i.e., reduction of >50% of all lesions), or “treatment failure” (i.e., any other responses). Complete and partial responses were considered “good responses.” The minimum period required for evaluation of a response was 7 days, except for 6 patients with rapidly progressive disease who died within 1 week of starting treatment, who were considered to have had treatment failures. For the purpose of this study, evaluations of response were determined retrospectively, and researchers were blinded to GM levels, regardless of the types, sequences, or combinations of treatments that were administered. Some patients had only 1 type of response, which was defined as “1 response episode” (e.g., “treatment failure”). Some patients had both “good” and “bad” responses at different times, which we defined as “[number] response episodes” (e.g., treatment failure followed by a good response and another treatment failure was considered “3 response episodes”).

Antigen detection technique. Serum GM levels were measured before transplantation and then weekly during episodes of neutropenia, episodes of acute or chronic graft-versus-host disease, and/or while the patients were receiving steroid treatment. Whenever IA was clinically or radiologically suspected, serum samples were obtained more frequently and GM values were measured. Serum GM was detected by use of a sandwich immunocapture ELISA (Platelia Aspergillus; Bio-Rad). The technique was performed as recommended by the manufacturer. Results were expressed as GM indices (GMI)—that is, the ratio of the optical density (OD) value of the sample to the OD value of a standard sample containing 1 ng of GM.

When a change in GMI value was observed, sequential serum samples were tested in parallel for optimum comparison. The reproducibility of the test results assessed under these conditions was examined by evaluating the results of 30 repeated tests of 3 pools of serum samples spiked with 0.65, 1.2, 2, 2.6, and 6.9 ng of purified GM per mL. The mean values of the GMI ± 2 SDs were 0.69 ± 0.22, 1.17 ± 0.22, 2.04 ± 0.36, 2.6 ± 0.38, and 4.04 ± 0.55, respectively. By use of this system, for a baseline GMI value of <3, an increase or decrease of 0.5 was considered significant at the 0.05 level when sequential serum samples obtained from the same patient were tested in parallel in the same run. For higher baseline GMI values, an increase or decrease of 1.0 was considered significant. Because one-third of the response episodes yielded a baseline GMI value of ≥3, to be on the safe side, the threshold of 1.0 was selected for statistical analysis.

Statistical analysis. Values and kinetics of serum GMI were analyzed and compared according to outcome. Each modification of the response status of the patient (for instance, from “good response” to “failure,” or vice-versa) was considered to
be a “new response episode” for this patient. For each new response episode, a new GMI baseline was used that corresponded to the most recent available GMI value before this episode. Then, all individual episodes of good response and of treatment failure were categorized as the “good response group” and the “treatment failure group,” respectively. In each group, median GMI values were calculated at baseline and then every 7 days thereafter, according to the episode duration, for a maximum period of 21 days. In each of the 2 groups, the GMI values at baseline were compared with those calculated at weekly intervals by use of the paired-signed Wilcoxon rank-sum test. Comparisons of GMI values between groups were made by means of a mixed-model analysis of variance (Proc Mixed). Survival curves from the day of transplantation and from the diagnosis of IA were computed using the Kaplan-Meier estimate. Statistical analysis was performed by use of SAS software, version 6.12 (SAS Institute), for IBM-compatible computers.

RESULTS

Fifty-eight response episodes were evaluable, representing a median of 1 episode (range, 1–3 episodes) per patient. There were 16 episodes of good response and 42 episodes of treatment failure (figure 1). The median duration of the 58 response episodes was 14 days (25th–75th percentiles, 7–40 days). Six hundred seventy-five serum samples were tested, with a median number of 18 serum samples (range, 3–32) per patient and 5 samples (range, 2–21) per response episode. Overall, at baseline, the good response group did not significantly differ from the treatment failure group with regard to GMI values. Thereafter, GMI values significantly increased in the treatment failure group, whereas no significant changes were observed in good response group (P = .002, by mixed analysis of variance).

Of the 58 response episodes, 47 could be evaluated at day 7 (figure 2A). At this time point, GMI values were not available for 11 patients. In the treatment failure group (39 episodes), GMI values increased significantly (P = .0008), whereas a statistically insignificant decrease was noted in the good response group (P = .5). Twenty-seven response episodes could be evaluated at day 14 (figure 2B). In the treatment failure group (19 episodes), GMI values increased significantly (P = .01), whereas, in the good response group (8 episodes), no significant variation was noted (P = .7). The same pattern of GMI variation was noted in the 15 response episodes that could be evaluated at day 21 (figure 2C). If only the first response episode is considered, 37 episodes were evaluable at day 7. In the treatment failure group (34 episodes), the median increase in the GMI value was 0.99 (25th–75th percentiles, −0.15 to 4.58), as compared with the baseline GMI value (P = .002). Of note, this pattern of GMI variation was also observed during the course of the 3 infections that were not due to A. fumigatus, all of which involved treatment failure.

We then examined the relationship between GMI increase and the outcome for IA, taking into account any increase in the GMI of 1.0 over the baseline value (i.e., a variation considered to be significant when sequential serum samples were tested in parallel in the same run). At day 7, an increase of 1.0 over the baseline level was found to be predictive of treatment failure, with a sensitivity of 44%, a specificity of 87%, and a

![Figure 1](image-url)
Figure 2. Box-and-Whiskers plots of galactomannan index (GMI) values, according to the type of response and the time points per response episode. A, Evaluation at week 1. B, Evaluation at week 2. C, Evaluation at week 3. F, treatment failure; N, number of evaluable episodes; R, good response (i.e., complete and partial response).

The positive predictive value of 94%. At day 14, the sensitivity for treatment failure was 55%, the specificity was 92%, and the positive predictive value was 92%.

DISCUSSION

In the present study, we show that serial determination of Aspergillus GM by use of sandwich ELISA is useful for prognosis of IA in allogeneic STC recipients during treatment. This finding is in agreement with observations of rabbit models of IA, in which GMI values increased in untreated animals but significantly decreased in those treated with amphotericin B [6, 8], voriconazole [9], and posaconazole [7]. Because antigen is released predominantly from hyphae, and because its level is directly correlated with the number of Aspergillus organisms in tissues [10], one can expect that the kinetics of GM variations...
would be a relevant marker of therapeutic response. The reduction of GMI values in serum and CSF specimens has been reported occasionally in patients with IA who were successfully treated [11, 12], whereas the persistence of a high GMI value has been associated with a poor prognosis [5, 11], which possibly justifies the modification of antifungal treatment [13]. Our results confirm and add to these findings.

The kinetics of GMI during treatment markedly differed between patients who experienced treatment failure and those who responded to treatment. Although baseline GMI values were not significantly different between these groups, the values became significantly higher in the treatment failure group during follow-up. Our data show that, in allogeneic SCT recipients, an increase in the GMI value of 1.0 over the baseline value during the first week of treatment was predictive of treatment failure. Although this finding was observed in <50% of patients with progressive disease, we think that it has clinical relevance, because the clinical and radiological assessment of treatment efficacy are often inconclusive until ≥2 weeks of treatment [14, 15].

Acknowledgments

We thank David Denning, for critical review of the manuscript, and Marc Tabouret, for testing the reproducibility of GM determinations on spiked samples.

References