Early Proinflammatory Cytokines and C-Reactive Protein Trends as Predictors of Outcome in Invasive Aspergillosis

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Background. Monitoring treatment response in invasive aspergillosis is challenging, because an immunocompromised host may not exhibit reliable symptoms and clinical signs. Cytokines play a pivotal role in mediating host immune response to infection; therefore, the profiling of biomarkers may be an appropriate surrogate for disease status.

Methods. We studied, in a cohort of 119 patients with invasive aspergillosis who were recruited in a multicenter clinical trial, serum interleukin (IL)–6, IL-8, IL-10, interferon-γ, and C-reactive protein (CRP) trends over the first 4 weeks of therapy and correlated these trends to clinical outcome parameters.

Results. Circulating IL-6 and CRP levels were high at initiation of therapy and generally showed a downward trend with antifungal treatment. However, subjects with adverse outcomes exhibited a distinct lack of decline in IL-6 and CRP levels at week 1, compared with responders (p < .02, for both IL-6 and CRP). Nonresponders also had significantly elevated IL-8 levels (p < .001).

Conclusions. High initial IL-8 and persistently elevated IL-6, IL-8, and CRP levels after initiation of treatment may be early predictors of adverse outcome in invasive aspergillosis. Cytokine and CRP profiles could be used for early identification of patients with a poor response to antifungal treatment who may benefit from more-aggressive antimicrobial regimens.

Invasive aspergillosis continues to be a leading cause of mortality and morbidity among patients treated for hematological malignancies [1, 2]. The adverse outcomes associated with acquisition of the disease may in part be attributable to the compromised immune system of at-risk patients. Securing a firm diagnosis of invasive aspergillosis remains difficult. Management of invasive aspergillosis is complicated by the absence of appropriate tools with which to assess progress and treatment response. First, monitoring symptoms and clinical signs is not a reliable indicator of improvement in the patient receiving therapy, because they can be influenced by the patient’s white blood cell counts and concurrently received medications. Second, it is recognized that ra-
diagnostic follow-up may not be an accurate or timely reflection of progress, because the appearance of pulmonary lesions on serial computed tomography scans may lag behind response in invasive pulmonary aspergillosis [3]. Third, a procedure-based microbiological test-of-cure or response is not feasible, because these patients are high-risk candidates for perioperative complications after invasive procedures.

Current understanding of the pathogenesis of invasive aspergillosis has shown that alveolar macrophages and monocytes form the first line of host defense after inhalation of Aspergillus conidia from the environment [4]. Upon recognition of the pathogen, the host innate immune defense is activated, resulting in phagocytic ingestion of conidia and release of microbicidal compounds [5, 6]. In the absence of adequate immune containment, conidial germination with development of hyphae heralds invasive disease. Neutrophils, which form the second line of defense, attack the hyphae through release of oxidants and degranulation. These are lacking in the neutropenic host. Meanwhile, antigen-presenting functions, as performed by mononuclear phagocytes and dendritic cells, facilitate the development of a T helper (Th) cell–based adaptive immune response [5]. During this process of immune activation and response, cytokines play a pivotal role in mediating the host inflammatory response. Interleukin (IL)–6 is a pleiotropic cytokine produced by macrophages and lymphocytes [7]. It facilitates lymphocyte differentiation and proliferation of hematopoietic progenitor cells and is an important mediator of the acute-phase response of which C-reactive protein (CRP) is a major product. IL-8, released by monocytes, macrophages, and endothelial cells, recruits neutrophils and induces chemotaxis to the site of infection [8]. T cell–derived cytokines also play an important role in the modulation of host defense. Interferon (IFN)–γ is the prototypic type 1 T helper lymphocyte (Th1) proinflammatory cytokine that activates cell-mediated immune responses, whereas IL-10 is an anti-inflammatory product mainly released by type 2 T helper lymphocytes (Th2) and T regulatory (Treg) lymphocytes that keeps in check the inflammatory cascades [4, 8].

We hypothesize that cytokine levels in the blood during invasive aspergillosis may form appropriate surrogate markers of disease progress and may also be predictive of treatment response. The availability of such a noninvasive means of monitoring would be of immense practical utility in the clinical setting. Herein, we report findings from a study involving a cohort of 119 patients with invasive aspergillosis who were enrolled in a multicenter clinical trial in which we assessed whether circulating cytokine levels correlate with the outcome of infection.

PATIENTS AND METHODS

Study patients and design. Study patients were from the Global Comparative Aspergillosis Study (protocol 150–307), a multicenter randomized trial that compared the efficacy of voriconazole versus amphotericin B in the primary treatment of invasive aspergillosis [9]. Selection of eligible patients for the trial, as well as the case definitions for definite or probable invasive aspergillosis, were as detailed elsewhere [9]. These were in accordance with the protocols as then approved by the European Organization for Research and Treatment of Cancer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>81 (68.1)</td>
</tr>
<tr>
<td>Female</td>
<td>38 (31.9)</td>
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<tr>
<td>Certainty of diagnosis</td>
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<tr>
<td>Definite</td>
<td>31 (26.1)</td>
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<tr>
<td>Probable</td>
<td>88 (73.9)</td>
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<td>Underlying disease*</td>
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<tr>
<td>Leukemia and lymphoma</td>
<td>88 (73.9)</td>
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<td>Post-HSCT</td>
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<tr>
<td>Infection site</td>
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<tr>
<td>Pulmonary</td>
<td>107 (89.9)</td>
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<tr>
<td>Extrapulmonary</td>
<td>12 (10.1)</td>
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<tr>
<td>Amphotericin B</td>
<td>56 (47.1)</td>
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<tr>
<td>Voriconazole</td>
<td>63 (52.9)</td>
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<td>Switch to OALT</td>
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<td>78 (65.5)</td>
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<tr>
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<td>41 (34.5)</td>
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<td>Week 12 responseb</td>
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<tr>
<td>Success</td>
<td>58 (48.7)</td>
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<tr>
<td>Failure</td>
<td>61 (51.3)</td>
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<tr>
<td>EORT response</td>
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<tr>
<td>Satisfactory</td>
<td>49 (41.2)</td>
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<tr>
<td>Unsatisfactory</td>
<td>70 (58.8)</td>
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<tr>
<td>Day 84 outcomec</td>
<td></td>
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<tr>
<td>Survival</td>
<td>84 (70.6)</td>
</tr>
<tr>
<td>Deathd</td>
<td>35 (29.4)</td>
</tr>
</tbody>
</table>

* The remaining 7 patients had human immunodeficiency virus infection or had received solid-organ transplant or corticosteroid treatment. 
* Clinical response at week 12, as assessed by a data review committee. 
* Survival or death at 84 days (12 weeks). 
* The majority of the deaths were classified as “unrelated to Aspergillosis,” and another 6 were classified as “unrelated to Aspergillosis but evidence of active Aspergillus infection was present.” The cause of death could not be determined for 5 cases.
In brief, patients were randomized to receive either voriconazole or amphotericin B as primary therapy. In patients with intolerance or no response to initial therapy, the initial randomized drug received could be switched to other licensed antifungal therapy (OLAT). The planned duration of therapy was 12 weeks, at which time outcome measurements, including survival, were assessed by an independent and blinded data review committee on the basis of reviews of the clinical, mycological, and radiological data [9, 10]. Response to therapy was determined by the data review committee at 2 time points: the primary end point at week 12 (W12) and the end of (initial) randomized therapy (EORT), which was a prespecified secondary end point reached when the patient stopped receiving the randomized therapy (namely, either voriconazole or amphotericin B) and had treatment switched to an OLAT. Satisfactory response at week 12 (known as “W12 success”) or at EORT (known as “EORT satisfactory”) was defined as a complete or partial response after treatment, whereas a poor response at week 12 (known as “W12 failure”) or at EORT (known as “EORT unsatisfactory”) was defined as treatment failure or an unchanged disease state at the preset end points, in accordance with the assessment criteria of the blinded data review committee [9].

Biomarker levels were measured in the serum samples serially obtained from trial patients at the following time points: before initiation of therapy (at baseline) and at weeks 1, 2, 4, 8, and 12 (end point) after commencement of antifungal treatment. All specimens were identified only by codes during assay for the respective biomarker levels, to blind investigators to the corresponding clinical data until the stage of data analysis. The serum specimens had been stored at −20°C before assay; multiple freeze-thaw cycles were avoided. IL-6, IL-8, IL-10, and IFN-γ levels were measured using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (Pelikine Compact; CLB), in accordance with the instructions of the manufacturer. The interassay coefficient of variation was 14% in this study. The lower detection limits of the assays

Figure 1. Interleukin (IL)–6, IL-8, and IL-10 trends between responders and nonresponders to treatment during the first 4 weeks, as determined at week 12 by a data review committee. *P < .05, for the change in the cytokine level from baseline to a specified time point between responders and nonresponders, as analyzed by generalized estimating equations. **P < .05, for the difference in cytokine level in responders vs. nonresponders at a specified time point. "W12 success" denotes the clinical response to treatment after 12 weeks; "W12 failure" denotes a poor response to treatment after 12 weeks.

Figure 2. Interleukin (IL)–6, IL-8, and IL-10 trends during the first 4 weeks of treatment between patients who had a satisfactory or unsatisfactory end-of-randomized therapy response (EORT satis or EORT unsatis, respectively) to initial primary treatment, as determined by a data review committee. *P < .05, for the change in the cytokine level from baseline to a specified time point in responders vs. nonresponders, as analyzed by generalized estimating equations.
Figure 3. Interleukin (IL)-6, IL-8, and IL-10 trends during the first 4 weeks of treatment between survivors and nonsurvivors at 84 days after initiation of treatment for invasive aspergillosis. **P < .05, for the difference in the cytokine level between survivors and nonsurvivors at specified time point. There was no statistical difference, according to the generalized estimating equations approach analyzing the change in cytokine levels from baseline to any sampling time point.

were as follows: 3.1 pg/mL for IL-6, 16 pg/mL for IL-8, 4.7 pg/mL for IL-10, and 3.1 pg/mL for IFN-γ, respectively. CRP was measured by ELISA performed in accordance with the instructions of the manufacturer (Dako), and the lower detection limit was 20 mg/L.

**Statistical analysis.** Correlation between cytokine/biomarker trends over time and outcome (or primary therapy) were graphically presented by plotting the mean value per time point, with error bars denoting the standard error of the mean. Because patients had repeated measurements obtained at designated intervals over time, the generalized estimating equations approach was used to analyze differences in time trends. More specifically, differences in the change from baseline were tested: a significant difference at the specified time interval after initiation of antifungal treatment (eg, at week 2) implied that the change in the serum biomarker levels from baseline to week 2 was significant in one group (the responders), compared with that in the other group (the nonresponders). Correlation between biomarker levels (at baseline and specified time points) and outcome were also assessed. Receiver operating characteristic analysis was performed using the biomarker readouts of individual patients to derive prediction rules for clinical outcome on the basis of cytokine/biomarker levels at baseline or the change from baseline to week 1. Statistically significant results were reported whenever \( P < .05 \).

Hence, the strategy adopted for interpreting the results was
Figure 4. Interleukin (IL)–6, IL-8, and IL-10 trends of patients who were randomized to receive either amphotericin B or voriconazole and who had remained on the primary trial drug at the specified time points.

to assess first whether trends of the summary measurement profiles (namely, mean biomarker levels) between the responders and nonresponders differ. Only when there was a difference in trends and when this difference was significant would an attempt be made to formulate a clinical prediction rule through receiver operating characteristic analysis. Reliability of the prediction rule was reported by the area under the curve (AUC), sensitivity, and specificity.

RESULTS

A total of 539 serum specimens serially collected from 119 patients enrolled in the clinical trial were available for analysis. Missing specimens or specimens with inadequate serum volumes for assay constituted 1.6% of all specimens to be measured, and these were treated as missing values during data analysis. Table 1 summarizes the demographic characteristics of this study cohort and the treatment-related outcomes of interest.

Serum IFN-\(\gamma\) levels were low and were barely detectable in the first 300 specimens measured. The median IFN-\(\gamma\) level was 3.1 pg/mL (range, 3.1–12.0 pg/mL). This was verified with the use of another assay (Luminex). Given the low yield of almost undetectable IFN-\(\gamma\) levels in the serum of these patients, IFN-\(\gamma\) measurements were discontinued after 300 specimens.

Serially measured IL-6, IL-8, and IL-10 levels of patients were assayed at the intervals designated above, as long as the patients remained enrolled in the study. After reviewing the results, it was decided that only cytokine readings obtained between baseline and 4 weeks after therapy would be analyzed. Beyond this time point, there were concerns that the cytokine trends might not be representative because of the diminishing patient numbers (ie, the denominator) caused by deaths and withdrawals over time, resulting in wide variances of some results.

**Cytokine trends and clinical response at 12 weeks.** Generally, the circulating cytokine levels in the patients were elevated at baseline and displayed a trend toward a decrease over time while the patients were receiving therapy.

Serum IL-6 levels revealed a distinct difference between patients who did not show clinical response to treatment after 12 weeks (W12 failure) and those who did (W12 success) (Figure 1). Nonresponders had transiently elevated IL-6 levels 1 week after initiation of antifungal therapy, in contrast to responders, who had a progressive decrease in this inflammatory cytokine (ie, there was a delayed decrease in IL-6 levels in the nonresponders) \((P = .02)\). IL-8 levels were significantly higher in the nonresponders through the first 2 weeks of therapy for IA \((P < .02, \text{at both weeks 1 and 2})\). The serum IL-10 levels decreased more precipitously from baseline to weeks 1 and 2 in nonresponders, compared with responders \((P = .03\) and \(P = .045\), respectively).

**Cytokine trends and clinical response at EORT.** At the EORT end point, trends in serum IL-6 levels were similar to those found for the response at week 12 (Figure 2). Patients who were deemed as having an unsatisfactory response at EORT showed a delayed decrease in circulating IL-6 levels, compared with responders \((P = .045)\). The variations in IL-8 levels were wide, and the differences between the patients with an EORT-satisfactory or EORT-unsatisfactory response to treatment were not distinct. Likewise, there was no significant difference in IL-10 trends between the EORT responders and EORT nonresponders.

**Cytokine trends and mortality at day 84.** The association between cytokine trends and the overall mortality rate at 84 days (12 weeks) is presented in Figure 3. A prompt decrease in the IL-6 level after 1 week of receiving antifungal therapy was associated with survival at the study end point (survivors at day 84). Patients who did not survive until day 84 displayed a trend of persistently elevated IL-6 levels at week 1. Low IL-8 levels at baseline and week 1 were associated with survival to day 84 \((P = .001\) and \(P = .01\), respectively). The observed differences in cytokine profiles between survivors and nonsurvivors paralleled the trends observed for clinical response at week 12.
**Cytokine trends and primary antifungal therapy.** Because the primary aim of the trial was to compare the efficacy of voriconazole versus amphotericin B as initial therapy for invasive aspergillosis, we were also interested in the effects of the 2 drugs on the cytokine profile of patients with invasive aspergillosis. Because the design of the original study had permitted a switch from the initial trial drug to an OALT in the event of intolerance or poor response, patient data were only used up to the point of switching/discontinuation in the analysis of cytokine trends. As illustrated in Figure 4, IL-6, IL-8, and IL-10 profiles through the first 4 weeks of therapy were not significantly different between patients receiving either voriconazole or amphotericin B.

**CRP trends in relation to clinical outcomes and primary therapy.** CRP is a classical marker of inflammation, and its induction is initiated by IL-6. Given the trends observed with IL-6, we proceeded to ascertain CRP levels in our specimens. A steady decrease in the CRP profile was predictive of both clinical response and survival at week 12, as well as a satisfactory EORT response (Figure 5). Of note, elevated or minimally changed CRP levels at weeks 1, 2, and 4 after the start of therapy were all significantly associated with mortality by day 84 or a poor response at EORT. Patients who had received voriconazole as primary therapy showed a trend toward a more prompt reduction in CRP levels, compared with patients who had received amphotericin B (Figure 5D).

**Association between biomarker levels at baseline and outcome parameters.** IL-8 and IL-10 measurements at baseline were correlated with W12 clinical response and death. A high IL-8 level at baseline was associated with a poor clinical response ($P = .012$) and death ($P = .013$). On the other hand, a high initial IL-10 level was associated with survival ($P = .013$). In contrast, the CRP measurement at baseline did not predict either response or survival. The aforementioned usefulness of IL-8 as a point-of-care prognostic marker was further evaluated. As demonstrated by the receiver operating characteristic diagram (Figure 6A), IL-8 had moderate capacity as a prognostic marker for eventual outcomes. An IL-8 cutoff >135 pg/mL at baseline provides sensitivity of 85% and specificity of 55% for poor clinical response. In contrast, outcome measurements could not be correlated with the CRP readout at baseline, as further demonstrated by the receiver operating characteristic diagram.

**Threshold of change between baseline and week-1 measurements associated with adverse outcomes.** Persistently elevated or minimally changed IL-6 and CRP levels between baseline and week 1 were associated with adverse outcomes, as described above. Receiver operating characteristic analyses as-
Figure 6. A, Receiver operating characteristic diagrams comparing the baseline interleukin (IL)-8 and C-reactive protein (CRP) levels in relation to the prediction of a poor clinical response at 12 weeks (W12 response) and death by 84 days (D84 mortality). B, Receiver operating characteristic diagrams for the percentage decrease in serum IL-6 and CRP levels from baseline to week 1 in relation to a poor clinical response at week 12 (W12 response) and death by 84 days (D84 mortality).

Assessing the percentage decrease in IL-6 and CRP levels between baseline and week 1 were performed as a test to predict the W12 clinical response or death by day 84 (Figure 6B). Changes in the CRP level were more strongly correlated with the clinical outcomes than were changes in the IL-6 level. Treatment failure at 12 weeks may be anticipated if the decrease in the serum CRP level from baseline to week 1 is <48% (sensitivity, 83%; specificity, 50%). There is also increased likelihood of death if the reduction in the CRP level is <20% after 1 week of antifungal therapy (sensitivity, 78%; specificity, 70%). As has been demonstrated, the magnitude of the decrease in the CRP level between baseline and week 1 after antifungal treatment is a sensitive, simple, and economical test that is predictive of clinical outcome in invasive aspergillosis over existing diagnostic means.

**DISCUSSION**

The monitoring of response to therapy remains a major challenge in the management of invasive aspergillosis. We have demonstrated the usefulness of biomarker monitoring as a non-invasive and convenient tool, not only for the follow-up of treatment response but, even more importantly, as a predictive marker of adverse outcomes. The persistence of elevated IL-6 and CRP levels after 1 week of therapy was associated with an increased likelihood of treatment failure and death. A high circulating IL-8 level at baseline was also a predictor of poor prognosis in terms of response to treatment.

It has been proposed that measurements of IL-6 and IL-8 levels could be used to identify low-risk febrile patients with neutropenia, compared with a high likelihood of acquiring severe bacterial sepsis necessitating aggressive antimicrobial therapy [11–14]. Currently, CRP is the standard bedside inflammatory marker for the diagnosis of infection [15]. However, the potential of these biomarkers for the monitoring of response to therapy in patients with invasive aspergillosis has not been previously studied. Difficulties with definitive diagnosis of the disease, as well as a lack of consensus on outcome definitions for invasive aspergillosis, hampered case identification until recently [9, 16–18]. We have demonstrated, for what we believe is the first time, the prognostic value of these markers for assessing response to therapy in a well-characterized cohort of patients with invasive aspergillosis.

Our study shows that circulating cytokine and CRP levels were high in patients after acquisition of invasive aspergillosis and that they subsequently decrease during treatment. Notably, we show that trends in IL-6 and CRP levels during the first week after treatment initiation correlate with the outcome of infection. Patients who had a good clinical response showed a prompt and sustained decrease in serum IL-6 and CRP levels. This finding is in contrast to findings for nonresponders, whose IL-6 and CRP levels remained elevated at week 1 despite receiving treatment. In addition, patients with adverse therapeutic outcomes (those with W12 failure and those who died by day 84) had elevated IL-8 levels throughout the initial
weeks of treatment. We can account for these distinct cytokine trends on the basis of our current understanding on the pathophysiology of the host immune response during sepsis. It is thought that the outcome of sepsis is determined, to a significant extent, by the intensity of the host inflammatory response that entails a coordinated balance between proinflammatory and anti-inflammatory mediators. An exaggerated and uncontrolled proinflammatory response induces undue damage at the cellular and organ level [19, 20]. Recently, it has also been proposed that prolonged and unchecked proinflammatory responses may, paradoxically, facilitate the pathogenesis of *Aspergillus fumigatus* [21]. This may also explain why a higher anti-inflammatory IL-10 level, especially after week 1, may possibly be associated with a better clinical outcome, as seen in our study. On the other hand, a high cytokine level may also mirror the incapacity of the host to effectively eliminate the fungus, resulting in a prolonged stimulation and release of proinflammatory cytokines.

Our results are also consistent with 2 earlier studies of patients with sepsis and hematopoietic stem cell transplant (HSCT) patients. One study involving patients with sepsis in the intensive care unit had found that a limited decrease in the CRP level after 48 h following intervention was associated with treatment failure [22]. The second study observed that a high serum IL-6 level at week +1 after HSCT may be an early predictor of transplant-related complications in a cohort of 52 patients [23]. These findings highlight how the study of biomarker kinetics may yield potential benefit in inflammation-driven disease conditions beyond the setting of invasive aspergillosis.

Only one small previous study had investigated the cytokine profiles of patients with invasive aspergillosis [24]. Roilides and colleagues [24] reported a possible correlation between an elevated serum IL-10 level and an unfavorable outcome in 7 nonneutropenic patients with invasive aspergillosis. In their report, the 2 patients with poor outcomes had a distinct progressive increase in the IL-10 level before death. By contrast, our study revealed that an early, precipitous decrease in the IL-10 level by week 1 led to W12 treatment failure, and a higher IL-10 level at baseline was associated with survival by day 84. The reason for the differences between the IL-10 trends seen in our study and that of Roilides and colleagues are unclear. However, we used a much larger cohort of patients, many of whom were, or recently had been, neutropenic. Our larger patient population also permitted statistical analysis to avoid chance observation.

Although IFN-γ is a cytokine that is clearly involved in the host defense against aspergillosis [25–27], circulating levels were very low in our study. We have also observed low IFN-γ levels in bronchoalveolar lavage specimens from a separate cohort of patients with pulmonary invasive aspergillosis (unpublished data). Low IFN-γ levels in this patient cohort may be the result of the numerous immune suppressive treatments that these patients undergo [25, 28].

The criteria for definite and probable invasive aspergillosis in our study cohort had been prespecified in the protocol and strictly applied to all cases by a data review committee consisting of infectious diseases physicians, hematologists, and radiologists [9]. These criteria had similarities to the criteria established later, in 2002, by the European Organization for Research and Treatment of Cancer / National Institute of Allergy and Infectious Diseases Mycoses Study Group and updated in 2008 [16, 17]. We believe that the high degree of certainty of disease (as was seen with this study) is in tune with our aim to determine the usefulness of biomarkers as an indicator of disease progress in invasive aspergillosis. Another strength of the study is that, in addition to the case definitions, the end points and criteria for determination of clinical response were well established and objectively determined by a blinded panel of experts. However, caution should be exercised before extrapolating the findings of this study to all patients with invasive aspergillosis, regardless of underlying predisposing disease conditions, because in this study cohort, the majority (80%) were patients who had undergone chemotherapy for hematological malignancies. HSCT patients constituted the remaining 20% of the cohort, and this limited attempts at further meaningful analysis of the HSCT patient subcohort. This study remains a retrospective analysis involving patients in a landmark clinical trial that had not been designed with the purpose of correlating the performance of circulating biomarkers with outcome parameters. Nonetheless, it is unlikely that a prospective trial with such intent alone will be conducted in the foreseeable future, and therefore the robust original trial design and sizable patient cohort gives much weight to the results of this study.

In conclusion, we demonstrated that inflammatory markers, such as cytokines and CRP, may be used to monitor response and predict clinical outcome to invasive aspergillosis at an early stage of the disease. Measurement of these biomarkers (such as CRP and IL-8) is economical and can be easily performed. These assays will be valuable to physicians who are challenged with the difficult management of patients with invasive aspergillosis in hematology-oncology services.

**Acknowledgments**

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**References**

1. Neofytos D, Horn D, Anaissie E, et al. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant...


23. Min CK, Lee WY, Min DJ, et al. The kinetics of circulating cytokines including IL-6, TNF-α, IL-8 and IL-10 following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2001; 28:935–940.


