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Assessment of Measuring Circulating Levels of Interleukin-6, Interleukin-8, C-Reactive Protein, Soluble Fcγ Receptor Type III, and Mannose-Binding Protein in Febrile Children with Cancer and Neutropenia

Th. Lehrnbecher, D. Venzon, M. de Haas, S. J. Chanock, and J. Kühl

Circulating levels of interleukin (IL)-6, IL-8, soluble Fcγ receptor type III (sFcγRIII), mannose-binding protein (MBP), and C-reactive protein (CrP) were assessed among febrile children with cancer and neutropenia. Levels of IL-6, IL-8, sFcγRIII, MBP, and CrP were measured in serum from 56 pediatric cancer patients at the time of admission for 121 episodes of febrile neutropenia (88 febrile episodes without identifiable source, 20 episodes of bacteremia due to gram-positive and 5 due to gram-negative organisms, and 3 fungal infections). IL-6 and IL-8 levels were higher in patients with either bacteremia due to gram-negative organisms or fungal infections than in patients with febrile episodes without an identifiable source ($P < 0.0001$ for each). IL-6 and IL-8 levels were higher in children with bacteremia due to gram-negative organisms than in those with bacteremia due to gram-positive organisms ($P = 0.0011$ and $P = 0.0003$, respectively). The measured levels of CrP, MBP, and sFcγRIII were not useful for identifying the type of infection. These preliminary results show the potential usefulness of IL-6 and IL-8 as early indicators for life-threatening infections in febrile cancer patients with neutropenia.

Infectious complications represent a significant cause of morbidity and mortality in children with cancer undergoing chemotherapy [1]. Neutropenia (defined as an absolute neutrophil count of $<500/μL$) is a major risk factor for serious bacterial and fungal infections [1]. In most cases, fever is an important, early indication of serious infection, particularly in children with neutropenia. Discrimination between serious and inconsequential infection in febrile children with neutropenia at the time of presentation is difficult. Several groups have proposed risk categories based on clinical tests [2, 3]. However, determining the profile of a febrile child with neutropenia as either high or low risk for serious infection does not predict the likelihood of a specific infection in a given patient. It is the standard of care that all febrile patients with neutropenia receive systemic antibacterial therapy, primarily because of the deleterious effects of withholding therapy until confirming a diagnosis [4, 5].

Although the paradigm of treating all patients with neutropenia with broad-spectrum antibiotics at the first sign of fever has drastically reduced morbidity and nearly eliminated mortality, currently nearly two-thirds of these children are treated without having a source of the fever identified [6, 7]. Until recently, hospitalization for empirical antibiotic therapy and monitoring has been the accepted standard of care [8, 9]. This has resulted in exposing families to high costs in money, diagnostic studies, and disruption of family life. Antibiotics are generally given for a minimum of 3 days but often longer, despite the inability to establish a microbiological diagnosis. Extended time in the hospital also subjects children to acquisition of resistant organisms, a trend clearly encouraged by liberal use of antibiotics on oncology wards.

Recently, attention has focused on identifying serum markers of the immunologic response that may be useful for therapeutic intervention. For example, laboratory measurement of C-reactive protein (CrP) in serum has been investigated as a tool for the early diagnosis of a bacterial or fungal infection and a possible marker for severity of infection [10, 11]. Pediatric studies have been reported for children without neutropenia, but only a handful have looked at children with neutropenia [12–17]. Unfortunately, the increase in CrP is often delayed, and consequently, measurement of CrP may be of limited value in the early recognition of life-threatening infections in patients with neutropenia [18]. The main value of CrP is related to its negative predictive value after several determinations [14, 16].

Increases in circulating levels of the cytokines IL-6 and IL-8 are apparent early in the course of infection, indicating a rapid series of host response mechanisms, such as fever and chemo-
taxis of white blood cells [19]. IL-6 levels have been measured in several studies of patients with neutropenia. The preliminary results are promising, yet definitive studies are lacking [12, 13, 15, 18, 20–23]. Fewer studies have been published addressing the usefulness of measuring IL-8 to identify severe infection in febrile patients with neutropenia [20, 24–26]. Others have investigated the significance of circulating levels of molecules of innate immunity, such as the mannose-binding protein (MBP), which is increased during the acute phase of inflammation [27]. MBP is a pattern recognition molecule that recognizes complex carbohydrates on the surface of pathogens and activates the classical complement pathway [28]. Of interest is the observation that MBP levels are enhanced by IL-6 [29]. In a similar manner, the Fcγ receptor type III (FcγRIII), a low-affinity neutrophil surface membrane IgG receptor, has been studied, partly because a soluble form is released into circulation by neutrophils, and the concentration of soluble (s) FcγRIII has been shown to increase at the site of inflammation [30–32].

On the basis of these observations, we investigated the potential role of one or more soluble factors as indicators of serious infection in febrile children with cancer and neutropenia. The identification of one or more predictive factors may be useful for tailoring therapy but, naturally, will have to be substantiated in a prospective, randomized trial.

**Materials and Methods**

The study was performed at the Children’s Hospital of the University of Würzburg, Germany; 56 children with a confirmed malignancy and chemotherapy-induced neutropenia were admitted. There were a total of 121 episodes of fever. No more than four episodes of fever were included for any patient. The mean age was 8 years (range, 3 months to 20 years) in a population made up of 28 boys and 28 girls. The following distribution of underlying diagnoses was observed: acute lymphoblastic leukemia in 17, lymphoma in 5, myeloid leukemia in 7, and solid tumors in 27. Patients were eligible for the study if they had not received antibiotics for 72 hours before admission to the hospital; almost all patients were receiving thrice-weekly prophylaxis for *Pneumocystis carinii* infection with trimethoprim-sulfamethoxazole, which was not an exclusion criterion. Nearly all patients had an indwelling central venous catheter.

Patients were eligible for study if both fever and neutropenia were documented. Neutropenia was defined as <500 neutrophils/µL (absolute neutrophil count) at the time of admission. Patients who recently received chemotherapy and had evidence of rapidly dropping neutrophil counts within 72 hours were also included. All patients who had absolute neutrophil counts of >500/µL at enrollment had absolute neutrophil counts of <500/µL within 48 hours. Fever was documented if one axillary temperature measurement was >38.5°C or two measurements of 38–38.4°C were made within a 4-hour interval. The temperature was taken for at least 2 minutes with a digital thermometer (Hartmann, Heidenheim, Germany). Children who had been febrile for >24 hours before admission were excluded from the analysis.

All patients were examined at the time of admission and daily thereafter. As part of the initial evaluation, each patient had blood and urine drawn before iv antibiotic therapy was administered. In the case of a documented infection, diagnostic and/or clinical evidence was gathered within 48 hours of admission. Capillary blood was drawn daily for measurement of hemoglobin level, platelet count, white blood cell count (including the differential cell count), and CrP level. Additional study blood samples included serum and plasma, extracted immediately by centrifugation, at the time of admission and 24 hours later. Collected material was stored at −70°C as serum and EDTA-treated plasma for analysis of IL-6, IL-8, MBP, and sFcγRIII. After blood samples were collected, antibiotic therapy was initiated and continued until fever and neutropenia resolved or until a clinically appropriate time for discontinuation if a source was identified. Intravenous antibiotic therapy in the hospital included ceftazidime or imipenem, in combination with teicoplanin when indicated.

IL-6 and IL-8 were measured by commercially available EIAs (Dianova-Immunotech, Hamburg, Germany) with a detection limit of 3 and 8 pg/mL, respectively. Measurements of sFcγRIII and MBP were made according to previously published methods [33, 34]. Each assay was verified against reference standards. CrP was measured by a nephelometric assay by use of a commercial kit (Behringwerke, Marburg, Germany).

Statistical analysis of the data was done after entry into an Excel spreadsheet (Microsoft, Redmond, WA). The Wilcoxon rank sum test was used for comparisons of the individual groups categorized by the type of infection established at the time of admission. The *P* values reported are two-tailed and are not corrected for multiple comparisons. In the pairwise comparisons of a cytokine among all the diagnosis categories, *P* values of <.005 can be considered significant and those between .05 and .005 should be considered suggestive but requiring confirmation in independent data.

**Results**

The study measured IL-6, IL-8, MBP, sFcγRIII, and CrP levels in 121 febrile episodes among 56 pediatric oncology patients. Retrospectively, each episode was assigned to one of five categories on the basis of microbiological and clinical data: documented bacteremia with a gram-positive organism (*n* = 20; *Staphylococcus epidermidis* [13], *Staphylococcus aureus* [6], *Bacillus cereus* [1]); documented bacteremia with a gram-negative organism (*n* = 5; *Escherichia coli* [4], *Klebsiella* species [1]); fungal infection (*n* = 3; *Candida* species [2], *Aspergillus* species [1]); clinically documented infection.
(n = 5; pneumonia [3], sinusitis [1], and osteomyelitis [1]); and febrile episode without identifiable source (n = 88). All but two pathogens were isolated from culture of an initial blood specimen, which was obtained in nearly all of the cases through the central catheter. There was one episode of pleural infection due to *B. cereus* and a second case in which *Aspergillus* species was isolated from sputum from a patient with pulmonary infiltrates. The clinical characteristics of the patients were comparable for each group. There were no deaths, and no patients presented with septic shock. Of the patients with a febrile episode without an identifiable source, 59 (67%) were afebrile within 3 days of initiation of antibiotic treatment, whereas 29 patients (33%) were febrile for ≥4 days after initiation of treatment; however, no significant differences in measured levels of any of the molecules were seen between these two groups (data not shown).

Measured levels of IL-6 and IL-8 from the day of admission and 24 hours later are presented in figure 1. The highest levels for IL-6 and IL-8 measured at admission were observed in patients with bacteremia due to a gram-negative organism. For IL-6 and IL-8 levels, the differences between those with bacteremia due to a gram-negative organism and those with a febrile episode without an identifiable source were highly significant (P < .00001 for each). Significant differences were also seen for both cytokine levels between patients with life-threatening infections (gram-negative and fungal) and those with a febrile episode without an identifiable source (P < .00001). IL-6 and IL-8 levels were useful for discriminating bacteremia due to gram-negative organisms from that due to gram-positive organisms (P = .0011 and P = .0003, respectively). In addition, for IL-6, higher levels were observed among patients with bacteremia due to a gram-positive organism than among those with a febrile episode without identifiable source (P = .021). Analysis of the cases of bacteremia due to gram-positive organisms indicates that the measured levels of circulating IL-6 and IL-8 did not differ between documented episodes of bacteremia due to *S. epidermidis* and *S. aureus* (data not shown). Measured levels of CrP, MBP, and sFcyRIII displayed a broad overlap among the different groups, indicating that they are not useful for discrimination between life-threatening infections and febrile episodes without identifiable source or probable catheter-associated bacteremias (the majority of bacteremias due to gram-positive organisms) (data not shown).

Because of the severe consequences of inappropriate treatment of life-threatening infection in a patient with cancer and neutropenia, the predictive ability of one or more tests has to be accurate and highly reproducible. In table 1, we present an analysis of levels of IL-6 and IL-8 in serum at admission as well as the higher of two consecutive measurements of CrP in serum within 24 hours and suggest that these values are useful in differentiating patients with bacteremia due to gram-negative organisms or fungal infection from patients with febrile episodes without identifiable source, bacteremia due to gram-positive organisms, or clinically documented infection. Although the study is preliminary, it does suggest that either IL-6 or IL-8 might be a useful measurement at the time of admission in a febrile child with cancer and neutropenia.

Within 24 hours after admission, the elevated levels of IL-6 and IL-8 indicated a tendency to decrease (see figure 1), whereas levels of CrP rose, especially in the patients with bacteremia due to gram-negative organisms (data not shown). For the levels of MBP and sFcyRIII, no particular pattern was observed on the second day, implying that these measurements do not correlate with clinical outcomes (data not shown). Day
Table 1. Ability of serum IL-6 and IL-8 (measured on admission) and serum C-reactive protein (highest levels of two consecutive measurements within 24 hours after admission) to differentiate patients with bacteremia due to gram-negative organisms or fungal infection from patients with febrile episodes without identifiable source, bacteremia due to gram-positive organisms, or clinically documented infection.

<table>
<thead>
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<th>Marker</th>
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<th>Limit 2</th>
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<td>Specificity</td>
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<td>Level (pg/mL)</td>
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NOTE. Negative predictive value = percentage of children below threshold with febrile episodes without identified source, bacteremia due to gram-negative organisms, or clinically documented infection; positive predictive value = percentage of children above threshold with bacteremia due to gram-negative organisms or fungal infection; sensitivity = percentage of children with bacteremia due to gram-negative organisms or fungal infection above threshold; specificity = percentage of children with febrile episodes without identified source, bacteremia due to gram-positive organisms, or clinically documented infection below threshold.

* Lowest level measured in patients with fungal infection.
† Lowest level measured in patients with bacteremia due to gram-negative organisms.

Discussion

This single-institution study of children demonstrates that at the time of admission for fever and neutropenia, serum IL-6 and IL-8 levels might be useful markers for discrimination of more serious infections (i.e., bacteremia due to gram-negative organisms and fungal infections) from febrile episodes without identifiable source, clinical syndromes that are not acutely life-threatening, or bacteremia due to gram-positive organisms. On the basis of our preliminary data and published studies, we believe that further investigation of measurements of levels of IL-6 and IL-8 in serum is warranted. The potential to tailor therapy according to more sensitive diagnostic tests at the time of admission might permit more judicious use of antimicrobial agents. Clearly, this would lessen the selective pressure contributing to the spread of virulent, multiresistant strains of bacteria, especially in frequently hospitalized children.

The highest levels of IL-6 and IL-8 were measured in children who were diagnosed with bacteremia due to gram-negative organisms and these were significantly higher levels than those measured in patients with neutropenia who had bacteremia due to gram-positive organisms, febrile episodes without identifiable source, or clinically documented infection. Our study supports previous studies that have shown that IL-6 levels in patients with neutropenia are higher in those with bacteremia due to gram-negative organisms than in those with bacteremia due to gram-positive organisms [13, 18, 21]. On the other hand, this difference was not observed in patients without neutropenia [35]. A plausible explanation for the higher levels of IL-6 in patients with neutropenia may be related to the fact that gram-negative and gram-positive bacteria elicit IL-6 production through different mechanisms. Gram-negative bacteria release high concentrations of endotoxin, which directly activates monocytes via the CD14 receptor, whereas gram-positive bacteria require the intercession of T lymphocytes, which could be deficient or defective in children receiving cytoreductive chemotherapy [36–39].

In our study, we observed that the level of IL-8 at the time of presentation with fever and neutropenia might be a useful marker for identifying children with serious infection. To our knowledge, this has not been shown in hosts with neutropenia. Other groups have suggested that IL-8 levels are increased in febrile patients with neutropenia, but none of these studies looked at a large enough cohort to assess the importance of levels with respect to specific infectious outcomes [20, 24–26]. Hynninen et al. [24] reported higher levels of IL-8 in bacteremic patients with neutropenia than in those without neutropenia. Waage et al. [26] studied IL-8 levels in eight patients with acute myeloid leukemia and concluded that IL-8 can be detected in patients with neutropenia in the absence of infection as well as in patients with serious bacterial and fungal infections. The study was too small to comment on the usefulness of IL-8 for identifying infected patients.

It is important to note that most patients with febrile episodes without identifiable source had levels of IL-6 and IL-8 that were minimally elevated at the time of admission (median values, 119 and 70 pg/mL, respectively), which is in agreement with other reports [12, 13, 20]. It is plausible that low-grade endotoxemia may be measured in patients with neutropenia because of the concurrent mucositis, which serves as a portal of entry for colonizing gram-negative bacteria, even if clinically significant infection is not apparent [21]. The decrease in levels of both IL-6 and IL-8 within 24 hours after initiation of therapy (median values, 65 and 52 pg/mL, respectively) supports the concept that a significant number of febrile episodes without identifiable source in patients with neutropenia could be due to “subclinical” infection, but further studies are needed [12, 23]. In addition, within the first 24 hours after admission, a decrease
in IL-6 and IL-8 levels in patients with bacteremia due to gram-negative organisms (median values on admission, 3,715 and 3,084 pg/mL, vs. median values at 24 hours, 722 and 399 pg/mL) as well as in patients with fungal infection (median values on admission, 969 and 375 pg/mL, vs. median values at 24 hours, 233 and 202 pg/mL) was observed. The usefulness of subsequent measurements is unclear; they do not appear to enhance the identification of children with serious infection nor are they adequate for determining prognosis. In one preliminary study, falling IL-6 values in patients with persistent symptoms of endotoxic shock were reported [40].

We further analyzed the episodes of bacteremia due to gram-positive organisms. We separated infections due to S. epidermidis from those due to S. aureus on the basis of the clinical observation that infection with S. epidermidis is generally associated with a catheter device and, more important, with less severity [6, 41]. We did not find a significant difference in the circulating levels of IL-6 and IL-8 measured on the day of admission. In contrast, IL-6 and IL-8 levels in patients with S. epidermidis infection differed significantly from levels found in patients with infection due to gram-negative and fungal pathogens grouped with S. aureus (P = .0009 and P = .0072, respectively).

Determination of the CrP level is commonly used to assess and monitor the acute-phase response. We found a high sensitivity of serial CrP measurements, which is in agreement with other reports [14, 16, 17], whereas we and others [17, 23] could not observe the high specificity reported by Santolaya et al. [16]. The main value of CrP measurement is reported to be its negative predictive value (after two determinations), which approaches 100% in several studies [16, 17]. We found comparable results in our study (table 1). However, the low specificity and the low positive predictive value of serial CrP measurements limit the overall utility of CrP measurements in the initial empirical management of febrile children with cancer and neutropenia [17]. It has been suggested that CrP measurements might be helpful in monitoring the response to antibiotic therapy, but our study was not designed to address this question [21].

On the basis of previous reports that MBP and sFcγRIII levels correlate with infection in selected populations, we measured levels in febrile pediatric patients with neutropenia. MBP is an acute-phase reactant secreted by the liver and recognizes carbohydrate moieties on the surface of pathogens [28]. The soluble form of the FcγRIII is released from neutrophils following activation [30]. Although MBP levels are reported to be significantly higher in patients with signs of infection than in healthy controls, our data indicate that MBP levels may not be useful for discriminating between different types of pathogens in children with neutropenia [27].

Soluble FcγRIII levels have been shown to be elevated at inflammatory sites, but in our study, no correlation was observed between levels and a specific type of infection at diagnosis [30–32, 42]. This is not surprising, because serum levels of sFcγRIII, which have a long half-life, have been suggested to correlate with changes in the total number of neutrophils as opposed to neutrophil activation [42, 43]. Because of our study design, we were not able to assess the value of recovery of absolute neutrophil count and sFcγRIII level. In our data, subgroups of patients with high and low sFcγRIII levels did not correlate with specific infectious outcome. Koene et al. [42] reported that in patients with idiopathic neutropenia, measured levels of sFcγRIII above 100 arbitrary units were associated with a decreased risk for bacterial infection.

Before designing studies to determine whether IL-6 or IL-8 levels may be useful for tailoring therapy, further studies are needed to validate the suggested high sensitivity and negative predictive value of our preliminary findings. One goal will be to define reliable and reproducible thresholds for measured levels of IL-6 and IL-8 that might be informative in discriminating life-threatening infection from febrile episodes without identifiable source or bacteremia due to gram-positive organisms. The variable positive predictive values shown in table 1 underscore the need to look at a larger cohort of pediatric patients.

In summary, these preliminary results show the potential usefulness of measured levels of IL-6 and IL-8 as early indicators for documented, life-threatening infections in febrile patients with cancer and neutropenia. The results require confirmation in large, prospective studies before use for stratification of antibiotic therapy.

Acknowledgments

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References