


Association between cytokine concentration kinetics and prolonged fever in febrile neutropenic children with bacteremia

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Abstract

Introduction: Although prolonged fever in patients with neutropenic fever (NF) during empirical antibiotic therapy could be caused by dysregulated immune responses, its association with cytokine concentrations has rarely been investigated. This study determined the kinetics of cytokine concentrations in pediatric patients with NF and bacteremia and evaluated the impact of cytokine concentration kinetics on prolonged fever. **Methods:** Concentrations of 13 cytokines were measured on the initial day of NF (Day 1) and 3 days (Day 4) and 7 days (Day 8) later in 10 patients with NF with bacteremia, and their kinetics was determined. The results for each cytokine concentration on each sampling day were compared for patients with fever that lasted ≥ 3 days and those with fever that lasted < 3 days. **Results:** Interleukin (IL)-6 ($p < .001$) and IL-10 ($p = .001$) concentrations were significantly higher on Day 1 than on Days 4 and 8. However, the increased IL-6 ($p = 1.000$) and IL-10 ($p = 1.000$) concentrations on Day 1 were not associated with prolonged fever (≥ 3 days). For other cytokines, the concentrations measured on Days 1, 4, and 8 were similar regardless of fever duration. **Conclusion:** Prolonged fever in patients with NF and bacteremia was not associated with a prolonged increase in a specific cytokine concentration.

Keywords

cytokine, bacteremia, neutropenia, child

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Introduction

Neutropenic fever (NF) after chemotherapy and hematopoietic cell transplantation (HCT) for hematological malignancies increases morbidity and mortality rates; thus, empirical broad-spectrum antibiotic therapy is recommended.¹ For prolonged fever beyond 2–4 days of empirical antibiotic therapy, additional broad-spectrum antibiotic or antifungal therapy should be considered.¹ However, considering that bacteremia is identified in about 20% of patients with NF and invasive fungal infection is identified in $< 20\%$ of patients with NF

receiving empirical antifungal therapy,¹ this practice may cause inappropriate use of broad-spectrum antibiotics and increase antibiotic resistance and adverse effects associated with antibiotic and

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antifungal agents. If prolonged fever is caused by a dysregulated immune response and uncontrolled cytokine release rather than uncontrolled bacterial or fungal infection, immune-modulating therapy might be rather suitable. Previous studies investigated cytokines for the early prediction of bacterial infection, bacteremia, or severe sepsis in pediatric patients with NF.^{2,3} However, most previous studies measured cytokine concentrations at the development of NF, and the kinetics of cytokine concentrations and the associations with prolonged fever and severe complications of bacteremia, including septic shock and acute respiratory distress syndrome (ARDS), which are caused by dysregulated immune responses, have rarely been studied.⁴⁻⁶ This study aimed to investigate the changing trends in cytokine concentrations during the first week of bacteremia-induced NF in pediatric patients with hematological malignancy and evaluate its impact on prolonged fever.

Patients and methods

Subject and study design

From January to December 2016, pediatric patients aged <19 years, who received chemotherapy or HCT for hematological malignancies at the Department of Pediatrics of Seoul St Mary's Hospital (Seoul, Republic of Korea), were prospectively monitored for the development of NF and bacteremia during hospitalization. Among them, patients in whom both NF and bacteremia were identified were considered for this study and were enrolled if the patient and parents agreed to participate. Blood culture was performed as soon as possible if NF was identified, and bacterial growth was reported within 24–48 h of blood culture in most patients in our hospital. In our hospital, residual serum samples were kept in a refrigerator at the Department of Laboratory Medicine for 2 weeks after routine testing, and residual serum samples acquired for routine blood tests were collected three times for the enrolled patients: on the first day of NF (Day 1) and 3 days (Day 4) and 7 days (Day 8) after the onset of NF. These serum samples were stored at -70°C until testing. Neutropenia was defined as absolute neutrophil count (ANC) $<500/\text{mm}^3$, and fever was defined as tympanic membrane temperature $\geq 38.0^{\circ}\text{C}$ or axillary temperature $\geq 37.5^{\circ}\text{C}$ for

more than 1 h.⁷ Concentrations of 13 cytokines (tumor necrosis factor [TNF]- α , interferon- γ , interleukin [IL]-2, IL-6, IL-9, IL-4, IL-5, IL-10, IL-13, IL-17A, IL-17F, IL-21, and IL-22) were measured in each serum sample using a commercial multiplex fluorescent bead assay kit (LEGENDplex™ Human Th Cytokine Panel, BioLegend Inc., San Diego, CA, USA) as previously reported.⁸ All tests were performed in duplicate, and the mean concentration was used in the statistical analyses. Cytokine concentrations and C-reactive protein (CRP) levels were compared according to underlying hematological malignancy, administered chemotherapy type, complete remission (CR) status, and type of identified bacteria on each sampling day. Concentrations of cytokines and CRP levels on each sampling day were compared between patients with fever lasting for ≥ 3 days and those with fever lasting for <3 days. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Seoul St Mary's Hospital (approval no: KC15TISI0943). Because all study subjects were minors, written informed consent was acquired from a legally authorized representative (parent) of the patient. Additional written informed consent was acquired from the patient if he/she was aged ≥ 7 years.

Statistical analysis

Comparisons of cytokine concentrations and CRP levels between patient groups were performed using a Mann–Whitney test. For each cytokine and CRP, the concentrations on Days 1, 4, and 8 were compared using Friedman's test. The SPSS 21 program (IBM Corporation, Armonk, NY, USA) was used for the statistical analyses, and two-sided p -values $<.05$ were considered statistically significant.

Results

A total of 10 pediatric patients (six males, four females; median age, 11 years [range, 3–18 years]) with bacteremia-induced NF were enrolled in this study. Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB) were identified in six and four patients, respectively (Table 1). The demographic and clinical characteristics of the enrolled patients are summarized in Table 1. Fever

Table 1. Characteristics of the whole study subjects.

Patient number	Sex	Age, years	Status of underlying hematological malignancy	Type of chemotherapy	Fever interval, days ^a	Fever duration, days	Neutropenia before fever, days	Neutropenia after fever, days	Focal inflammation	Identified pathogen
1	Male	7	AML, 2nd CR	Consolidation	23	6	6	8	None	<i>Viridans streptococcus</i>
2	Female	3	AML, 1st CR	Consolidation	16	4	6	7	GI tract	<i>Viridans streptococcus</i>
3	Male	4	Burkitt lymphoma, naïve	Induction	14	3	2	2	None	<i>Klebsiella pneumoniae</i>
4	Male	16	AML, post-allogeneic HCT, 1st relapse	Re-induction	11	1	7	20	None	<i>Klebsiella pneumoniae</i>
5	Male	16	AML, 1st CR	Consolidation	14	2	5	4	None	<i>Viridans streptococcus</i>
6	Female	6	ALL, naïve	Induction	9	5	18	5	URT	<i>Aeromonas sobria</i>
7	Male	17	ALL, 1st CR	Consolidation	1	1	1	3	None	<i>Staphylococcus epidermidis</i>
8	Female	10	AML, 1st CR	Allogeneic HCT	2	6	27	14	None	<i>Klebsiella pneumoniae</i>
9	Female	18	AML, 1st relapse	Re-induction	8	1	4	31	GI tract	<i>Pseudomonas aeruginosa</i>
10	Male	12	AML, 1st CR	Consolidation	13	1	5	7	GI tract	<i>Neisseria</i> spp.

AML: acute myeloid leukemia; CR: complete remission; GI: gastrointestinal; HCT: hematopoietic cell transplantation; ALL: acute lymphoblastic leukemia; URT: upper respiratory tract.

^aFever interval was defined from initiation of chemotherapy to development of fever.

developed at a median of 12 days (range, 1–23 days) after initiation of chemotherapy and at a median of 6 days (range, 1–27 days) after neutropenia and persisted for a median of 2.5 days (range, 1–6 days). Neutropenia recovered at a median of 7 days (range, 2–31 days) after fever, and the ANC became over 1000/mm³ at a median of 15 days (range, 4–55 days) after fever: two patients (patient no. 4 and patient no. 9) died of uncontrolled leukemia before the restoration of ANC over 1000/mm³. No severe complications of bacteremia, such as septic shock, hypoxia, ARDS, or death, occurred. The bacteria were eradicated before Day 4 in all patients except patient no. 6 (on Day 10) and patient no. 7 (on Day 6).

For all study subjects, among the 13 cytokines, concentrations of all but IL-6 and IL-10 were not significantly different on Days 1, 4, and 8 (Figure 1(a)). IL-6 ($p < .001$) and IL-10 ($p = .001$) concentrations on Day 4 were significantly lower than those on Day 1 but comparable to those on Day 8 (Figure 1(a)). Median CRP levels on Days 1, 4, and 8 were 3.70 mg/dL (range, 0.04–14.48 mg/dL), 7.74 mg/dL (0.31–15.77 mg/dL), and 2.53 mg/dL (0.07–19.98 mg/dL), respectively ($p = .143$). On Day 1, there were no significant differences in underlying hematological malignancy, administered chemotherapy type, CR status, or fever duration for any cytokine concentrations or CRP levels. However, on Day 1, the median concentration of IL-2 was higher in patients with GPB infection than in those with GNB infection (6.05 pg/mL vs 3.00 pg/mL, $p = .038$), while median concentrations of IL-2 (5.51 pg/mL vs 1.77 pg/mL, $p = .019$) and TNF- α (7.48 pg/mL vs 5.08 pg/mL, $p = .010$) were higher in patients without focal inflammation than in those with focal inflammation. On Day 4, the median concentration of IL-21 was higher in patients with a CR status than in those without a CR status (142.49 pg/mL vs 74.65 pg/mL, $p = .038$). On Day 8, the median concentration of IL-22 was higher in patients with a GNB infection than in those with a GPB infection (76.46 pg/mL vs 47.32 pg/mL, $p = .010$). Fever duration was not significantly associated with any specific cytokine concentrations on any sampling day (Figure 1(b)).

Discussion

In this study, 13 cytokine concentrations were measured during the early phase of NF. IL-6 and IL-10 concentrations were elevated at NF

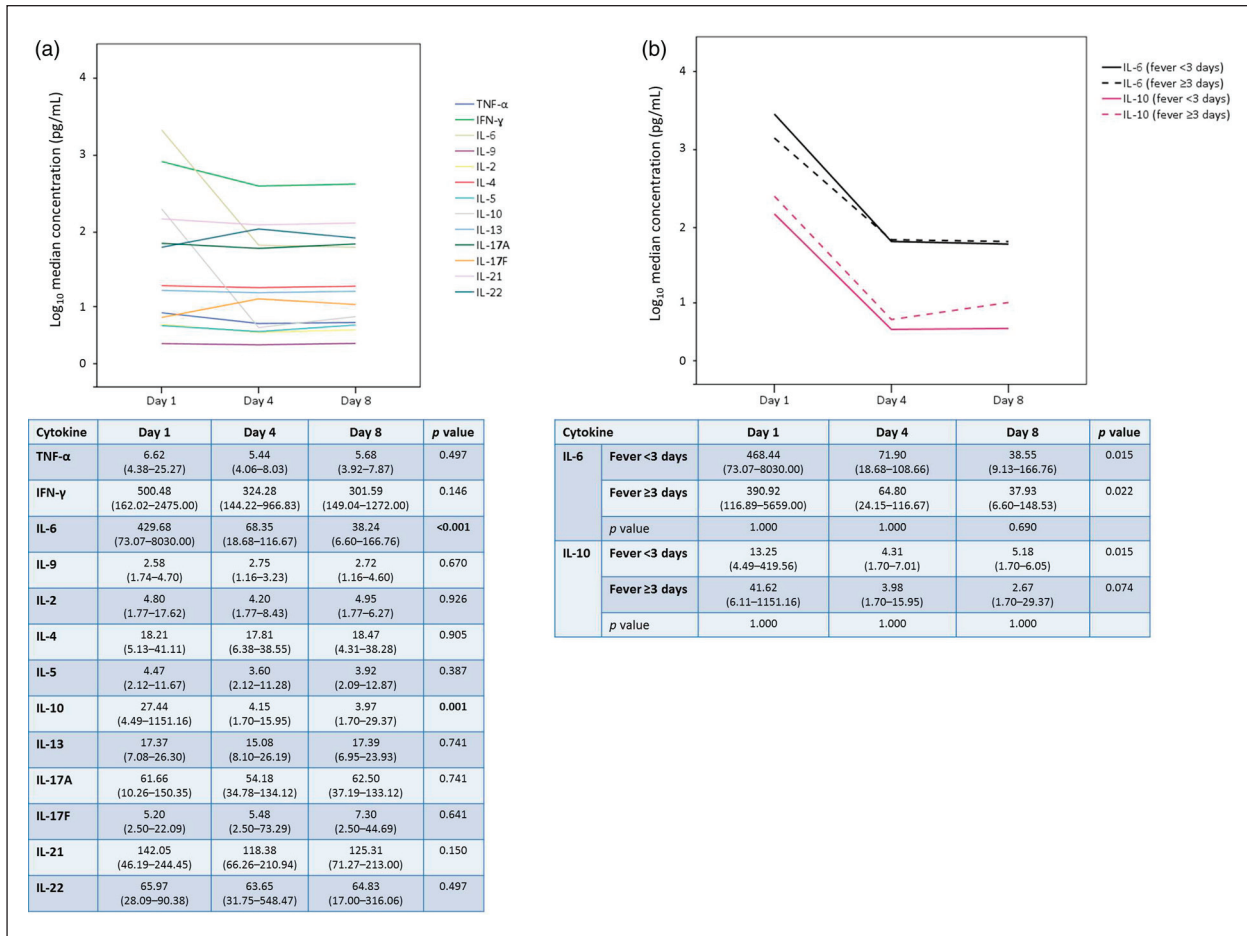


Figure 1. Kinetics of cytokine concentrations among all study subjects (a); kinetics of IL-6 and IL-10 concentrations among patients with fever that lasted for ≥ 3 days and those with fever that lasted for < 3 days (b). Data are presented as median (range).

development; however, the concentrations rapidly decreased within 4 days. Prolonged fever was not associated with specific cytokine concentrations.

Most previous studies of patients with NF have investigated the association between specific cytokine concentrations at the development of NF and bacteremia, reporting higher IL-6, IL-8, and IL-10 concentrations in patients with bacteremia than in those without bacteremia.^{2,3,6} However, no single cytokine has been identified as a perfect predictor for bacterial infection or associated severe complications.^{2,6} IL-6 and IL-10 concentrations for patients with NF and bacteremia in this study were significantly higher at NF development compared to those with an afebrile states. However, the concentrations decreased within 4 days after NF onset. Previous studies also reported significant decreases in IL-6 and IL-8 concentrations within 5 days after NF onset in patients with NF and bacteremia.^{5,6,9}

In this study, cytokine concentrations in patients with fever lasting for < 3 days were compared to those in patients with fever lasting for ≥ 3 days. Prolonged fever after bacterial eradication was suspected to be caused by prolonged elevation of pro-inflammatory cytokine concentrations, and immune-modulating treatment was expected to be effective. However, no cytokine concentrations were significantly different between the two patient groups. For patient no. 6, bacterial eradication was not achieved, while the patient was febrile on Day 4; however, the IL-6 concentration decreased significantly from Day 1 to Day 4. Previous studies of patients with NF and bacteremia showed higher IL-6 and IL-8 concentrations at NF development in cases of fever lasting for > 3 days compared with cases of fever lasting for ≤ 3 days, whereas IL-6 and IL-8 concentrations on Day 3 were not significantly different between the two patient groups.^{6,10}

Deceased patients showed persisting higher concentrations of IL-6 and IL-8 in cases of severe sepsis without underlying immunocompromised disorders compared to surviving adults.¹¹ Therefore, information about the cytokine kinetics might be more useful for predicting severe complications of bacteremia including mortality than simply prolonged fever. Cytokine kinetics in bacteremia patients with underlying diseases that could develop NF might differ for that of patients without underlying disease. Types of underlying malignancies and identified bacteria might affect the cytokine kinetics. Therefore, the inclusion of NF patients with severe bacteremia complications might influence the cytokine kinetics, considering that no patients with severe complications of bacteremia were included in this study.

This study had some limitations. First, this was a pilot study that aimed to identify specific cytokines related to prolonged fever, which might represent dysregulated immune responses in NF patients. A subsequent study to confirm the significant relationship between the specified cytokines and immune dysregulation was planned. The appropriate sample size could not be calculated for this study because cytokines of interest have not been specified, and unfortunately, only a small number of patients were enrolled. A larger number of patients may help define specific cytokines. Moreover, comparisons between NF patients with and without bacteremia were not conducted. The impact of cytokine kinetics on the development of severe complications of bacteremia could not be determined. Some cytokines not tested in this study might exhibit varying concentrations in NF patients with versus without severe complications of bacteremia. Moreover, some lipid metabolites and peptides were reportedly more reliable for predicting fever origin and duration than cytokines in adult patients with NF.⁴ Future studies including more NF patients should evaluate the impact of the kinetics of several organic materials on prolonged fever and severe complications of bacteremia.

Conclusion

The changes in cytokine concentrations were restored within 4 days after fever development in pediatric patients with NF and bacteremia. Prolonged fever in these patients was not associated

with specific cytokine kinetics. Therefore, immunomodulating therapy might not be useful for prolonged fever in patients with NF.

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Declaration of conflicting interests

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Ethical approval

Ethical approval for this study was obtained from the Institutional Review Board of Seoul St Mary's Hospital (Approval Number: KC15TISI0943).

Informed consent

Because all study subjects were minors, written informed consent was acquired from a legally authorized representative (parent) of the patient. Additional written informed consent was acquired from the patient if he/she was aged ≥ 7 years.

Trial registration

This randomized clinical trial was not registered because this study was a non-interventional pilot study.

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