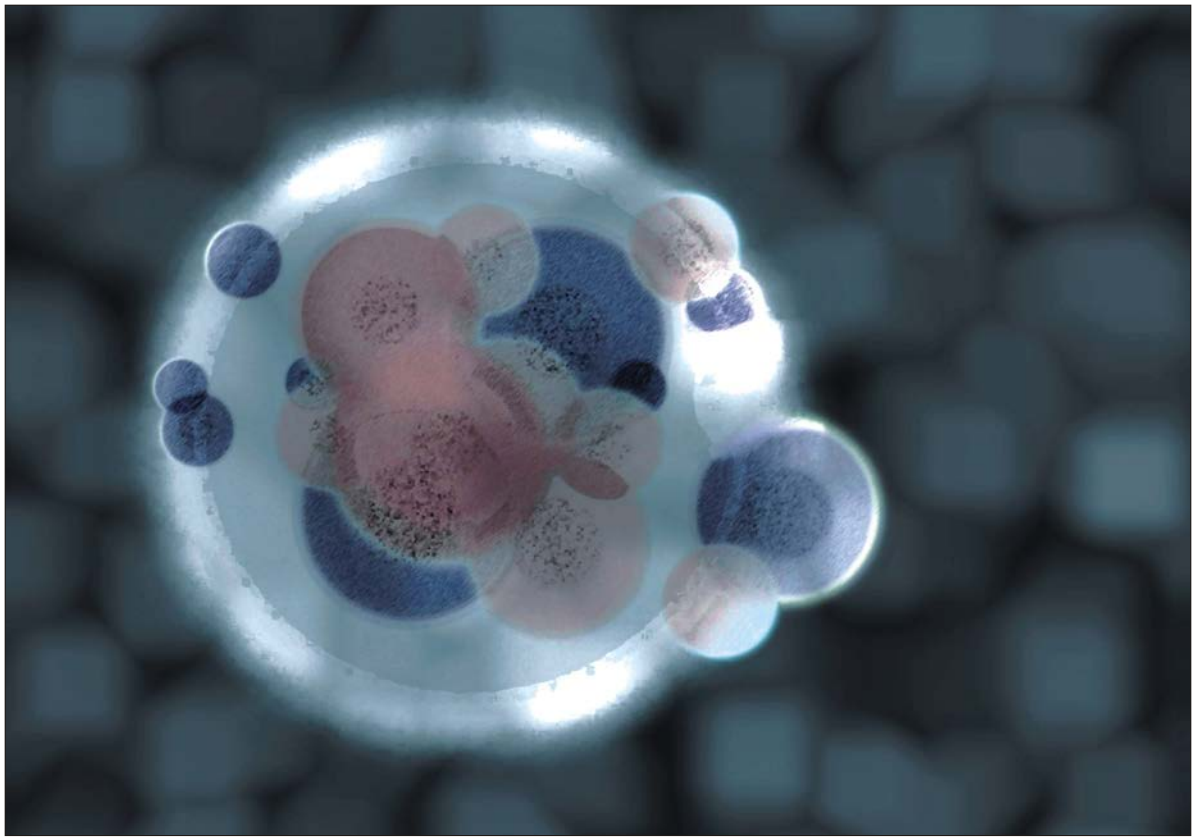




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Diagnostic Relevance of Interleukin-6 and Tumor Necrosis Factor Alpha in Discriminating High Risk and Low Risk Groups in Febrile Patients with Neutropenia

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Abstract: The present study was designed to determine diagnostic value of IL-6 and TNF- α in patients with fever and neutropenia. This is a prospective study of 133 patients admitted to two university hospital in Tehran, Iran with fever and neutropenia. Patients were divided two groups as low risk and high-risk groups. Cytokines level compared with Mann-Whitney test in study groups of patients and ROC curves used to determine best cut-off points level for cytokines discriminating risk groups. Mean age of patients was 26.8 ± 2.5 years and 7.5% of patients allocated in low risk group. The mean IL-6 and TNF- α serum level below 17 pg mL^{-1} was defined as best cut-off point determining low risk group patients with sensitivity and specificity of 70 and 67.5% respectively. However, we cannot define a statistically significant cut-off point for TNF- α to use as a diagnostic test. 13.5% of patients of our study have positive blood cultures (6% gram-negative, 6% gram- positive, 1.5% fungi), but no statistical difference had found in serum IL-6 and TNF- α levels in blood culture groups. Despite our findings about IL-6 diagnostic value in neutropenic patients with fever and its advantages in discriminating risk groups of patients it seem necessary to design a randomized controlled trial before use of this marker.

Key words: IL-6, TNF- α , neutropenia, fever, risk group

INTRODUCTION

In cancer patients, morbidity and mortality because of infectious complications following aggressive chemotherapy remain a major clinical problem. The treatment-related neutropenia (defined as a neutrophil count $<0.5 \times 10^9 \text{ L}^{-1}$) following antineoplastic chemotherapy is associated with a high risk of infections. The prediction of deterioration or else, of a stable course during the febrile episode is ambiguous. It has therefore been recommended to reassess anti-microbial therapy from fever 3-5 days onward. In the majority of neutropenic fever episodes, causative infectious agents cannot be identified (Hughes *et al.*, 2002). A specific, rapid and cost-effective marker indicating ongoing infection or indicating deterioration during the febrile neutropenic period would therefore be highly warranted (Persson *et al.*, 2005).

Patients with febrile neutropenia require hospitalization and prompt empirical treatment with broad-spectrum antibiotics. Patients experiencing severe febrile neutropenia episodes need a longer hospitalization period and close follow-up, but those with mild episodes might derive limited benefit from a lengthy hospital stay (Karan, 2002). In addition, the sequential assessment of febrile episodes, the early diagnosis of patients at high risk of deterioration or secondary infectious complications and recognition of patients at low risk of complications are essential factors in hospitalization (Flischhack *et al.*, 2000). Early discharge of a defined group of patients at low risk for septicemia would be of great advantage. For high-risk patients, hospital treatment is appropriate considering the possibility of rapid deterioration. However, prolonged hospitalization of patients with fever and neutropenia result in risk of selection for resistant

flora and risk of exposure to nosocomial pathogens. Early discharge or no admission at all of low risk patients would be interest since the costs of prolonged hospitalization are substantial (Talcott *et al.*, 1992).

Several groups have proposed based on clinical data for discrimination of high risk and low risk patients (Escalante, 2004; Kern, 2006; Kamana *et al.*, 2005; Talcott *et al.*, 1992). Unfortunately, this criterion is mostly based on clinical data and the number of patients in high-risk group is still very large. The crucial question in this setting is which test is reliable diagnostic marker to identify a low risk group of febrile patients with neutropenia.

Recently attention has focused on identifying serum markers of immunologic response that may be useful for therapeutic intervention (Kitanovski *et al.*, 2006; Stryjewski *et al.*, 2005). In response to inflammatory stimuli, a number of macrophage/monocyte-derived cytokines mediate a number of metabolic changes that are known as acute phase reactions. Two of the most important mediators are interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α). IL-6 and TNF- α were identified as early and sensitive markers of severe infections in both non-neutropenic patients and immunosuppressed cancer patients. There have been few cytokines whose role in trauma, sepsis and burns has been studied more and understood less than interleukin (IL)-6. IL-6 is a highly pluripotent cytokine. Produced by a variety of different cell types, including inflammatory cells, epithelial cells and fibroblasts (Taub, 2004; Engel, 1998).

Some studies have suggested a possible role for IL-6 as a diagnostic test in the differentiation between fever due to sepsis or non-bacterial conditions in neutropenic patients (Kitanovski *et al.*, 2006; Persson *et al.*, 2005, 2004; Stryjewski *et al.*, 2005), whereas others could not confirm this (Hammond and Potgieter, 1996).

The present study was designed to determine the diagnostic value and relevance of IL-6 and TNF- α in patients with fever and neutropenia at the start of febrile episode. The identification of one or more predictive factors may be useful for tailoring therapy but naturally, will have to be substantiated in the prospective randomized trials.

MATERIALS AND METHODS

This study was performed at emergency and hematology/oncology ward of university hospitals: Imam Khomeini and Shariati, Tehran, Iran. Patients with fever and neutropenia were included in the study unless they were pregnant or received systemic antibiotics during last 72 h. Eligible patient with chemotherapy relate

neutropenia (ANC<500 μL) and fever (body temperature $>38^{\circ}\text{C}$ for 6 h or $>38.5^{\circ}\text{C}$ once) were entered. University medical research committee approved the study protocol. All patients gave informed consent.

This was a prospective study; a full medical history was taken from all enrolled patients. Baseline investigations included complete blood cell counts with differential white blood cell counts, chest radiographs, culture of blood and urine were collected. Blood specimens were collected for cytokines IL-6 and TNF- α analysis. All samples were collected at the time of admission before any antibiotic use.

To discriminate patients to high risk and low risk group for septicemia we used some criterion based in previous studies and patients based on this criterion divided into two groups mentioned above. The criterion components were: having ANC $>100 \mu\text{L}^{-1}$, Absolute monocyte count (AMC) $>100 \mu\text{L}^{-1}$, duration of fever less than 7 days, fever $<39^{\circ}\text{C}$, not ill appearance, no abdominal pain, systolic blood pressure (SBP) $>90 \text{ mmHg}$, no sign of renal failure (serum creatinin $<1.5 \text{ mg dL}^{-1}$), no sign of liver failure (serum aminotransferases level more than two times normal), no uncontrollable bleeding, no mucositis, no dysuria, no diarrhea, O_2 saturation in arterial blood $>90\%$, no loss of consciousness or no sign of neurological deficit, no hypocalcemia (serum calcium level $>7.5 \text{ mg dL}^{-1}$), no vomiting, malignancy in remission, sign of bone marrow recovery, normal chest radiographs, no folliculitis. Patients with all fourteen ROLSTON criterion defined as low risk and other without even one of them defined as high risk.

Considering the standard deviation (δ) of 880 pg mL^{-1} for IL-6 levels and considering standard difference (d) of 150 pg mL^{-1} with first type error (α) equal to 0.05, we calculate the sample size about 133 patients.

Measurement of plasma IL-6 and TNF- α levels: blood specimens were used to measure IL-6 and TNF- α plasma concentrations with quantitative sandwich enzyme immunoassay (Roche Biochemicals Corp. Cat No. -1-5 34-475) according to the manufacturer's instructions.

Data collected from patients were analyzed with SPSS 10.05 program. Data on IL-6 and TNF- α were summarized as mean and percentiles. For crude comparisons of these parameters in various groups, we used the Mann-Whitney U and Kruskal-Wallis tests and Chi and Fisher's exact tests. To determine a cut-off point level for IL-6 and TNF- α to discriminate risk groups of patients, we used ROC-curve method then sensitivity and specificity of calculated cut-off points of IL-6 and TNF- α using ROLSTON criterion as gold standard test, were calculated. We also used EPI-info 6 software to power calculation. Statistical tests were carried out at a significance level of 0.05.

RESULTS

The study measured IL-6 and TNF- α and level in 133 febrile patients with chemotherapy that induced neutropenia. Mean age $\pm$ 2SE was 26.8 $\pm$ 2.5 years and male to female ratio was 72/61. Patients assigned into two groups based on selected criterion. Low risk group consist of 7.5% of patients and high risk group of 92.5% patients, characteristics are depicted in Table 1.

21.1% of patients had ANC<100 μ L⁻¹ and 30.1% had AMC<100 μ L⁻¹ in their blood samples. In 18% of patients, there was the probability of fever lasting more than 10 days and for 56.4% of them body temperature was above 39°C. 15.8% of patients seem ill and 3% complaint of abdominal pain. Twelve percent had diarrhea and 3.3 had positive urine cultures. There is no sign of neurological deficit in 97% of patients and 5.3% of patients had SBP<90 mmHg.

Blood culture results showed that 13.5% of neutropenic patient with fever had positive blood culture, 6% gram-negative microorganisms, 6% Gram positive microorganisms and in 1.5% fungi has been reported. At the time of admission for fever during chemotherapy induced neutropenia, the mean IL-6 level was 128.5 $\pm$ 44.5 pg mL⁻¹ and mean TNF- α level was 37.2 $\pm$ 11.6 pg mL⁻¹. The IL-6 levels among low risk group of patients had no statistically significant difference with high risk group patients (p-value = 0.286).

The levels of 17 pg mL⁻¹ for IL-6 and 20 pg mL⁻¹ for TNF- α have defined as best level determining risk group using ROC curve method. The sensitivity and specificity of having IL-6 above 17 pg mL⁻¹ to state high risk group were 70 and 67.5%, respectively. In addition, they were statistically significant (p-value<0.05). but not for TNF- α levels above 20 pg mL⁻¹. Its sensitivity and specificity were 52.8 and 80% respectively and not significant statistically (Table 2).

Table 1: Baseline characteristics (age and sex) of 133 study patients Groups are assigned based on selected criterion

Characteristics	Total	High risk	Low risk
Age, Mean ($\pm$ 2 SE)	26.8 $\pm$ 2.5		
<15 years	10.2%	10%	12.58%
16-50 years	84.5%	85%	75.0%
>50 years	5.5%	5%	12.5%
Sex Male/Female	72/61	67/56	5/5

Table 2: Diagnostic value of IL-6>17 pg mL⁻¹ and TNF- α >20 pg mL⁻¹ to distinguish risk group in febrile neutropenic patients

Diagnostic value	Low risk	High risk	Sensitivity (%)	Specificity (%)	Accuracy (95% CI*)	p-value
IL-6			70	67.5		0.017
<17**	7	40			65.8	
$\geq$ 17	3	83			52.3-79.2	
TNF- α			52.8	80		NS***
<20	8	77			64.4	
$\geq$ 20	2	46			50.4-78.5	

* CI: Confidence interval ** All units: pg/ml *** N.S.: Not significant

DISCUSSION

Patients with neutropenia (absolute neutrophil count $\geq$ 500 mm³) continue to be at increased risk of developing infections, despite recent advances in supportive care, including infection prevention (Hughes *et al.*, 2002).

The leading cause of treatment-related mortality among patients with hematological malignancies undergoing chemotherapy is multiorgan failure due to systemic infection during neutropenia (Buchheidt *et al.*, 2003). Most neutropenic patients with infection present with fever as the first symptom. However, there is a variety of causes for febrile conditions. Apart from infection, reactions to drugs or blood products as well as tumor-associated fever are all possible underlying mechanisms. If the fever is not accompanied by clinical or microbiological evidence of infection, it is classified as Fever of Unknown Origin (FUO) (Link *et al.*, 2003).

Since bacterial infections are life-threatening for neutropenic patients, empiric antibiotic therapy is started immediately when fever occurs, before microbiological proof of an infection is obtained. In this setting, reliable and readily available parameters to diagnose or rule out infection are needed. The most common parameter is Creactive protein (CRP), an acute-phase protein produced after proinflammatory cytokine release. The CRP concentration rises within 24 h and is elevated in almost all cases of microbial infection, but its reliability as a marker of infection is hampered by very low specificity (Sudhoff *et al.*, 2000).

A diagnostic test to define the low risk group of neutropenic patients with fever would be of great value. In neutropenic cancer patients, early markers are needed that are regulated or released independently of the leukocytes count and activity of underlying disease. They have to reflect the severity of infection and distinguish episodes of high risk and low risk for septic complications. More recently, proinflammatory cytokines, especially interleukin-6 (IL-6), have been evaluated as parameters for infections. IL-6 is a polypeptide that quickly reaches high levels after stimulation with endotoxin, tumor necrosis factor alpha or IL-1 and it has a much shorter half-life than CRP (Engervall *et al.*, 1995a,b). The value of inflammatory cytokines IL-6 and TNF- α in diagnosis and assessment of infection in nonneutropenic and neutropenic patients had well established in clinical studies during the past decade (Flach *et al.*, 1999; De Bonte *et al.*, 1999; von Lilienfeld-Toal, 2004; Kitanovski *et al.*, 2006; Stryjewski *et al.*, 2005; Persson *et al.*, 2005; Karan, 2002).

In the present study, we evaluated the value of IL-6 and TNF- α in 133 patients with fever and neutropenia. We detect positive blood culture in 13% of patients and this

is fewer than amounts reported in previous studies 20-30% (von Lilienfeld-Toal, 2004; Persson *et al.*, 2005; Valenga *et al.*, 1996). In our study, percent of positive cultures were 12% that it result was similar with our study (Kamana *et al.*, 2005). In this study IL-6 serum levels was strongest and best predictor of high risk patients group in feverish neutropenic patients, but not TNF- α , this is consistent with similar studies in this field (Hynninen *et al.*, 1997; De Bonte *et al.*, 2001). In contrast to other studies that report difference in IL-6 serum levels in patients with fever and neutropenia based on blood culture results high levels in gram negative cultures compared with gram positive cultures (Lehrbecher, 1999; De Bonte *et al.*, 2001), we had not similar findings and it may be as results of fewer culture positive in our study.

One of our study goals was to define a reliable and reproducible threshold for measured levels of IL-6 and TNF- α based on selected criterion as gold standard. We suggested IL-6 level above 17 pg mL⁻¹ as a best cut-off point to determine high risk patients with sensitivity and specificity of 70 and 67.5%, respectively. But we must consider that before designing study to determine whether IL-6 levels may be useful for tailoring therapy, further studies are needed to validate the clinical criterion as a gold standard in studies about serum markers. We also must consider that none of studies assessing cytokines in feverish patients with neutropenia looked at a large enough cohorts to assess the importance of levels with respect to specific infectious outcomes (Persson *et al.*, 2004, 2005; Kitanovski *et al.*, 2006). In this study, result of TNF- α did not significant in discrimination of high risk and low risk groups in febrile patients with neutropenia.

As conclusion of this study it would seem reasonable to consider serum IL-6 levels below 17 pg mL⁻¹ to define low risk group of febrile neutropenic patients. But a prospective randomized study under strict conditions (e.g., hospitalization) and well defined boundaries for IL-6 plasma levels, will ultimately answer the question whether the observed parameters are of clinical relevance and might be used in the future for early discharge of selected group of patients and result in cost reduction. Additional studies are necessary before a clinical ward can safely discharge febrile neutropenic patients.

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