Use of inflammatory molecules to predict the occurrence of fever in onco-hematological patients with neutropenia

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Abstract

Febrile neutropenia remains a frequent complication in onco-hematological patients, and changes in the circulating level of inflammatory molecules (IM) may precede the occurrence of fever. The present observational prospective study was carried out to evaluate the behavior of plasma tumor necrosis factor alpha (TNF-α), soluble TNF-α I and II receptors (sTNFRI and sTNFRII), monocyte chemoattractant protein-1 [MCP-1 or chemokine (c-c motif) ligand 2 (CCL2)], macrophage inflammatory protein-1α (MIP-1α or CCL3), eotaxin (CCL11), interleukin-8 (IL-8 or CXCL8), and interferon-inducible protein-10 (IP-10 or CXCL10) in 32 episodes of neutropenia in 26 onco-hematological patients. IM were tested on enrollment and 24-48 h before the onset of fever and within 24 h of the first occurrence of fever. Eight of 32 episodes of neutropenia did not present fever (control group) and the patients underwent IM tests on three different occasions. sTNFRI levels, measured a median of 11 h (1-15) before the onset of fever, were significantly higher in patients presenting fever during follow-up compared to controls (P = 0.02). Similar results were observed for sTNFRI and CCL2 levels (P = 0.04 for both) in non-transplanted patients. A cut-off of 1514 pg/mL for sTNFRI was able to discriminate between neutropenic patients with or without fever during follow-up, with 65% sensitivity, 87% specificity, and 93% positive predictive value. Measurement of the levels of plasma sTNFRI can be used to predict the occurrence of fever in neutropenic patients.

Key words: Neutropenia; Cytokines; Oncological hematology

Introduction

A significant improvement in the outcome of patients with hematological malignancies has been observed, mainly due to progress in treatment and supportive care (1,2). In spite of the indisputable advance in the management of these patients, many clinical challenges remain, such as opportunistic infections, drug toxicity, and hemorrhages (3,4).

Infectious complications related to neutropenia are the leading cause of morbidity and mortality in onco-hematological patients. Neutropenia is classically defined as a neutrophil count below 0.5×10^9/L (5). Management of infections in neutropenic patients is especially challenging owing to their nonspecific signs and symptoms. Fever is regarded as an early warning sign in patients with neutropenia, and all guidelines recommend prompt use of broad-spectrum antibiotic therapy when it occurs (5-7).

However, fever might not be a sufficiently precocious marker of infection in neutropenic patients, leading to an undesirable delay in the use of antibiotic therapy. In addition, some patients do not present fever at all despite severe bacterial or fungal infections (8-11).

Cytokines and other inflammatory molecules have the ability to promote the onset of fever, to recruit different immune system cells to the site of infection, and to act on hematopoiesis and cell regulation (12). It appears that a change in plasma levels of some of these molecules precedes the occurrence of fever in neutropenic patients. Therefore, the aim of this study was to evaluate the ability of using the levels of eight different inflammatory molecules to predict the occurrence of fever in a population of onco-hematological neutropenic patients.

Material and Methods

This was a prospective observational pilot study with onco-hematological patients admitted to a 400-bed University Hospital in Southeast Brazil, a regional reference hospital for the management of patients with hematological diseases and hematopoietic stem cell transplantation (HSCT). The study protocol was approved by the Ethics Review Board of Universidade Federal de Minas Gerais (Process. No. 0250.0.203.000-08), and written informed consent was obtained from all patients or their legal representative.

All consecutive adult (≥18 years) patients, hospitalized from September 2008 to March 2009 and presenting neutropenia (defined as a neutrophil count $<1.0 \times 10^9$/L) were assessed for eligibility. Inclusion criteria were as follows: 1) to be afebrile for at least 48 h before enrollment; 2) to present no sign/symptom of clinically and/or radiologically active infection for at least 48 h before enrollment; 3) to be diagnosed with a malignant hematological disorder or severe aplastic anemia, and 4) to present neutropenia expected to last for at least 6 days. Fever was defined as a single axillary temperature ≥38.3°C or axillary temperature ≥37.8°C sustained for at least 1 h. Inclusion of more than one neutropenia episode per patient was allowed, provided it occurred in different hospitalizations and that neutropenia receded between hospitalizations. Patients receiving therapeutic antibiotics and/or with hospital discharge planned for the next 5 days were not included in the study.

Clinical evaluation and full medical history were obtained for all participants. Clinical data were recorded at baseline and during follow-up. Routine blood tests were recorded at inclusion and periodically thereafter, along with results from microbiologic cultures. In 10 HSCT patients, data on the type of transplant, HLA compatibility and graft source were also recorded. Axillary temperature was routinely measured every 4 h during hospitalization. Patients were followed for 28 days, or until the first episode of fever, death or discharge, whichever came first. Patients were divided into two groups: group 1, all patients presenting fever during the follow-up period, and group 2 (control group), patients that did not present fever during follow-up. All diagnostic and therapeutic interventions were performed by the attending physician as clinically indicated.

Laboratory tests

To measure plasma levels of different inflammatory molecules, peripheral blood samples were collected daily (early in the morning), using EDTA tubes (BD Vacutainer; Becton Dickinson Diagnostic Systems, Brazil). Blood samples were centrifuged and the resulting plasma was frozen and stored at -80°C. The following inflammatory molecules were tested: plasma tumor necrosis factor alpha (TNF-α), soluble TNF-α I and II receptors (sTNFRI and sTNFRII, respectively), monocyte chemotactic protein-1 (MCP-1/CCL2), macrophage inflammatory protein 1α (MIP-1α/CCL3), eotaxin (CCL11), interleukin 8 (IL-8/CXCL8), and interferon gamma-induced protein (IP-10/CXCL10). Plasma was thawed at room temperature in order to determine the circulating levels of each molecule using a sandwich-type ELISA method (Duoset, R & D Systems, USA) according to manufacturer instructions. The detection limit of the ELISA was approximately 5 pg/mL for all inflammatory molecules. Inter- and intra-assay coefficients of variation were below 10% and all assays were performed in duplicate.

Plasma levels of inflammatory molecules were analyzed at three different times in group 1 patients: on enrollment (T0); 48 to 24 h before the first onset of fever (T1), and within 24 h of the first occurrence of fever (T2). Final blood samples for each febrile patient were collected at a median time of 11 h (range: 1-15 h) before the onset of fever. Given the absence of fever as a benchmark, arbitrary points were defined to test inflammatory markers among group 2 (control group) subjects. Three time points were chosen according to neutropenia duration, as follows: upon inclusion (T0), at the median (T1), and on the final (T2) day of the neutropenia period, for each patient.

Statistical analysis

The primary end point was the occurrence of fever during neutropenia. We tested whether a difference in plasma levels of different inflammatory molecules among neutropenic patients predicted the occurrence of fever during the follow-up period. A subgroup analysis was performed including non-transplanted patients only. Discrete variables are reported as percentages, and continuous variables as median and range. Differences between groups were tested by the chi-square or the Fisher exact test for categorical variables, and the Mann-Whitney U-test was used for continuous variables. Results are presented as crude (unadjusted) data with 95% confidence intervals. The diagnostic accuracy of each inflammatory plasma level presenting statistical significance was expressed as the area under the receiver operating characteristic curve (ROC), from which the best cut-offs and their related sensitivity, specificity, positive (PPV) and negative (NPV) predictive values were calculated. Statistical analysis was performed using the SPSS 12.0 software (Chicago University, USA). Significance was reported at a P value of 0.05 or less.

Results

A total of 211 episodes of neutropenia were assessed for eligibility, 41 episodes that occurred in 33 patients fulfilled the required criteria. Most neutropenia episodes were not considered to be eligible for this study because they did not fulfill the criteria for neutropenia duration (in

Table 1. Clinical characteristics of neutropenic patients presenting fever or not.

	All patients	Patients with fever episode	Patients without fever episode
Numbers of patients	26*[+]	20[+]	8[+]
Male gender	19 (73%)	15 (75%)	6 (75%)
Age (years)	36 (18-69)	35 (20-64)	36 (18-69)
Diagnosis			
Acute myeloid leukemia	9 (35%)	7 (35%)	3 (37.5%)
Multiple myeloma	4 (15%)	4 (20%)	-
Myelodysplastic syndrome	3 (11%)	2 (10%)	1 (12.5%)
Non-Hodgkin lymphoma	6 (23%)	4 (20%)	3 (37.5%)
Hodgkin lymphoma	2 (8%)	2 (10%)	-
Aplastic anemia	1 (4%)	1 (5%)	-
Acute lymphocytic leukemia	1 (4%)	0	1 (12.5%)

Data are reported as number (%) or median (range). *Two patients were included twice: once in the febrile group and once in the afebrile group for one patient and twice in febrile group for another. Two patients were included three times: twice in the febrile group and once in the afebrile group for one patient and three times in the febrile group for another. [+]Two patients were included in both groups.

most cases, short-term neutropenia in lymphoma, myeloma or solid tumor patients) and/or patients had already presented fever before inclusion. A further 9 of the 41 episodes were additionally excluded because a final plasma sample (T2) before fever was unavailable. Therefore, 32 episodes of neutropenia, observed in 26 patients, were included in the final analysis. The clinical characteristics of the patients included are summarized in Table 1.

As expected, all patients were neutropenic on inclusion, and median neutrophil count was 0.27 (0-0.99) 10^9/L. Median follow-up time was 3 (1-27) days. Twenty-four (75%) episodes of neutropenia developed fever during the follow-up period. Median time from enrollment to fever was 2 days (range 1-9 days). Blood samples for cultures were collected for 23 of 24 episodes of fever and 7 (29%) cultures were positive. There was no episode of fever related to drug infusion or blood transfusion. As shown in

Table 1, there were no differences in the clinical characteristics of patients presenting fever, or not, during follow-up.

Dynamic behavior of the different plasma markers

Circulating levels of the tested inflammatory molecules measured at three different times for the whole study population are summarized in Table 2. Significantly higher levels of sTNFRI were observed in the final sample before fever (within 24 h of fever) in group 1 compared to the levels measured on the corresponding neutropenia (T2) day in the control group (P = 0.029; Table 3 and Figure 1). No difference was observed for the remaining molecules.

In order to define the ability of plasma sTNFRI levels to be used to identify neutropenic patients who are at risk of presenting fever, an ROC curve was constructed and a cut-off value of 1514 pg/mL was identified as showing the

Table 2. Median plasma levels of inflammatory molecules measured at different times in the entire population of neutropenic patients.

Inflammatory molecules	T0 (n = 19)	T1 (n = 22)	T2 (n = 31)
TNF-α	110.03 (0-799.82)	158.8 (0-730.79)	95.74 (0-720.31)
sTNFRI	1261.6 (647.26-2381.56)	1316.8 (647.26-4593.31)	1557.26 (731.37-6306.12)
sTNFRII	2498.31 (1441.34-4036.09)	2610.4 (1510.03-4953.69)	2915.10 (1514.7-5546.53)
CXCL8	104.23 (33.21-223.85)	118.5 (48.05-631.21)	130.21 (13.49-1070.16)
CCL3	48.9 (5-1071.95)	107.7 (18-1068.14)	75.58 (5-1031.50)
CCL11	203.90 (95.12-659.98)	189.1 (115.66-524.82)	164.89 (121.75-601.00)
CXCL10	0.00 (0-641.74)	0.00 (0-650.60)	12.27 (0-269.35)
CCL2	1834.4 (0-6492.26)	1460.4 (0-3535.94)	1384.01 (0-6278.90)

Data are reported in pg/mL as median (range). T0 = on inclusion; T1 = 48 to 24 h before the first occurrence of fever; T2 = within 24 h of the first occurrence of fever. TNF-α = tumor necrosis factor-α; sTNFRI = soluble TNF-α I receptor; sTNFRII = soluble TNF-α II receptor; CXCL8 = interleukin-8; CCL3 = macrophage inflammatory protein-1α; CCL11 = eotaxin; CXCL10 = interferon-inducible protein-10; CCL2 = chemokine (c-c motif) ligand 2.

Table 3. Dynamic behavior of the different plasma inflammatory molecule levels in the entire population of neutropenic patients with fever or not.

Inflammatory molecules	T0		T1		T2	
	Fever (n = 8)	No fever (n = 11)	Fever (n = 7)	No fever (n = 15)	Fever (n = 8)	No fever (n = 23)
TNF-α	110.0	94.9	177.1	53.1	127.8	88.8
sTNFRI	1261.6	1241.4	1356.4	1089.1	1877.8*	1290.4
sTNFRII	2498.3	2567.0	2785.0	2599.6	3190.5	2657.2
CXCL8	77.6*	150.9	108.9	143.3	139.0	120.9
CCL3	30.3	70.5	107.5	125.0	83.6	49.5
CCL11	208.4	184.4	168.8	191.8	162.8	177.7
CXCL10	0.0	0.0	0.0	0.0	16.8	12.2
CCL2	1834.4	1625.9	1384.0	2189.8	1812.9	858.1

Data are reported in pg/mL as median. T0 = on inclusion; T1 = 48 to 24 h before the first occurrence of fever; T2 = within 24 h of the first occurrence of fever. For abbreviations, see legend to Table 2. *$P < 0.05$ compared to patients with no fever (Mann-Whitney U-test).

best discrimination between neutropenic patients presenting fever, or not, within 24 h, with 65% sensitivity, 87% specificity, 93% PPV, 46% NPV, and 70% accuracy.

Since about one third of our patients presented neutropenia after HSCT, a subgroup analysis was performed including only episodes in non-transplanted patients (N = 22). In this case, significantly higher plasma levels of sTNFRI and CCL2 were observed in the final sample before fever (within 24 h of fever) for patients in group 1 compared to control (P = 0.04 for both; Table 4). ROC curve analysis was also performed to evaluate the best cut-off value in this subgroup. Similar to the overall population, the best cut-off of sTNFRI for predicting the occurrence of fever among non-transplanted neutropenic

Figure 1. Plasma levels of soluble TNF-α I receptor (TNFRI) measured at enrollment, 24 to 48 h before the occurrence of fever, and within 24 h of fever onset (group 1) compared to the corresponding days in the group without fever (group 2). X2 = outliers. *$P < 0.05$ compared to group 2 (Mann-Whitney U-test).
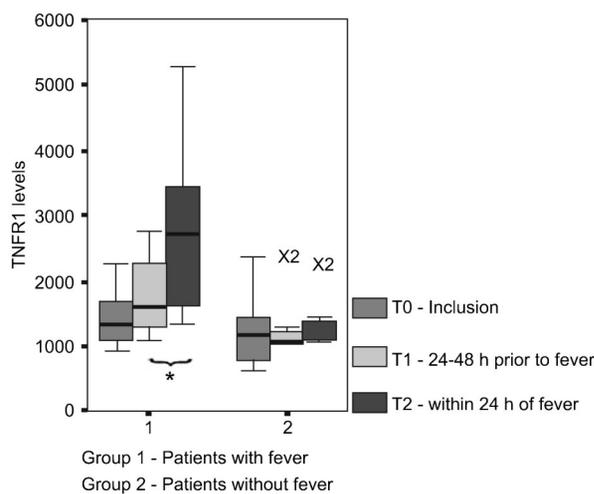
patients was 1514 pg/mL, with 71% sensitivity, 87% specificity, 91% PPV, 63% NPV, and 77% accuracy. CCL2 did not present a satisfactory discriminatory performance, with an accuracy of less than 50%.

Discussion

This exploratory study whose objective was to use the levels of circulating inflammatory molecules to predict the occurrence of fever in onco-hematological patients with chemotherapy-induced or disease-related neutropenia. We showed that, among the individuals studied, a significant elevation in circulating levels of sTNFRI preceded the onset of fever, with a median interval of 11 h. Similar results were observed in a subgroup of non-transplanted patients. A cut-off of 1514 pg/mL was shown to be the best discriminative value for sTNFRI in order to identify neutropenic patients who would present fever.

Fever is the main, and frequently the only, clinical sign used to guide antibiotic treatment in neutropenic patients. However, fever may also be a delayed sign, or even not occur at all, despite the presence of a life-threatening infection. Early use of antibiotic therapy was demonstrated to have improved outcome in different clinical scenarios (13-15). It is conceivable that a test that precociously detects the presence of infection, onset of fever and other clinical signs, could improve the outcome of neutropenic individuals (16). If so, the potential benefit of such a marker would be directly proportional to its accuracy and precocity.

Several biomarkers have been investigated for their utility in rapidly discriminating true infection from other inflammatory processes causing fever and two in particular, procalcitonin (17-20) and endotoxin (21,22), demonstrated their utility as an adjunctive diagnostic tool for discriminating severe bacterial infection in different clinical situations (i.e., sepsis, septic shock, and different

Table 4. Dynamic behavior of the different plasma inflammatory molecule levels in neutropenic patients not undergoing transplantation with fever or not.

Inflammatory molecules	T0		T1		T2	
	Fever (n = 8)	No fever (n = 7)	Fever (n = 7)	No fever (n = 11)	Fever (n = 8)	No fever (n = 14)
TNF-α	164.1	94.9	207.6	53.1	212.4	88.8
sTNFRI	1419.7	1241.4	1367.2	1089.1	1858.7*	1290.4
sTNFRII	2679.9	2567.0	2785.0	2599.6	3283.9	2657.2
CXCL8	100.3	150.9	108.9*	143.3	128.5	120.9
CCL3	78.9	70.5	107.9	125.0	100.0	49.5
CCL11	202.9	184.4	144.9	191.8	151.5	177.7
CXCL10	0.0	0.0	0.0	0.0	18.0	12.2
CCL2	2025.8	1625.9	1384.0	2189.8	2452.1*	858.1

Data are reported in pg/mL as median. T0 = on inclusion; T1 = 48 to 24 h before the first occurrence of fever; T2 = within 24 h of the first occurrence of fever. For abbreviations, see legend to Table 2. *$P < 0.05$ compared to patients with no fever (Mann-Whitney U-test).

kinds of bacterial infections). A large number of publications have also addressed the usefulness of inflammatory molecules to predict the outcome of febrile neutropenia episodes (23-25). However, very few studies have tested the ability of circulating inflammatory molecules to predict (or anticipate) the occurrence of fever in this population (11,26-29). Engel et al. (11) evaluated the ability of serum levels of CXCL8, IL-6 and C-reactive protein (CRP) to predict the occurrence of fever in oncology patients with neutropenia. The levels of none of the tested cytokines was able to anticipate fever 24 h before its onset. The same group evaluated the circulating levels of procalcitonin and CXCL8 in patients with febrile neutropenia, correlating them with the occurrence of infection. Median procalcitonin levels increased from 0.16 ng/mL, on the day before, to 0.34 ng/mL, on the day after fever (27). Goetz et al. (28) tested the utility of sTNFRII in 54 patients with acute myeloid leukemia to provide an early diagnosis of sepsis during neutropenia. Eleven patients did not develop a fever. Median circulating levels of sTNFRII, tested on the day before fever, and on the day of fever, were similar (28). Finally, Buyukberber et al. (29) analyzed the soluble receptor levels of IL-2 (sIL-2R), IL-6, CXCL8, CRP, IL-1b, and TNF-α in 22 patients with onco-hematological diseases before chemotherapy, and at different moments after treatment. No cytokine was found to be predictive of fever (29). These studies have dissimilarities in many key aspects, such as target population, tested molecules, sample size, study design, and blood sampling schedules. This heterogeneity may explain the different results and their inability to identify an inflammatory molecule able to predict fever in neutropenic individuals.

To be helpful to neutropenic patients, a fever-predictive tool must present elevated PPV, since a potential advantage of this test is to anticipate the commencement of antimicrobial therapy. Should fever

occur, antibiotics would be prescribed regardless of the level of an inflammatory marker. In this study, the PPV for sTNFRI cut-off in the entire population and in the subgroup of non-transplanted patients was higher than 90%.

Several limitations of this study must be mentioned. Firstly, most episodes of neutropenia assessed for eligibility were eventually not included in the study. As stated before, the main reason for exclusion was non-compliance with the criteria for duration of neutropenia and/or already having presented fever at assessment. In this study, we were particularly interested in a subgroup of long-duration neutropenia, usually considered to represent high-risk patients due to a higher risk of complication, even with antibiotic treatment. A selection bias, however, cannot be ruled out. Secondly, a small sample of patients was studied, which limited our statistical inferences. As noted, this was a pilot study designed to identify promising inflammatory markers to be studied in a large series of neutropenic patients. Thirdly, 10 patients underwent HSCT, and all of them presented fever at some point during neutropenia. It is well known that host tissues are directly damaged by conditioning chemotherapy used in transplants, or by different cytokines released after conditioning (IL-6, IL-1, TNF-α, etc.), the so-called "cytokine storm" (30). This particular cytokine profile, which occurs early after HSCT, could limit the use of some inflammatory molecules as predictors of neutropenic fever. In fact, sTNFRI seems to be a better predictor for fever in non-transplanted patients. Unfortunately, the number of transplanted patients became too small to be analyzed as a subgroup. Finally, fever might also be due to reasons other than infection during neutropenia. To minimize this problem, only patients for whom fever was a determining factor in the use of antibiotic therapy by the attending physician were included in the study.

To the best of our knowledge, this is the first study to

have evaluated sTNFRI, CCL2, CCL11, and CXCL10 in the context of neutropenia. Elevation of sTNFRI levels observed before fever has a biological background. TNF-α is a key inflammatory mediator in bacterial sepsis. It appears early following the event, initiating host response mechanisms, such as induction of other cytokines and fever (31-33). Virtually all cell types possess either sTNFRI or sTNFRII, or both, which mediate the vast range of the TNF-α effect. As TNF-α exerts its proinflammatory effect via two distinct cell surface receptors, on TNF-α stimulation, the extracellular domain of sTNFR can be shed into the blood (34-36). sTNFR can be measured earlier in serum than TNF-α

(37-39). Finally, sTNFRI concentration is easier to measure than TNF-α levels, which makes it a more reliable serum marker (40).

This study showed that plasma sTNFRI levels represent a promising marker to predict the occurrence of fever in onco-hematological patients with neutropenia. A larger study is needed to confirm the role of sTNFRI as a fever predictor in neutropenic patients with hematological diseases.

Acknowledgments

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