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Case report

Interaction between IL-6 and TNF- α genotypes associated with bacteremia in multiple myeloma patients submitted to autologous stem cell transplantation (ASCT)Fernanda M.B. Trigo^{a,*}, Marcelo R. Luizon^{b,1}, H elio S. Dutra^c,  ngelo Maiolino^c, M rcio Nucci^c, Belinda P. Sim es^a^a Department of Internal Medicine, Medicine School of Ribeir o Preto, University of S o Paulo, Ribeir o Preto, SP, Brazil^b Department of Pharmacology, Medicine School of Ribeir o Preto, University of S o Paulo, Ribeir o Preto, SP, Brazil^c University Hospital, Bone Marrow Transplantation Unit, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

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ABSTRACT

Stem cell transplantation affects patient's vulnerability to infections due to immunological changes related to chemotherapy. Multiple myeloma is characterized by susceptibility to infections, and IL-6 and TNF- α increased levels affect immune response (IR). Polymorphisms in promoter region of cytokine genes may alter expression levels and affect IR. We performed interaction analysis of IL-6 (–174G/C) and TNF- α (–308G/A) polymorphisms with infection susceptibility in 148 patients classified accordingly to infection status and found an interaction when compared groups with and without bacteremia ($p=0.0380$). The interaction may be more important than single effects for the IR associated with the infection susceptibility in ASCT.

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1. Introduction

Multiple myeloma (MM) is a plasma cell neoplasm characterized by the production of a monoclonal protein, bone destruction and susceptibility to infections [1,2]. Most infections in myeloma patients are caused by bacteria, and this susceptibility to infection is mostly due to the reduction in the production of normal immunoglobulins, typical of the disease [3]. The conditioning regimen for the autologous stem cell transplantation (ASCT) further increases patient's vulnerability to infections [1].

High levels of proinflammatory cytokines such as IL-6 and TNF- α are related to MM pathogenesis, and have a relationship with the immune response and the susceptibility to infections. Single nucleotide polymorphisms (SNPs) in the promoter region of cytokines genes are responsible for altering their levels of expression, thereby affecting the immune response [1,3,4]. Specifically, the SNP –174G > C (rs1800795) has been correlated with higher levels of IL-6 [5], and the –308G > A (rs1800629) is associated with higher serum levels of soluble TNF- α [6]. These SNPs have been previously studied in multiple myeloma [4,6].

However, gene–gene interactions have been successfully used to detect susceptibility to complex diseases, and it is possible that gene interactions may influence the outcome in patients with multiple myeloma [7,8]. Therefore, we aimed to evaluate the association of these SNPs with bacteremia in MM patients submitted to ASCT. Moreover, as IL-6 and TNF- α cytokines are known to biologically interact in immune response pathways, we also performed gene–gene interaction analyses between these SNPs.

2. Material and methods

We studied 148 MM patients submitted to ASCT from the Bone Marrow Transplantation unit of Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, from March 1997 to May 2007. The study was approved by the Ethics Research Committee of the Medicine School of Ribeir o Preto, University of S o Paulo, and the Federal University of Rio de Janeiro. Informed consent was obtained from each individual.

All patients received melphalan-based conditioning regimen and peripheral blood stem cells (PBSC) after mobilization with granulocyte colony-stimulating factor (G-CSF) with or without cyclophosphamide. Until 2006, no antibacterial prophylaxis had been given. Since then, all patients received ciprofloxacin (500 mg orally twice a day), started concomitantly with the conditioning regimen, and was maintained until bone marrow recovery or

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fever. In case of fever, blood cultures were obtained, and the patients were immediately started on intravenous cefepime. Blood cultures were repeated in case of persistent or recurrent fever, or as clinically indicated. Modifications in the empirical antibiotic regimen were performed according to the results of cultures and the clinical course of the patient.

Genomic DNA was extracted from mobilized peripheral-blood stem cells. Genotyping was carried out for the SNPs IL-6 (-174G > C) and TNF- α (-308G/A) using PCR followed by restriction length fragment polymorphism (PCR-RFLP). Clinical and genotypic database and genotypic analysis were performed with the Statistical Package for the Social Sciences version 15 (SPSS Inc., Chicago, Ill), using the Chi-square test. The gene-gene interaction analysis was performed by multifactor dimensionality reduction (MDR) method [8]. We used MDR to combine multilocus genotypes into high risk and low risk cells, which were evaluated for the ability to predict bacteremia through cross-validation and permutation testing. We considered as the best model of interaction the one that had the maximum testing accuracy and the maximum cross-validation consistency (CVC). Permutation testing was performed to assess the statistical significance of the testing accuracy of the best model [8]. Bivariate analysis (logistic regression) was performed to evaluate the impact of the use of quinolones on the risk of bacteremia.

3. Results

We evaluated 148 MM patients, 87 women and 61 men, age range 29–69 years, 58 of whom developed bacteremia during the early post-transplant period (Table 1). The genotypes were in

Hardy–Weinberg equilibrium. We did not observe any statistically significant association between the genotypes of TNF- α and IL-6 polymorphisms and bacteremia (Table 2). However, analyzing the association between bacteremia and the interaction of polymorphisms, we found a significant interaction between TNF- α -308G > A and IL-6 -174G > C polymorphisms, with a CVC of 10/10 and balanced accuracy of 0.6021 ($p=0.04$). Using this method, we could identify two risk groups: the high risk group comprised patients with wild genotype of TNF- α -308G > A and mutated genotype of IL-6 -174G > C, wild genotype of TNF- α -308G > A and heterozygous genotype of IL-6 -174G > C, and heterozygous for both genes (dark gray cells in Fig. 1). The low-risk group comprised all other combinations (light gray cells in Fig. 1). As shown in Table 3, the frequency of bacteremia overall (50.7% vs. 29.6%, $p=0.009$) and of Gram-positive bacteremia (34.3% vs. 12.5%, $p=0.002$) was significantly higher in the high risk group compared with the low risk group.

We analyzed the impact of quinolone use on the frequency of bacteremia. The frequency of bacteremia was significantly lower in patients receiving quinolone prophylaxis (16.7% vs. 43.5% in patients not receiving quinolones, $p=0.01$). We then run a bivariate analysis to evaluate the influence of quinolone use and the interaction of polymorphisms on the occurrence of bacteremia. Receipt of quinolones decreased the risk of bacteremia (odds ratio [OR] 0.25, 95% confidence interval [95% CI] 0.08–0.81, $p=0.02$) while the high risk group (as defined by the analysis of interaction of polymorphisms) was associated with an increased risk of bacteremia (OR 2.47, 95% CI 1.24–4.94, $p=0.01$). Therefore, the high risk group was associated with an increased risk of bacteremia independent of quinolone use.

Table 1
Characteristics of 148 patients with multiple myeloma undergoing autologous hematopoietic cell transplantation.

Characteristic	No.
Gender male: female	85: 63
Age (years), median (range)	54 (29–69)
Number of CD34 cells ($\times 10^6$ /kg), median (range)	3.89 (1.13–20.07)
Febrile neutropenia	147 (99.3%)
Duration (days) of neutropenia, median (range)	7 (3–25)
Duration (days) of antibiotic therapy, median (range) ^a	8 (1–44)
Quinolone prophylaxis	24 (16.2%)
Fluconazole prophylaxis	78 (52.7%)
Cefepime-based empiric antibiotic regimen ^a	135/147 (91.8%)
In monotherapy ^a	117/147 (79.6%)
Classification of the febrile episodes ^a	
Fever of unknown origin	70/147 (47.6%)
Bacteremia	58/147 (39.5%)
Gram-positive ^b	33 ^b /58
Gram-negative ^b	28 ^b /58
Polymicrobial ^b	4 ^b /58 ^b
Microbiologically documented without bacteremia	3/147 (2.0%)
Clinically documented	16 (10.9%)

^a In 147 patients who developed febrile neutropenia.

^b Within the 58 cases of bacteremia, 4 presented polymicrobial bacteremia.

Table 2
Genotype frequencies in patients with multiple myeloma and bacteremia due to Gram-positive or Gram-negative organisms.

	TNF- α -308G > A			P value	IL-6-174G > C			P value
	GG	GA	AA		GG	GC	CC	
Gram-negative bacteremia	23 (20.0)	5 (17.2)	0	0.58	17 (19.8)	10 (18.2)	1 (14.3)	0.92
Gram-positive bacteremia	25 (21.9)	8 (27.6)	0	0.45	18 (21.2)	12 (21.8)	3 (42.9)	0.41
All bacteremias ^a	46 (40.0)	12 (41.4)	0	0.26	34 (39.5)	20 (36.4)	4 (57.1)	0.57

^a The number of Gram-positive and Gram-negative bacteremia are higher than the number of total bacteremias because some patients had both Gram-positive and Gram-negative bacteremia.

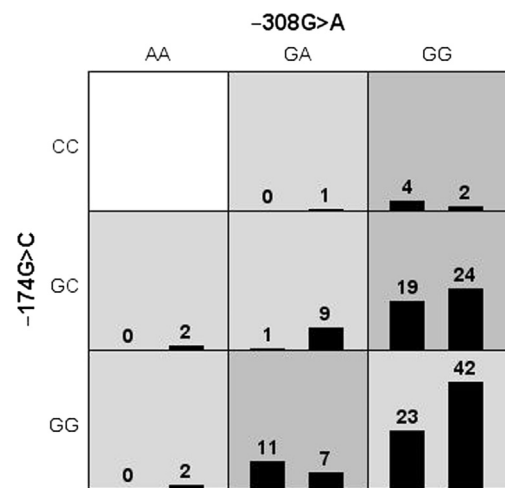


Fig. 1. Interaction model for TNF- α -308G > A and IL-6 -174G > C polymorphisms when compared the groups with (left bars) and without bacteremia (right bars), which are illustrated for each multilocus genotype combinations. The light gray cells are labeled as low risk and the dark gray cells are labeled as high risk.

Table 3

Frequency of bacteremia in low risk and high risk groups as defined by the analysis of the interactions of genetic polymorphisms for TNF- α –308G>A and IL-6 –174G>C.

Bacteremia	High risk N=67 (%)	Low risk N=81 (%)	P value
Gram-positive	23 (34.3)	10 (12.5)	0.002
Gram-negative	14 (20.9)	14 (17.3)	0.58
All bacteremias	34 (50.7)	24 (29.6)	0.009

4. Discussion

In the present study, we reported for the first time the possible association of TNF- α –308G>A and IL-6 –174G>C genotypes with bacteremia in MM patients submitted to ASCT. Interestingly, these analyses suggest that the interaction of polymorphisms may be more important than the effects of single polymorphisms for the immune response associated with the susceptibility to infection in ASCT.

The patterns of high risk and low risk cells shown in Fig. 1 differ across each of the different multilocus dimensions, which may be interpreted as evidence of epistasis, or gene–gene interaction [8]. TNF-stimulates the secretion of IL-6 in bone marrow stromal cells [9], which are associated with a worst prognosis in MM patients [1]. The analysis of gene interaction efficiently discriminated two risk groups, with very different incidences of bacteremia. Furthermore, the association of the interaction of polymorphisms and bacteremia was still present after controlling for the use of quinolones.

The present findings must be replicated in populations with different genetic backgrounds. Moreover, as they focused only on TNF- α and IL-6 polymorphisms, further studies should be conducted to examine whether interactions between polymorphisms

in other genes may affect the infection status in MM patients. Likewise, the association between these polymorphisms and Gram-negative and Gram-positive bacteremia needs to be evaluated in a larger number of patients.

4.1. Speculations

The present findings are relevant as they may contribute to the search for genetic markers associated with infection in MM patients submitted to ASCT, which may be potentially translate into clinical application, with appropriate preventive measures for high-risk patients.

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