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Gene Polymorphisms and Febrile Neutropenia in Acute Leukemia—No Association with IL-4, CCR-5, IL-1RA, but the MBL-2, ACE, and TLR-4 Are Associated with the Disease in Turkish Patients: A Preliminary Study

Mustafa Pehlivan,[1] Handan Haydaroğlu Sahin,[1] Kurşat Ozdilli,[2] Hüseyin Onay,[3] Ali Ozcan,[3] Ferda Ozkinay,[3] and Sacide Pehlivan[2,3]

Aims: The aim of this study was to investigate the mannose-binding lectin 2 (MBL-2), interleukin (IL)-4, Toll-like receptor 4 (TLR-4), angiotensin converting enzyme (ACE), chemokine receptor 5 (CCR-5), and IL-1 receptor antagonist (RA) gene polymorphisms (GPs) in acute leukemias (ALs) and to evaluate their roles in febrile neutropenia (FN) resulting from chemotherapy. *Methods:* The study included 60 AL patients hospitalized between the period of July 2001 and August 2006. Polymorphisms for the genes ACE(I/D), CCR-5, IL-1RA, MBL-2, TLR-4, and IL-4 were typed by polymerase chain reaction (PCR) and/or PCR–restriction fragment length polymerase. Genotype frequencies for these genes were compared in the patient and control groups. The relationships between the genotypes and the body distribution of infections, pathogens, the duration of neutropenia, and febrile episodes in AL patients were evaluated. *Results:* No significant differences in either the genotype distribution or the allelic frequencies of TLR-4, IL-4, CCR-5, IL-1RN GPs were observed between patients and healthy controls. The AB/BB genotype (53.3%) in the MBL-2 gene was found to be significantly higher in the AL patients compared with control groups. There were correlations between the presence of MBL-2, TLR-4, and ACE polymorphisms and clinical parameters due to FN. Overall, bacteremia was more common in MBL BB and ACE DD. Gram-positive bacteremia was more common in ACE for ID versus DD genotype. Gram-negative bacteremia was more common for both the MBL-2 AB/BB genotype and TLR-4 AG genotype. Median durations of febrile episodes were significantly shorter in ACE DD and MBL AB/BB. *Conclusion:* Although TLR-4, ACE, and MBL-2 GPs have been extensively investigated in different clinical pictures, this is the first study to evaluate the role of these polymorphisms in the genetic etiopathogenesis of FN in patients with ALs. As a conclusion, TLR-4, ACE, and MBL-2 genes might play roles in the genetic etiopathogenesis of FN in patients with ALs.

Introduction

Infections are major causes of morbidity and mortality in patients with acute leukemia (AL) undergoing chemotherapy. Attendant prolonged and severe neutropenia makes these subjects susceptible to infections (Bucaneve *et al.*, 2005; Roongpoovapatr and Suankratay, 2010). Fever is present in 85% of patients during neutropenia caused by chemotherapy, and microbiologically documented infections are seen in 45% of the patients (Bucaneve *et al.*, 2005). Before 2002, gram-negative bacteria were considered to be the most common causative pathogen of febrile neutropenia (FN), whereas gram-positive bacteria have taken the lead since 2005 and fungal infections are being seen more commonly in the last years (Roongpoovapatr and Suankratay, 2010). Several authors have claimed that using prophylactic antibiotics lowered the risk of infections in leukemia patients undergoing chemotherapy (Bucaneve *et al.*, 2005; Vekemens *et al.*, 2007; Roongpoovapatr and Suankratay, 2010). Therefore, some of the gene polymorphisms (GPs) influencing normal immune system function have been studied to analyze if giving prophylactic antibiotics to a group with susceptibility is more efficient. This has been done in cancer patients in a limited number of groups (Dahmer *et al.*, 2005; Nachtigal *et al.*, 2014).

[1]Department of Hematology, School of Medicine, Gaziantep University, Gaziantep, Turkey.
[2]Department of Medical Biology, Medipol University Hospital, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.
[3]Department of Medical Genetics, Ege University School of Medicine, Izmir, Turkey.

The body's response to bacterial infection requires first, recognition of the presence of pathogen-associated bacterial products through receptors (Klostergaard *et al.*, 2010). Polymorphisms in genes coding for proteins involved in the recognition of bacterial pathogens (Toll-like receptor 4 [TLR-4], mannose-binding lectin 2 [MBL-2], etc.) and the response to bacterial pathogens (tumor necrosis factor-alpha [TNF-α], interleukin [IL]-1 receptor antagonist [RA], IL-6, IL-10,and angiotensin converting enzyme [ACE]) can influence the amount or function of the protein produced in response to bacterial stimuli (Vaschetto and Protti, 2010).

MBL-2 is a calcium-dependent lectin that plays an important role in innate immunity by activating the complement pathway and phagocytosis. Decreased MBL-2 in the circulation may cause predisposition to infections in patients treated with chemotherapy for hematological diseases and autoimmune diseases (Turner and Hamvas, 2000). In addition to this, it has been found that MBL-2 GPs increase the risk of severe infections and their occurrence rate (Vekemens *et al.*, 2007; Dommett *et al.*, 2013).

TLR-4 is one member of a class of pattern recognition receptors (PRRs) that play a significant role in the physiologic innate immune response. TLR-4 has been reported to recognize not only the lipopolysaccharide (LPS) component of gram-negative bacteria but also the mouse mammary tumor virus and vesicular stomatitis virus proteins. Variant TLR-4 might be associated with fungal infection, gram-negative bacterial infection, and/or sepsis (Turner and Hamvas, 2000). Furthermore, the TLR-4 (Asn299Gly) variant was found to be responsible for increased risk of mucosa associated lymphoid tissue lymphoma and Hodgkin lymphoma (Nuolivirta *et al.*, 2009).

IL-4 has multiple immune response modulatory functions on a variety of cell types (Vannier *et al.*, 1992). IL-4 suppresses also the IL-1 and IL-2 activation pathways at the level of signal transduction and increases the expressions of IL-1RA (Vannier *et al.*, 1992; Pehlivan *et al.*, 2009). The IL-1 cluster of genes is located on chromosome 2q and contains three related genes, IL-1A, IL-1B, and IL-1RA, which code for IL-1α, IL-1β, and the IL-1RA, respectively (Hoeft *et al.*, 2008). In some studies, but not in others, individuals who are homozygous for the rare allele IL-1RA*2 have high circulating IL-1RA levels and even more elevated IL-1β levels, resulting in a heightened, prolonged inflammatory response (Bruserud, 1996).

ACE's main function is to control blood pressure. An insertion/deletion (I/D) polymorphism of a 287-bp sequence in intron 16 of the ACE gene accounts for most of the variability of serum ACE activity, with DD genotypes having the highest and II genotypes having the lowest ACE activity (Cogulu *et al.*, 2008). An elevated activity of the renin–angiotensin system due to ACE I/D polymorphism has been connected with increased susceptibility and illness severity in the acute respiratory distress syndrome in adults, meningococcal meningitis in children, and severe circulatory compromise in febrile neutropenic children with cancer (Jin *et al.*, 2004; Bárdi *et al.*, 2005).

The macrophage (M)-tropic HIV-1 uses the b-chemokine receptor 5 (CCR-5) to enter into macrophages. A 32 basepair deletion (Δ32) within the CCR-5 gene found on chromosome 3p21, results in a truncated protein leading to lack of integration into the cell membrane (Nkenfou *et al.*, 2013). The relationship between FN and CCR-5 (Δ32) polymorphism has not been investigated before, but a negative association between CCR-5 (Δ32) and rheumatoid arthritis has been described (Pang and Yu, 2010). Because of this paradoxical affect on autoimmune diseases, the role of CCR-5 (Δ32) polymorphism on FN was investigated in this study.

We aimed to investigate MBL-2, IL-4, TLR-4, ACE, CCR-5, and IL-1RA gene polymorphisms in ALs and to evaluate their roles in FN resulting from chemotherapy for AL.

Materials and Methods

Study population

From July 2001 to August 2006, all febrile episodes ($n = 100$) occurring in 60 neutropenic patients with AL (13 acute lymphoblastic leukemia and 47 acute myeloid leukemia [AML]) and 60 healthy controls were investigated. All participants were informed about the nature of the study and all consented to participate. The study was approved by the Ege University's Ethics Committee.

Basic data acquisition

We analyzed 60 patients who received 100 cycles of chemotherapy. The mean age of the AL patients was 42 (16–72) and 31 of the 60 patients were female and 29 were male. The median time to neutrophil count recovery (defined as $> 0.5 \times 10^9$/L [500×10^6/L] for 2 consecutive days) was 16 days (range, 2–56) and the median days of fever (defined as temperature above 38°C) was 6 (range, 1–30). The mean age of healthy controls was 38 (16–66). Established criteria for fever and infection were defined (Huges *et al.*, 2002). Patients, who developed febrile episodes, were examined and investigated for the cause of the fever. This included sampling of blood for bacterial culture according to standard protocols. Empirical first-line antibiotic therapy followed the above-mentioned guidelines and consisted of a third-generation cephalosporin or carbapenem with an aminoglycoside in most cases. The treatment was modified according to microbiological results and clinical evolution. In the event that pulmonary infiltrates were detected or fever persisted and cultures were negative after 5–7 day of antibiotics, empirical antifungal treatment with liposomal amphotericin B was started after obtaining new samples for cultures. Defervescence was defined as a body temperature < 37.5°C for > 24 h. Bacteremia was defined by microbial growth in one blood culture bottle, but for coagulase-negative *Staphylococcus* and *Corynebacteria* species, two positive blood culture bottles with samples from different venepunctures were required; otherwise, the result was regarded as possible contamination. The criteria proposed by Ascioglu *et al.* (2002) were used for the diagnosis of invasive fungal infection.

DNA extraction and genotyping analysis

Genomic DNA was extracted from peripheral blood leukocytes by a salting-out procedure (Miller *et al.*, 1998). Polymorphisms (genotypes) for the genes ACE I/D, CCR-5 (Δ32), IL-1RA (VNTR in intron 2), IL-4 (−590), MBL-2 (codons 54 and 57), and TLR-4 (A896G) were typed by polymerase chain reaction (PCR) and/or PCR–restriction fragment length polymerase and agarose gel electrophoresis (Scarel-Caminaga *et al.*, 2003; Shi *et al.*, 2004; Vardar *et al.*, 2007; Pehlivan *et al.*, 2009; Serdaroglu *et al.*, 2009; Zheng *et al.*, 2009).

Statistical analyses

Statistical analyses of data were performed using the computer software SPSS for Windows (version 13.0; SPSS, Inc., Chicago, IL). The statistical significance of the differences between the patient and control groups was estimated by logistic regression analysis. Adjusted odds ratios were calculated with a logistic regression model that controlled for gender and age and are reported at 95% confidence intervals (CIs). Differences in MBL-2, IL4, ACE, CCR-5, IL-1RA, and TLR-4 allele frequencies between the control group and patients were compared using the chi-square test and when needed, the Fisher's exact test was used. For statistical comparison of groups, the median test was used. A p-value < 0.05 was considered statistically significant.

Results

Genotype frequencies for MBL-2, ACE, CCR-5, IL-1RA, IL-4, and TLR-4 genes found in healthy controls and patients with FN are shown in Table 1. No significant differences in the genotype distribution of CCR-5, IL-1RA, IL-4, TLR-4, and ACE GPs were observed between FN patients and healthy controls. The AB/BB genotype (53.3% and 8.3%, respectively) frequencies in MBL-2 (codon 54), which are important in susceptibility to infections, were found to be significantly high in the AL group compared with control groups (Table 1).

Gram-negative bloodstream infection due to FN was more common in the AG genotype in TLR-4 when compared to the AA genotype. Whereas there was no relationship between the presence of TLR-4 GP and the duration of neutropenic episodes, bloodstream infection, mortality, candidemia, and the presence of fungal pneumonia due to FN (Table 2).

There was no relationship between the presence of MBL-2 (codon 54) GP and the duration of febrile episodes, candidemia, gram-positive bloodstream infection, and the presence of fungal pneumonia due to FN. Whereas bloodstream infection, gram-negative bloodstream infection, and mortality due to FN were more common in the AB/BB genotype when compared with the AA genotype (Table 3).

There was no relationship between the presence of ACE GP and candidemia, gram-negative bacteremia, and the presence of fungal pneumonia due to FN. Whereas the duration of neutropenic episodes, the duration of febrile episodes, bloodstream infection, gram-positive bacteremia, and mortality due to FN were more common in the ID and/or DD genotype when compared with the II genotype (Table 4).

There was no relationship between the presence of CCR-5, IL-4, and IL-1RA GPs and the body distribution of infections, pathogens, the duration of neutropenia, and febrile episodes in AL patients (data not shown).

Discussion

Innate immunity is the earliest response to invading microbes and acts to contain the infection in the first minutes to hours of challenge. Innate immune mechanisms are

Table 1. Comparison of MBL-2, IL-4, ACE, CCR-5, IL-1RN, and TLR-4 Gene Polymorphism Frequencies Between Patients with Acute Leukemia and Control Subjects

	Genotype	*Acute leukemia* n = *60 (%)*	*Healthy control* n = *60 (%)*	*OR*	*95% CI*	p
MBL-2 (codon 54)	AA	28 (46.7)	55 (91.7)	0.080[a]	0.028–0.227[a]	**0.001**[a]
	AB	24 (40)	5 (8.3)	0.136[a]	0.048–0.390[a]	**0.001**[a]
	BB	8 (13.3)	– (0)	1.154[a]	1.045–1.274[a]	**0.006**[a]
	AB/BB	32 (53.3)	5 (8.3)	0.080[b]	0.028–0.227[b]	**0.001**[b]
CCR-5 (Δ32)	NN	50 (83.3)	55 (91.7)	0.455[a]	0.145–1.421[a]	0.269[a]
	ΔN	9 (15)	4 (6.7)	0.400[b]	0.115–1.393[b]	0.150[b]
	ΔΔ	1 (1.7)	1 (1.7)	0.867[b]	0.052–14.573[b]	0.921[b]
ACE (I)/(D)	DD	20 (33.3)	18 (30)	1.070[b]	0.327–3.500[b]	0.911[b]
	ID	30 (50)	33 (55)	1.271[b]	0.434–3.721[b]	0.662[b]
	II	10 (17.7)	9 (15)	0.882[a]	0.331–2.354[a]	1.000[a]
IL-4 (−590)	CC	35 (58.3)	33 (55)	1.227[a]	0.594–2.534[a]	0.712[a]
	TC	22 (36.7)	23 (38.3)	1.124[b]	0.519–2.432[b]	0.768[b]
	TT	3 (5)	4 (6.7)	1.411[b]	0.293–6.798[b]	0.668[b]
IL-1RN (VNTR in intron 2)	2/2	2 (3.4)	– (0)	1.034[a]	0.987–1.084[a]	0.496[a]
	2/3	13 (21.6)	19 (31.6)	0.597[a]	0.263–1.356[a]	0.302[a]
	2/5	1 (1.7)	1 (1.7)	1.000[a]	0.061–16.366[a]	1.000[a]
	3/3	34 (56.6)	35 (58.3)	0.934[a]	0.453–1.927[a]	1.000[a]
	3/4	1 (1.7)	1 (1.7)	1.000[a]	0.061–16.366[a]	1.000[a]
	3/5	8 (13.3)	4 (6.7)	2.154[a]	0.612–7.579[a]	0.362[a]
	4/4	1 (1.7)	– (0)	1.017[a]	0.984–1.051[a]	1.000[a]
TLR-4 (A896G)	AA	57 (95)	58 (96.6)			
	AG	3 (5)	2 (3.4)	1.638[b]	0.247–10.866[b]	0.609[b]
	GG	– (0)	– (0)			

The bold values are statistically significant.
[a]Fisher's exact test.
[b]OR (95% CI) was adjusted by age and sex.
ACE, angiotensin converting enzyme; CCR-5, chemokine receptor 5; CI, confidence interval; IL, interleukin; MBL-2, mannose-binding lectin 2; OR, odds ratio; TLR-4, Toll-like receptor 4.

Table 2. Comparison of TLR-4 (A896G) Genotypes with the Febrile Neutropenic Patients and Episodes

	All patients	*TLR-4 AA allele*	*TLR-4 AG allele*	p[a]
Number of patients	60	57	3	
Age*	42 (16–72)	42 (16–72)	21 (19–21)	0.312[#]
Sex (male/female)	29/31	28/29	1/2	1.000[&]
Diagnosis				
AML	47	45	2	
ALL	13	12	1	0.526[&]
Number of febrile neutropenic episodes	100	93	7	
Chemotherapy				
Ara-c/İda (7 + 3)/(6 + 3)	46/17	44/14	2/3	
FLAG-IDA	20	19	1	
GMALL phase I–II	6/11	6/10	0/1	0.887[&]
Duration of neutropenia* (< 500/μL)	16 (2–56)	17 (2–56)	12 (8–39)	1.000[#]
Duration of fever*	6 (1–30)	6 (1–30)	4 (1–14)	0.571[#]
Bloodstream infection	45 (45%)	40 (43%)	5 (71.4%)	0.238[&]
Gram positive	19 (19%)	19 (20.4%)	0 (0%)	0.341[&]
Coagulase-negative *Staphylococci*	3	3	–	
Staphylococcus aureus	11	11	–	
Enterococcus spp.	5	5	–	
Gram negative	27 (27%)	22 (23.7%)	5 (71.4%)	**0.015**[&]
Pseudomonas aeruginosa	4	3	1	
Klebsiella pneumonaie	6	4	2	
Escherichia coli	8	7	1	
Acinetobacter spp.	5	4	1	
Enterobacter spp.	2	2	-	
Stenotrophomonas maltophlia	2	2	-	
Polymicrobial infection	5 (5%)	4 (4.3%)	1 (14.3%)	0.310[&]
Candidemia	3 (3%)	2 (2.2%)	1 (14.3%)	0.197[&]
Proven/probable invasive aspergillosis	21 (21%)	20 (21.5%)	1 (14.3%)	1.000[&]
Mortality due to febrile neutropenia	19 (194%)	18 (19.4%)	1 (14.3%)	1.000[&]

The bold values are statistically significant.
[a]Statistics between AA genotype and AG genotype.
*Median.
[#]Median test.
[&]Fisher's exact test.
ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ara-c/İda, cytarabine, idarubicin; FLAG-IDA, fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin; GMALL, German multicenter study group for treatment of adult ALL (03/87 and 04/89), phase I, prednisolone, vincristine, daunorubicin, L-asparaginase, methotrexate; phase II, cyclophosphamide, cytarabine, 6-mercaptopurine, methotrexate.

important determinants of the prognosis for cancer patients undergoing chemotherapy. FN, resulting from chemotherapy, is an important cause of mortality and morbidity. There are some articles that support the role of different GPs in FN (Vekemens *et al.*, 2007). It is now nearly four decades since the relationship between the degree of neutropenia and the risk of bacterial and fungal infections was first recognized in patients treated for cancer (Bucaneve *et al.*, 2005; Vekemens *et al.*, 2007; Klostergaard *et al.*, 2010; Roongpoovapatr and Suankratay, 2010). Infections were noted to be worse during relapse of the underlying disease and the failure of leukocytes to recover following an infection has a very poor prognosis. It is now clear that this is largely determined by both the underlying disease and potency of chemotherapy. Interestingly, however, it is now also apparent that patients differ in their susceptibility to infection in the context of neutropenia. This indicates that other factors are operating to protect patients from infection in this immunocompromised state (Huges *et al.*, 2002; Dahmer *et al.*, 2005; Vaschetto and Protti, 2010).

Turner and Hamvas show that MBL-2 and the innate immune signaling triggered by the canonical PRRs, the TLRs, are linked by their spatial localization to the phagosome. These observations demonstrated a novel role for MBL-2 as a TLR coreceptor and establish a new paradigm for the role of opsonins, which we propose to function not only to increase microbial uptake but also to spatially coordinate, amplify, and synchronize innate immune defense mechanisms. This intracellular MBL is found localized with coat protein complex II (COPII) vesicles and the endoplasmic reticulum and has been suggested to play a role in protein quality control (Turner and Hamvas, 2000; Vekemens *et al.*, 2007; Frakking *et al.*, 2009; Ip *et al.*, 2009).

MBL-2 is a C-type serum lectin that plays a central role in the innate immune response. Clinical studies have shown that MBL-2 insufficiency is associated with bacterial infection in patients with neutropenia and meningococcal sepsis (Vardar *et al.*, 2007; Frakking *et al.*, 2009). Mullighan *et al.* (2002) stated that in patients having undergone allogeneic transplantation, invasive bacterial, viral, and fungal infection occurred more frequently among those with variant MBL-2 alleles (Bárdi *et al.*, 2005). In another study, in which 113 patients were treated with high-dose chemotherapy and autologous stem cell transplantation, MBL-2 deficiency was

Table 3. Comparison of MBL-2 (Codon 54) Genotypes with the Febrile Neutropenic Patients and Episodes

	MBL-2 AA[a] *allele*	*MBL-2 AB*[b] *allele*	*MBL-2 BB*[c] *allele*	p
Number of patients	28	24	8	
Age*	42 (17–72)	46 (16–69)	36 (18–56)	$0.275^{\#ab}$, $0.184^{\#ac}$
Sex (male/female)	13/15	13/11	5/3	$0.689^{\&}$
Diagnosis				
AML	22	19	7	
ALL	6	5	1	$0.969^{\&}$
Number of febrile neutropenic episodes	47	41	12	
Chemotherapy				
Ara-c/İda (7 + 3)/(6 + 3)	22/8	22/8	22/8	
FLAG-IDA	7	7	7	
GMALL phase I–II	3/7	3/7	3/7	$0.686^{\&}$
Duration of neutropenia* (< 500/μL)	17 (2–42)	15 (6–56)	17 (6–28)	$0.819^{\#ab}$, $0.702^{\#ac}$
Duration of fever*	5 (1–30)	7 (1–16)	6 (3–16)	$0.531^{\#ab}$, $0.425^{\#ac}$
Bloodstream infection	13 (27.7%)	24 (58.5%)	8 (66.7%)	**$0.001^{\&a/bc}$, $0.005^{\&ab}$, $0.018^{\&ac}$**
Gram positive	7 (14.9%)	9 (22%)	3 (25%)	$0.420^{\&ab}$, $0.409^{\&ac}$
Gram negative	8 (17%)	14 (34.1)	5 (41.7%)	**$0.043^{\&a/bc}$**, $0.085^{\&ab}$, $0.113^{\&ac}$
Polymicrobial infection	3 (6.4%)	3 (7.3%)	0 (0%)	$1.000^{\&ab}$, $1.000^{\&ac}$
Candidemia	–	3 (7.3%)	–	$0.097^{\&ab}$
Proven/probable invasive aspergillosis	7 (14.9%)	9 (22%)	5 (41.7%)	$0.420^{\&ab}$, $0.055^{\&ac}$
Mortality due to febrile neutropenia	4 (8.5%)	11 (26.8%)	4 (33.3%)	**$0.020^{\&a/bc}$, $0.044^{\&ab}$, $0.046^{\&ac}$**

The bold values are statistically significant.
[ab]Statistics between AA genotype and AB genotype.
[ac]Statistics between AA genotype and BB genotype.
[a/bc]Statistics between AA genotype and AB + BB genotype.
[#]Median test.
*Median.
[&]Fisher's exact test.

Table 4. Comparison of ACE Genotypes with the Febrile Neutropenic Patients and Episodes

	ACE II[a] *allele*	*ACE ID*[b] *allele*	*ACE DD*[c] *allele*	p
Number of patients	10	30	20	
Age*	28 (16–67)	42 (16–69)	48 (21–72)	$0.121^{\#ab}$, $0.249^{\#ac}$
Sex (male/female)	5/5	16/14	8/12	$0.648^{\&}$
Diagnosis				
AML	7	24	16	
ALL	3	6	4	$0.782^{\&}$
Number of febrile neutropenic episodes	19	48	33	
Chemotherapy				
Ara-c/İda (7 + 3)/(6 + 3)	7/4	24/5	15/8	
FLAG-IDA	3	12	5	
GMALL phase I–II	¼	3/4	2/3	$0.615^{\&}$
Duration of neutropenia* (< 500/μL)	13 (7–42)	18 (2–56)	16 (6–39)	**$0.029^{\#ab}$**, $0.463^{\#ac}$
Duration of fever*	3 (1–11)	7 (1–30)	6 (1–16)	**$0.020^{\#ab}$, $0.036^{\#ac}$**
Bloodstream infection	4 (21.1%)	22 (45.8%)	19 (57.6%)	$0.094^{\&ab}$, **$0.019^{\&ac}$**
Gram positive	0 (0%)	11 (22.9%)	8 (24.2%)	**$0.026^{\&ab}$, $0.021^{\&ac}$**
Gram negative	3 (15.8%)	13 (27.1%)	11 (33.3%)	$0.526^{\&ab}$, $0.209^{\&ac}$
Polymicrobial infection	0 (0%)	3 (6.3%)	2 (6.1%)	$0.533^{\&ab}$, $0.527^{\&ac}$
Candidemia	–	1 (2.1%)	2 (6.1%)	$1.000^{\&ab}$, $0.527^{\&ac}$
Proven/probable invasive aspergillosis	2 (10.5%)	11 (22.9%)	8 (24.2%)	$0.321^{\&ab}$, $0.293^{\&ac}$
Mortality due to febrile neutropenia	1 (5.3%)	7 (14.6%)	11 (33.3%)	$0.424^{\&ab}$, **$0.037^{\&ac}$**

The bold values are statistically significant.
[ab]Statistics between II genotype and ID genotype.
[ac]Statistics between II genotype and DD genotype.
[#]Median test.
*Median.
[&]Fisher's exact test.

also shown to significantly increase the risk of serious infection. Peterslund *et al.* (2001) described 54 adults with hematological malignancies. No differences were observed between the distribution of MBL-2 levels in the patient and control groups. However, in 16 patients, who developed either bacteremia, pneumonia, or both within 3 weeks of the commencement of chemotherapy, MBL-2 levels were significantly lower compared with the patients without serious infections. In our study, the AB/BB genotype (53.3%) frequency in MBL-2, which is important in susceptibility to infections, was found to be significantly high in the AL group compared with the control groups. Moreover, we also found out that having an AB/BB genotype increased the risk of susceptibility toward AML progression 12.5-fold (95% CI, 0.028–0.227) (Table 1). This is the first study in adults in which MBL-2 AB/BB genotype is found to be correlated with AML. Confirming these studies, we found that patients with bloodstream infections, gram-negative bloodstream infection, and mortality due to FN had the AB/BB genotype more often when compared with the AA genotype (Table 3) and that patients with bloodstream infections have MBL-2 polymorphisms when compared with the control group. It has been shown that MBL-2 deficiency was associated with a twofold increase in the duration of febrile episodes (Neth *et al.*, 2001; Garred *et al.*, 2003; Frakking *et al.*, 2009). However, correlation was determined between MBL-2 polymorphisms and the duration of febrile episodes in our study. Some studies found no differences between the frequency of infections and MBL-2 GPs (Frakking *et al.*, 2009; Klostergaard *et al.*, 2010). The second major part of our discussion is on the effect of MBL-2 variants on bacterial subclasses. Sutherland *et al.* (2005) stated that the cluster of differentiation (CD) 14 single-nucleotide polymorphisms was associated with gram-negative bacteria and TLR-2 with gram-positive bacteria, whereas MBL was not associated with a particular organism class. MBL-2 variants were also associated with increased prevalence of positive bacterial cultures, but not with a specific organism class on critically ill adults (Sutherland *et al.*, 2005). However, Shi *et al.* (2004) provided evidence that MBL-2 plays a key role in restricting the complications associated with *Staphylococcus aureus* infection in mice and that the MBL-2 gene might act as a disease susceptibility gene against staphylococcal infections in humans. Confirming this statement, in our study, single-nucleotide polymorphisms in MBL-2 are associated with increased prevalence of gram-negative bacterial cultures and bloodstream infections, but not with altered prevalence of fungal pneumonia or increased 28-day mortality on FN.

The discovery of genetic variations in the genes encoding for TLRs has highlighted a potential link between genomic variation of the host and susceptibility to infections (Nuolivirta *et al.*, 2009). Most studies, however, have focused on the highly polymorphic TLR-4 gene, which encodes the receptor recognizing bacterial LPS (Carvalho *et al.*, 2009). TLR-4 signaling is triggered by the interaction with LPS, the major cell wall component of gram-negative bacteria (Yoon *et al.*, 2006; Bochud *et al.*, 2008). Mammalian TLR-4 was first recognized as the transmembrane receptor for LPS, a key component for the detection of gram-negative bacteria (Bochud *et al.*, 2008; Roger *et al.*, 2009). TLR-4 has been reported to recognize not only the LPS component of gram-negative bacteria but also the mouse mammary tumor virus, and vesicular stomatitis virus proteins (Beutler, 2002). Apetoh *et al.* (2007) demonstrated that TLR-4 of dendritic cells play a key role in the antitumor response of the branches of the immune system, which is evoked following radiation and chemotherapy modalities. Webb *et al.* (2009) show that the TLR-4 expression in both leukemic groups was decreased compared with normal controls. As the levels of TLR-4 expression in both leukemic groups of this study were lower than that found in the normal controls, depressed resistance to the challenge of leukemic transformation could therefore quite possibly be associated with the lack of sufficient host TLR-4. The decreased expression of TLR-4 in leukemic samples observed in this study might indicate a novel functional role for this receptor, which has predominately been recognized for its recognition of microbial ligands, such that when altered allows or contributes to leukemic transformation and maintenance. If reduced TLR-4 expression indeed specifically factors into the pathogenesis of leukemia, it is possible that tailored treatment toward activating this receptor might be of therapeutic value (Webb *et al.*, 2009). In our study, there was no relationship between the presence of TLR-4 GP and the duration of neutropenic episodes, bloodstream infection, mortality, and the presence of fungal pneumonia due to FN. Gram-negative bloodstream infection due to FN was more common in the AG genotype when compared with the AA genotype (Table 2).

ACE is involved not only in intracellular volume regulation but also in proliferation control. ACE converts angiotensin I to angiotensin II (AT2) and inactivates bradykinin (Lee *et al.*, 2005). The I/D polymorphism of the human ACE gene, characterized by the presence (I) or absence (D) of a 287 bp fragment in intron 16, has been shown to modulate ACE activity both in the circulation and in the tissue. Subjects, homozygous for the deletion (DD), exhibit about two times higher plasma ACE activity than homozygotes for the insertion (II) (Hajek *et al.*, 2003) (ACE3). In our study, there is a relationship between the presence of ACE GP and the duration of neutropenic episodes, bloodstream infection, mortality, and gram-positive bacteremia due to FN (Table 4).

In patients, who carry variant MBL-2, ACE, and TLR-4 genotypes with FN, it can be useful to arrange empiric antibiotic treatment. To elucidate the role of MBL-2, ACE, and TLR-4 genes in gram-positive and/or -negative bacteremia in FN, there needs to be further clinical research with larger patient populations.

Author Disclosure Statement

The authors declare that they have no conflicts of interest.

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Address correspondence to:
Sacide Pehlivan, MSc, PhD
Department of Medical Biology
Istanbul Faculty of Medicine
Istanbul University
Capa–Fatih
Istanbul 34080
Turkey

E-mail: sacide.pehlivan@istanbul.edu.tr; psacide@hotmail.com