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Original article

The association of HLA-DRB1 alleles and MBL2 gene variant in pediatric acute lymphoblastic leukemia patients

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ABSTRACT

Introduction: Epidemiologic studies on pediatric acute lymphoblastic leukemias (ALL) have been conducted to evaluate the possible risk factors including genetic, infectious and environmental factors with the objective of idenfying the etiology. Mannose-binding lectin 2 (MBL2) plays an important role in first-line immune defense. HLA DRB1 alleles play a role in presentation of peptides to T cells and in activation of the adaptive immune response.

Objective: In our study, we aimed to investigate both the MBL2 gene variant and HLA-DRB1 alleles in pediatric ALL patients.

Materials: In this study, 86 high-risk ALL patients and 100 controls were included. Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (PCR-RFLP) and PCR-sequence specific primer (SSP) methods were used for detection of polymorphism of the MBL2 and HLA-DRB1 alleles, respectively.

Results: The frequency of the MBL2 AB genotype was lower in female ALL patients, compared to male ALL patients (p = 0.034). An association was found between the MBL2 BB genotype and DRB1*07 and among patients with the MBL2 BB genotype; those who also carried the DRB1*07 and *04 alleles were significantly higher than those without the DRB1*07 and *04 alleles. (p = 0.048, p = 0.022, respectively).

Conclusion: This is the first study suggesting that the MBL2 BB genotype in association with the DRB1*07 or co-inheritance of the HLA-DRB1*04 and HLA DRB1*07 may have an impact on the etiopathogenesis of the disease.

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Introduction

Acute lymphoblastic leukemia (ALL) accounts for 75% of childhood leukemias and 25% of childhood cancers. It has been reported that preleukemic cells can usually be detected at birth in pediatric ALL. Epidemiological studies suggest that it may occur in patients with ALL as a result of an abnormal interaction between the immune system and unspecified infections.[1-5] The relationship between "human leucocyte antigen (HLA) class II alleles" and pediatric leukemia has been demonstrated in different studies. This may be due to the role of the HLA allele-restricted peptide binding and T cell activation.[6] The mannose-binding lectin (MBL) is involved in the lectin pathway, which is one of the pathways in the complement system. The MBL contributes to the innate immunity through opsonization and complement activation.[7,8] In humans, the MBL gene is named MBL2, is located on chromosome 10q21-23 and contains 6321 base pairs, 4 exons and 3 introns.[9-12] The normal "wild type" MBL2 gene is defined as allele A. There are 3 single nucleotide polymorphisms (SNPs) all identified for the MBL2 gene.[12] The mutant alleles B, C and D alleles are linked to the SNP in the first exon of the MBL2 gene. The codons 54, 57 and 52 interfere with the assembly of the protein, causing decreased functional circulating MBL. In addition, the −221 X/Y promoter SNP affects the protein expression, with the −221Y variant being associated with high MBL levels in the serum.[13] The functional gene variant MBL2-rs1800450 has been shown to be associated with MBL2 activity.[14] Genetically determined MBL2 insufficiency is associated with increased susceptibility to both bacterial and viral infections. In individuals who are heterozygous for these alleles, the amount of protein is reduced by 10-fold, whereas the protein cannot be detected at all in individuals who are homozygous or combined heterozygous.[14,15]

The aim of this study was to analyze the association between the MBL2 genotype and the HLA-DRB1 alleles in ALL patients who may be candidates for allogeneic hematopoietic stem cell transplantation (HSCT), and to investigate the possible role of the MBL2 genotype in the gaft versus host Disease (GvHD).

Materials and methods

Patients

This retrospective association study included 86 high-risk pediatric ALL patients and 100 healthy controls who had consecutively applied to the Istanbul Medical Faculty, Pediatric Hematology Unit and Yeni Yuzyıl Medical Faculty, Pediatric Hematology Department. As part of routine practice, leukemia patients were classified as 'high-risk' at the time of diagnosis according to the Berlin Frankfurt-Munster (BFM) study group risk factors and were HLA-typed in preparation for possible bone marrow transplantation.[16] Twenty transplant patients were analyzed for MBL2 genotypes after allogeneic HSCT.

Molecular method

The genomic DNA was isolated from the peripheral blood using the Thermo Fisher PureLink Genomic DNA Mini Kit™isolation kit (Thermo Fisher Scientific, MA, USA). The DNA concentration was measured using a Thermo Fisher Nanodrop Spectrophotometer.

Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RLFP)

The SNPs for the MBL2 gene (rs:1800450) were determined by the PCR-RLFP.[15] We analyzed the codon 54 A/B (gly54asp: rs rs1800450) variation in exon 1 of the MBL2 gene. In the genotyping of the MBL2 gene (gly54asp-rs1800450), the polymerase chain reaction (PCR) was performed using forward (5′TAGGACAGAGGGCATGCTC-3′) and reverse (5′-CAGG-CAGTTTCCTCTGGAAGG-3′) primers. The product's 349 base pairs (bp) were digested with the restriction enzyme *Ban*I (Fermentase) to identify the codon 54 polymorphism. The *Ban*I digestion was performed at 37°C for overnight incubation. After enzyme digestion, products were visualized by electrophoresis in 3% agarose gel. The BanI restriction site is present on the wild type allele A (269 and 89 bp) and absent on the variant allele B (349 bp).[15]

All patients and controls were typed by the sequence specific primer (PCR-SSP) typing method. All patients and controls were typed at the Tissue Typing Laboratory, Istanbul Faculty of Medicine, which is accredited to perform clinical tissue typing by the European Federation of Immunogenetics (EFI).

Polymerase chain reaction-sequence specific primer (PCR-SSP)

The HLA-DRB1 typing was performed using the PCR-SSP (Olerup SSP AB).

Statistical analysis

The data analysis was performed using the Statistical Package for the Social Sciences software version 21 (SPSS, Chicago, IL, USA). The patients and controls complied with the ''Hardy-Weinberg equilibrium'' ($p > 0.05$). The allele and genotype frequencies for MBL2 polymorphisms were calculated by direct counting and the associations of alleles and genotypes were compared with the chi-square test (χ^2) and Fisher's exact test using the SPSS 21. The Bonferroni correction test was analysed as well. The p-values of <0.05 were considered statistically significant.

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Results

Demographics and clinical characteristics

Eighty-six patients (62 males/24 females), aged 2–17 years, and 100 healthy controls (36 males/64 females), aged 20–35 years, were included in this study. Twenty of the 86 patients were transplanted (23%) and 8 of these patients developed GvHD (40%).

In the pediatric ALL patient group, the frequency of DRB1*04 and *07 alleles (34.3% and 16.9%, respectively) was significantly higher, compared to the healthy control group (18.0% and 9.5%, respectively) ($p < 0.0001$ and $p = 0.043$, respectively). The frequencies of the DRB1*03 and DRB1*11 alleles (11.0% and 21.5%, respectively) were significantly lower in the pediatric ALL patients, compared to the control group ($p = 0.018$ and $p = 0.008$, respectively) (Table 1).

The frequencies of alleles/genotypes of the MBL2 variants in patients with pediatric ALL and healthy controls are shown in Table 2. The AA (73.3%), AB (18.6%) and BB (8.1%) genotypes were detected in the patients. The genotypic/allelic frequencies of MBL2 variants in the pediatric ALL patients, who had heterozygous and homozygous DRB1 alleles, and in healthy controls, are shown in Table 3. A significant difference was not found between pediatric ALL patients and healthy controls in terms of HLA-DRB1 alleles (Tables 2 and 3).

In pediatric ALL patients, we showed that the relationship between the MBL2 BB genotype and the DRB1*07 allele was found to be significantly frequent in those without the DRB1*07 ($p = 0.048$; OR = 3.933). In addition, the relationship between the MBL2 BB genotype and presence of the DRB1*07 and DRB1*04 was found to be more significantly frequent, compared to those without the DRB1*07 ($p = 0.022$; OR = 9.125) (Table 4).

Moreover, the association between the MBL genotypes and the HLA-DRB1*04/*07/*09 alleles separately, and with the individuals having together the HLA-DRB1*04/*07 and the DRB1*04/*09 alleles was investigated, however, no statistical significance was detected (Table 5).

The GvHD developed in 8 (40%) of 20 transplant patients. Among these patients, the frequency of the AA genotype was 50% in GvHD-negative patients. In patients with GVHD, the frequency of the AB genotype was 50%, the frequency of the AB genotype was 50% and the frequency of the BB genotype was 8.3% (Table 6).

Discussion

The mannose-binding lectin deficiency has been associated with some infectious and autoimmune diseases. The fact that the MBL could affect infectious and autoimmune processes was the starting point for clinical research.[7,8]

In the fetal immune system, the MBL2 recognizes carbohydrates in bacteria, viruses, fungi or parasites and, when bound to them, activates the MBL2 lectin pathway in the complement system.[17-19] The results related to the impact of SNPs in the MBL2 gene in hematological malignancies are controversial.[20] According to the hygiene hypothesis related to pediatric ALL, a lack of infection in the infant may lead to failure of normal immune system modulation, which may cause susceptibility to leukemogenic bacterial or viral infections in individuals.[10,20]

The MBL2 gene is an important component of the innate immune system with 4 main functions, including the activation of the complement, direct promotion of opsono-phagocytosis, modulation of the inflammatory response and promotion of apoptosis.[21] There are also other promoter variants that may affect gene expression.[22,23]

Zerhnder et al. showed that the MBL2 serum level was not associated with the overall survival or event-free survival in a retrospective multi-center study on 372 pediatric hematologic malignancies.[24] High levels of MBL-associated serine protease (MASP)-2, however, were associated with better event-free

Table 1 – HLA-DRB1 and HLA-DRB3/4/5 frequencies (%) in pediatric ALL patients and age-matched healthy controls.

HLA-DRB1	ALL (2*n*:172) %	Healthy controls (2*n*:200) %	*p*-value	OR (95% CI)
DRB1*01	(6) 3.5 %	(13) 6.5%	0.239	0.519 (0.193–1.399)
DRB1*03	(7) 4.1%	(22) 11.0%	**0.018**[a]	**0.343 (0.142–0.824)**
DRB1*04	(59) 34.3%	(36) 18.0%	**<0.0001**[a]	**2.379 (1.473–3.840)**
DRB1*07	(29) 16.9%	(19) 9.5%	**0.043**[a]	**1.932 (1.041–3.587)**
DRB1*08	(0) 0.0%	(3) 1.5%	0.252	0.163 (0.008–3.191)
DRB1*09	(3) 1.7%	(1) 0.5%	0.339	3.533 (0.363–34.259)
DRB1*10	(1) 0.6%	(6) 3.0%	0.128	0.189 (0.022–1.587)
DRB1*11	(19) 11.0%	(43) 21.5%	**0.008**[a]	**0.453 (0.252–0.813)**
DRB1*12	(0) 0.0%	(3) 1.5%	0.252	0.163 (0.008–3.191)
DRB1*13	(13) 7.6%	(22) 11.0%	0.288	0.661 (0.322–1.357)
DRB1*14	(7) 4.1%	(14) 7.0%	0.264	0.536 (0.222–1.431)
DRB1*15	(16) 9.3%	(9) 4.5%	0.095	2.177 (0.936–5.061)
DRB1*16	(12) 6.9%	(9) 4.5%	0.369	1.592 (0.653–3.875)
	100%	**100%**		

Abbreviations: ALL: acute lymphoblastic leukemia; OR: odds ratio; CI: confidence interval; HLA: human leukocyte antigen

[a] Statistically significant values ($p < 0.05$) are indicated in bold.

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Table 2 – Comparison of genotype and allele frequencies of MBL2 gene variants between pediatric ALL patients and healthy controls.

MBL2	ALL	Healthy controls	OR	95% CI	*p*-value
Genotype	*n*: 86 (%)[a]	*n*: 100 (%)[b]			
AA	63 (73.3%)	76 (76.0%)	0.865	0.446–1.678	0.735
AB	16 (18.6%)	16 (16.0%)	1.200	0.559–2.572	0.698
BB	7 (8.1%)	8 (8.0%)	1.019	1.353–2.936	0.999
Allele					
A	142 (82.6%)	168 (84.0%)	1.127	0.667–1.902	0.691
B	30 (17.4%)	40 (16.0%)			
HWEp	0.000	0.129			

Abbreviations: ALL: acute lymphoblastic leukemia; MBL2: mannose-binding lectin 2; HWEp: Hardy–Weinberg equilibrium; OR: odds ratio; CI: confidence interval; HLA: human leukocyte antigen.
[a]*n* = 86, [b]*n* = 100, *OR (95% CI) was adjusted by age, [&]Fisher's exact test.

Table 3 – Comparison of MBL2 gene variants between healthy controls and pediatric ALL patients with heterozygous and homozygous HLA-DRB1 alleles.

MBL2	HLA-DRB1 alleles (heterozygosity) (ALL)	HLA-DRB1 alleles (heterozygosity) (healthy controls)	OR	95%CI	*p*-value
Genotype	n: 57[a]	n: 86[b]			
AA	41 (71.9%)	66 (76.7%)	0.776	0.361–1.668	0.558
AB	11 (19.3%)	12 (14.0%)	1.475	0.601–3.617	0.486
BB	5 (8.8%)	8 (9.3%)	0.937	0.290–3.025	1.000
Allele					
A	93 (81.6%)	144 (83.7%)	0.861	0.461–1.606	0.634
B	21 (18.4%)	28 (16.3%)			
HWEp	0.000	0.129			

[a]n= 57, [b]n= 86 *: OR (95%CI) was adjusted by age, [&]Fisher's Exact Test.

MBL2	HLA-DRB1 alleles (homozygosity) (ALL)	HLA-DRB1 alleles (homozygosity) (Healthy controls)	OR	95%CI	*p*-value
Genotype	n: 29[c]	n: 14[d]			
AA	22 (75.9%)	10 (71.4%)	1.257	0.298–5.297	1.000
AB	5 (17.2%)	4 (28.6%)	0.520	0.115–2.353	0.441
BB	2 (6.9%)	0 (%0.0)	2.636	0.118–58.700	1000
Allele					
A	49 (84.5%)	24 (85.7%)	0.907	0.253–3.248	1.000
B	9 (15.5%)	4 (14.3%)			
HWEp	0.247	0.129			

Abbreviations: ALL: acute lymphoblastic leukemia; MBL2: mannose-binding lectin 2; HWEp: Hardy–Weinberg equilibrium; OR: odds ratio; CI: confidence interval.
[c]n= 29, [d]n= 14*:OR (95%CI) was adjusted by age, [&]Fisher's Exact Test.

survival in this study.[24] Frakking et al. showed that MBL plasma levels increased during febrile neutropenia in "wild-type" MBL2 individuals in the first pediatric oncology cohort study.[25] Allogeneic stem cell transplantation (allo-SCT) is being increasingly used worldwide for treating hematologic and nonhematologic diseases.[26] The prolongation of the state of neutropenia, the presence of GvHD and steroid and/or other immunosuppressant treatments have been identified as important risk factors for the development of invasive fungal infection following allo-SCT.[27] Granell et al. used 3 groups in their genotyping study for the SNPs reported in the promoter and exon 1 of the MBL2 gene. Of these groups, 102 were allo-SCT recipients, 106 were HLA-identical sibling donors, and 104 were healthy volunteer blood donors. There was no significant difference in the prevalence of MBL2 SNP in these three groups.[28] Low serum MBL levels, related to promoter polymorphism and structural variants, have been associated with an increased risk of infection.[29] Puente et al. showed that MBL genotypes of recipients and donors did not influence the severity of the acute graft versus host disease (aGVHD) or

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Table 4 – Comparison of frequencies of MBL2 gene variants and HLA-DRB1*04, DRB1*07 and DRB1*09 alleles in pediatric ALL patients.

	Genotype	HLA-DRB1*04 (+) (n:43)	HLA-DRB1*04 (-) (n:43)	OR	95%CI	*p*-value
MBL2 (n:86)	AA (n:63) AB (n:16) BB (n:7)	33 (76.8%) 5 (11.6%) 5 (11.6%)	30 (69.8%) 11 (25.6%) 2 (4.6%)	1.430 0.382 2.697	0.546–3.740 0.120–1.218 0.493–14.745	0.626 0.164 0.433
		HLA-DRB1*07 (+) (n:24)	HLA-DRB1*07 (-) (n:62)	OR	95 %CI	*p*-value
MBL2 (n:86)	AA (n:63) AB (n:16) BB (n:7)	15 (62.5%) 5 (20.8%) 4 (16.7%)	48 (77.4%) 11 (17.7%) 3 (4.8%)	0.486 1.220 3.933	0.176–1.346 0.324–3.976 0.809–19.114	0.181 0.762 **0.048**[a]
		HLA-DRB1*09 (+) (n:3)	HLA-DRB1*09 (-) (n:83)	OR	95%CI	*p*-value
MBL2 (n:86)	AA (n:63) AB (n:16) BB (n:7)	3 (100%) 0(0.0%) 0(0.0%)	60 (72.3%) 16 (19.3%) 7 (8.4%)	2.719 0.584 1.457	0.135–54.721 0.028–11.885 0.068–30.996	0.561 0.999 0.999
		HLA-DRB1*04+07 (+) (n:9)	HLA-DRB1*04+07 (-) (n:77)	OR	95%CI	*p*-value
MBL2 (n:86)	AA (n:63) AB (n:16) BB (n:7)	4 (44.4%) 2 (22.3%) 3 (33.3%)	59 (76.6%) 14 (18.2%) 4 (5.2%)	0.244 1.286 9.125	0.059–1.007 0.240–6.865 1.645–50.615	0.053 0.671 **0.022**[a]
		HLA-DRB1*04+09 (+) (n:1)	HLA-DRB1*04+09 (-) (n:85)	OR	95%CI	*p*-value
MBL2 (n:86)	AA (n:63) AB (n:16) BB (n:7)	1 (100%) 0(0.0%) 0(0.0%)	62 (73.0%) 16 (18.8%) 7 (8.2%)	0.792 1.404 3.489	0.030–20.366 0.054–36.070 1.130–93.457	1.000 1.000 1.000

Abbreviations: ALL: acute lymphoblastic leukemia; MBL2: mannose-binding lectin 2; HWEp: Hardy–Weinberg equilibrium; OR: odds ratio; CI: confidence interval; HLA: human leukocyte antigen.

[a] Statistically significant values ($p < 0.05$) are indicated in bold.

total respiratory morbidity (TRM) and stated that low pre-transplant MBL levels, but not the MBL2 genotype, may predispose patients undergoing Allo-HSCT to increased risk of viral infection.[30]

In our study, we found that no significant difference was observed in terms of genotype or allele frequencies, when we compared MBL2 genotypes between ALL patients and healthy controls. The MBL-deficient genotypes were associated with another type of leukemia in children in Schmiegielow's study. Insufficient levels of MBL2 may, therefore, be involved in the development of both acute myoblastic leukemias (AML and ALL), in spite of differences in their etiopathogenesis.[31]

The importance of individual non-HLA encoded genetic variability in the development of infections has recently been recognized.[32] Thus, polymorphisms of several genes, namely myeloperoxidase and MBL, have been associated with a higher incidence of infections.[33] Von Fliedner et al. reported that the HLA-DRB1 alleles are associated with familial inheritance factors for acute malignant diseases of childhood.[34] The significant increase in the frequency of the HLA-DRB1*04 allele, both in the overall ALL and male ALL populations, and the significantly lower frequency of the HLA-DRB1*13 allele in female patients, compared with female controls found in the study conducted by Ozdilli et al., are consistent with results reported by others.[4,35,36]

In this study, the association of the MBL2 BB genotype and the HLA-DRB1*07 was found to be high and significant, compared to those without the HLA-DRB1*07 in the patient group. At the same time, the association of the MBL2 BB genotype and the co-inheritance of the HLA-DRB1*04 and HLA DRB1*07 was found to be high and significant, compared to those without the HLA-DRB1*07. No association between the MBL genotypes and the HLA-DRB1 allele was detected in healthy controls in the study. Although there is some evidence indicating that there is a relationship between HLA class II alleles and ALL, there are few studies to support the link between the common polymorphisms in the immune system.[33] The SNPs of the MBL2 gene reduce the amount of MBL2 circulating in the body, resulting in changes in the immune response. Briefly, the MBL2 has an important role in different types of inflammatory diseases and cancer.[37]

It is known that fetal MBL produced by the fetal liver may play a role in the defense against pathogens and/or in the modulation of immune response during pregnancy. Changes in the MBL serum concentration can affect susceptibility to different types of infections and their course. Individuals carrying variant genotypes may be predisposed to recurrent infections, so we can speculate that the risk of ALL might have increased in individuals having the MBL2 BB genotype together with the HLA-DRB1*04 and

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Table 5 – Comparison of frequencies of MBL2 gene variants and HLA-DRB1*04, DRB1*07 and DRB1*09 alleles in healthy controls.

	Genotype	HLA-DRB1*04 (+) (n:36)	HLA-DRB1*04 (-) (n:64)	OR	95%CI	*p*-value
MBL2 (n:100)	AA (n:76)	24 (66.6%)	52 (81.3%)	1.279	0.597–2.374	0.162
	AB (n:16)	6 (16.7%)	10 (15.6%)	0.246	0.187–1.109	0.882
	BB (n:8)	6 (16.7%)	2 (3.1%)	1.983	0.437–12.049	0.054
		HLA-DRB1*07 (+) (n:18)	HLA-DRB1*07 (-) (n:62)	OR	95%CI	*p*-value
MBL2 (n:100)	AA (n:76)	11 (61.1%)	65 (79.3%)	0.675	0.201–1.317	0.102
	AB (n:16)	4 (22.2%)	12 (14.6%)	0.947	0.337–3.467	0.426
	BB (n:8)	3 (16.7%)	5 (6.1%)	1.794	0.794–16.138	0.139
		HLA-DRB1*09 (+) (n:1)	HLA-DRB1*09 (-) (n:99)	OR	95%CI	*p*-value
MBL2 (n:100)	AA (n:76)	1 (100%)	75 (75.8%)	0.347	0.124–9.227	0.647
	AB (n:16)	0 (0.0%)	16 (16.2%)	0.276	0.089–13.107	1.000
	BB (n:8)	0 (0.0%)	8 (8.1%)	0.976	0.068–30.996	1.000
		HLA-DRB1*04+07 (+) (n:3)	HLA-DRB1*04+07 (-) (n:97)	OR	95%CI	*p*-value
MBL2 (n:100)	AA (n:76)	2 (66.7%)	74 (76.3%)	0.376	0.157–2.438	0.700
	AB (n:16)	1 (33.3%)	15 (15.5%)	1.108	0.349–4.334	0.405
	BB (n:8)	0 (0.0%)	8 (8.2%)	2.317	1.846–22.467	0.069
		HLA-DRB1*04+09 (+) (n:0)	HLA-DRB1*04+09 (-) (n:100)	OR	95%CI	*p*-value
MBL2 (n:100)	AA (n:76)	0 (0.0%)	76 (100.0%)	–	–	–
	AB (n:16)	0 (0.0%)	16 (100.0%)	–	–	–
	BB (n:8)	0 (0.0%)	8 (100.0%)	–	–	–

Abbreviations: ALL: acute lymphoblastic leukemia; MBL2: mannose-binding lectin 2; HWEp: Hardy–Weinberg equilibrium; OR: odds ratio; CI: confidence interval; HLA: human leukocyte antigen.

Table 6 – The association of MBL2 and GvHD.

MBL2 (rs:1800450)	With GvHD (n:8) 40%	Without GvHD (n:12) 60%	*p*-value
Genotype			
AA	(4) 50.0%	(6) 50.0%	0.648
AB	(4) 50.0%	(5) 41.7%	0.926
BB	(0) 0.0%	(1) 8.3%	0.337

Abbreviations: MBL2: mannose-binding lectin 2; GvHD: graft versus host disease.

HLA-DRB1*07 or those having the MBL2 BB genotype with the HLA-DRB1*07.

Conclusion

The association between the MBL2 (rs:1800450) BB genotype and the DRB1*07 or co-inheritance of the HLA-DRB1*04 and HLA DRB1*07 was demonstrated for the first time in pediatric patients with ALL in this study. Further analysis in multicenter studies is needed to confirm the association of the MBL2 and the HLA-DRB1 as a potential biomarker of a more individualized risk for bacterial infections in children with leukemia.

Author contributions

All authors designed the study. The material preparation and data collection were performed by Rustu Oguz, Yeliz Ogret, Muge Gökce, Serap Kahraman, Zeynep Karakas, analysis of data was performed by Hayriye Senturk Ciftci, Sedat Karadeniz, literature search was performed by Rustu Oguz, Kursat Ozdilli and the first draft of the manuscript was written by Rustu Oguz, Hayriye Senturk Ciftci, Sacide Pehlivan and critically reviewed and edited by Rustu Oguz. All authors read and approved the final manuscript.

Ethics committee approval

This study was supported by the Clinical Research Ethics Committee of Istanbul University (13.11.2020, no.: 28). This

study was approved by the ethical review boards of the Istanbul University, Istanbul, Turkey and conducted in accordance with the standards of the Declaration of Helsinki.

Informed consent

Informed consent form was not obtained because the study was retrospective.

Data availability statement

Data supporting the findings of this study are available upon request from the corresponding author (Rustu Oguz).

Conflicts of interest

The authors declare that there is no conflict of interest for the study.

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