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Mannose Binding Lectin and Ficolin-2 Polymorphisms are Associated With Increased Risk for Bacterial Infections in Children With B Acute Lymphoblastic Leukemia

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Background. We aimed to investigate whether the presence of mannose binding lectin (MBL2), ficolin 2 (FCN2) polymorphisms or the combined deficiency significantly influence the risk and subsequently the frequency of chemotherapy-induced bacterial infections in children with B acute lymphoblastic leukemia (B-ALL). ***Procedure.*** MBL2 polymorphisms for exon 1 and FCN2 polymorphisms for promoter regions −986, −602, −557, −64, −4 and exon 8 regions +6,359, +6,424 were determined in children with B-ALL. FCN2 haplotype was determined by gene sequencing. Number and duration of FN episodes as well as number of bacterial infections were recorded during induction chemotherapy. ***Results.*** Forty-four children with B-ALL (median age 4.3 years, 65.9% males) suffered from 142 FN episodes and 92 bacterial infections (40.2% Gram positive and 59.8% Gram negative). MBL2 low-risk genotype was found in 59.1%, medium-risk in 31.8% and high-risk in 9%. FCN2 low-risk haplotypes were detected in 38.2%, medium-risk in 44.1% and high-risk in 17.6%. MBL2 genotype and FCN2 haplotype were not associated with increased frequency of FN episodes. MBL2 medium/high-risk genotype and FCN2 medium/high-risk haplotype were associated with prolonged duration of FN ($P = 0.007$ and $P = 0.001$, respectively) and increased number of bacterial infections ($P = 0.001$ and $P = 0.002$, respectively). The combined MBL2/FCN2 medium/high-risk genotype was associated with an increased number of bacterial infections ($P = 0.001$). ***Conclusions.*** MBL2 and FCN2 single or combined deficiencies are associated with increased duration of FN episodes as well as increased number of bacterial infections in children with B-ALL suggesting a prognostic role of these genes. Pediatr Blood Cancer 2014;61:1017–1022.

Key words: B-ALL ;bacterial infections; ficolin L gene polymorphisms; immunosuppression; MBL polymorphisms; pattern recognition receptors

INTRODUCTION

Bacterial infections are major complications of antineoplastic therapy resulting in excessive morbidity and mortality in pediatric hematology–oncology patients. A significant variability has been reported in the frequency and severity of infections among patients with the same predisposing factors (underlying malignancy, chemotherapeutic regimen, age) implying an individualized pattern of susceptibility to infections [1]. Furthermore, while about 70% of children with acute lymphoblastic leukemia (ALL) are at high risk for severe bacterial infections, the remaining patients are at low risk and can be treated orally with continuous monitoring at an outpatient setting [2–5]. Thus, current research focuses on detecting genetic markers with prognostic value that may contribute to an adoption of a more personalized risk-adjusted antimicrobial therapy [1].

Both mannose-binding lectin (MBL) and ficolin are important humoral components of the innate immune system and therefore MBL2 and FCN2 genes have been proposed as significant genetic determinants in regulating the immune response [6–8]. Polymorphisms of these genes affect either the plasma levels of the lectins encoded or the binding affinity to their substrates [9]. Genetic polymorphisms of MBL2 and FCN2 are commonly detected in several populations; however, their true impact on the susceptibility to infections in immunocompetent individuals remains insignificant, since alternative defense/immunosurveillance mechanisms compensate this common immunologic disorder [1,10,11].

Hence, the prognostic role of MBL2 and FCN2 polymorphisms on the risk of immunocompromised patients for bacterial infections is still questionable [12]. While the chemotherapy period is a unique situation for studying the role of lectins in the context of an iatrogenic immunodeficiency, results of previous studies have been rather controversial [13–19]. The difficulty of comparing these results has been attributed to the heterogeneity and different degree of immunosuppression of the patients enrolled. In contrast, current reports suggest that lectins may play a role on the susceptibility to infections in immunosuppressive conditions with sufficient absolute neutrophil count (ANC) and opsonophagocytosis of microbes. Under intensive chemotherapy, however, prolonged severe

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neutropenia without opsonophagocytic activity may significantly affect the clinical role of lectins [20].

Our research question was to elucidate whether the presence of mannose binding lectin (MBL2), ficolin 2 (FCN2) polymorphisms or the combined deficiency significantly influence the risk and subsequently the frequency of chemotherapy induced bacterial infections in children with B acute lymphoblastic leukemia (B-ALL).

METHODS

Setting

Children with newly diagnosed B-ALL admitted to the Pediatric Hematology–Oncology Unit of the 2nd Pediatric Department during 2005–2010 were enrolled. For the purpose of sample homogeneity, patients suffering from the same leukemia type and treated with the same chemotherapy protocol ALLIC-BFM were selected. Their follow-up extended from the beginning of induction until maintenance chemotherapy. The study was approved by the Ethics Committee and written consent was given by the parents/guardians. Gender, age, B-ALL type, and BFM stratification were recorded.

Febrile Neutropenic Episodes

For each patient, the number of FN episodes, duration of the first FN episode and total duration of FN during follow-up period were recorded. Furthermore, the rate of febrile episodes per 30 days of neutropenia was calculated. As we wanted to evaluate the role of MBL2 and FCN2 under mild neutropenia, a FN episode was defined as fever with a single axillary temperature of $\geq$38.5°C for >2 hours, or two episodes of >38°C within 12 hours or a single episode >39°C with ANC $<1 \times 10^9$/L. Fever that occurred following transfusion of blood and blood products was not considered. Prolonged FN was defined as fever for >10 days until recovery of ANC ($\geq 1 \times 10^9$/L).

Bacterial Infections

A microbiologically documented bacterial infection was defined if one or both of the following criteria were met: (1) occurrence of bacteremia ($\geq$1 blood cultures positive for any organism except for coagulase-negative staphylococci, for which $\geq$2 positive blood cultures were required); or (2) a positive culture obtained from another sterile site, such as urine. Data collected were: number of microbiologically documented bacterial infections for each patient, identification of bacteria and antimicrobial susceptibility. A strain was defined as multiresistant if it was phenotypically resistant to >3 different classes of antimicrobials. A child was considered to have an increased number of infections if $\geq$2 infections occurred during follow-up. The rate of bacterial infections per 30 days of neutropenia was calculated.

MBL2 Genotype

Polymorphisms of exon 1 of MBL2 gene, referred as allele B for codon 54, C for codon 57, and A for the wild type, were evaluated. From each patient, two 2-ml blood samples were collected in ethylene–diamine–tetracetic acid tubes. DNA was extracted according to the protocol of QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). Exon 1 of MBL gene was amplified by PCR. The primer's sequences were Eo3 (forward primer): 5′-TAGGACAGAGGGCATGCTC-3′ and Eo4 (reverse primer) 5′-CAGGCAGTTTCCTCTGGAAGG-3′, respectively. PCR was performed in a final volume of 50 μl using 1.5 mM $MgCl_2$, 2 mM for each deoxyribonucleotide triphosphate (dNTP), and 16 U/μl Ampli Taq DNA polymerase (TaKaRa Biotechnology; Dalian Co., Ltd, Shiga, Japan). The following cycling conditions were used: 5 minutes at 94°C and 35 two-step cycles consisting of 60 seconds of denaturation at 56°C, followed by annealing at 72°C for 1 minutes and elongation at 72°C for 5 minutes. The 349 bp PCR product was digested with *Ban*I and *Mbo*II for codon 54 and 57, respectively. *Ban*I digestion was performed at 50°C for 60 minutes with five units of the enzyme and *Mbo*II digestion was performed at 37°C for 90 minutes with 3.5 units of the enzyme. The normal allele (allele A) was cut into two fragments with *Ban*I, 260 and 89 bp. The variant allele (allele B) remained uncut. *Mbo*II cleaved the variant allele (allele C) into 270 and 79 bp fragments. The fragments were visualized by electrophoresis on 2% (w/v) agarose gel with EtBr 0.5 μg/ml under UV light. Three MBL2 genetic subgroups were defined: group I (low risk genotype) including A/A MBL patients (homozygous for the wild type), group II (medium risk genotype) including A/O (heterozygous for the mutant allele B or C), and group III (high risk genotype) including O/O (homozygous for the mutant alleles B, C).

FCN2 Haplotype

Two novel polymorphisms in exon 8 (+6,359, +6,424) and three in the promoter region (−987, −602, −4) of FCN2 gene were evaluated. DNA extraction was performed using QIAampTM DNA extraction kits following the manufacturer's instructions (QIAGEN). For each site two primers were used (Supplemental Table SI). For each reaction, 100 ng of genomic DNA was amplified in a 20 μl total volume of reaction mixture obtaining 2 μl reaction 10x buffer (20 mM Tris pH 8.8, 10 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 mM of each primer and 1.0 U Taq polymerase (QIAGEN). The following cycling conditions were used: for 2 minutes at 95°C 32 two-step cycles consisting of 60 seconds of denaturation at 58°C followed by 1 minutes of annealing at 72°C and elongation at 72°C for 10 minutes. The amplified PCR fragments were stained and visualized on a 1% (w/v) agarose gel. PCR products were then purified, sequenced and analyzed on an automated sequencer (Prism 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA). The resulting DNA sequences were aligned using Bio-Edit software and Multialign software, and DNA polymorphisms were confirmed using NCBI-BLAST software [21].

For a better interpretation of functionally relevant FCN2 polymorphisms at both promoter and exon 8 regions, haplotypes of FCN2 gene were determined, according to Ruskamp et al. [22] and Munthe-Fog et al. [23]. The FCN2 (−986/−602/−4/+6,359/+6,424) haplotype included the following risk groups: AGGTG (low risk), AAACG (low risk), GGACG (medium risk), AGACG (medium risk), GGATG (medium risk), and GGACT (high risk).

Combined MBL2 and FCN2 Genetic Deficiency

The combined MBL2/FCN2 deficiency was recorded as follows: High/medium risk combined deficiency included the groups: [(A/O or O/O) + GGATG], [(A/O or O/O) + AGACG], [(A/O or O/

O) + GGACG], and [(A/O or O/O) + GGACT]. Low risk combined deficiency included the groups: (A/A) + AGGTG and (A/A) + AAACG.

Statistical Analysis

The program "Statistical Package for the Social Science SPSS for Windows" (edition 15.0, Chicago, IL) was used. Mean, median, standard deviation, and standard error of mean were calculated. Comparisons were performed using a Student's *t*-test for parametrical and Mann–Whitney for non-parametrical variables. For categorical variables chi square test was used. Statistical significance was assigned to two-sided *P*-values <0.05.

RESULTS

Patient Characteristics

Forty-four children (65.9% males) were enrolled. Their median age was 4.3 years (range 0.5–13). In the 2–6 years subgroup, 34 children (77.3%) were enrolled. According to BFM ALL stratification, in 26 patients ALL was classified as standard risk (SR; 59.1%), in 12 as intermediate risk (MR; 27.3%) and in six as high risk (HR; 13.6%).

FN Episodes and Duration

A total of 142 FN episodes were recorded. The median number was 3 FN episodes per child (range 1–8). The rate of FN episodes per 30 days of neutropenia was 0.9 (95% CI: 0.81–1.08). The median FN duration during the follow-up period was 9.5 days (range 4–37).

Bacterial Infections

Ninety-two microbiologically documented bacterial infections were recorded. The median number of bacterial infections per child was two (range 1–6). In particular, one to two infections were observed in 28 children (63.6%), three infections in eight patients (18.2%), and four to six infections in eight children (18.2%). The rate of bacterial infections per 30 days of neutropenia was 0.55 (95% CI: 0.49–0.71). In total, 78 (84.8%) bacteremias with or without an identified site of infection were recorded, and 14 (15.2%) urinary tract infections (UTI) without bacteremia. Bacteremias occurred after a mean $\pm$ SE 8.1 ± 3.0 days of neutropenia as compared to 4.3 ± 1.9 days for UTIs ($P < 0.0001$).

Gram negative bacteria were isolated in 59.8% of bacteremias, while Gram positive in 40.2% (Table I). Among 7 *Staphylococcus aureus* isolates, 4 (57%) were methicillin-resistant (MRSA). No resistance to vancomycin or linezolid was noticed. Among 55 Gram negative bacteria, resistance to ceftazidime, amikacin, and imipenem was observed in 24%, 10%, and 2%, respectively. In total, the prevalence of multidrug-resistant Gram negative isolates was observed in 9%.

MBL2 Genotype and Infections

No significant association between age, sex, leukemia risk stratification (standard versus intermediate/high B-ALL risk), number and rate of FN episodes and MBL2 genotype was found (Table II). In particular, a comparison of the rate of febrile episodes per 30 days of neutropenia between MBL2 low risk versus MBL2 medium/high risk genotypes did not reveal any difference (0.90 versus 0.91, $P = 0.50$). On the contrary, MBL2 genotype was significantly associated with the duration of FN. The median duration of overall FN was 18 days in children with medium/high risk MBL2 genotype and 8 days in children with low risk MBL2 genotype ($P = 0.01$). Children with the medium/high risk MBL2 genotype presented a significantly increased risk for prolonged FN (>10 days) compared to the low risk genotype group (OR 2.1; 95% CI: 1.1–3.7, $P = 0.007$; Table III).

The median number of bacterial infections was one (range 1–4) for the children with low risk MBL2 genotype and four (range 1–6) for those with medium/high risk MBL2 genotype ($P = 0.001$). The rate of bacterial infections was 0.42 per 30 days of neutropenia in children with low risk MBL2 genotype as compared to 0.69 in children with medium/high risk MBL2 genotype, respectively ($P = 0.06$). Children with medium/high risk MBL2 genotype exhibited significantly increased risk to experience ≥ 2 bacterial infections during immunosuppression compared to children with low risk genotype (OR 2.9; 95% CI: 1.3–5.0, $P = 0.001$; Table III).

FCN2 Haplotype and Infections

No significant association was found between age, sex, leukemia risk stratification, number, and rate of FN episodes and FCN2 haplotype (Table II). Similarly, the rate of febrile episodes per 30 days of neutropenia between the FCN2 low risk and medium/high risk haplotype subgroups did not differ significantly (0.9 vs. 1.0 respectively, $P = 0.52$). FCN2 haplotype was, however, associated with the duration of FN. The median duration of overall

TABLE I. Microorganisms Isolated From 92 Documented Bacterial Infections

Gram (+)	No. of strains	%	Gram (−)	No. of strains	%
Staphylococcus epidermidis	18	21.2	*Escherichia coli*	19	20.6
Staphylococcus aureus	7	7.6	*Pseudomonas aeruginosa*	12	13
Staphylococcus hominis	3	3.3	*Klebsiella pneumoniae*	13	14.1
Staphylococcus hemolyticus	3	3.3	*Proteus mirabilis*	5	5.4
Enterococcus faecium	4	4.3	*Enterobacter cloacae*	2	2.2
Streptococcus viridans	2	2.2	*Acinetobacter baumanii*	1	1.1
Total	37	40.2	*Corynobacterium*	1	1.1
			Pseudomonas fluroscents	1	1.1
			Providencia rustiganii	1	1.1
			Total	55	59.7

TABLE II. Frequency of Three MBL2 Genotypes and Six FCN2 Haplotypes (−986/−602/−4/+6,359/+6,424) Detected in the Study

MBL2 genotype	No. of patients (%)	Type of MBL2 genotype
A/A (normal allele B)[a]	26/44 (59.1)	Low risk genotype
A/0 (heterozygous for mutant allele B)	14/44 (31.8)	Medium risk genotype
O/O (homozygous for mutant allele B)	4/44 (9.9)	High risk genotype
FCN2 haplotype	No. of patients (%)	Type of FCN2 haplotype[b]
AGGTG (−986/−602/−4/+6,359/+6,424)	4/34 (11.8)	Low risk haplotype
AAACG (−986/−602/−4/+6,359/+6,424)	9/34 (26.5)	Low risk haplotype
GGATG (−986/−602/−4/+6,359/+6,424)	9/34 (26.5)	Medium risk haplotype
GGACT (−986/−602/−4/+6,359/+6,424)	6/34 (17.6)	High risk haplotype
GGACG (−986/−602/−4/+6359/+6,424)	5/34 (14.7)	Medium risk haplotype
AGACG (−986/−602/−4/+6,359/+6,424)	1/34 (2.9)	Medium risk haplotype

[a]None of the children in the study were carriers ofallele C. [b]Types of FCN2haplotypeshave been determined according to Ruskamp et al. [21] and Munthe-Fog et al. [22].

FN for children with haplotype AGGTG was 8.5 days (range 2–17), for children with AAACG 6 days (range 2–9), for GGATG 21 days (range 12–37), for GGACT 11.5 days (range 7–26), and for haplotype GGACG 12 days (range 4–16; $P = 0.031$ for comparisons between FCN2 groups). Patients with medium/high risk FCN2 haplotype presented an increased risk for prolonged FN compared to low risk FCN2 haplotype patients (OR: 3.2; 95% CI: 1.5–4.6, $P = 0.001$; Table III).

The median number of bacterial infections in children with haplotype AGGTG and AAACG was one (range 1–4), for children with haplotype GGATG and GGACT 3 (ranges 3–6 and 2–4, respectively), and for haplotype GGACG 2 (range 1–3; $P = 0.0001$). The rate of bacterial infections was 0.36 per 30 days of neutropenia in children with low risk FCN2 haplotype as compared to 0.65 in children with medium/high risk FCN2 haplotype, respectively ($P = 0.009$). Children carrying the medium/high risk FCN2 haplotype showed an eightfold higher risk to suffer from ≥2 infections compared to children with low risk FCN2 haplotype (OR: 8.0; 95% CI: 1.1–54.9, $P = 0.002$; Table III).

TABLE III. Associations of MBL2/FCN2 Polymorphisms With (a) Prolonged Duration of FN (>10 Days) and (b) Increased Number of Infections (>2 Infections)

	I. Prolonged FN duration (no. of patients)			
	0–10 days	>10 days	*P*-value	OR (95% CI)
MBL2 genotype				
MBL2 low risk	18	8	0.007	2.1 (1.1–3.7)
MBL2 high/medium	5	13		
FCN2 haplotype				
FCN2 low risk	12	1	0.001	3.2 (1.48–4.65)
FCN2 high/medium	5	16		
Combined				
MBL2/FCN2 low risk	15	5	0.001	3.4 (1.5–7.5)
MBL2/FCN2 high/medium risk	2	12		
	II. Increased number of bacterial infections (no. of patients)			
	0–1 infections	≥2 infections	*P*-value	OR (95% CI)
MBL2 genotype				
MBL2 low risk	22	4	0.001	2.9 (1.3–5)
MBL2 high/medium	6	12		
FCN2 haplotype				
FCN2 low risk	12	1	0.002	8 (1.1–54.9)
FCN2 high/medium	8	13		
Combined				
MBL2/FCN2 low risk	6	8	0.0012	5.2 (1.8–15.4)
MBL2/FCN2 high/medium risk	14	4		

Combined MBL2/FCN2 Deficiency and Infections

The rate of febrile episodes per 30 days of neutropenia between MBL2/FCN2 deficiency subgroups (low vs. medium/high risk) did not differ (0.94 vs. 0.99 respectively, $P = 0.72$). The mean $\pm$ SE duration of overall FN in children with the combined medium/high risk MBL2/FCN2 genetic deficiency was 19.6 ± 8.7 days, while in children with the combined low risk MBL2/FCN2 genetic deficiency was 7.8 ± 4.3 days ($P = 0.0001$). The detection of the combined medium/high risk MBL2/FCN2 genetic deficiency showed a 3.4-fold increase in the risk for a prolonged duration of FN compared to low risk genetic deficiency group (OR: 3.4; 95% CI: 1.5–7.5; $P = 0.001$; Table III).

The median number of bacterial infections in children with the combined medium/high risk MBL2/FCN2 deficiency was three (range 2–6), while in those with the low risk the median number was one (range 1–4; $P = 0.001$). In particular,the rate of bacterial infections was 0.4 per 30 days of neutropenia in children with low risk MBL2/FCN2 deficiency as compared to 0.77 in children with medium/high risk MBL2/FCN2 deficiency, respectively ($P = 0.018$). The detection of medium/high risk MBL2/FCN2 genetic deficiency was associated with a 5.2-fold risk to present $\geq$2 bacterial infections (OR: 5.2; 95% CI: 1.8–15.4), $P = 0.001$; Table III).

Furthermore, in children with medium/high risk MBL2/FCN2 deficiency, ALL risk stratification (standard vs. intermediate + high risk ALL patients) did not influence the risk for bacterial infections ($P = 0.91$), while for children with low risk MBL2/FCN2 deficiency, the ALL risk stratification tended to influence the risk for bacterial infections ($P = 0.07$). Finally, MBL2/FCN2 deficiency was not associated with the type of bacteria (gram positive vs. gram negative) isolated ($P = 0.81$).

DISCUSSION

This study demonstrates that MBL2 genotype and FCN2 haplotype affect the duration, but not the frequency, of febrile neutropenia episodes as well as the frequency of bacterial infections in children with B-ALL under mild neutropenia. Furthermore, the combined MBL2 and FCN2 genetic deficiency is shown to increase the frequency of bacterial infections in children with ALL during antineoplastic chemotherapy.

We focused only to the determination of gene polymorphisms and not to lectin plasma levels because: first, both MBL and ficolin-2 protein plasma levels present a wide variability among different geographic populations and age subgroups [24,25]. Previous attempts have been undertaken in order to define the normal lectin concentration levels in the pediatric setting, but until definite normal cut-off values are determined, lectin plasma levels could not be easily utilized in disease-association studies. Secondly, both lectins are produced in the liver and therefore any liver dysfunction may also affect the lectin production. Antineoplastic chemotherapy may cause liver toxicity and subsequently impaired lectin production. Nevertheless, a recent systematic review has reported that low MBL plasma levels in children with malignancies may be associated with an increased risk for FN episodes and multiple serious bacterial infections [12].

Data from previously published studies evaluating the role of MBL polymorphisms or plasma levels and the risk of infection in children with cancer are rather conflicting [13,16–19,26,27]. Most of these studies have failed to show a significant impact of MBL2 genotype on the risk for bacterial infections [13,15]. These partially conflicting results may be attributed to differences in sample size, definition of variables and outcomes, treatment protocols, period of follow-up, heterogeneity of study populations in terms of age, underlying disease, geographical–regional distribution of MBL2/FCN2 gene polymorphisms, and most importantly degree of immunosuppression [12]. Our results support and extend those of the single similar study previously published, in which MBL2 genotype under mild immunosuppression was associated with increased risk FN episodes and bacterial infections [14]. These observations strengthen the hypothesis that the impact of lectins may be only present under mild immunosuppression, but further multi-center studies are required to confirm this observation. The putative mechanism by which MBL2 contributes to the increased frequency of FN episodes or prolongation of neutropenia is not fully elucidated. The genetic deficiencies of C-type lectins seem to impact either lectin plasma concentrations or the lectins' binding affinity to their substrates, both required to combat an invading microorganism during neutropenia. In that sense, the presence of this genetic immunodeficiency in neutropenic patients may significantly hamper the control of an infection. The increased frequency of FN episodes or the prolongation of neutropenia may be an indirect way of demonstrating the impaired clearance of bacteria.

Data regarding FCN2 polymorphisms or ficolin-2 plasma levels in children with ALL are lacking. On the contrary, two previously published studies have tried to associate plasma levels of ficolin-1 and -3 with increased risk for infections and FN episodes [28,29]. In the first, ficolin-1 levels did not differ significantly between patients and controls and were not associated with FN episodes [29]. In the second, low plasma ficolin-3 levels were significantly associated with increased risk for FN episodes, prolonged hospitalization, prolonged antimicrobial treatment and increased number of bacteremic episodes [28]. Among the three ficolins (−1,−2,−3) identified in humans, we selected to evaluate the polymorphisms of ficolin-2 because, as a plasma-circulating pattern recognition receptor, it exerts a more systemic activity, while ficolin-1 and -3 exert more localized activities [30]. Furthermore, in the present study, the FCN2 gene haplotype was evaluated instead of the single FCN2 polymorphisms alone, since two previous studies have shown that for the better association of all functional FCN2 polymorphisms with clinical outcomes, the haplotype prevails over single polymorphisms [22,23]. Moreover, the putative importance of FCN2 haplotype in immune response has been previously assessed in other diseases, such as rheumatic fever, schistosomiasis, cutaneous leishmaniasis, and hepatitis B and in adults with underlying diseases and various infections [31–36].

According to our findings, the combined MBL2/FCN2 genetic deficiency increases the risk for prolonged duration of FN and multiple bacterial infections during immunosuppression. However, this combined deficiency was not strongly associated with the type of bacteria isolated. These genetic factors are considered to be determinants of the bacterial infectious "burden" in immunocompromised children [14]. Further *in vivo* and *in vitro* analyses concerning MBL2/FCN2 genotype and specific pathogen recognition or ligand specificity are needed.

The microbiological spectrum in patients with malignancies and FN presents epidemiological significantly differs among institutions. The rate of FN episodes per 30 days of neutropenia in our study population (0.9, 95% CI: 0.81–1.08) was slightly higher than that found by Castagnola et al. [37] (0.76, 95% CI: 0.70–0.81). Nevertheless, it is difficult to compare these results since the latter

study also included children with lymphomas and solid tumors. After stratification, children receiving aggressive treatment for ALL presented a frequency of >1 FN episode per 30 days at risk [37]. On the other hand, the incidence of bacterial infections in children with ALL depends on the treatment intensity. In another study the number of bacteremias per 100 days at risk in children with ALL was 0.092 (or approximately 0.3 per 30 days) [38] as compared to 0.55 (95% CI: 0.49–0.71) in our study. Furthermore, the increasing incidence of antimicrobial resistance as a complication of the widespread use of antibiotics in this population raises major concerns in the last years [39–41].

Our findings may help to stratify children with B-ALL according to their risk for bacterial infection in a more reliable manner. Evaluation of genetic factors in combination with clinical and laboratory parameters may eventually make possible to tailor supportive care according to individual risk profiles in order to ultimately improve quality of life, reduce costs, avoid unnecessary aggressive antibiotic therapy, and possibly also reduce antibiotic resistance rates.

A limitation of this study is its relatively small sample size. We believe however, that this is partially compensated by the homogeneity of the patients based on the same type of hematological malignancy, the same chemotherapy protocol, the same immunosuppressive status, and hospitalization area.

In conclusion, MBL2 genotype and FCN2 haplotype affect the duration but not the frequency of febrile neutropenia episodes in children with B-ALL. Patients with MBL2 and ficolin 2 genetic single or combined deficiencies are at increased risk for bacterial infections. Further analysis in multicenter studies is warranted to confirm their role as surrogate markers of a more individualized risk for bacterial infections in children with leukemia.

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