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ORIGINAL ARTICLE

The role of mannose binding lectin on fever episodes in pediatric oncology patients

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Abstract Despite significant changes in pediatric oncological therapy, mortality is still high, mainly due to infections. Complement system as an ancient immune defense against microorganisms plays a significant role in surmounting infections, therefore, deficiency of its components may have particular importance in malignancies. The present paper assesses the effect of promoter (X/Y) and exon 1 (A/0) polymorphisms of the *MBL2* gene altering mannose binding lectin (MBL) serum level in pediatric oncological patients with febrile neutropenia. Furthermore, frequency distribution of *MBL2* alleles in children with malignancies and age-matched controls was analysed. Fifty-four oncohematological patients and 53 children who had undergone pediatric surgery were enrolled into this retrospective study. No significant differences were found in the frequency of *MBL2* alleles between the hemato-oncologic and control group. The average duration of fever episodes was significantly shorter ($p = 0.035$) in patients carrying genotypes (AY/AY and AY/AX) that encode normal MBL level, compared to individuals with genotypes associated with lower functional MBL level (AX/AX, AY/0, AX/0, or 0/0) (days, median (IQ range) 3.7(0–5.4) vs. 5.0(3.8–6.6), respectively). In conclusion, our data suggest that *MBL2* genotypes may influence the course of febrile neutropenia in pediatric patients with malignancies, and may contribute to clarification of the importance of MBL in infections.

Keywords MBL · Polymorphism · Febrile neutropenia · Oncohematology

Introduction

Modern treatment of childhood malignancies have been markedly changed leading to a higher life expectancy; the overall 5-year survival rate is 70–80 %. Nonetheless, the mortality is still significant, as the chemotherapy-induced immunosuppression increases susceptibility to infections, which contributes to about 10–20 % of mortality in pediatric oncology.

As a sequel of treatment, pediatric oncology patients may often become neutropenic, leukopenic or pancytopenic that emphasizes the importance of innate immune defense against microbes. The complement system, activated through the classical, alternative or lectin pathways, is an essential component of the ancient immune response to infections caused by a wide variety of pathogens. The lectin pathway can be initiated by a circulating protein called mannose binding lectin (MBL) that binds to carbohydrates found on the surface of many pathogens [1, 2]. MBL binds with high affinity to microbes often detected in hematology departments and can cause severe sepsis, such as diverse *Candida* species, *group A Streptococci* or *Staphylococcus aureus* with specific antibiotic resistance (MRSA) [3].

The MBL protein is encoded by the *MBL2* gene (10q11.2-q21) comprising 4 exons. The promoter region of the gene contains a single nucleotide polymorphism (SNP) at position −221 denoted as Y/X in the literature. The most widely studied

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Table 1 Allele frequencies of *MBL2* polymorphisms in children with and without hemato-oncologic disorders

Allele		children with hemato-oncologic disorder	children without hemato-oncologic disorder
Promoter	Y	78.7 %	85.8 %
	X	21.3 %	14.2 %
Exon-1	A	79.6 %	76.5 %
	B	11.1 %	16 %
	C	1.9 %	1.8 %
	D	7.4 %	5.7 %

variations of the gene are three polymorphisms in the first exon causing aminoacid substitutions in the protein. The wild type allele without any polymorphic variant is named A, while the alleles with amino acid changes at codon 54 (Gly54Asp), 57 (Gly57Glu) or 52 (Arg52Cys) are termed as B, C or D, respectively and any of these variants on a chromosome is referred to as a "0 allele". Serum concentration of functional mannose binding protein shows close correlation with the genotype of *MBL2* polymorphisms. The wild type *A* allele is associated with normal plasma level, while all variant alleles (*B*, *C* and *D*) have a dominant effect lowering the level of functional MBL. Polymorphism of the promoter region also influences the circulating MBL level in particular the variant allele (X) is associated with lower MBL expression [4, 5].

A growing body of evidence suggests that functional MBL deficiency may be associated with an increased risk of infections especially in malignancies; however, contradictory results have also been reported [6, 7]. Present paper deals with the possible role of polymorphisms influencing MBL serum level on the incidence, frequency and duration of febrile neutropenia (FN) in oncohematological patients. Moreover, frequency of *MBL2* alleles was compared in children with vs. without malignancies.

Materials and Methods

Fifty-four patients (24 girls, 30 boys) diagnosed with malignant diseases and treated between 2001 and 2008 at the 2nd Department of Pediatrics of the Budapest Semmelweis University were enrolled into our retrospective clinical study. Inclusion criteria were oncohematological disease and age of 18 years or younger at the date of diagnosis. The average age at diagnosis was 9.4 years (range 3 months-17 years). The diagnoses of enrolled participants were: acute lymphocytic leukemia (ALL) (N = 30); acute myelocytic leukemia (AML) (N = 2); Hodgkin's disease (N = 7); non-Hodgkin lymphoma (NHL) (N = 9), and osteosarcoma (N = 6). Each patient received chemotherapy according to protocols ALL (IC) BFM 95/2002, AML BFM 98, COSS 96, Interfant 98, NHL BFM 95 or HD 95 and chemotherapy was the only treatment modality used in the study population.

To assess the frequency of the *MBL2* polymorphisms in an age-matched population, 53 children of average age of 6.9 years (range 1–17 years) without malignancies were enrolled as controls with following diagnoses: phymosis; adhesion of preputium; hernias (inguinal, umbilical and abdominal); pectus excavatum; major labial adhesion; acute appendicitis; acute gastroenteritis; celiac disease; carpal ganglion; fractures; verrucas; gland mycosis; varicocele or testicular hydrocele. The study was approved by the National Ethical Committee (TUKEB 180/2007), and parents or guardians of all participants gave informed consent.

Fever episodes occurred during chemotherapy or shortly after treatment were followed up for 2 years after the diagnosis of patients with hemato-oncologic disorders. Febrile neutropenic episode (FN) was defined as an axillary temperature exceeding 38 °C for at least 2 days and granulocyte count under 0,5G/l. Several parameters were recorded during each episode, such as the date of first and last day of fever, certain clinical parameters (WBC, Neutrophils and CRP) determined at the onset of the episode, at the time of hemoculture test, and on the first day of normal body temperature. In case of positive hemoculture, the identified microbe, its antibiotic resistance and the treatment (antibiotic and/or citokin) were also registered.

EDTA-anticoagulated blood samples were obtained for genomic DNA preparation using a salting-out procedure. Genotyping of *MBL2* C (rs1800451), D (rs5030737) and Y/X (rs7096206) polymorphisms was carried out by real-time PCR with commercially available TaqMan® SNP Genotyping Assays (Applied Biosystems, CA, USA), while the B allele (rs1800450) was determined by PCR-RFLP [8]. In our study patients were assessed into three groups according to the expected serum level of MBL protein encoded by the carried genotype as repoterted by Garred et al. [5, 9] Group 1: patients carrying genotypes (YA/YA and YA/XA) encoding normal MBL level; group 2: patients with genotypes associated with low protein levels (XA/XA and YA/0) and goup 3: MBL-deficient (XA/0 and 0/0) subjects.

Statistical analysis was performed with the GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA) and SPSS 13.0 (SPSS Inc., Chicago, IL) software. Categorical data were analyzed by the χ^2 test and between-group differences were evaluated by the Mann-Whitney or Kruskal-Wallis tests. Multivariate analyses were performed by multiple logistic regression adjusted to the diagnosis of the patients or the applied chemotherapy protocol.

Results

Allele frequencies of the studied SNPs of the *MBL2* gene were compared in the groups of children with or without hemato-oncologic disorders (Table 1). There were no significant

Table 2 Frequencies of different diseases in the three groups of patients formed according to the carried *MBL2* genotype

	Genotypes associated with normal MBL level (YA/YA and YA/XA)	Genotypes associated with low MBL level (XA/XA and YA/0)	Genotypes associated with MBL-deficiency (XA/0 and 0/0)
ALL	16	10	4
	51.6 %	*59 %*	*66.6 %*
AML	2	0	0
	6.5 %	*0 %*	*0 %*
Osteosarcoma	4	2	0
	12.9 %	*12 %*	*0 %*
Hodgkin's disease	4	2	1
	12.9 %	*12 %*	*16.7 %*
NHL	5	3	1
	16.1 %	*17 %*	*16.7 %*
Sum	31	17	6
	100 %	*100 %*	*100 %*

differences in the allele frequencies in either the promoter, or the exon 1 polymorphisms of this gene.

As the incidence of infections and their treatment is different in distinct childhood malignancies, the ratio of different diseases was evaluated in the three groups of patients according to the carried *MBL2* genotype (Table 2). The difference between all groups was not significant ($p = 0.85$).

The analysis of the features of febrile neutropenia during the first 2 years following the diagnosis in 3 genotype groups (Table 3), have revealed a shorter time interval between diagnosis and the first episode in individuals with low MBL level (Group 2) and in MBL-deficient patients (Group 3), than in subjects with genotypes encoding normal MBL level (Group 1), however, this difference was not significant ($p = 0.196$). There was a trend ($p = 0.052$) that patients with a lower expected MBL level based on the *MBL2* genotype have a longer average duration of FN, that indicates an inverse relationship between MBL level and duration of FN. Individuals with genotypes associated with lower MBL levels had slightly higher ratio of febrile days during chemotherapy in the first 2 years following the diagnosis, but this difference was not significant ($p = 0.690$). Frequency of FN episodes was similar among the genotype groups (median 1–1.25 FN/year).

In the following analyses patients carrying the variant allele of exon 1 polymorphism (A/0, 0/0) and those homozygous for the promoter allele associated with lower MBL expression level (XA/XA) were merged (group 2 and 3 in Tables 2 and 3). Average duration of fever episodes was significantly shorter ($p = 0.035$) in those carrying the AA genotype and maximum one X allele (YA/YA and YA/XA) than in patients with genotypes associated with lower functional MBL level (group 2 and 3). The median (IQ range) of average fever episode length was 3.7 days (0–5.4) in group 1 and 5.0 days (3.8–6.6) in the merged group of 2 and 3.

Next, we performed a multiple logistic regression analysis in order to assess the strength of the association between *MBL2* genotype groups and the average duration of FN (dichotomized at the median: ≤4 days vs. >4 days). The carrier state of genotypes associated with low or deficient functional MBL level was found to be a significant risk factor for longer average duration (>4 days) of fever episodes after adjustment for the diagnosis (OR (95 % confidence interval), 1.84 (1.04–3.25), $p = 0.037$) or the applied chemotherapy protocol (OR: 1.86 (1.05–3.28), $p = 0.033$) or the duration of chemotherapy (days) (OR: 3.34 (1.06–10.56), $p = 0.040$) as possible confounding variables.

Table 3 Data on fever episodes experienced by patients in the first 2 years after the diagnosis in the three *MBL2* genotype groups

	N	Duration between diagnosis and the first fever episode (days) (median (IQ range))	Average length of fever episodes (days) (median (IQ range))	Ratio of days with fever during chemotherapy (median (IQ range))
YA/YA, YA/XA	31	53 (12–730)	3.7 (0–5.4)	2.9 (0–6.7)
XA/XA, YA/0	17	38 (22.5–161)	4.5 (3.4–6.2)	3.2 (1.7–5.9)
XA/0, 0/0	6	23.5 (3.2–85.8)	5.3 (4.5–8.7)	3.5 (1.9–6.1)

Discussion

Our study evaluated the influence of *MBL2* gene polymorphisms on the incidence, frequency and duration of febrile neutropenia in oncohematological patients. Our results showed that genotypes encoding high MBL level are associated with shorter duration of fever episodes in the first 2 years after the diagnosis of malignancy.

Frequency of variant alleles of Y/X and A/0 polymorphisms in our patients was 21.3 and 20.4 that is similar to that found in the general population. A previous paper reported that *MBL2* variant alleles occur significantly more frequently in children with ALL compared to healthy individuals, however only adults comprised the control group [10]. In our study oncologic pediatric patients were compared with non-oncologic age-matched controls and no difference was found in the allele distribution.

Analyzing the characteristics of fever episodes in the first 2 years after the diagnosis of malignancy, we have found that patients carrying high MBL level coding genotypes (YA/YA and YA/XA) had shorter average duration of febrile neutropenia than individuals with genotypes coding for lower MBL serum levels (XA/XA, XA/0, YA/0 and 0/0). Differences were also found in time interval between the diagnosis and the first fever episode and the ratio of days with fever during chemotherapy among patients grouped by *MBL2* genotypes, but none of these were significant.

Previous studies analyzing the role of MBL in infections in children with cancer showed contradictory results. Neth et al. found that the median duration of febrile neutropenic episodes was longer in MBL deficient children receiving chemotherapy than in patients with normal MBL coding genotypes [11]. Similarly, other studies also showed association between low concentrations of MBL or low-producing *MBL2* genotypes and serious infections related to chemotherapy [12–15], while a recent paper showed that MBL deficiency was associated with decreased event-free survival in children with cancer [16]. However, several reports failed to find relationship between the incidence or duration of fever episodes and MBL levels in patients with different malignancies [17–21]. Another recent paper retrieving data from six cohorts studies failed to identify MBL deficiency as an independent risk factor for febrile neutropenia or infection in pediatric oncology patients [22]. Although inconsistent results have been published, MBL therapy for MBL deficient immunocompromised patients is an area of ongoing research [23–25]. Phase I and II trials have already been performed with plasma-derived MBL in small populations and as recombinant human MBL has recently become available, more resultes are expected to be published in the near future.

We are aware of the limitations of our study, namely low sample size and the heterogenous patient group in terms of diagnosis. However, as the multivariate analyis including diagnosis or the applied chemotherapy has confirmed that low MBL level coding genotypes confer risk of longer FN episodes, our results may contribute to clarify previous controversial findings. Further studies on the role of MBL as a clinical prognostic factor in febrile neutropenia are needed to verify present results on a larger population of pediatric oncologic patients and to assign candidates for MBL replacement therapy.

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Conflict of Interest The author(s) declare that they have no conflict interests.

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