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The role of mannose-binding lectin (MBL) in paediatric oncology patients with febrile neutropenia

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ABSTRACT

Children with cancer often have fever during chemotherapy-induced neutropenia, but only some develop serious infectious complications. Mannose-binding lectin (MBL) deficiency might increase infection susceptibility in these children. MBL genotype and phenotype were prospectively determined in 110 paediatric oncology patients. During febrile neutropenia, MBL concentrations were measured longitudinally in time. MBL genotype and phenotype were correlated to clinical and laboratory parameters. Structural exon-1 *MBL2* mutations and the *LX* promoter polymorphism lead to deficient MBL concentrations. The capacity to increase MBL concentrations during febrile neutropenia was associated with *MBL2* genotype. Infectious parameters did not differ between MBL-deficient and MBL-sufficient neutropenic children ($n = 66$). In contrast, MBL-sufficient patients had a greater risk of Intensive Care admittance (Relative Risk 1.6, 95% Confidence Interval 1.3–2.0, $P = 0.04$). MBL-deficient neutropenic children did not have more severe infections. However, most patients (61%) were severely neutropenic (<100 cells/μL), compromising the opsonophagocytic effector function of MBL. MBL substitution might still be beneficial in patients with phagocytic activity.

1. Introduction

Although the treatment of paediatric oncology patients has dramatically improved over the past 20 years, infections still play a major role in morbidity and mortality.[1,2] Many patients experience severe and prolonged neutropenia but not all patients suffer from the same infectious complications during a neutropenic episode. The reasons for this are not clear but low concentrations of mannose-binding lectin (MBL) might play a role.

MBL is a collagenous lectin of the innate immune system that binds to sugars on the surface of many micro-organisms. Once bound, it activates the lectin pathway of complement activation through a MBL-associated serine protease, MASP-2. The result is direct complement mediated lysis and opsonization of the micro-organism followed by phagocytic killing.[3]

A single functional gene (*MBL2*) on chromosome 10q25 codes for the human protein MBL.[4] Three autosomal dominantly inherited structural gene mutations at codons 52, 54, and 57 (D, B, and C variants, respectively) in exon-1 have

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been described, resulting in low concentrations of circulating MBL.[3] The A variant represents the "wild-type" MBL. In addition, three autosomal recessive polymorphisms (termed *H/L*,*X/Y*, and *P/Q*) within the promoter region of the *MBL2* gene determine the plasma concentration due to altered transcriptional efficiency.[5] The structural exon-1 mutations are in linkage equilibrium with the promoter polymorphisms and every individual will express two out of seven possible haplotypes: *HYPA*, *LYQA*, *LYPA*, *LXPA*, *LYPB*, *LYQC*, and *HYPD*.[6] The *HY* haplotype induces high MBL concentrations while exon-1 mutations (*O* variant) and the *LX* haplotype cause reduced MBL concentrations.[7] Therefore, patients can be classified into high (*HYA/HYA*, *HYA/LYA*, *HYA/LXA*, *LYA/LYA*, and *LYA/LXA*), medium (*LXA/LXA*, *HYA/O* and *LYA/O*), and low (*LXA/O* and *O/O*) MBL genotype expression groups.[5,8,9] Mean MBL concentrations of 1860–3840, 670–1570, and 100–690 μg/L were described in these genotype groups, respectively.[8]

Reduced MBL concentrations are associated with increased frequencies of infection, particularly in children and patients with co-existing immune defects, including primary and secondary immunodeficiencies.[10,11] Therefore, MBL deficiency might also increase infection susceptibility in chemotherapy-induced neutropenic children. To date three prospective and four retrospective studies have been performed in oncology and transplant patients, of which only one was performed in children (Table 1). These paediatric MBL-deficient oncology patients experienced longer episodes of febrile neutropenia.[12] In three retrospective studies, MBL-deficient adults had more severe infections.[13–15] However, patients with different malignancies and (transplant) treatment regimens were included and infection was not strictly defined by culture results. In contrast, no association between MBL deficiency and infections was found in adult cancer patients on chemotherapy in the three remaining studies.[16–18]

If MBL deficiency does increase infection susceptibility in (paediatric) cancer patients, supplying MBL to patients might be beneficial.[19] In this prospective study, the role of MBL was studied in a cohort of paediatric oncology patients with febrile neutropenia.

2. Patients and methods

2.1. Study design

A prospective cohort study was performed during a 2-year period (February 2003–January 2005). Out of 308 newly diagnosed and relapsed oncology patients, 110 patients, expected to become neutropenic during chemotherapy treatment, were included. The study protocol was approved by the local ethics committee. Written informed consent from parents and children (>12 years) was obtained.

2.2. Procedures

Prior to the start of chemotherapy a blood sample was taken for *MBL2*-genotyping. A "day 0" MBL plasma concentration was measured when the patient was not febrile or neutropenic. During one febrile neutropenic episode, clinical and laboratory parameters were collected on days 1, 3, and 5. During the entire chemotherapeutic treatment the number of febrile episodes was recorded.

Vital parameters, pattern and duration of fever, (prophylactic) antibiotic use, Intensive Care Unit (ICU) admittance and mortality were recorded. Clinical signs and symptoms were scored concerning catheter infection, pneumonia, and sepsis, using the common toxicity criteria (http://ctep.cancer.gov/forms/CTCAEv3.pdf). The routine laboratory investigations consisted of a full blood count, C-reactive protein (CRP), liver enzymes and microbiological tests. Other diagnostic procedures such as chest X-ray were included when clinically indicated. The clinical outcome measures were: severity and duration of neutropenia, duration and pattern of fever, CRP level, blood culture results, sepsis, ICU admittance and mortality. Clinical data were collected independently of MBL measurements.

Fever was defined as a single ear-temperature >38.5 °C. The definition for neutropenia was an absolute neutrophil count <500 cells/μL;[1] for severe neutropenia <100 cells/μL. Sepsis was defined as the presence of clinical signs and symptoms of infection (tachypnea: mean respiratory rate >+2 SD for age, or tachycardia: mean heart rate >+2 SD for age) and

Table 1 – Literature summary on mannose-binding lectin (MBL) and infections in cancer and transplant patients

	Trial	Patient	Malignancy	Duration febrile neutropenia	Frequency infections	Severity infections
Neth[12]	Prospect	Child *N* = 100	Various	Sign. longer duration	=	=
Horiuchi[13]	Retrospect	Adult *N* = 113	Haematologic; Autologous PBSCT	=	=	Sign. more severe infections
Mullighan[14]	Retrospect	Adult *N* = 97	Allogeneic BMT	=	=	Sign. more severe infections
Peterslund[15]	Retrospect	Adult *N* = 54	Haematologic	=	=	Sign. more severe infections
Bergmann[16]	Prospect	Adult *N* = 80	ANLL	=	=	=
Kilpatrick[17]	Prospect	Adult *N* = 128	Haematologic	=	=	=
Rocha[18]	Retrospect	Adult *N* = 107	Haematologic; Allogeneic BMT	=	=	=

Abbreviations: Sign.: significantly; BMT: bone marrow transplantation; PBSCT: peripheral blood stem cell transplantation; ANLL: acute non-lymphoblastic leukaemia; (=): no influence of MBL deficiency.

a positive blood culture (fungus or bacteria).[12,20] For coagulase-negative staphylococci, *Corynebacterium* spp. and *Bacillus* spp., at least two positive blood cultures were required.[16] A diagnosis of pneumonia required combined clinical and radiological findings. Septic shock was defined as sepsis with cardiovascular organ dysfunction, e.g. hypotension (blood pressure <+2 SD for age) despite adequate fluid resuscitation.[20]

2.3. Assays

Six single nucleotide polymorphism (SNP) variants of the *MBL2* gene (AD, AB, AC, HL, XY, and PQ) were analysed using Taqman Allelic Discrimination assays (ABI Prism® 7000 Sequence Detection System, Applied Biosystems, USA). Primer and probe sequences were taken from the NCBI website (http://snp500cancer.nci.nih.gov/snplist.cfm). Probe concentration: 200 nM, primer concentration: 900 nM. The assay was performed in 15 μl, with 20 ng DNA. A pre-read was performed at 60 °C. Thermocycling conditions for all assays were 2 min 50 °C, 10 min 95 °C, 50 cycles of 30 s 92 °C and 1 min 60 °C (except for AB, which was performed at 62 °C), followed by 10 min 60 °C. After the polymerase chain reaction, a post-read was performed. Fluorescent signal was corrected for pre-read fluorescent background. Analysis of Allelic Discrimination was performed using sequence detection software (ABI Prism 7000 software version 1.1, Applied Biosystems, USA).

Functional MBL concentrations were determined in a solid phase ELISA with mannan coated to the solid phase and a monoclonal antibody (biotinylated mouse-anti-MBL IgG). Microtiter plates were coated with 100 μl (10 mg/L) mannan in 0.1 M $NaHCO_3$, pH 9.6 overnight at room temperature. The plates were washed 5 times with H_2O between incubation steps. Plasma samples and MBL standards (standard plasma, 1870 μg/L MBL) were diluted in TTG/Ca^{2+} (20 mM Tris pH 7.4/150 mM NaCl/0.02% TWEEN-20/0.2% gelatin/10 mM $CaCl_2$), with 10,000 U/L heparin and incubated with shaking at room temperature for 1 h. After washing, the plates were incubated for 1 h with biotinylated αMBL-1 in TTG/Ca^{2+}, followed by incubation for 30 min with streptavidin pHRP 1:5000 in TBS/Ca^{2+} (20 mM Tris pH 7.4/150 mM NaCl/10 mM $CaCl_2$)/2% milk. Colour developed using tetramethyl-3.3,5.5-benzidin (TBM)/H_2O_2 in 0.1 M NaAc pH 5.5 and stopped with 2 M H_2SO_4. A Bio-Assay Reader, Sunrise (Tecan, Austria, Salzburg) measured spectrophotometric absorbances at 405 nm.

2.4. Statistics

Frequencies between groups were compared by the Mann–Whitney U, Kruskall–Wallis, and χ^2 tests, where appropriate. If the number was smaller than five in one of the cells we performed a Fisher's exact test. Concerning the infection-related parameters, the medium and low MBL genotype expression groups were combined for statistical comparison with the high group. Longitudinal MBL concentrations were compared by the Friedman test. An optimal cut-off plasma concentration for defining MBL deficiency was calculated by a receiver–operator characteristic (ROC) curve. SPSS 11.5 computer software was used.

3. Results

3.1. Total cohort

3.1.1. Patient characteristics

The cohort consisted of 110 patients (71 boys, 39 girls). This skewed sex distribution was independent of tumour type or MBL geno- and phenotype. The median age was 5.8 years (interquartile range (IQR) 3.4–11.8 years). Seventeen patients had a lymphoma and 41 had haematological malignancies, of which 37 had acute lymphoblastic leukaemia (ALL). The most frequent solid tumours ($n = 52$) were rhabdomyosarcoma, neuroblastoma, Ewing sarcoma and Wilms tumour (Table 2). The median follow-up period after diagnosis was 13 months (range 2–46). Relapse occurred in 16 patients. Until January 2005, 14 patients died (two following a febrile neutropenic episode). Thirteen died due to their malignancy or treatment and one due to infection (Table 2).

3.1.2. MBL genotype and phenotype

In 109 (99%) patients, a complete genotype was obtained in combination with day 0 MBL concentrations. The genotype of the remaining patient was lacking, but his MBL concentrations were measured during febrile neutropenia. The MBL haplotypes were used to make three categories as described before,[5,8,9] i.e. high, medium, and low genotype expression groups of 64 (58%), 30 (27%), and 15 (14%) patients, respectively (Table 2). The median MBL concentrations of patients in the high, medium, and low genotype expression groups were 3326 μg/L (IQR 1980–4900 μg/L), 710 μg/L (IQR 370–1870 μg/L), and 80 μg/L (IQR 40–210 μg/L), respectively (Fig. 1). The difference between the three groups was significant ($P < 0.001$).

An optimal ROC cut-off value for dividing the cohort into those with sufficiently high MBL concentrations from those with insufficient concentrations, corresponding with the medium and low groups, was calculated at 1000 μg/L. Because the MBL concentrations of the patient without determined genotype were repeatedly >1000 μg/L, this patient was allocated to the MBL-sufficient group. Thirty-eight patients (35%) had concentrations under 1000 μg/L, defining MBL deficiency in further analysis. The remaining 65% had concentrations >1000 μg/L (Table 2).

3.2. Febrile neutropenia group

3.2.1. Patient characteristics

Upon admission for fever during neutropenia, 66 patients (40 boys, 26 girls) were followed prospectively. Their median age was 6.3 years (IQR 3.4–11.3 years). Patient characteristics did not differ between the febrile and non-febrile group (Table 2).

3.2.2. MBL genotype and phenotype

Of these 66 patients, 65 *MBL2* genotypes were known. According to their haplotypes, 39 (59%) were categorized in the high, 15 (23%) in the medium and 11 (17%) in the low expression group. Twenty-three patients (35%) had insufficient (<1000 μg/L) day 0 MBL concentrations (Table 2).

Table 2 – Patient characteristics

	Total group N = 110	Non-febrile group (I) N = 44	Febrile neutropenic group (II) N = 66	P-value between I and II
Age				
Median years (IQR)	5.8 years (3.4–11.8)	5.4 years (3.3–12.3)	6.3 years (3.4–11.3)	P = 0.98
Sex				
Male	71 (65%)	31 (71%)	40 (61%)	P = 0.32
Female	39 (35%)	13 (29%)	26 (39%)	
Malignancy				
Haematological	41 (37%)	16 (36%)	25 (38%)	
Solid	52 (47%)	22 (50%)	30 (45%)	P = 0.87
Lymphoma	17 (16%)	6 (14%)	11 (17%)	
Relapse patients	16 (14%)	4 (9%)	12 (18%)	P = 0.27
Mortality				
Infection-related	1 (1%)	0 (0%)	1 (1%)	P = 0.25
Other cause	13 (12%)	3 (7%)	10 (15%)	
MBL genotype expression groups				
High	64 (58%)	25 (57%)	39 (59%)	P = 0.31[a]
HYA/HYA	6 (5%)	2 (4%)	4 (6%)	
HYA/LYA	27 (25%)	10 (23%)	17 (26%)	
HYA/LXA	12 (11%)	6 (14%)	6 (9%)	
LYA/LYA	5 (4%)	1 (2%)	4 (6%)	
LYA/LXA	14 (13%)	6 (14%)	8 (12%)	
Medium	30 (27%)	15 (34%)	15 (23%)	
LXA/LXA	5 (4%)	2 (5%)	3 (5%)	
HYA/O	14 (13%)	5 (11%)	9 (14%)	
LYA/O	11 (10%)	8 (18%)	3 (4%)	
Low	15 (14%)	4 (9%)	11 (17%)	
LXA/O	10 (9%)	3 (7%)	7 (11%)	
O/O	5 (5%)	1 (2%)	4 (6%)	
Missing	1 (1%)	0 (0%)	1 (1%)	
MBL conc. Day 0				
>1000 μg/L	72 (65%)	29 (66%)	43 (65%)	P = 1.0
<1000 μg/L	38 (35%)	15 (34%)	23 (35%)	

Abbreviations: IQR: interquartile range (p25–p75); MBL: mannose-binding lectin; conc.: concentration.
a P-value between group I and II and the aggregated high, medium, and low genotype expression group.

In 55 patients, MBL concentrations were measured longitudinally in time during hospitalization. In the low genotype expression group median MBL concentrations remained extremely low (<100 μg/L) during the whole febrile period. In the medium and high groups MBL concentrations increased in 5 out of 14 (36%) and 21 out of 30 (70%) patients, respectively (Table 3). In the medium expression group median MBL concentrations were 480 μg/L (IQR 150–740 μg/L), 830 μg/L (IQR 330–2320 μg/L), and 1240 μg/L (IQR 530–2010 μg/L), on days 1, 3, and 5, respectively (P = not significant [NS]). MBL concentrations in the high genotype expression group increased statistically significant from 2210 μg/L (IQR 1500–3800 μg/L) to 2960 μg/L (IQR 1710–5590 μg/L) and 3810 μg/L (IQR 2100–6980 μg/L) between days 1 and 5 (P = 0.025) (Fig. 2).

3.2.3. *Clinical outcome during febrile neutropenia*

The median number of febrile neutropenic episodes was similar in the three expression groups (NS) (Table 4). In 55 patients, the first febrile episode was studied, the other 11 patients already experienced a febrile neutropenic episode before onset of the study. The median (IQR) time patients had received chemotherapy before onset of the febrile neutropenic episode was 3 (1–5) months. The median leukocyte count on day 1 was 0.6×10^9/L (IQR 0.2–1.4×10^9/L). On the first day of febrile neutropenia, 40 patients (61%) had a severe neutropenia. As routinely applied at our center,[21] 61 patients had started with oral prophylactic antibiotics (trimethoprim or quinolones) before the onset of neutropenia. At the onset of fever, patients were started on broad-spectrum intravenous antibiotics (either vancomycin or cefuroxim combined with gentamicin).

Of the 66 patients, 43 (65%) had febrile neutropenia without positive blood cultures or complications. They all recovered completely. Five children (8%) did not have positive blood cultures or proven viral infections, but did have pneumonia (n = 1), or were admitted to the ICU (n = 4) for other reasons. Of these four, one child was admitted after surgery for typhlitis. He recovered within a few days. The other three were admitted with signs and symptoms of septic shock. One of them died, the other two recovered completely.

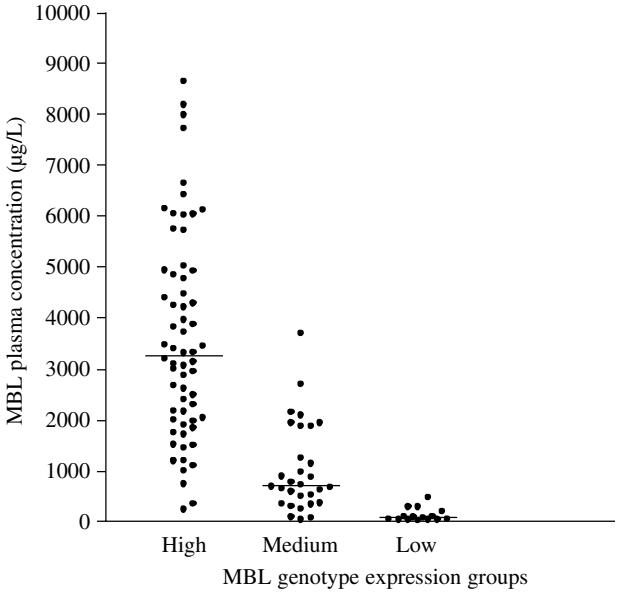

Fig. 1 – Scatter plot representing "day 0" MBL plasma concentrations according to MBL genotype expression groups (n = 109). The high group consists of the haplotypes *HYA/HYA*, *HYA/LYA*, *HYA/LXA*, *LYA/LYA*, and *LYA/LXA*, the medium group of *LXA/LXA*, *HYA/O* and *LYA/O*, and the low group of *LXA/O* and *O/O*. Median is illustrated, $P < 0.001$ between groups.

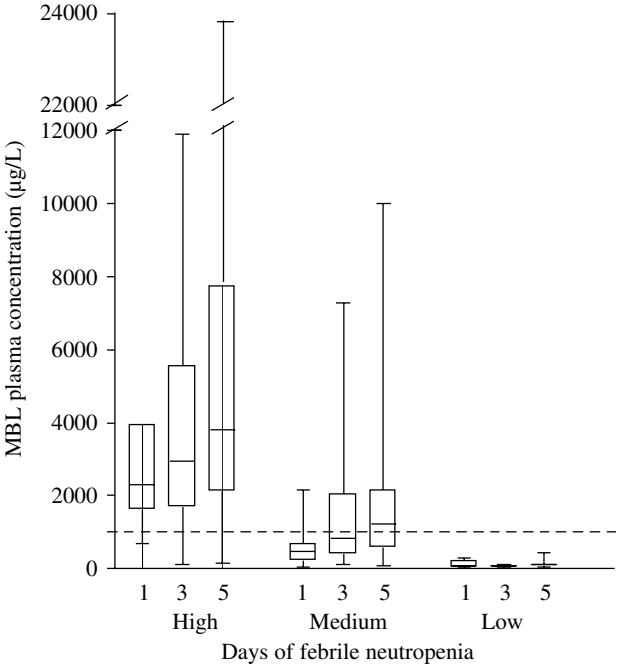

Fig. 2 – Box plot representing the MBL plasma concentrations over the febrile neutropenic episode (days 1, 3, and 5) comparing the low, medium, and high genotype MBL expression groups. Dashed line represents the cut-off value of 1000 μg/L, defining MBL deficiency.

Table 3 – The association between MBL2 haplotypes and the course of mannose-binding lectin (MBL) concentrations during the febrile neutropenic episode

	Increasing MBL concentrations (N = 26)	Stable MBL concentrations (N = 18)
	N (%)	N (%)
MBL genotype expression groups		
High		
HYA/HYA	3 (11%)	0 (0%)
HYA/LYA	11 (42%)	2 (11%)
HYA/LXA	2 (8%)	2 (11%)
LYA/LYA	1 (4%)	1 (6%)
LYA/LXA	4 (15%)	4 (22%)
Medium		
LXA/LXA	3 (12%)	0 (0%)
HYA/O	2 (8%)	8 (33%)
LYA/O	0 (0%)	3 (17%)
Low		
LXA/LXA *O/O*	Not applicable	Not applicable

The remaining 18 patients (27%) had positive blood cultures (12 Gram-positive, 6 Gram-negative organisms, no fungi) (Table 4). The most frequent organisms were: 9 coagulase-negative staphylococci (CNS), 4 *Streptococcus* spp and, 3 *E. coli*. Eight patients recovered quickly (although one had pneumonia); the other 10 developed sepsis (6 Gram-positive, 4 Gram-negative organisms) (Table 4). Of these, 7 recovered without complications and 3 developed septic shock (2 CNS, 1 *Bacteroides*), for which they were admitted to the ICU. One of them died due to severe cardiomyopathy which was diagnosed just after he started his chemotherapy treatment, the other two recovered completely.

The high genotype expression group was compared with the combined medium and low groups. At the onset of febrile neutropenia, vital parameters, routine laboratory investigations and clinical signs and symptoms were not significantly different (data not shown). The number of patients with severe neutropenia, the duration of fever (1–3 days, 3–7 days, or >7 days) or neutropenia, and the fever pattern (peaking, continuous or rapidly normalising) were not different either ($P = 1.00$, $P = 0.57$, $P = 0.96$, $P = 0.99$, respectively). Patients in the medium and low group did not have more positive blood cultures, sepses, or ICU-admissions than patients in the high group ($P = 0.27$, $P = 0.73$, $P = 0.23$, respectively) (Table 4). Furthermore, comparing patients with sufficient and insufficient MBL plasma concentrations (at the cut-off MBL value of 1000 μg/L), infectious parameters did not differ either. However, MBL-sufficient patients had a greater risk of ICU-admittance (Relative Risk (RR) 1.6, 95% Confidence Interval (CI) 1.3–2.0, $P = 0.04$, data not shown). All ICU-patients ($n = 7$) had MBL concentrations >1000 μg/L (corresponding to 6 high and 1 medium MBL genotype expression group patients) (Table 4). When all end-points were studied in 26 patients with neutrophil counts >100 cells/μL and when the patients with haematological and solid malignancies were analysed separately, similar results were obtained (data not shown).

Table 4 – Comparison of MBL genotype expression groups and (infectious) clinical and laboratory parameters in the febrile neutropenic episode (FNE) group

	High group (I)	Medium/low group (II)		P-value between I and II
	High N = 39	Medium N = 15	Low N = 11	
Median time from diagnosis to FNE	2.5 months Range 0.1–23.2	2.4 months Range 0.2–14.4	3.4 months Range 0–14.6	P = 0.41
Median number of FNE's	2.0 Range 1–7	2.0 Range 1–4	1.0 Range 1–3	P = 0.53
Neutrophil count				
<100 cells/μL	23 (59%)	11 (73%)	5 (45%)	P = 1.00
>100 cells/μL	16 (41%)	4 (27%)	6 (55%)	
Duration neutropenia				
1–5 days	18 (46%)	4 (27%)	6 (55%)	P = 0.57
5–10 days	17 (44%)	7 (47%)	4 (36%)	
>10 days	4 (10%)	4 (27%)	1 (9%)	
C-reactive protein				
<150 mg/L	26 (68%)	11 (73%)	9 (82%)	P = 0.58[a]
>150 mg/L	12 (32%)	4 (27%)	2 (18%)	
Duration of fever				
1–3 days	26 (67%)	8 (53%)	10 (91%)	P = 0.96[b]
3–7 days	11 (28%)	7 (47%)	0 (0%)	
>7 days	2 (5%)	0 (0%)	1 (9%)	
Fever pattern				
Peaking	11 (28%)	6 (40%)	1 (9%)	P = 0.99
Continuous	6 (15%)	2 (13%)	2 (18%)	
Rapidly normal	22 (57%)	7 (47%)	8 (73%)	
Blood-culture				
Bacterial growth:	13 (33%)	3 (20%)	2 (18%)	P = 0.27[c]
Gram-positive	8 (20%)	3 (20%)	1 (9%)	
Gram-negative	5 (13%)	0 (0%)	1 (9%)	
Fungal growth	0 (0%)	0 (0%)	0 (0%	
No growth	26 (67%)	12 (80%)	9 (82%)	
Sepsis				
Yes	7 (18%)	2 (13%)	1 (9%)	P = 0.73
No	32 (82%)	13 (87%)	10 (91%)	
ICU admission				
Yes	6 (15%)	1 (7%)	0 (0%)	P = 0.23
No	33 (85%)	14 (93%)	11 (100%)	

P-values represent the difference between the high versus the combined medium/low group (N = 65 as 1 genotype is missing). Abbreviations: n.a.: not applicable; ICU: intensive care unit.

a N = 64.

b P-values not exact because >20% of the cells have an expected count <5.

c P-value between bacterial growth versus no growth.

Because not all patients in the high and medium groups had increasing MBL concentrations during febrile neutropenia, we compared haplotypes and clinical parameters of those with increasing MBL concentrations and those without. In the high genotype increasing group, significantly more *HYA/HYA* and *HYA/LYA* haplotypes were found (P = 0.046). In the medium group, all three *LXA/LXA* haplotype patients showed increasing MBL concentrations, whereas 9 out of 11 exon-1 mutation patients did not (P = 0.027) (Table 3). No significant association between increasing MBL concentrations and infection was found in the high or medium group (data not shown).

4. Discussion

In contrast to Neth and colleagues,[12] we did not find an association between MBL deficiency and severity of infection in 66 febrile neutropenic children. Infectious parameters and other clinical signs did not differ between children in the high MBL genotype expression group and children in the medium or low genotype expression group, as was found by others.[16–18] Children with MBL-deficient plasma concentrations (<1000 μg/L) did not show more severe infections either. Because several studies including that of Neth and colleagues [12] used, although arbitrarily chosen, the same cut-off value

(1000 μg/L) this appears to be a good value for studying clinical MBL deficiency in (paediatric) cancer patients.

An explanation for this contradicting result may be that 61% of our patients had a severe neutropenia (<100 cells/μL), while this percentage was probably smaller in the study of Neth and colleagues.[12] In their cohort, 55 children had ALL (for which chemotherapy is given with relatively mild bone-marrow suppressive effects) and the cut-off value for neutropenia was <1000 cells/μL instead of <500 cells/μL. Because neutrophils are required for enhanced phagocytosis after MBL-induced complement activation, the effector functions of MBL are probably severely compromised in neutropenic patients.[16] In patients with a primary phagocytic disorder, such as chronic granulomatous disease, MBL deficiency was also not associated with severe infections.[22] Although analysis of patients with neutrophil counts >100 cells/μL yielded similar results, this small group ($n = 26$) probably lacked sufficient power. Another factor may be that in our study only one febrile neutropenic episode per patient was studied, while the other prospective studies analysed the total number of febrile days of several neutropenic periods.[12,16,17] Different patient groups (with respect to age and chemotherapy regimen) in previous studies may also account for contradicting results.

Surprisingly, all children admitted to the ICU had MBL concentrations >1000 μg/L (RR 1.6, $P = 0.04$), septic shock being the main reason for admission. The reason for this is not clear, but high MBL concentrations might play a role. Possibly, MBL has beneficial effects in inflammation and host defence but host damaging effects in sepsis, just like tumour necrosis factor.[23] ICU-admission may be associated with detrimental MBL depositions or excessive complement-activation by MBL during sepsis. Other studies also reported an association between pathology and normal or elevated MBL concentrations. For instance, MBL deposited in the glomeruli of patients with renal disease and activation of the lectin pathway by high MBL concentrations induced vascular tissue damage in myocardial ischaemia-reperfusion injury and diabetes type 1.[24–27] Besides, deficiency or depletion of MBL and other complement factors prevents complement activation and subsequent systemic inflammation in both patients and animal models.[28,29] However, we did not measure complement activation or cytokines in these patients to support this hypothesis. This might be a focus for further research.

Because MBL is an acute phase protein, one would expect to see a rise in MBL concentrations over time during a febrile neutropenic episode. Indeed, individual MBL concentrations increased during the febrile episode in 36% and 70% of the patients in the medium and high group, respectively. In healthy controls, the influence of promoter polymorphisms and exon-1 mutations has been well defined.[7] Here it is shown that the haplotypes also influence MBL concentrations during the acute phase response. The *HY* haplotype is associated with increasing MBL concentrations, whereas most patients with exon-1 mutations do not show a rise. The MBL concentrations in the low genotype expression group remained <100 μg/L during the whole episode, due to inability to synthesize functional MBL.

In agreement with the conclusion of Klein and Kilpatrick,[30] MBL deficiency appeared not to be associated with infections in this heterogeneous cohort of children undergoing chemotherapy. However, we did show that *MBL2* haplotypes influence MBL increasing capacity during febrile neutropenia, which may confirm lectin pathway activity. Although it was not possible to define specific oncological patient groups that might benefit from MBL supplements, the role of MBL during febrile neutropenia can not be neglected as yet. Children receiving chemotherapy with mild bone-marrow suppressive effects (for instance those with ALL) will usually maintain more than 100 neutrophils/μL. Concomitant granulocyte colony-stimulating factor treatment might even ameliorate the remaining phagocytic activity. Probably, these children will be the most likely to benefit from MBL substitution.

Conflict of interest statement

None declared.

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