Prediction and prevention of infectious complications in children with cancer
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Mannan-Binding Lectin (MBL) serum levels in pediatric oncology patients: a pilot-study

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The Netherlands

Study in progress
Abstract

The febrile neutropenic patient with cancer forms a heterogenous group. A small group of these patients will be at high risk for serious infectious complications. It is thought that Mannan-Binding Lectin (MBL) deficiency as part of the innate immunity could lead to serious infections in this group of patients.

A prospective cohort study was performed in a single pediatric oncology unit genotyping the patients who were expected to become neutropenic. Data were collected on day 1, 3 and 5 of the febrile neutropenic episode correlating the MBL genotype and the MBL serum level to the severity of the infection.

We included 40 children with cancer and of these 24 could be followed during a neutropenic febrile episode. The incidence of MBL exon-1 gene mutations and MBL serum level deficiency (<800 µg/L) was found to be 32.5%. In the deficient group there was no relation found with severity of infection, but there was a trend towards a prolonged duration of neutropenia in the deficient group (p=0.07). A possible explanation is that 70% of patients were severely neutropenic (<100 cell/µL) and 22.5% of patients were relapse patients. Because of the severe neutropenia the effector function of MBL might be severely compromised. Extending the cohort of patients might clarify the significance of the prolonged duration of neutropenia. This will answer the question in which group of patients MBL substitution will be of benefit.
**Introduction**

Although the treatment of pediatric oncology patients has dramatically improved over the past 20 years, infections still play a major role in morbidity and mortality\(^1\). Although many patients experience severe and prolonged neutropenia not all patients suffer from the same complications due to infection during a neutropenic episode. The reasons for this are not clear but low levels of MBL might play a role.

MBL is a serum protein produced in the liver, and plays a critical role in the innate immune response. It is a collaginous lectin with two roles in host defense\(^4\). First, it binds to sugars, in particular N-acetylglucosamine and mannose, on the surface of many different micro-organisms and facilitates their opsonization.

Secondly MBL activates the classical route of the complement system via the so-called lectin route by means of two MBL-associated serine proteases (MASP’s). The result is direct complement mediated lysis and opsonization followed by uptake by phagocytes.

A single functional gene (**MLB**2) at chromosome 10q25 codes for human MBL\(^5\). This **MBL** gene consists of four exons. MBL deficiency is due to structural gene mutations in exon-1 i.e. at codon 52 (D variant), codon 54 (B variant) and codon 57 (C variant). The A variant represents the "wild type" or normal MBL. In addition to the structural gene mutations there are several polymorphisms within the promoter region of the **MBL** gene. The four promoter haplotypes most commonly found are LXP, LYP, LYQ and HYP. The gene mutations are in linkage with the promoter polymorphisms and every individual will express two of the seven possible haplotypes-HYPA, LYQA, LYPB, LXPA, LYPB, LYQC and HYPD\(^6\). Serum levels are dominantly influenced by the 3 mutations in exon 1 and modulated by the recessive promoter region polymorphisms.

The concentration of MBL possessed by an individual is genetically determined by the two haplotypes inherited from the parents. MBL deficiency is thought to be clinically important in patients with co-existing immune-defects, including primary and secondary immune deficiencies. Deficiency of MBL was first identified in children with an opsonization defect\(^10\). Subsequent studies focussed on the role of MBL deficiency in relation with severity of infection. To date 3 prospective studies have been performed in oncology patients, of which one was performed in children\(^17\) and 2 studies only included adult oncology patients\(^12\). Three retrospective studies were performed, 2 in allogenic transplant patients\(^14,15\) and one study including patients with various hematological malignancies\(^16\) (Table 1). However, there is controversy about the results of these studies. In the study of Neth *et al*\(^11\) MBL-deficient pediatric oncology patients were shown to experience longer episodes of febrile neutropenia. Peterslund *et al*\(^16\) and Mullighan *et al*\(^14\) found more severe infections in a group of adult oncology patients. Possibilities are now available to start replacement MBL, with a plasma-derived product, the first clinical applications have shown no adverse effects, no antibody response to MBL, and a biological half life of 5-7 days\(^17\). A prospective study needs to gain insight in the patients who will benefit the most from replacement therapy with MBL.
Table 1: Literature summary

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patient</th>
<th>Malignancy</th>
<th>Duration FN</th>
<th>Freq. Infections</th>
<th>Severity Infections</th>
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</thead>
<tbody>
<tr>
<td>Neth'10</td>
<td>Prospect.</td>
<td>Child N=100</td>
<td>All Malignancies</td>
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<td>Bergmann'11</td>
<td>Prospect</td>
<td>Adult N=80</td>
<td>ANLL</td>
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<td>=</td>
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<tr>
<td>Kilpatrick'12</td>
<td>Prospect</td>
<td>Adult N=128</td>
<td>Hematologic Malignancies</td>
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<td>=</td>
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<tr>
<td>Peterslund ' 13 Retrospect</td>
<td>Adult N=54</td>
<td>Hematologic Malignancies</td>
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<td>=</td>
<td>Sign. more severe inf.</td>
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<td>Mullighan '14 Retrospect</td>
<td>Adult N=97</td>
<td>Allogenic BMT</td>
<td>=</td>
<td>=</td>
<td>Sign. more severe inf.</td>
</tr>
<tr>
<td>Rocha '15 Retrospect</td>
<td>Adult N=107</td>
<td>Allogenic BMT</td>
<td>=</td>
<td>=</td>
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</tbody>
</table>

Patients and methods

Study design

From February 2003 until November 2003 we included pediatric oncology patients admitted to the pediatric oncology unit of the Emma Children’s Hospital in Amsterdam. Patients between one and 18 years of age, who were expected to become neutropenic on base of the chemotherapy given, were considered for inclusion.

Patients admitted for an autologous bone-marrow transplant were excluded.

The study protocol was approved by the local ethics committee. Informed consent from parents and children (>12 years) was obtained.

Procedures

At the start of chemotherapy a blood-sample was taken for MBL-genotyping and the serum level of MBL was determined.

The patient was followed during episodes of febrile neutropenia. Both clinical parameters and laboratory investigations were done on day 1, 3 and 5, and MBL-serum levels were determined on these days. The clinical outcome-parameters included were duration of fever, duration of neutropenia, signs of septicemia, intensive-care admission, and mortality due to infection. The routine laboratory investigations consisted of a full blood count, CRP, liver enzymes and (blood)-cultures. Other investigations such as chest X-ray were included when clinically indicated.

Febrile neutropenia was defined as a single temperature of greater than 38.5°C, and neutropenia was defined as an absolute neutrophil count < 500 cells /mm³. Bacteremia was defined as the presence of clinical signs and symptoms of infection together with the isolation of bacterial pathogens from the blood.
Clinical parameters
Vital parameters (respiration rate, heart rate, blood pressure) and clinical signs were recorded on a case record form. Clinical signs scored included symptoms of illness (signs of sepsis, signs of airway problems, signs of abdominal complaints) and the fever pattern. All clinical signs were scored using the common toxicity criteria (CTC). The investigators who scored the clinical signs were blinded to the MBL genotype and serum level of the patient.

Laboratory investigations
Full blood count, CRP, liver enzymes, creatinine, and blood cultures were all performed, according to standard laboratory procedures.

MBL genotyping
Codon 52Cys, 54Asp and 57Glu on exon 1 were genotyped using the polymerase chain reaction and sequence-specific primers as previously described. In this technique alleles with each of the coding polymorphisms are directly amplified using forward and reverse allele-specific primers. Genotyping was performed independently of clinical data collection.

MBL serum level
Serum 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 5E12, 10 μg/mL). Briefly, microtiter plates were coated with 100μL (10 μg/mL) mannan in 0.1 M NaHCO₃, pH 9.6 overnight at room temperature. The microtiter plates were washed 5 times with H₂O. Serum samples and MBL standards (standard serum, 1.5 μg/mL MBL) were diluted in TTG/Ca (20 mM Tris pH 7.4/150 mM NaCl/0.02% TWEEN-20/0.2% gelatin/10mM CaCl₂), with 10 U/mL heparin for testing serum, and incubated shaking at room temperature for 1 hour. After washing, the plates were incubated for 1 hour with biotinylated MAb5E12 in TTG/Ca and washed 5 times with H₂O and were then incubated at room temperature for 30 minutes with streptavidin pHRP 1:10000 in TBS/Ca/2% (20mM Tris pH 7.4/150 mM NaCl/10 mM CaCl₂/2% milk). After washing, color was developed using tetramethyl-3,3',5,5'-benzidin (TBM)/H₂O₂ in 0.1 M NaAc pH 5.5 and stopped with 2M H₂SO₄ A BioAssay Reader, HTS 7000 plus (Perkin Elmer) measured spectrophotometric absorbances at 405 nm. The cut-off point for MBL deficiency was a plasma concentration of 800 μg/L (i.e. above the lower limit of the 95% CI from 200 control individuals without exon-1 mutations).

Statistics
Patients were classified according to the genotype of MBL and divided in a MBL-sufficient (>800 μg/L) and a MBL-insufficient group (<800 μg/L) The number of days of fever, duration of neutropenia and outcome of the febrile neutropenic episode in patients with structural gene mutations were compared with patients without structural gene mutations by means of the
Mann-Whitney U test, or the chi-square test where appropriate. If the number was less than five in one of the cells we performed a Fisher’s exact test.

Changes in concentrations of MBL in serum during febrile neutropenic episodes were analysed by the paired t-test.

To evaluate the prognostic value of the MBL serum level we used both the cut-off mentioned in the literature (above 800 µg/L) and also an optimal cut-off point selected from our own data, obtained with a receiver-operator characteristic curve (ROC). We used SPSS 11.0 computer software.

Table 2: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total Group N=40</th>
<th>Not febrile group N=16 (Group1)</th>
<th>Febrile neutropenia N=24 (Group 2)</th>
<th>p-value between Group 1 and 2</th>
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<tr>
<td><strong>Age median</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>years</td>
<td>8.7 years</td>
<td>9.7 years</td>
<td>8.1 years</td>
<td>0.22</td>
</tr>
<tr>
<td>(IQR')</td>
<td>(1.8-16.7)</td>
<td>(1.8-16.7)</td>
<td>(1.9-16.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>27 (67.5%)</td>
<td>13 (81.3%)</td>
<td>14 (58.3%)</td>
<td>0.12</td>
</tr>
<tr>
<td>female</td>
<td>13 (32.5%)</td>
<td>3 (18.8%)</td>
<td>10 (41.7%)</td>
<td></td>
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<tr>
<td><strong>Malignancy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hematological</td>
<td>17 (42.5%)</td>
<td>6 (37.5%)</td>
<td>11 (45.8%)</td>
<td>0.42</td>
</tr>
<tr>
<td>solid</td>
<td>23 (57.5%)</td>
<td>10 (62.5%)</td>
<td>13 (54.2%)</td>
<td></td>
</tr>
<tr>
<td><strong>Relapse patients</strong></td>
<td>9 (22.5%)</td>
<td>3 (18.7%)</td>
<td>6 (25.0%)</td>
<td>0.45</td>
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<td><strong>Gene mutation</strong></td>
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</tr>
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<td>AA</td>
<td>21 (52.5%)</td>
<td>8 (50.0%)</td>
<td>13 (54.2%)</td>
<td></td>
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<tr>
<td>AO</td>
<td>10 (25.0%)</td>
<td>3 (18.8%)</td>
<td>7 (29.0%)</td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>3 (7.5%)</td>
<td>2 (12.5%)</td>
<td>1 (4.1%)</td>
<td>0.66</td>
</tr>
<tr>
<td>missing</td>
<td>6 (15.0%)</td>
<td>3 (12.5%)</td>
<td>3 (12.5%)</td>
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</tr>
<tr>
<td><strong>MBL level Day 0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;800 µg/L</td>
<td>13 (32.5%)</td>
<td>6 (40.0%)</td>
<td>7 (29.2%)</td>
<td>0.36</td>
</tr>
<tr>
<td>&gt;800 µg/L</td>
<td>26 (65.0%)</td>
<td>9 (60.0%)</td>
<td>17 (70.8%)</td>
<td></td>
</tr>
<tr>
<td><strong>MBL level Day 0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000 µg/L</td>
<td>15 (37.5%)</td>
<td>6 (37.5%)</td>
<td>9 (37.5%)</td>
<td>0.57</td>
</tr>
<tr>
<td>&gt;1000 µg/L</td>
<td>24 (60.0%)</td>
<td>9 (56.4%)</td>
<td>15 (62.5%)</td>
<td></td>
</tr>
</tbody>
</table>

* 1 MBL serum level day 0 missing, *IQR=interquartile range

Results

Patient characteristics of the total group:

In total, 40 patients were entered in the study. The median age was 8.6 years (IQR 4.3-13.5 years). There were 27 males (67.5%) and 13 females (32.5%). Hematological malignancies were seen in 17 patients (42.5%) (16 patients ALL, 1 patient ANLL) and 23 patients (57.5%) had solid malignancies, of which rhabdomyosarcoma, neuroblastoma and Ewing sarcoma were the most frequent. A total of 9 patients were relapsed patients (table 2).
Mannan-Binding Lectin (MBL) serum levels in pediatric oncology patients

**MBL gene and serum level:**
Of these 40 patients, 34 gene sequences were performed. Twenty one patients (52.5%) were found to have a normal MBL gene, and 13 patients (32.5%) showed exon 1 mutations (Table 2). In 39 patients the MBL-level was done together with the geno typing at a time the patient was not febrile or neutropenic. In 13 patients (32.5%) levels <800 µg/L were found and 26 patients (65%) had levels >800 µg/L. With the use of a ROC curve on our own data the cut-off point was 1000 µg/L. There were 15 patients (37.5%) with a level <1000 µg/L, and 24 patients (60%) with a level >1000 µg/L. The median level of MBL in patients with no gene mutation was 3010 µg/L (IQR 1900-4385 µg/L). In patients with an MBL gene mutation in exon 1 the median level of MBL is 340 µg/L (IQR 90-715 µg/L). As expected, the difference of the MBL serum level between the patients with wild type MBL and the group with exon-1 mutations was significantly different (Mann-Whitney U test p=0.001)(Fig. 1).

![MBL serum level scatter plot](image)

**Figure 1:** Scatter plot representing MBL-serum level in wild-type MBL group and exon-1 mutation group (n=40). All values are plotted, mean is illustrated, p=0.001.

**No febrile neutropenic episode:**
During this study-period there were 16 patients (40%) who became neutropenic during the study-period but did not need to be admitted with a febrile neutropenic episode. In this group, there were 13 males (81.3%) and 3 females (18.8%). Six patients (37.5%) had hematological malignancies (ALL), 10 patients (62.5%) had solid malignancies. Eight patients (50.0%) had a wild-type MBL, 5 patients had exon 1 mutations (31.2%), 3 gene mutations were missing. Six patients (40%) had MBL levels <800 µg/L, and 9 patients (60%) had levels >800 µg/L. Using our own cut-off level the number of patients in each group did not change. There were no significant differences between this group and the group who did experience a febrile neutropenic episode (see Table 2).
Febrile neutropenic episode:

**Patient characteristics:**
Of the 40 patients, 24 were followed prospectively upon admission of the episode of febrile neutropenia (60%). The median age of this group of patients was 5.7 years (IQR 3.9-12.7 years). There were 14 males (58.3%) and 10 females (41.7%). Hematological malignancies were seen in 10 patients (45.8%) (9 patients had ALL, 1 patient ANLL) and 13 patients (54.2%) had solid malignancies (Table 2). Of the patients treated for a febrile neutropenic episode there were 6 relapse patients.

**MBL gene and serum level:**
Of the 24 patients 21 were genotyped. Of these patients 13 had a normal MBL gene (54.2%) and 8 patients had exon-1mutations (33.3%) (Table 2).
Seven patients (29.2%) had MBL levels <800 µg/L, and 17 patients (70.8%) had MBL levels >800 µg/L, measured before the febrile neutropenic episode. Using the cut-off measured with the ROC, 9 patients (37.5%) had MBL-levels <1000 µg/L and 15 patients (62.5%) had MBL-levels >1000 µg/L. Of this group of patients MBL levels were measured prospectively on day 1, 3 and 5 of the febrile neutropenic episode during hospitalization. The MBL levels of MBL-deficient children did not rise during this time period. On day 1 the median MBL level was 370 µg/L (IQR 145-850 µg/L), 485 µg/L (IQR 100-1470 µg/L) on day 3, and 635 µg/L (IQR 120-2317µg/L) on day 5, respectively (t-test for paired samples N.S.). In the MBL-sufficient children there was a slight yet nonsignificant rise in MBL-levels measured between day 1, 3 and 5. On day 1 the median MBL level was 2300 µg/L (IQR 1750-3910 µg/L), 3060 µg/L (IQR 2000-5700 µg/L) on day 3 and 2500 µg/L (IQR 2170-4150 µg/L) on day 5, respectively (N.S.) (Figure 2).

**Severity of the febrile neutropenic episode:**
Of the 24 patients 16 patients (66.6%) had a neutrophil count <100 cells/mm³ (defined as severe neutropenia) on the first day of the febrile neutropenic episode. The median WBC on day 1 was 0.6 x 10⁹/L (IQR 0.2-1.1 x10⁹/L). All patients were started on selective gut decontamination before the onset of neutropenia. At the onset of fever during neutropenia all patients were admitted and started on broad spectrum intravenous antibiotics (either vancomycin or a second generation cephalosporin combined with gentamicin). Concerning the outcome of the neutropenic episode, 5 patients (20.8%) had a positive blood culture (4 Gram-positive organisms and 1 Gram-negative organism), 4 patients (16.6%) were admitted to the intensive care unit, 2 patients (8.3%) died (one patient died because of infectious complications, the other patient developed a severe cardiomyopathy during the induction phase of ANLL treatment).

**Wild-type MBL patients compared with patients with exon-1 mutation:**
The number of patients with a neutrophil count <100 cells/mm³ was not significantly different
between the 2 groups, however, the duration of neutropenia showed a trend towards significance (p=0.07). Even in this small cohort of patients (n=24) there are more patients who experience a longer duration of neutropenia in the exon-1 mutation group (Table 3).

Clinical characteristics (respiratory rate, lung problems, gastrointestinal problems) at the onset of the febrile neutropenic episode were not significantly different between the 2 groups (not shown). The fever pattern (peaking, continuous or rapidly normalising) and the duration of fever (quick recovery (1-3 days), moderate (3-7 days), severe (>7 days)) were not significantly different between both groups (Table 3).

Also the number of positive blood cultures, ICU admissions and death of the patient were not significantly different between both groups (Table 3).

If all analyses were done comparing the MBL-deficient group (MBL<1000 μg/L) to the patients with an MBL level >1000 μg/L, no significant differences were found. There was however a trend that more positive blood cultures were found in the MBL-insufficient group (37.5%) compared to 13.3% in the MBL-sufficient group (chi square, N.S. p=0.29). The same trend was observed comparing the duration of fever in the 2 groups. In the MBL-insufficient group 50% of patients had a duration of fever >3 days, in the MBL-sufficient group this concerned 26.6% (chi square, N.S. p=0.25).

**Laboratory parameters in patients related to the MBL genotype:**

The Hb level, platelets, leucocytes, and liver enzymes were not significantly different between the MBL-deficient and the MBL-sufficient group (not shown).

However, the CRP values on day 1 of the FNE between both groups was significantly different (t-test p=0.015). It was shown that patients with an exon-1 mutation had a significantly lower
Table 3: Comparison of the MBL wild type group and exon-1 mutation group in the febrile neutropenic episode group (n=24)

<table>
<thead>
<tr>
<th></th>
<th>MBL wild type</th>
<th>MBL mutation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time from diagnosis to febrile neutrophil episode</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median 6.6 months</td>
<td>Median 12 months</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Range 0-23</td>
<td>Range 2-19</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Relapse patients</strong></td>
<td>2 (15.4%)</td>
<td>3 (42.8%)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Neutrophil counts/μL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100 cells/μL</td>
<td>9 (69.3%)</td>
<td>5 (71.4%)</td>
<td>0.98</td>
</tr>
<tr>
<td>&gt;100 cells/μL</td>
<td>4 (30.7%)</td>
<td>2 (28.5%)</td>
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</tr>
<tr>
<td><strong>Duration neutropenia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 days</td>
<td>7 (53.8%)</td>
<td>3 (42.8%)</td>
<td>0.07</td>
</tr>
<tr>
<td>5-7 days</td>
<td>6 (46.2%)</td>
<td>4 (57.2%)</td>
<td></td>
</tr>
<tr>
<td>&gt;7 days</td>
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<td><strong>Fever pattern</strong></td>
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<td>Peaking</td>
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<td>1 (14.2%)</td>
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<td>Continuous</td>
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<td>1 (14.2%)</td>
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<tr>
<td>Rapidly normal</td>
<td>7 (54.0%)</td>
<td>5 (71.6%)</td>
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</tr>
<tr>
<td><strong>Duration of fever</strong></td>
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<td></td>
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<tr>
<td>1-3 days</td>
<td>9 (69.2%)</td>
<td>5 (71.4%)</td>
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<tr>
<td>3-7 days</td>
<td>2 (15.4%)</td>
<td>2 (28.6%)</td>
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<td>&gt;7 days</td>
<td>2 (15.4%)</td>
<td>0 (0%)</td>
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<td><strong>Blood-culture</strong></td>
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<td>Gram-positive</td>
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<td>2 (28.5%)</td>
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<tr>
<td>Gram-negative</td>
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<td>0 (0%)</td>
<td>0.69</td>
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<td>5 (71.5%)</td>
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<td><strong>CRP</strong></td>
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<td>&gt;150 mg/L</td>
<td>4 (30.8%)</td>
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<td>&lt;150 mg/L</td>
<td>9 (69.2%)</td>
<td>7 (100%)</td>
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<td><strong>ICU admission</strong></td>
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<td>Yes</td>
<td>4 (30.8%)</td>
<td>0 (0%)</td>
<td>0.13</td>
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<tr>
<td>No</td>
<td>9 (69.2%)</td>
<td>7 (100%)</td>
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<tr>
<td><strong>Death</strong></td>
<td>2 (15.4%)</td>
<td>0 (0%)</td>
<td>0.39</td>
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</table>

* 1 relapse patient genotype missing

CRP (median 50 mg/L IQR 12-86 mg/L) than patients with the wild type MBL genotype (median 93 mg/L IQR 60-210 mg/L Figure 3). This difference did not remain during the total febrile neutropenic episode.

**Discussion**

In this small prospective cohort study in pediatric oncology patients, the incidence of mutations of the MBL exon 1 gene was found to be 32.5% and the incidence of MBL-deficiency (<800 μg/L) was 32.5%. These findings are in agreement with earlier findings of prospective studies in cancer patients.\textsuperscript{11-13} Although the patients with an exon 1 mutation had low serum levels of
MBL, during febrile neutropenic episodes these patients did not have a more severe infections as was found before. There was a trend however to a prolonged duration of neutropenia as was found in the study of Neth et al. All our other findings on severity of infection were in keeping with Bergmann et al and Kilpatrick et al who found that there was no strong relationship between MBL and chemotherapy-related infections. Bergmann et al who studied 80 adult ANLL patients, hypothesized that these patients have such a severe and prolonged neutropenia that the effector function of MBL is severely compromised. The main effector functions of MBL enhance phagocytosis through complement receptors expressed on macrophages, monocytes and neutrophils and through complement activation this in turn will influence the intracellular fate and the inflammatory response. Because of the existing severe neutropenia, the possible lack of MBL may be completely overshadowed. This same result was shown in patients with a primary phagocytic disorder, such as chronic granulomatous disease. These patients also did not show a significant relation between MBL deficiency and severity of infections. The above could well be the explanation for this hemato-oncological cohort of children used. Most of the included patients were not “newly diagnosed” patients but had been on chemotherapy at least several months (median 8 months, range 0-59 months). The early induction phase is the worst period in which MBL-deficiency may be more relevant. Six patients in the febrile neutropenic episode group were relapsed patients who were now experiencing chemotherapy for the second time. Of all patients, 70% presented with a severe neutropenia (<100 cells/mm³). However, we are aware that we are dealing with a small cohort of patients in this study, in which the exon-1 mutations are known but the polymorphisms of the promoter region are not performed as yet. The results of the promoter polymorphisms might change the results, but we do not expect major changes in outcome because of the
strong linkage disequilibrium between the promoter region polymorphisms and exon-1 variants of the MBL2 gene. If we analyzed the results according to a cut-off level found in this cohort of patients (ROC curve level 1000 μg/L) there is a non-significant trend towards more positive blood-cultures in the deficient group (37.5%) compared to the sufficient group (13.3%), and longer duration of fever >3 days in the deficient group (50%) compared to the sufficient group (26.6%). Extending the cohort of patients might clarify the meaning of above results. Power analysis would predict significance if 150 patients were included in the total cohort.

In our cohort of patients the MBL-deficient patients did not show a rise in their MBL serum levels during the febrile episode. The MBL-sufficient patients did show a slight but not significant increase in MBL serum level during the febrile episode. As MBL is known as a protein of the acute phase response one expected to see a rise in MBL levels over time. Consistent with this finding is the fact that the CRP values of the MBL-deficient patients were relatively low (<150 mg/L) at the start of the febrile episode. This cut-off point was chosen because in earlier studies this was shown to be a good predictor of bacteremia’s in patients with cancer. In the MBL-deficient group no ICU admissions were observed and no deaths. This is probably the explanation why no high CRP values was found.

Of course it is known that both MBL and CRP act as acute phase proteins, and bind to specific ligands found on the surface of certain bacteria, and both may separately activate the complement system. MBL activates the complement cascade via the lectin pathway, and CRP activates complement via the classical route, and at the same time inhibits the activation of the alternative pathway. The complement activating functions of CRP and MBL may be coordinated in the acute-phase response. This could be one of the explanations to find low CRP values in the MBL-insufficient group, even though septicemia occurred relatively more often.

These preliminary data in a relatively small cohort of hemato-oncological children does not show significant impact of MBL genotype or serum levels on the chosen end-points. Yet, the data can not exclude that MBL suppletion, starting prior to the occurrence of bone-marrow depression may work by mechanisms and principles different from the end-points studied. Possible ways might still be, prevention or shorter duration of fever in the MBL-deficient patients, shorter duration of neutropenia, limitation of the toxic effects of chemotherapy on neutrophil development resulting in an earlier recovery, and reduction of toxic effects on gastro-intestinal leakage and permeability, thus enhancing barrier function of mucosa.

In sum, these effects may promote the well-being or recovery of the patient at a level different from infectious disease. Of course the success of MBL may be overshadowed by the increased intensity of the chemotherapy regimens during the last 5 years. A prospective trial on MBL substitution will necessitate strict inclusion criteria to determine the benefit of MBL substitution in this group of patients.
Reference List
