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Short Communication

Safety and pharmacokinetics of plasma-derived mannose-binding lectin (MBL) substitution in children with chemotherapy-induced neutropaenia

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ABSTRACT

Mannose-binding lectin (MBL)-deficient children with cancer may benefit from substitution of the innate immune protein MBL during chemotherapy-induced neutropaenia. We determined the safety and pharmacokinetics of MBL substitution in a phase II study in MBL-deficient children.

Twelve MBL-deficient children with cancer (aged 0–12 years) received infusions of plasma-derived MBL once, or twice weekly during a chemotherapy-induced neutropaenic episode (range: 1–4 weeks). Four patients participated multiple times. Target levels of 1.0 μg/ml were considered therapeutic.

In total, 65 MBL infusions were given. No MBL-related adverse reactions were observed, and the observed trough level was 1.06 μg/ml (range: 0.66–2.05 μg/ml). Pharmacokinetics were not related to age after correction for body weight. The half-life of MBL, for a child of 25 kg, was 36.4 h (range: 23.7–66.6 h). No anti-MBL antibodies were measured 4 weeks after each MBL course.

Substitution therapy with MBL-SSI twice weekly was safe and resulted in trough levels considered protective.

1. Introduction

Mannose-binding lectin (MBL) is a collagenous plasma protein that is part of the innate immune system. After binding to sugar residues on the surface of various micro-organisms, it activates the lectin pathway of the complement system through MBL-associated serine proteases (MASPs).[1]

MBL concentrations are genetically determined.[2] MBL is encoded by the *MBL2* gene.[3] In general, individuals with a wild-type (denoted A) *MBL2* gene have MBL levels above

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1.0 μg/ml.[4] Three single nucleotide polymorphisms (SNPs) in codons 52, 54 and 57 of exon-1 of the *MBL2* gene (termed D, B and C, respectively) induce reduced or deficient MBL levels.[1] In addition, three polymorphisms at –550 (termed H/L), –221 (termed Y/X) and –66 (termed P/Q) in the promoter region influence MBL expression.[2] The X variant is associated with reduced MBL levels.[5]

MBL deficiency is associated with increased infection susceptibility, particularly in children and immunocompromised patients.[6,7] Duration and severity of febrile neutropaenia were increased in MBL-deficient children and adults with cancer.[8–10] Therefore, neutropaenic oncology patients were proposed to possibly benefit from MBL substitution. MBL substitution has proven to be safe in phase I trials on both plasma-derived (n = 20) and human recombinant MBL (n = 40) in MBL-deficient adults.[11,12] Therapeutic serum levels of >1.0 μg/ml were reached after infusion of plasma-derived MBL. Peak levels were 1.2–4.5 μg/ml, but the half-life was highly variable with a mean of about 3 d (69.6 h; range: 14.6–114.9 h).[11] Reanalysis of the pharmacokinetic data from this trial with a population pharmacokinetic approach enabled us to design a relatively small phase II study to gather the data required for a future randomised placebo-controlled phase III efficacy study. We performed an open, uncontrolled phase II clinical trial on MBL substitution in 12 MBL-deficient paediatric oncology patients with chemotherapy-induced neutropaenia. In this report, we describe the safety, pharmacokinetics and clinical course of these patients.

2. Material and methods

2.1. Study design and protocol

Between April 2004 and August 2006 a prospective, open, uncontrolled study was performed in 12 children admitted to the oncology unit of the Emma Children's Hospital, Amsterdam, The Netherlands. All parents gave written informed consent in accordance with the Medical Research Involving Human Subjects Act (WMO). The study was conducted according to the declaration of Helsinki and Good Clinical Practice. The protocol was approved by the Local Ethical Committee. Sanquin Plasma Products, Amsterdam, were responsible for monitoring the trial. Trial registration number: NCT00138736.

After the end of a chemotherapy course patients received an MBL infusion, which was repeated twice weekly until patients had recovered from chemotherapy-induced neutropaenia (neutrophil count <500 cells/μl) (Fig. 1). Dosages of 0.2 mg/kg alternated with dosages of 0.3 mg/kg. Patients were allowed to participate more than once. To increase the willingness of patients to participate, the study protocol was changed after the first seven patients. Interim analysis had demonstrated that single MBL infusions would be sufficient to determine pharmacokinetic parameters. Therefore, patients could also participate with only a single infusion followed by an observation period of 3 or 4 d (Fig. 1).

2.2. Patient selection

Participants were children treated for cancer in the Emma Children's Hospital, Amsterdam, The Netherlands. Inclusion

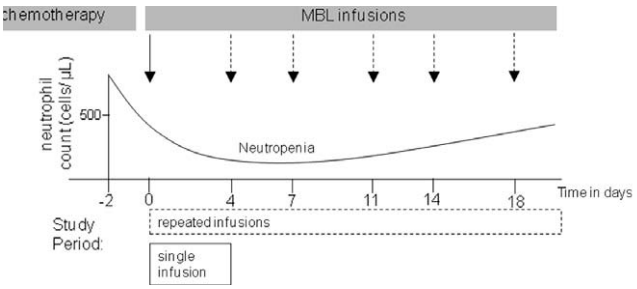

Fig. 1 – MBL treatment regimen. Each patient received a MBL infusion (—) following a neutropaenia-inducing chemotherapy course (day 0). Seven patients received repeated MBL infusions afterwards (- - - -).

criteria were: (1) ⩽12 years of age; (2) mutation in exon-1 of the *MBL2* gene or plasma MBL level <0.10 μg/ml and (3) cancer for which they were treated with chemotherapy expected to induce neutropaenia. Exclusion criteria consisted of the known allergic reactions against human plasma products, participation in other investigational drug studies within the last month and clinically relevant abnormalities in serum immunoglobulins (IgG, IgA and IgM) or complement factors (measured by AP50 and CH50).

The clinical condition of 9 of 34 eligible children did not allow them to participate in this clinical trial, e.g. palliative treatment setting. Parents of 10 patients gave informed consent. The remaining parents (n = 15) refused consent because of the required twice weekly visits to the hospital or because their child had not yet experienced infections during neutropaenia.

Furthermore, two MBL-deficient patients were treated despite violation of the inclusion criteria. One patient had a plasma MBL level of 0.35 μg/ml, but no concomitant exon-1 mutation. Another patient was 15-years-old. He was treated on compassionate grounds during Glivec therapy, by an amendment in the protocol.

2.3. End-points

The primary end-points of our trial were: (1) pharmacokinetics, i.e. determination of the half-life of MBL-SSI and the achievement of plasma trough levels > 1.0 μg/ml, (2) safety, i.e. lack of adverse events and (3) biological efficacy, i.e. reconstitution of MBL-dependent complement activation and opsonophagocytosis *in vitro*. Data on the occurrence and duration of fever and infections, the use of antibiotics/antifungal medication and oxygen and/or immediate circulatory support were recorded. Due to the small number of patients, clinical efficacy was not considered a realistic end-point.

2.4. Data collection

MBL levels were measured before infusion, 15 min (″), 2, 4, 6 and 16–24 h (′) after the first infusion, and before each following MBL infusion. All patients had a central venous catheter (port-a-cath), which was used for MBL infusions and blood sampling. Vital signs (blood pressure, temperature and heart rate) were measured before and after each MBL infusion. Full

blood cell counts, creatinin and liver enzymes were monitored before and 24 h after infusion.

Blood cell products and granulocyte colony-stimulating factor were permitted during the study period. Patients measured their temperature twice daily at home and were hospitalised when fever >38.5 °C developed.

2.5. MBL-SSI production and dosage

SSI produced MBL from a pool of plasma from non-remunerated voluntary Danish donors as described previously.[13] Based on the previously collected data in MBL-deficient patients, adult volunteers and simulation studies, we calculated that administration of 0.2 mg/kg MBL-SSI for a 3-d interval between infusions and 0.3 mg/kg MBL-SSI for a 4-d interval between infusions, would increase MBL serum level to the therapeutic level above 1.0 μg/ml.[11]

2.6. Assays

MBL measurements were performed at Sanquin Research and the Landsteiner Laboratory, AMC, Amsterdam. Genotyping was performed by a Taqman assay, as previously described.[14] For screening purposes, MBL plasma levels were measured by enzyme-linked immunosorbent assay (ELISA) technique as previously described.[14,15] Briefly, mannan was coated to the solid phase and incubated with plasma. Afterwards, biotinylated mouse-anti-MBL (anti-MBL-1, 10 μg/ml, Sanquin) was used as a detection antibody.[15] During the trial, MBL serum levels were determined by the same ELISA at the Department of Immunochemistry, Sanquin Diagnostics, Amsterdam.

2.7. Detection of anti-MBL antibodies

MBL antibodies were assessed by ELISA.[11] In brief, purified human MBL-coated microtitre plates (1 μg/well) were incubated for 2 h at RT with serial dilutions of the patient sera followed by a dilution series of rabbit anti-MBL. After washing, horseradish peroxidase-conjugated rabbit anti-human IgG or swine anti-rabbit immunoglobulins (DakoCytomation, Denmark) were added to the wells and incubated for 1 h. Anti-serum from rabbits immunised with purified human MBL served as a positive reference. If the response of the 1/10 dilution of the patient's sample was below the response of the 1/163,840 dilution of the rabbit anti-MBL serum (the highest dilution showing a positive response), the patient was considered free of anti-MBL activity.

2.8. Pharmacokinetics

For the population pharmacokinetic analysis, NONMEM version VI (GloboMax LLC, Hanover, MD, USA) was used, applying the first order conditional estimation method with an interaction throughout the analysis. An open single compartment model was used. Pharmacokinetic parameters estimated were clearance, volume of distribution and baseline MBL level, which was assumed to be constant during the treatment period.

Precision of the parameters was estimated by using the covariance step of NONMEM. Individual Bayesian parameter values were obtained using the posthoc step of NONMEM. Since from four patients data of several occasions were available, both interindividual and interoccasion variabilities were estimated using proportional models.[16] Residual error was estimated with a proportional error model.

Weight was incorporated into the basic pharmacokinetic model according to allometric scaling.[17] Weight was scaled at 25 kg to provide a relevant estimate of clearance and volume of distribution for a child of 25 kg. For diagnostic purpose, we attempted to evaluate the power estimates of the allometric scaling functions.

Age and gender were included in the pharmacokinetic model on clearance and volume of distribution, and significance was evaluated with the likelihood ratio test (p-value of <0.01). The half-life and mean residence time were estimated from the primary pharmacokinetic parameters.

Model evaluation was based on both numerical and graphical diagnostics. The R-based model building aid Xpose (version 4, Uppsala, Sweden)[18] was used for graphical model diagnostics. The model was evaluated using basic goodness-of-fit plots (e.g. predicted versus observed level and several residual based diagnostics). Furthermore, a case-deletion procedure was executed to evaluate whether the parameter values were driven by a single influential individual. Finally, a visual predictive check was performed.[19]

Since not all patients received MBL infusion during the whole neutropaenic period, the time above 1.0 μg/ml was estimated for each individual assuming that patients received the twice weekly dosing strategy using the individual pharmacokinetic parameters of each patient. Similarly, the trough level and the maximal level were estimated.

Based on the population pharmacokinetic model developed, a simulation study with >10,000 individuals was conducted to investigate whether the proposed dosing strategy would yield adequate MBL substitution. The time above 1.0 μg/ml was estimated.

2.9. Statistical analysis

Continuous variables were presented by descriptive statistics, whereas categorical variables were summarised by frequency counts. Because of the limited number of patients, data were analysed descriptively. The occurrence of (serious) adverse events possibly related to the study drug was described.

3. Results

3.1. Baseline characteristics

Patient characteristics are described in Table 1. The median age of the 12 patients (7 males) was 8.8 years (range: 6 months–15.4 years). The underlying malignancy varied. Median baseline MBL plasma level was 0.40 μg/ml (range: <0.04–1.0 μg/ml).

Each patient received a unique identification letter, and each inclusion a unique identification number. Seven patients (A–G) received repeated MBL infusions, varying from 2 to 8 infusions in total (Table 1, Fig. 2). Five patients (H–L) received single MBL infusions (Fig. 2). Patients C and I were excluded in the per-protocol analysis due to violation of the original inclu-

Table 1 – Patient characteristics.

Patient	Sex	Age	*MBL2* genotype	Tumour	MBL level (μg/ml)	Infusions	Neutropaenic fever
A-01	F	2	HYPA/LYPB	AML	0.87	5	Yes, sepsis
B-02	M	8	LYQA/HYPD	Common ALL	0.66	2	Yes, eci
C-03	M	12	LYQA/LXPA	T cell ALL	0.35	2	Not applicable
D-04	F	1	LYQA/LYPB	Neuroblastoma	0.51	3	Yes, eci
D-08	=	2	=	=	=	4	Yes, eci
D-09	=	2	=	=	=	5	Yes, eci
D-11	=	2	=	=	=	3	Yes, eci
E-05	F	9	LXPA /LYPB	Ewing sarcoma	0.48	4	Yes, eci
F-06	M	1	LXPA/LYPB	B-ALL	0.09	5	No
G-07	M	0	HYPD/HYPD	Pro B-ALL	0.08	8	No
G-13	=	1	=	=	=	2 + 5[a]	No
G-18	=	1	=	=	=	7	Yes, sepsis
H-10	M	10	LXPA /LYPB	T cell lymphoma	0.09	1	Not applicable
I-12	M	15	LXPA/HYPD	Gastro-intestinal stromal tumour	0.47	1	Not applicable
J-14	F	7	LXPA /HYPD	Malign peripheral nerve sheath tumour	0.38	1	Not applicable
J-16	=	7	=	=	=	1	Not applicable
K-15	F	11	LXPA /LYPB	Osteosarcoma	0.13	1	Not applicable
K-17	=	11	=	=	=	1	Not applicable
K-19	=	11	=	=	=	1 + 2[b]	Not applicable
L-20	M	9	LYPA/LYPB	Ewing sarcoma	0.54	1	Not applicable
Total	7M					65	

F, female; M, male; and =, same value as above.

a There was an interval of 9 d between the second and third MBL infusion chemotherapy was given in between.

b Six days after a single MBL infusion, the patient was admitted with neutropaenic fever and MBL infusions were resumed; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; and eci, e causa ignota.

sion criteria. Patient D was included four times, patients G and K three times and patient J two times. In total, 65 MBL infusions were given in 20 MBL courses (trial number 1–20) to 12 patients (A–L).

3.2. *Safety*

No MBL-related serious adverse events were reported during 20 MBL courses. Two patients experienced serious adverse events considered unrelated to the study drug, both 3 d after the last MBL infusion. Patient C developed permanently disabling convulsions and aphasia. The MRI showed two infarctions, as a result of thrombosis, a possible complication of concomitant sepsis or chemotherapy. Patient G experienced an anaphylactic reaction to asparaginase. Two mild, one moderate and two severe MBL-unrelated adverse events (port-a-cath removal) occurred. Only one, mild, adverse event was considered 'possibly' MBL-related. This patient developed a short-lasting temperature of 38 °C without allergic reactions 1 h after the second MBL infusion. No adverse events were suggestive of infusion reactions or resulted in either discontinuation or reduction in the dose of MBL. The MBL infusions did not affect vital signs or laboratory parameters in any of the patients. None of the patients withdrew from the study. No anti-MBL antibodies were detected four weeks after each final MBL infusion.

3.3. *Pharmacokinetics*

Fig. 3 shows MBL level versus time after the first course of MBL infusions in 12 patients. Patients D, G, J and K were included multiple times. In the per-protocol pharmacokinetic analysis, data on all occasions (multiple inclusions) of 10 patients (A–B, D–G and J–L) were simultaneously included. No significant differences in PK parameters within patients treated at different occasions were observed. Table 2 shows the final parameter estimates of the basic model. Estimation of the power coefficients of the allometric scaling did not improve the model. The final estimates of the power coefficients were near the expected values of 0.7 and 1 for clearance and volume of distribution, respectively. Inclusion of interoccasion variability on clearance did not improve the fit, and the estimate of interoccasion variability was not significantly different from 0. Basic goodness-of-fit plots did not reveal any relevant structural model misspecification (data not shown).

As calculated from the primary pharmacokinetic parameters, the half-life of MBL for a typical child of 25 kg was 36.4 h (range: 23.7–66.6 h). Variability was moderate for clearance and volume of distribution (up to 27%). The alternate dosing strategy resulted in an adequate substitution of MBL in the included patients, since the median fraction of time above the threshold of 1.0 μg/ml was 1 (range: 0.8–1.0) for a two-week treatment period (Table 2). Only patient G had a somewhat lower fraction of time above 1.0 μg/ml, but this patient was still above this threshold for 80% of the time. Median trough and maximum MBL levels were 1.1 and 5.8 μg/ml, respectively (Table 2). No significant relation between pharmacokinetics of MBL and sex or age was demonstrated. In the simulation study, the median time above 1.0 μg/ml MBL was over 99%, indicating adequate MBL substitution with the dosing strategy as proposed in the study protocol. After inclusion of patients C and I, the half-life remained similar: 34.0 h (range: 22.2–62.9 h).

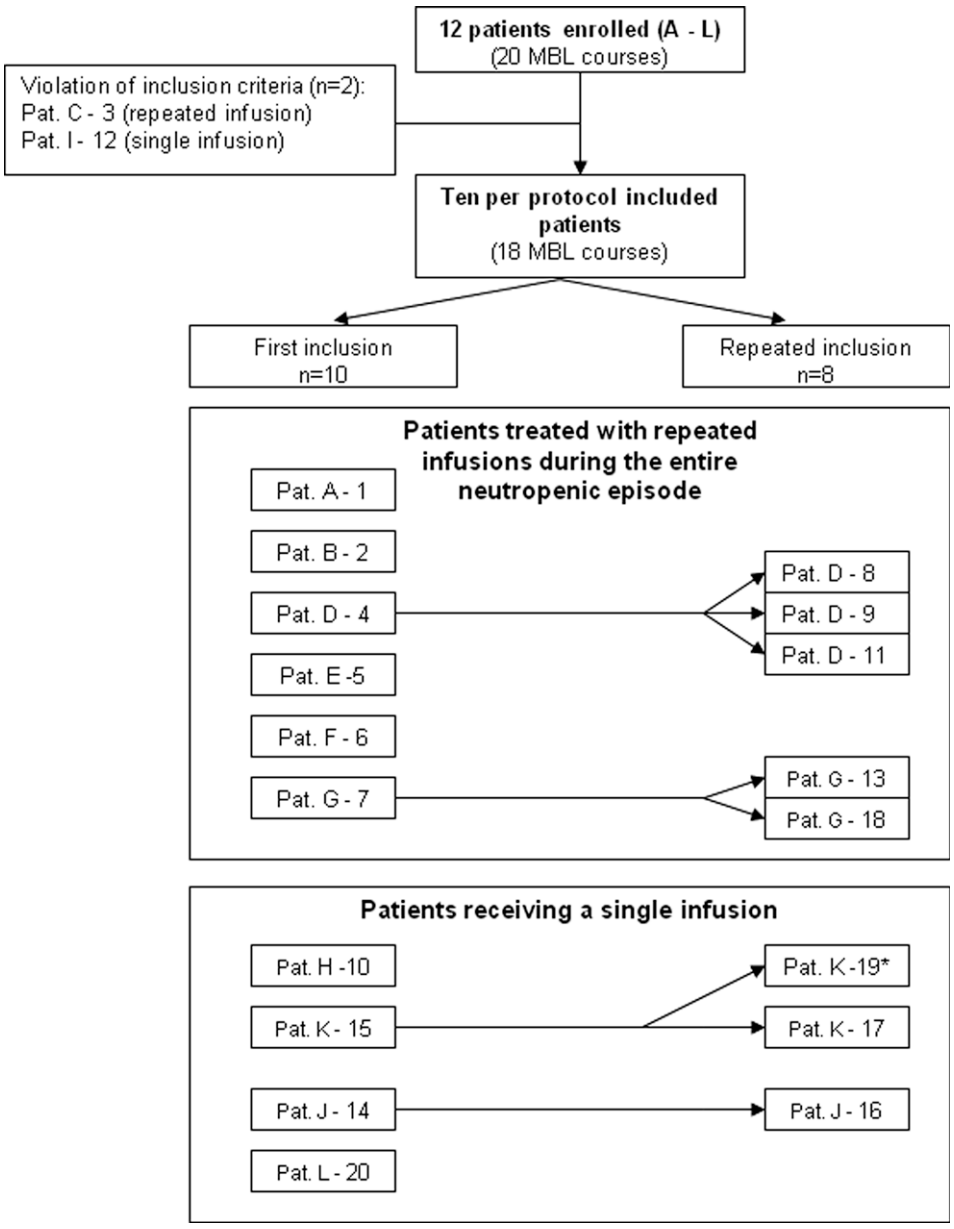

Fig. 2 – Flowchart of included patients. Each individual patient (pat.) is named A, B, etc. Patients D, G, K and J were included more than once. *Patient K-19 initially received a single infusion (0.2 mg/kg) followed by two more repeated infusions after admission due to neutropaenic fever (interval 5 d).

3.4. *Clinical parameters*

Clinical parameters were evaluated in 11 MBL courses of six patients (A–B and D–G), who received repeated infusions during a neutropaenic episode (Fig. 2). In 8 of 11 MBL courses (73%), neutropaenic fever occurred (Table 1). In four patients, fever resolved within 72 h after starting antibiotics (vancomycin/gentamycin and/or ceftazidime) and four needed prolongation or change of antibiotic therapy after persistence of fever. Two patients developed sepsis (positive blood cultures: *Comamonas acidovorans* (A-1) and *Streptococcus mitis* (G-18)) with persistent fever for which their port-a-cath was removed. For the latter infection immediate circulatory support was required. None of the patients needed oxygen support or intensive care during the study. All patients used oral prophylactic antibiotic and antifungal medication (selective gut decontamination consisting of co-trimoxazole, amphotericin or nystatin and colistin or ciprofloxacin).

None of the four patients with one or more single MBL infusions developed fever or infection within 3 or 4 d after the MBL infusion.

4. Discussion

We demonstrated that twice weekly infusions of plasma-derived MBL resulted in therapeutic MBL trough levels in neutropaenic children with cancer. The results of our population pharmacokinetic analyses are in agreement with

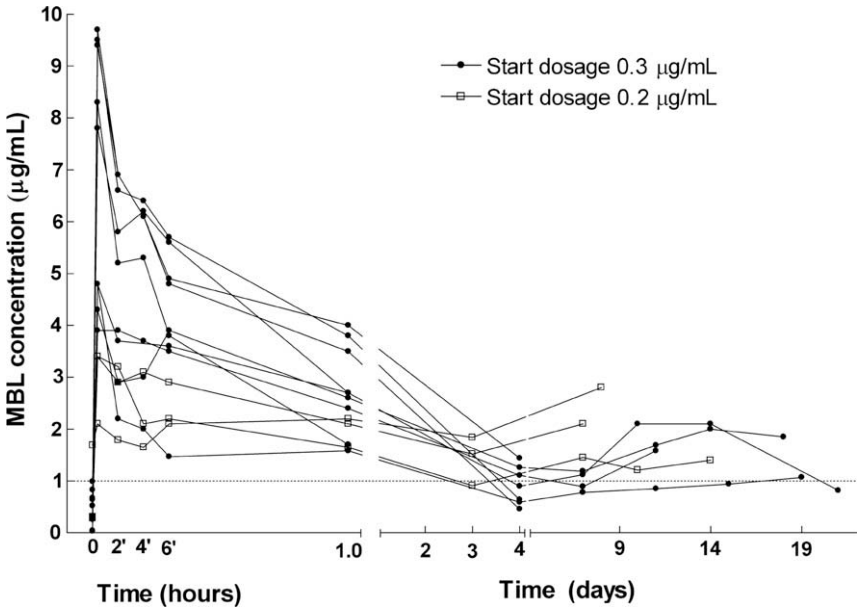

Fig. 3 – Observed MBL concentrations versus time after the first course of MBL infusion(s) of each patient. Three patients started with a dosage of 0.2 mg/kg (□) and nine patients started with a dosage of 0.3 mg/kg (●). After day 4 trough levels of alternate dosages are shown of all patients who received repeated (two or more) infusions of MBL (patients A–G).

Table 2 – Final parameter estimates of the basic pharmacokinetic model of MBL.

PK parameter	Value	IIV (%)	IOV (%)	RSE (%)	Range[d]	
Clearance (l/h)[a]	0.0329	22.1		8.39	0.0122	0.0513
V (l)[a]	1.73	27.5	27.0	14.1	0.736	4.35
Baseline MBL (μg/ml)	3.29	85.7		28.8	0.07	1.12
Weight (kg)	29.8				7.6	43.2
Length (cm)	129				66	152
Mean residence time (h)[b]	52.6				34.1	96.1
$t_{1/2}$ (h)[b]	36.4				23.7	66.6
Fraction of time above 1.0 μg/ml[c]	1				0.804	1
Maximal MBL level (μg/ml)	5.75				4.89	7.59
Trough MBL level (μg/ml)	1.06				0.66	2.05

IIV = interindividual variability, IOV = interoccasion variability, RSE = relative standard error of estimate, V = volume of distribution, and $t_{1/2}$ = half-life.

a Typical values reported for a patient with a body weight of 25 kg.

b Secondary parameters are calculated from primary parameters, therefore, no parameter precision can be reported.

c Median value of individual estimates provided.

d Range of individual values as obtained from Bayesian estimation.

the retrospective population pharmacokinetic analysis of MBL in 20 healthy MBL-deficient adults.[11] The clearance we reported for a patient of 25 kg translates into a clearance of 0.071 l/h for a 75 kg patient, which is close to the estimate we obtained from adults (0.052 l/h), keeping in mind that both study populations were relatively small. The half-life of 36 h is also close to the half-life in adults of 45 h, as calculated from the results of our pharmacokinetic analysis. According to allometric scaling, pharmacokinetics were not related to age after correction for body weight. This indicates that calculation of the optimal dose based on the body weight is a reasonable strategy for MBL substitution in MBL-deficient patients. In the simulation study, the proposed dosing strategy led to adequate MBL substitution.

The half-life range in our trial is smaller (23.7–66.6 h) than in the phase I trial in healthy adults and is closer to that observed with recombinant MBL, i.e. approximately 30 h,[11,12] which can be explained by weight-adjusted dosing. Plasma-derived MBL differs from recombinant MBL because it contains MASPs and higher degree of oligomerisation.[13,20]

In agreement with the experience in adults,[11,12,21] the infusion of plasma-derived MBL appears to be safe in children. Dosages up to 14 mg can be injected within 10 min without any adverse effects. The only adverse event possibly related to the study drug was probably caused by a viral upper respi-

ratory tract infection. The absence of anti-MBL antibodies up to one year after MBL substitution suggests that no immune response against the plasma-derived MBL is initiated.

Interestingly, most of the patients that received repeated MBL infusions developed neutropaenic fever and two even sepses, despite MBL trough levels > 1.0 μg/ml. Although the number of patients is too small to determine the clinical efficacy, these observations should not be neglected. MBL substitution appeared to be beneficial in case reports and pre-clinical studies in knock-out mice,[22,23] but it has not been tested in a controlled trial in neutropaenic cancer patients previously. MBL substitution has been proposed to be beneficial in these patients, because in a number of studies the duration and severity of febrile neutropaenia were increased in MBL-deficient as compared to MBL-sufficient children and adults.[8–10] However, infection frequency or severity was not increased in more recent studies.[14,24,25] This variation may be related to the depth and duration of the chemotherapy-induced bone-marrow suppression,[14,24] disabling an optimal phagocytic function in the host, necessary for MBL-induced opsonophagocytosis. In this regard, neutropaenic cancer patients may not be the suitable target group for MBL substitution. Also, the target level of 1.0 μg/ml may be too low. Since the two youngest patients did not develop neutropaenic fever, we can speculate that there is an age-dependent effect. Based on the available cohort studies in neutropaenic patients and this trial, MBL substitution therapy may be more suitable in other paediatric patient groups, such as neonates or children with sepsis or recurrent (airway) infections.[21,26,27]

In sum, we have demonstrated that therapeutic MBL trough levels can be predicted and attained with twice weekly infusions with plasma-derived MBL in children with cancer. Repeated MBL substitution treatment appears to be safe. The pharmacokinetics of MBL in MBL-deficient children are comparable to adults, after correction for body weight. The half-life of MBL was estimated to be 36 h with a smaller range than in adults. After definition of a suitable target patient group, clinical efficacy of MBL should be investigated in multicentre phase III clinical trials.

Conflict of interest statement

G. Houen and I. Laursen work for the Plasma Products Department of the Statens Serum Institut (Denmark), where the plasma-derived MBL was produced. The study was performed and written by doctors and researchers of the Emma Children's Hospital, Amsterdam, The Netherlands. They do not have a conflict of interest to declare.

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