Swiss Medical Weekly
Formerly: Schweizerische Medizinische Wochenschrift
The European Journal of Medical Sciences

Original article | Published 29 April 2011, doi:10.4414/smw.2011.13191
Cite this as: Swiss Med Wkly. 2011;141:w13191

Mannan-binding lectin (MBL) and MBL-associated serine protease-2 in children with cancer

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Summary

QUESTIONS UNDER STUDY: Mannan-binding lectin (MBL) and MBL-associated serine protease-2 (MASP-2) are two key components of the lectin-pathway of complement-activation. Information on the potential role of lectin-pathway components in carcinogenesis versus immune surveillance of cancer is scarce. This study aimed to determine if serum concentrations of MBL and MASP-2 differ between children with cancer and healthy age-matched controls.

METHODS: In this retrospective multicentre study, MBL and MASP-2 were measured by commercially available ELISA in frozen remnants of serum taken at diagnosis in paediatric patients with cancer. For six diagnostic groups, these concentrations were compared with serum concentrations of age-matched healthy controls using exact Wilcoxon signed-rank tests.

RESULTS: MBL and MASP-2 were measured in serum of 372 patients. MBL was significantly higher in patients with solid tumours vs. controls (median, 2,799 vs. 1,917 µg/L; $P = 0.008$), and MASP-2 was significantly higher in patients with acute lymphoblastic leukaemia (406 vs. 317 µg/L; $P = 0.009$), Non-Hodgkin lymphoma (361 vs. 293 µg/L; $P = 0.037$) and CNS tumors (463 vs. 296 µg/L; $P = 0.002$).

CONCLUSIONS**:** These results may indicate a role of MBL and MASP-2 in the initiation or progression of specific paediatric cancers, while other mechanisms remain possible as well. Larger, disease-specific studies are warranted for confirmation and for elucidation of the underlying mechanisms.

Key words: *cancer; innate immunity; lectin pathway of complement activation; mannan-binding lectin (MBL); MBL-associated serine protease-2 (MASP-2)*

Introduction

In contrast to adaptive immunity and to certain components of innate immunity, only little is known on the influence of the complement system on cancer suppression and promotion [1, 2]. The lectin pathway of complement activation is an important part of innate immunity. It is mainly involved in opsonisation and lysis of pathogens and in recruitment of inflammatory cells. Mannan-binding lectin (MBL) binds mannose and N-acetylglucosamine structures on the surface of pathogens and builds complexes with MBL-associated serine protease (MASP-2). Bound to MBL or ficolins, MASP-2 activates the complement system cascade by generating the C3 convertase C4bC2b [3]. Due to single nucleotide polymorphisms, deficiencies of MBL and MASP-2, two key components of the lectin pathway, are frequent in Caucasians, with an incidence of about 10% [3]. In adults, such deficiencies or the underlying genotypes seem to be associated with the incidence, or the prognosis, of different types of carcinoma both for MBL [4–8] and MASP-2 [9, 10]. In young children, where adaptive immunity is still maturing, innate immunity may play a more important role in carcinogenesis or immunosurveillance. Despite this, nearly nothing is known on components of the lectin pathway in children with cancer. We are aware of only one exception, a study on MBL genotypes in children with acute lymphoblastic leukaemia (ALL), who were found to have an increased frequency of low-producing MBL genotype compared to healthy adults [11]. The role of MASP-2, and of MBL phenotypes, in children with cancer versus healthy controls has not yet been studied.

This study aimed to explore if serum concentrations of MBL and MASP-2 differ between children with cancer and healthy age-matched controls.

Methods

Patients

In this retrospective multicentre cohort study, patients diagnosed below 18 years of age, and between January 1, 2002 and December 31, 2006, at one of four participating paediatric oncology centres (Bern, Bonn, Geneva, Zurich) with a malignancy requiring chemotherapy were investigated. Patients with relapsed or second malignancies and those in which serum was not accessible were excluded. Required was ≥100 µL serum taken within two weeks of diagnosis and before starting chemotherapy. This study was approved by the respective Institutional Review Boards.

Characteristics of patients and malignancies [12] were extracted from patient charts. Diagnoses were classified into six groups: ALL, acute myeloid leukaemia, Hodgkin's disease, non-Hodgkin lymphoma (NHL), tumour of the central nervous system (CNS; hereafter called CNS-tumour), and solid tumour outside the CNS (hereafter called solid tumour).

Determination of MBL and MASP-2 serum concentrations

Remnants of patients' serum which had been taken for clinical purposes at time of diagnosis were stored at -20 °C until analysis. MBL and MASP-2 serum concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. The minimal detection limit was 2 µg/L for MBL (MBL oligomer ELISA kit, Antibodyshop, Gentofte, Denmark) and 6 µg/L for MASP-2 (HK326, HyCult, Uden, The Netherlands). The serum usually underwent two freeze/thaw cycles, for measurement of MBL and MASP-2, respectively. Additional freeze/thaw cycles were needed if the first ELISA result was not valid, because it was below or above the calibration range, or because the coefficient of variation of the duplicate determinations exceeded the limit defined in the manufacturer's instructions.

Controls and matching process

MBL and MASP-2 serum concentrations in children vary substantially with age [13, 14], whereas adult serum concentrations are considered to be stable over time [3, 15]. Thus, patients were age-matched with healthy children (hereafter called controls) in whom MBL and MASP-2 serum concentrations had been determined using the same ELISA kits [14]. These children had had outpatient consultations at the Infectious Diseases Clinic, Department of Paediatrics, University of Bern, Switzerland. They had been found not to have any relevant acute or chronic infection in the last three months before consultation, and their C-reactive protein, if determined for clinical reasons, was below 30 mg/L [14].

Matching, allowing for a relative age difference of 20% between patients and controls, was performed by one of the authors (UP.F.) who was blinded for MBL and MASP-2 serum concentrations. Because of the relatively low number of controls available, the same control could be matched to two patients within each diagnostic group.

Statistical analysis

Exact Fisher's and Fisher-Freeman-Halton tests were used to compare demographic characteristics. Within each diagnostic group, median differences of MBL and MASP-2 between patients and controls were calculated, together with their exact Hodges-Lehmann 95% confidence intervals (CI), and exact Wilcoxon signed-rank test statistics. Sensitivity analyses were performed in diagnostic groups where some controls had been matched to two patients by randomly eliminating one of the respective two patient-control pairs. Because of heterogeneity of diagnoses, we deliberately refrained from comparing MBL and MASP-2 serum concentrations between all patients and controls. Because of the exploratory nature of this study, we did not apply corrections for the multiple comparisons performed.

Two-sided test were used throughout. *P*-values <0.05 were considered significant. The analyses were performed using StatXact 8.0 (Cytel Software Corp., Cambridge, MA).

Results

Patients

Of 718 paediatric malignancies potentially eligible for the study, 37 (5.2%) relapsed and 1 (0.1%) second malignancy were excluded. Of the 680 patients with a non-relapsed first malignancy, 308 (43%) were excluded because sufficient serum was not accessible. While sex and age were not significantly associated with inclusion versus exclusion, patients with CNS-tumors were less often included into the study than those with other malignancies (46 of 127, 36%, versus 326 of 591, 55%; table 1; further details of this cohort have been described elsewhere [16].). Finally, 372 (52%) patients were included in the study. Their median age at diagnosis was 7.4 years (range, 0.05 to 17.8; interquartile range (IQR), 3.4 to 12.8), and 157 (42%) were female.

The median MBL serum concentration of patients at time of diagnosis was 2,808 µg/L (range, 2 to 10,060; IQR, 620 to 4,062). The median MASP-2 serum concentration was 391 µg/L (range, 46 to 2,771; IQR, 247 to 591). Sex and duration of serum storage were not significantly associated with MBL and MASP-2 [16].

Controls

The median age of 120 controls available for matching was 3.4 years (range, 0.06 to 14.7, IQR, 1.2 to 7.2), 62 (52%) of them were female. While the sex distribution was thus comparable between controls and patients studied, the controls had a significantly different age distribution (table 1).

The median serum concentration of controls was 2,110 µg/L (range, 8 to 7,957; IQR, 441 to 3,389) for MBL, and 334 µg/L (range, <12.5 to 1,980; IQR, 231 to 489) for MASP-2. The intended age range for matching could always be respected. In the three largest diagnostic groups (ALL, CNS-tumors, solid tumours), controls were used for matching of two patients in ≤28% of patients.

MBL serum concentration in patients versus controls

In all six diagnostic groups, the median MBL serum concentration was higher in patients than in their age-matched

controls. This difference was significant only for patients with solid tumours ($P = 0.008$), which was confirmed in sensitivity analysis (table 2).

MASP-2 serum concentration in patients versus controls

In all six diagnostic groups, the median MASP-2 serum concentration was higher in patients than in their age-matched controls. This difference was significant in patients with ALL ($P = 0.009$), NHL ($P = 0.037$), and CNS-tumors ($P = 0.002$). These differences were confirmed in sensitivity analyses for ALL and CNS-tumors, while no sensitivity analysis was required for NHL (table 3).

Table 1: Characteristics of patients and controls.

	Patients potentially eligible, n = 718				
Characteristic	Excluded*, n = 346	Studied*, n = 372	*P*-value†	Controls, n = 120	*P*-value‡
Sex			0.49		0.073
Female	137 (40%)	157 (42%)		62 (52%)	
Male	209 (60%)	215 (58%)		58 (48%)	
Age at diagnosis			0.12		<0.001
<4 years	107 (31%)	120 (32%)		63 (53%)	
≥4 and <8 years	87 (25%)	79 (21%)		31 (26%)	
≥8 and <12 years	68 (20%)	58 (16%)		14 (12%)	
≥12 years	84 (24%)	115 (31%)		12 (10%)	
Diagnostic group			0.002		-
Acute lymphoblastic leukemia	82 (24%)	114 (31%)		-	
Acute myeloid leukaemia	24 (7%)	29 (8%)		-	
Hodgkin's disease	20 (6%)	23 (6%)		-	
Non-Hodgkin lymphoma	25 (7%)	41 (11%)		-	
Tumour of CNS	81 (23%)	46 (12%)		-	
Tumour outside CNS	114 (33%)	119 (32%)		-	

* Excluded were 37 patients with relapsed malignancies, 1 with second malignancy, and 308 because of missing serum; † *P*-values from exact Fisher's and Fisher-Freeman-Halton tests, comparison of excluded versus studied patients; ‡ *P*-values from exact Fisher's and Fisher-Freeman-Halton tests, comparison of studied patients versus controls.

Table 2: MBL Serum concentration in patients and age-matched controls.

Diagnostic groups	Number of patients	Median (IQR) concentration [µg/L]		Difference between patients and controls		
		Patients	Controls	Median	Exact 95% CI*	*P*-value†
Acute lymphoblastic leukaemia	114	2,976 (760 to 4,130)	2,423 (441 to 3,577)	473	–38 to 1,026	0.07
ALL sensitivity analysis‡	*82*	*2,976 (797 to 4,342)*	*2,150 (409 to 3,434)*	*680*	*15 to 1,338*	*0.05*
Acute myeloid leukemia	29	2,230 (414 to 3,532)	2,016 (677 to 2,858)	154	–863 to 1,199	0.78
Hodgkin's disease	23	3,323 (483 to 4,003)	2,445 (932 to 3,209)	870	–462 to 2,307	0.15
Non-Hodgkin lymphoma	41	2,649 (388 to 3,902)	2,445 (874 to 3,367)	200	–708 to 1,284	0.70
CNS-tumour	46	2,004 (459 to 3,817)	1,985 (441 to 3,091)	397	–468 to 1,414	0.37
CNS-tumour sensitivity analysis‡	*43*	*2,073 (506 to 3,969)*	*2283 (483 to 3,153)*	*458*	*–547 to 1,471*	*0.41*
Solid tumour outside CNS	119	2,799 (790 to 4,126)	1,917 (450 to 3,339)	753	200 to 1,230	0.008
Solid tumour sensitivity analysis‡	*89*	*2,799 (1,002 to 4,197)*	*1,785 (383 to 3,199)*	*808*	*202 to 1,401*	*0.009*

* Hodges-Lehmann exact 95% confidence interval; † two-sided exact Wilcoxon signed-rank test; ‡ by randomly eliminating one of the respective two patient-control pairs when controls had been matched to two patients. No sensitivity analysis was made for groups in which no control had been matched to two patients, i.e., acute myeloid leukemia, Hodgkin disease, and Non-Hodgkin lymphoma.
Abbreviations: MBL, mannan-binding lectin; 95% CI, 95% confidence interval; ALL, acute lymphoblastic leukemia; CNS, central nervous system; IQR, interquartile range.

Table 3: MASP-2 serum concentration in patients and age-matched controls.

Diagnostic groups	Number of patients	Median (IQR) concentration [µg/L]		Difference between patients and controls		
		Patients	Controls	Median	Exact 95% CI*	*P*-value†
Acute lymphoblastic leukaemia	114	406 (282 to 608)	317 (194 to 517)	95	24 to 157	0.009
ALL sensitivity analysis‡	*82*	*471 (298 to 633)*	*323 (211 to 514)*	*126*	*48 to 201*	*0.002*
Acute myeloid leukaemia	29	356 (241 to 591)	325 (235 to 453)	52	–48 to 197	0.35
Hodgkin's disease	23	365 (211 to 466)	290 (171 to 364)	49	–118 to 210	0.64
Non-Hodgkin lymphoma	41	361 (205 to 646)	293 (182 to 437)	131	8 to 277	0.037
CNS-tumour	46	463 (268 to 676)	296 (192 to 376)	178	72 to 295	0.002
CNS-tumor sensitivity analysis‡	*43*	*459 (269 to 673)*	*317 (201 to 415)*	*159*	*55 to 269*	*0.005*
Solid tumour outside CNS	119	380 (236 to 552)	312 (192 to 453)	53	–11 to 117	0.10
Solid tumor sensitivity analysis‡	*89*	*382 (243 to 556)*	*325 (203 to 548)*	*34*	*–43 to 110*	*0.39*

* Hodges-Lehmann exact 95% confidence interval. † two-sided exact Wilcoxon signed-rank test; ‡ by randomly eliminating one of the respective two patient-control pairs when controls had been matched to two patients. No sensitivity analysis was made for groups in which no control had been matched to two patients, i.e., acute myeloid leukemia, Hodgkin disease, and Non-Hodgkin lymphoma.
Abbreviations: MASP-2, mannan-binding lectin-associated serine protease-2; 95% CI, 95% confidence interval; ALL, acute lymphoblastic leukaemia; CNS, central nervous system; IQR, interquartile range.

Discussion

In this study, children with malignancies of all diagnostic groups had higher median MBL and MASP-2 serum concentrations compared to age-matched controls. For MBL, the differences were significant in patients with solid tumours, and marginally for those with ALL. For MASP-2, the differences were significant in patients with ALL, NHL and CNS-tumours.

Regarding MBL, these results contrast with the finding that children with ALL have more frequently low-producing genotypes of the *MBL2* gene compared to healthy adults [11]. Methodological differences, i.e., genotype versus phenotype, may at least partially explain this discrepancy. We do not know of further reports on MBL in children with cancer versus controls. In line with our results, MBL was found to be higher in adult patients with colorectal cancer [4] compared to healthy adults, and MBL was higher in women with tumours of the reproductive system compared to healthy controls with corresponding genotypes [6].

Regarding MASP-2, this is - to the best of our knowledge – the first study comparing MASP-2 serum concentrations in paediatric patients with malignancies versus age-matched controls. In adult patients with colorectal cancer, higher MASP-2 serum concentrations than in healthy adults have as well been described [9]. Notably, high MASP-2 was associated with poor prognosis in adult colorectal cancer patients [9]. In contrast, high MASP-2 at diagnosis has been reported to be associated with a better prognosis in children with acute leukaemia and lymphoma [16].

This study cannot answer why MBL and MASP-2 serum concentrations in children with certain malignancies are higher than in controls. Several mechanisms are conceivable. First, individual MBL or MASP-2 concentrations might facilitate or hinder the development of malignancies. Specifically, both high MBL and MASP-2 might act via increased complement factor C5a in the tumour microenvironment, which has been shown to enhance tumour growth in a mouse model of cervical cancer [17]. Second, MBL – but not MASP-2 – is an acute-phase component [18, 19] and might be increased during infections. Infections, however, are frequent at diagnosis of leukemia, but not of solid tumors [20], which makes this hypothesis less plausible. Third, MBL or MASP-2 might be increased as part of the immunological response to malignancies themselves. Fourth, MBL or MASP-2 might be actively expressed by malignant cells, as has been shown for oesophageal squamous cell carcinoma cells [10].

This study has several limitations. First, a selection bias due to non-random availability of serum from diagnosis might be possible. Such a bias could be excluded for sex and age, but was confirmed for patients with CNS-tumors. This bias did not distort the results in a relevant way, because the analyses were performed separately for each diagnostic group. Second, this study focused only on phenotype (serum concentration), not genotype. Third, controls were partially used for matching more than once, but the sensitivity analyses addressing this issue confirmed the main results. Despite the significant differences in age distribution between patient and controls, it was possible to always respect the predefined maximum relative age difference of 20% between patients and controls for matching. The strengths of this study include the broad spectrum of diagnoses, reflecting current epidemiological data [21], the relatively large number of paediatric oncology patients from four independent centers, and the use of age-matched controls.

In conclusion, MBL serum concentration was significantly higher in pediatric patients with solid tumours compared to age-matched healthy controls, as was MASP-2 serum concentration in patients with ALL, NHL and CNS-tumours in this study. These results may indicate a role of MBL and MASP-2 in the initiation or progression of specific paediatric cancers, while other mechanisms remain possible as well. Larger, disease-specific studies are warranted for confirmation and for elucidation of the underlying mechanisms.

The authors thank A. Wasem (RN, Bern), N. Beusch (RN, Bern), and G. Wisznievsky (CRA, Bonn) for their help in data extraction from patient charts; the Institute for Infectious Diseases, University of Bern, Switzerland, and the Institute for Infectious Diseases, University of Zurich, Switzerland for providing us with serum samples; M. Otth in the laboratory of Prof. C. Aebi, MD, in Bern for performing of and instruction on ELISA measurements, and S. Sallenbach for kindly providing us with information on controls.

Study funding/potential competing interests

This study was supported by an unrestricted research grant from the Prof. E. Rossi Foundation for Research in Pediatrics, University Children's Hospital, Bern, Switzerland. No conflict of interest was declared by any of the authors.

References

1 Finn OJ. Cancer immunology. N Engl J Med. 2008;358:2704–15.

2 Markiewsky MM, Lambris JD. Is complement good or bad for cancer patients? A new perspective on an old dilemma. Trends Immunol. 2009;30:286–92.

3 Thiel S. Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. Mol Immunol. 2007;44:3875–88.

4 Ytting H, Jensenius JC, Christensen IJ, Thiel S, Nielsen HJ. Increased activity of the mannan-binding lectin complement activation pathway in patients with colorectal cancer. Scand J Gastroenterol. 2004;39:674–9.

5 Baccarelli A, Hou L, Chen J, Lissowska J, El-Omar EM, Grillo P, et al. Mannose-binding lectin-2 genetic variation and stomach cancer risk. Int J Cancer. 2006;119:1970–5.

6 Swierzko AS, Florczak K, Cedzyński M, Szemraj J, Wydra D, Bak-Romaniszyn L, et al. Mannan-binding lectin (MBL) in women with tumours of the reproductive system. Cancer Immunol Immunother. 2007;56:959–71.

7 Bernig T, Boersma BJ, Howe TM, Welch R, Yadavalli S, Staats B, et al. The mannose-binding lectin (MBL2) haplotype and breast cancer: an association study in African-American and Caucasian women. Carcinogenesis. 2007;28:828–36.

8 Wang FY, Tahara T, Arisawa T, Shibata T, Yamashita H, Nakamura M, et al. Mannan-binding lectin (MBL) polymorphism and gastric cancer risk in Japanese population. Dig Dis Sci. 2008;53:2904–8.

9 Ytting H, Christensen IJ, Thiel S, Jensenius JC, Nielsen HJ. Serum mannan-binding lectin-associated serine protease 2 levels in colorectal cancer: relation to recurrence and mortality. Clin Cancer Res. 2005;15:1441–6.

10 Verma A, Matta A, Shukla NK, Deo SV, Gupta SD, Ralhan R. Clinical significance of mannose-binding lectin-associated serine protease-2 expression in esophageal squamous cell carcinoma. Int J Cancer. 2006;15:2930–5.

11 Schmiegelow K, Garred P, Lausen B, Andreassen B, Petersen BL, Madsen HO. Increased frequency of mannose-binding lectin insufficiency among children with acute lymphoblastic leukemia. Blood. 2002;15:3757–60.

12 Steliarova-Foucher E, Stiller CA, Lacour B, Kaatsch P. International classification of childhood cancer, third edition. Cancer. 2005;103:1457–67.

13 Aittoniemi J, Miettinen A, Laippala P, Isolauri E, Viikari J, Ruuska T, Soppi E. Age-dependent variation in the serum concentration of mannan-binding protein. Acta Paediatr. 1996;85:906–9.

14 Sallenbach S, Thiel S, Aebi C, Otth M, Bigler S, Jensenius JC, et al. Serum concentrations of lectin-pathway components in healthy neonates, children and adults: mannan-binding lectin (MBL), M-, L-, and H-ficolin, and MBL-associated serine protease-2 (MASP-2). Pediatr Allergy Immunol. (in press, DOI 10.1111/j.1399-3038.2010.01104.x)

15 Ytting H, Christensen IJ, Thiel S, Jensenius JC, Svendsen MN, Nielsen L, et al. Biological variation in circulating levels of mannan-binding lectin (MBL) and MBL-associated serine protease-2 and the influence of age, gender and physical exercise. Scand J Immunol. 2007;66:458–64.

16 Zehnder A, Fisch U, Hirt A, Niggli FK, Simon A, Ozsahin H, Schlapbach LJ, Ammann RA. Prognosis in pediatric hematologic malignancies is associated with serum concentration of mannose-binding lectin-associated serine protease-2 (MASP-2). Pediatr Blood Cancer. 2009;53:53–7.

17 Markiewski MM, DeAngelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, Gerard C, et al. Modulation of the antitumor immune response by complement. Nat Immunol. 2008;9:1225–35.

18 Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol. 1992;90:31–5.

19 Herpers BL, Endeman H, de Jong BA, de Jongh BM, Grutters JC, Biesma DH, van Velzen-Blad H. Acute-phase responsiveness of mannose-binding lectin in community-acquired pneumonia is highly dependent upon MBL2 genotypes. Clin Exp Immunol. 2009;156:488–94.

20 Schlapbach LJ, Aebi C, Otth M, Ridolfi Luethy A, Leibundgut K, Hirt A, Ammann RA. Serum levels of mannose-binding lectin and the risk of fever in neutropenic pediatric cancer patients. Pediatr Blood Cancer. 2007;49:11–6.

21 Gurney JG, Bondy ML. Epidemiology of childhood cancer. In: Pizzo PA, Poplack DG, editors. Principles and practice in pediatric oncology, 5th ed. Philadelphia: Lippincott; 2006. p 1–13.