

## Relationship between mannose-binding lectin and febrile neutropenia in acute leukemia patients

Relationship between mannose-binding lectin and febrile neutropenia

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### Abstract

**Aim:** Mannose-binding lectin (MBL) is an important component of the natural immune system. Its low levels have been linked to increased frequency of opportunistic infections. This study aimed to determine the association of serum MBL levels and duration of febrile neutropenia (FN) after cytotoxic chemotherapy in patients with acute leukemia. **Material and Methods:** Seventy patients aged 15-75 years with acute leukemia (40 AML (Acute myeloid leukemia), 30 ALL (Acute lymphoblastic leukemia)), and 30 age-matched healthy subjects were included in this study. Blood MBL levels were measured using ELISA Kit before chemotherapy (MBL1) and at FN (MBL2). **Results:** The MBL1 levels of the patient group (Median 466 ng/ml; interquartile range (iqr): 4507) were higher than that of the control group (Median 485 ng/ml; iqr: 1872), but this difference was statistically insignificant ( $p=0.92$ ). During FN, MBL levels of 49 (70%) patients increased and 21 (30%) patients remained at the same level. The MBL2 levels (Median 772 ng/ml; iqr: 5870) of the patients were significantly higher than the MBL1 levels ( $p<0.001$ ). Patients with very low ( $<100$  ng/ml) MBL2 levels had significantly longer FN duration than patients with normal ( $>1000$  ng/ml) MBL2 levels ( $p=0.016$ ). **Discussion:** Our results suggest that duration of FN is longer when MBL level is low especially less than 100 ng/ml. These patients seem to have the highest risk for infection-related morbidity and mortality and deserve interest for trials of MBL replacement.

### Keywords

MBL; Acute Leukemia; Febrile Neutropenia

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## Introduction

At the present time, it has been possible to obtain complete remission of the underlying disease in many patients with hematological malignancies and even prolong their survival times with appropriate treatment approaches. However, the aggressive and invasive treatment modalities applied to these patients cause defects in their immune systems and result in opportunistic infections. Approximately 85% of the leukemia patients who undergo aggressive cytotoxic chemotherapy have opportunistic infections with or without fever [1,2].

Various studies have described new factors increasing the risk of infections in patients with malignancies. Mannose-binding lectin (MBL), which is an important component of the natural immune system, is bound to the carbohydrate structures on cell walls of bacteria, fungi or viruses and activates the complement system. Mannose-binding lectin acts like an opsonin and enhances phagocytosis [3,4,5].

Mannose-binding lectin level in the human bloodstream is mainly genetically determined and ranges from 0.1 ng/ml to 10.000 ng/ml [6]. However, the MBL level may increase up to 1.5-3 folds after an infection or trauma because it is an acute-phase reactant [7]. It has been stated that MBL synthesis is insufficient in one-third of the normal population and low serum levels may result in a predisposition to recurrent infections [8]. For these reasons, there has been an increased interest in the role of MBL replacement in some patients with sepsis, septic shock or for the immunosuppressed patients due to treatment [9,10].

High levels of MBL may be important for protection of the patients from sepsis and septic shock. On the other hand, its lower levels have been reported to lengthen the duration of FN after chemotherapy and serious opportunistic infections [8,9]. However, it has also been claimed in other reports that MBL levels do not affect the duration of FN and the developing of pneumonia or sepsis in patients undergoing chemotherapy [9,11-14].

Conflicting results were obtained in clinical studies regarding the effects of MBL levels and/or low MBL producing polymorphisms on the infections occurring during the neutropenic period in cancer patients undergoing chemotherapy. Thus, the present study was planned to determine the association of serum MBL levels and the duration of FN after cytotoxic chemotherapy in patients with acute leukemia.

## Material and Methods

Seventy patients aged 15-75 years who admitted to a single hematology center from August 2017 to August 2018 and diagnosed with acute leukemia were included in the study. The patients who had metabolic disease or solid organ tumor were excluded. Blood samples of 5 ml were obtained from each patient before chemotherapy (MBL1) and during the first 2 hours at the first FN episode after chemotherapy (MBL2), and stored at -80°C. Mannose-binding lectin levels of all patients were measured using ELISA kit (E1480Hu) (USCN Life Science Inc. Wuhan, P.R. China). Febrile neutropenia was defined as fever  $\geq 38.30^{\circ}\text{C}$  on a single measurement or fever between  $38.0-38.2^{\circ}\text{C}$  lasting at least for an hour in a patient who has an absolute neutrophil count  $< 500/\text{mm}^3$  or between  $500-1000/$

$\text{mm}^3$  and expected to fall below  $500/\text{mm}^3$  in the following 24-48 hours. All patients received the standard FN protocol of the institution, starting with piperacillin/tazobactam and expanding the coverage according to the IDSA (Infectious Diseases Society of America) guidelines [15]. Multinational Association for Supportive Care in Cancer (MASCC) scoring system was used to categorize the patients as low or high risk according to clinical parameters [16]. The duration of FN was calculated from the day the patient had the first FN episode until the feverless recovery of neutrophils  $\geq 1500/\text{mm}^3$  for two consecutive days. In order to compare the MBL levels of the patients with healthy subjects, 30 age-matched healthy subjects without known systemic disease were used as control. Informed consent was obtained from each subject included in this study. Ethic approval was obtained from the local ethical committee of the University.

## Statistics

All statistical evaluations were performed using the SPSS software package (SPSS for Windows XP, version 20, SPSS Incorporated, Chicago, IL, USA). Since the distribution of samples was not normal in groups, non-parametric tests were used to compare the MBL levels of control group and patients group (Mann-Whitney U test) and also within the patient group (MBL1 versus MBL2) (Wilcoxon signed ranks test). Multiple comparisons between the subgroups of MBL2 (Very low, low, normal) and duration of FN were done by using LSD test. A p-value less than 0.05 was accepted as statistically significant.

## Results

In the study group, 40 patients (57.1%) had acute myeloid leukemia (AML), and 30 patients (42.9%) had acute lymphocytic leukemia (ALL). The numbers of the male and female patients were 47 (67.1 %) and 23 (32.9%) in the study group and 19 (63.3%) and 11 (36.7%) in the control group, respectively. After chemotherapy, FN developed in all patients. The patients and the control subjects were divided into three groups according to their MBL levels as follows: less than 100 ng/ml (very low), between 100-999 ng/ml (low), and more than 1000 ng/ml (normal). The mean MBL level of the patients before chemotherapy (MBL1) was 2032 ng/ml (95% CI 1441-2623 ng/ml). The percentages of patients with very low, low, and normal MBL1 levels were 23%, 36%, and 41%, respectively.

The mean MBL value in the control group was 1553 ng/ml (95% CI 766-2340 ng/ml). In the control group, MBL levels were very low, low, and normal in 20%, 40%, and 40% of the patients, respectively. The MBL1 levels of the patient group (Median 466 ng/ml; interquartile range: 4507) were higher than that of the control group (Median 485 ng/ml; interquartile range: 1872), but this difference was statistically insignificant ( $p=0.92$ ). During the FN period, MBL levels of 49 (70%) patients increased and 21 (30%) patients remained at the same level. The MBL2 levels (Median 772 ng/ml; interquartile range: 5870) of the patients were significantly higher than the MBL1 levels ( $p < 0.001$ ). The increase in the MBL level remained in the same subgroup in 63 patients. Only 7 patients were upgraded to a higher subgroup. MBL2 subgroups were compared with each other with respect to age, CRP levels, ferritin levels, and the MASCC Scores and no statistically significant difference was found between the

groups (Table 1).

The duration of FN ranged from 6 to 40 days, and the mean duration was  $11.6 \pm 5.9$  days. As shown in the Figure 1, the FN duration lengthened as the MBL2 level decreased and the difference was very close to significance ( $p=0.052$ ). Patient groups with different MBL2 levels were compared with each other with regard to the duration of FN (Table 2). Patients with very low (<100 ng/ml) MBL2 levels had significantly longer FN duration than patients with normal (>1000 ng/ml) MBL2 levels ( $p=0.016$ ).

### Discussion

The association of the MBL levels or different MBL genotypes with the duration of FN in patients receiving chemotherapy has been a challenge for many investigators for more than a decade [11,12,17,18]. However, controversial results have been reported. First of all, there is a great interindividual difference in MBL levels which has resulted in difficulty in finding a cut-off level for MBL deficiency. Clinical trials have indicated that at least 200 ng/ml MBL level is required for reconstituting in vitro functional activity. On the other hand, studies have also indicated that leukemia patients may require higher levels [19,20]. According to the results of the present study, the mean level of MBL was 2032 (95% CI 1441-2622) ng/ml and 2232 (95% CI 1634-2829) ng/ml before chemotherapy and at FN, respectively. However, other studies have demonstrated higher MBL levels in similar patient groups [11-14]. For example, Vekemans et al. assessed MBL concentration as a risk factor for infection in 255 patients with hematological malignancy receiving chemotherapy. The authors defined the levels below 500 ng/ml as MBL deficiency and demonstrated that 62 patients (24%) had MBL deficiency [9]. Schlapbach et al. analyzed 94 neutropenic pediatric cancer patients and found that very low MBL levels were associated with more frequent FN episodes, mainly due to severe bacterial infections. These investigators used the cut-off value for the MBL level similar to ours [17].

In the present study, mean MBL1 levels of the study group were higher than the control group, but this was not statistically significant. This signifies that having the diagnosis of leukemia does not necessarily increase the MBL levels in a patient. MBL is an acute phase reactant and the levels may increase up to three fold after an insult. However, the MBL levels are generally genetically determined and the influence of genotype seems to have much higher effect on the MBL level of each individual. Thus, the difference between the patient and the control groups might have also resulted from diversity in the patient group. Klostergaard et al. investigated the distribution of polymorphism in MBL gene in cancer patients and the polymorphism in this gene (MBL2) was associated with low, medium, and high MBL levels in 53%, 32%, and 15% patients, respectively. They could not demonstrate any statistically significant relationship between MBL2 polymorphism and sepsis developing after chemotherapy [12]. Dahl et al. conducted a large study and genotyped an ethnically homogenous Caucasian population for three MBL alleles (B,C, and D) known to be associated with MBL deficiency as opposed to the normal A allele, and could not find any evidence for significant differences in infectious disease

**Table 1.** Comparison of the different MBL2 subgroups according to various parameters

Parameters	MBL2 levels (ng/ml)	Patient number	Mean $\pm$ SD	P- value
Age (Years)	<100	13	35.77 $\pm$ 18.76	0.226
	100-1000	25	44.20 $\pm$ 19.28	
	$\geq$ 1000	32	36.81 $\pm$ 16.08	
	Sum	70	39.26 $\pm$ 17.90	
CRP (mg/L)	<100	13	13.28 $\pm$ 6.81	0.375
	100-1000	25	11.42 $\pm$ 5.75	
	$\geq$ 1000	32	10.25 $\pm$ 6.75	
	Sum	70	11.23 $\pm$ 6.43	
Febrile Neutropenia duration (days)	<100	13	15.08 $\pm$ 9.96	0.052
	100-1000	25	11.36 $\pm$ 5.07	
	$\geq$ 1000	32	10.38 $\pm$ 3.75	
	Sum	70	11.60 $\pm$ 5.96	
Ferritin (ng/ml)	<100	13	980.46 $\pm$ 604.62	0.241
	100-1000	25	1199.64 $\pm$ 567.00	
	$\geq$ 1000	32	942.25 $\pm$ 552.47	
	Sum	70	1041.27 $\pm$ 571.72	
MASCC Score	<100	13	20.00 $\pm$ 3.21	0.236
	100-1000	25	20.84 $\pm$ 1.95	
	$\geq$ 1000	32	21.16 $\pm$ 1.82	
	Sum	70	20.83 $\pm$ 2.19	

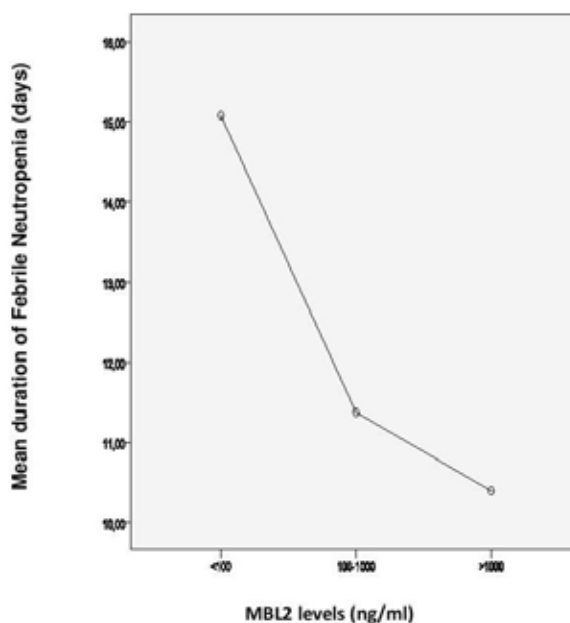
MBL: Mannose-binding lectin, CRP: C-reactive protein, SD: standard deviation  
MASCC score: Multinational Association of supportive Care in Cancer score

**Table 2.** Comparisons of different MBL2 subgroups with each other with respect to duration of febrile neutropenia

Dependent variable	MBL levels (ng/ml)	MBL levels (ng/ml)	Mean difference (day)	Standard Error	P- value
Febrile Neutropenia Duration	<100	100-1000	3.702	1.992	0.068
		>1000	4.683	1.894	0.016
	100-1000	<100	-3.702	1.992	0.068
		>1000	0.981	1.552	0.529
	>1000	<100	-4.683	1.894	0.529
		100-1000	-0.981	1.552	

MBL: Mannose-binding lectin  
MASCC score: Multinational Association of supportive Care in Cancer score

**Figure 1.** Duration of febrile neutropenia with respect to MBL2 levels



or mortality in MBL-deficient individuals versus controls [18]. In the present study, we have found that the duration of FN was statistically significantly longer in patients whose MBL levels were less than 100 ng/ml than those of the patients whose MBL levels were more than 1000 ng/ml ( $p=0.016$ ). The relationship between MBL levels and the frequency and duration of FN has been investigated in various studies. Neth et al. demonstrated in children with ALL that the duration of FN was longer in patients having polymorphic MBL alleles and in patients whose MBL levels were less than 1000ng/ml [20]. On the contrary, other studies could not demonstrate any association between the MBL levels and the duration, frequency, and severity of FN. In one of these studies, MBL, L-ficolin, and H-ficolin were measured in 128 patients with hematological malignancies treated with chemotherapy alone or combined with bone marrow transplantation. MBL, L-ficolin, and H-ficolin, independently or in combination, did not have a major influence on susceptibility to infection in these patients rendered neutropenic by chemotherapy [11]. A study by Lausen et al. could not demonstrate an increased frequency of infection after induction chemotherapy in children with genotypes encoding low serum levels of MBL [20]. Bergmann et al. investigated the clinical significance of low serum concentrations of MBL in 80 consecutive, newly diagnosed, and unselected adult AML patients undergoing remission induction chemotherapy. They showed that low levels of MBL did not affect overall survival or morbidity in terms of incidence or duration of fever, or occurrence of septicemia or pneumonia [14]. Vekemans et al. also could not demonstrate that MBL deficiency predisposed adults with hematological cancer to more-frequent or more-prolonged febrile episodes during myelosuppressive chemotherapy. However, they found that MBL-deficient patients had a greater number of severe infections and experience their first severe infection earlier, compared with non-deficient patients [9]. According to a prospective study on 110 pediatric oncology patients, the MBL deficient neutropenic children did not have more severe infections than children with normal MBL levels [13].

#### Conclusion

Our results suggest that the duration of FN is longer when MBL level is low, especially less than 100 ng/ml. Different results from various studies might have originated from the differences in patient groups, different chemotherapy regimens, the use of antibiotics and granulocyte-colony stimulating factor and also MBL measurements. New studies are required to better understand how MBL levels affect the duration or severity of FN. If the clear association can be established, MBL replacement for the patients who are known to be MBL deficient may be a promising approach in cancer patients undergoing cytotoxic chemotherapy.

#### Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

#### Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical

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#### Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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