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TITLE PAGE

Evaluation of Mannose-Binding Lectin is a Useful Approach to Predict the Risk of Infectious Complications Following Autologous Hematopoietic Stem Cell Transplantation

My manuscript is submitted as an original work.

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*Abstract

Abstract and keywords

Hematopoietic stem cell transplantation (HSCT) associated immuncompromised state carries high risk of infectious complications. Mannose-binding lectin (MBL) is an acute phase protein involved in innate immune response. Serum MBL level is genetically determined and quite stable. According to literature, significant association was shown between low MBL concentrations and serious infections.

The association between serum MBL level and frequency, severity of infections was studied in 186 patients following autologous HSCT.

Double-monoclonal antibody sandwich ELISA was used to determine MBL antigen level in sera. MBL levels were measured around 100 days following transplantation, in a period without active infection.

21 patients (11%) were MBL deficient. The median time of first infection and number of infections during the first posttransplant year were not significantly different between MBL deficients and non-MBL deficients. Occurrence and number of infections after HSCT correlated with MBL/CRP ratio. Number of severe infections was not higher among MBL deficients. Occurrence of infections after pre-engraftment period in first posttransplant year were significantly different in patient-groups separated by MBL cut-off level.

MBL/CRP ratio might be a useful marker of infectious complications. MBL measurement may be helpful in antibiotic treatment, in case of MBL deficiency earlier and more intensive treatment may be indicated.

mannose-binding lectin, autologous hematopoietic stem cell transplantation, infectious complication

***Research Highlights**

Highlights

- Immuncompromised state carries high risk of infectious complications.
- Time of first infection and number of infections during the first posttransplant year were not significantly different between MBL deficients and non-MBL deficients.
- Occurrence and number of infections after HSCT correlated with MBL/CRP ratio.
- Occurrence of infections after pre-engraftment period in first posttransplant year were significantly different in groups separated by MBL cut-off level.
- MBL/CRP ratio might be a useful marker of infectious complications.

Introduction

The innate immune system means immediate defence against infections and activates an adequate specific immune response [1]. When the adaptive immune response is immature or compromised, the innate immune system constitutes the principle defense against infection [2]. Mannose-binding lectin (MBL) is a C-type serum lectin that plays a central role in the innate immune response. MBL is produced by liver and is an acute phase protein [3,4]. The opsonic activity of MBL was first described in relation to immune deficiency in 1968 [5]. In plasma, MBL is associated with MBL-associated serine proteases (MASPs). MASP-2 is the enzyme of MBL/MASP complex needed for activation of complement factor C4 [6].

The subunit of MBL consists of an N-terminal cross-linking region, a collagen-like domain, and a C-terminal carbohydrate-recognition domain (CRD) [7]. The oligomeric configuration permits to have multiple CRDs [8]. MBL binds microbial surface carbohydrates and mediates opsonophagocytosis directly and by activation of the lectin complement pathway [9,10]. Staphylococcus aureus and β-hemolytic group A streptococci bind MBL, but only a part of several species (E. coli, Klebsiella species, Haemophilus influenzae, etc.) showed significant binding [11]. MBL binding is inhibited by encapsulated organisms [10]. MBL allows opsonization of Aspergillus fumigatus, Candida albicans and Criptococcus neoformans, the main microorganisms involved in invasive fungal infections (IFI) [11,12].

MBL is also involved in the recognition of self-targets, such as apoptotic and necrotic cells [13]. The endothelial cells exposed to oxidative stress bind MBL [14]. Neoplastic diseases are often associated with altered glycosylation patterns, so surfaces of malignant cells might be recognised by MBL as non-self [15].

The reason of low MBL level may be the actual MBL concentration or the level of functional activity. If the goal is to estimate the activity of MBL/MASP complex, so MBL pathway

activity, anti-C4 antibody is needed to determine the amount of C4b bound to the surface [1,16]. The results of this assay correlate well with assay for MBL as antigen, except in case of MASP-2 deficiency [17,18].

Serum MBL concentrations vary from 5 to 5000 ng/ml, because of genetic mutations within the gene and its promoters [19,20]. More than 10% of the general population may be classified as MBL deficient [1]. The majority of MBL-deficients are healthy without higher susceptibility for infections [21]. MBL deficiency may increase risk of infection when additional impairments of the immune system are present [22].

There is a strong correlation between MBL concentration and genotype [23,24]. Individuals with the same genotypes may differ by 10-fold in MBL levels [25]. The capacity to increase MBL concentration during febrile neutropenia is associated with MBL2 genotype [26]. There is a small increase during acute phase responses [4]. This increase is slow (1-2 weeks after the inducing event) and modest (up to three-fold increase) [1].

The variant monomers have less complement fixation capability and higher turnover [27]. The impairment of polymerization causes low serum levels of high molecular weight MBL and impaired MBL function [28].

Gram-positive cocci are responsible for the majority of post-bone-marrow transplant bloodstream infections. The most common Gram-positive species are coagulase-negative Staphylococcus, Streptococcus viridans, MRSA, enterococci and Staphylococcus epidermidis [29,30]. Fluoroquinolones prophylaxis reduced the rate of Gram negative infections but it has a lower efficacy against Gram positive microorganisms [31]. The frequency of resistant Gram negative bacteraemia increases [32]. This may be associated with wider use of intravascular devices and fluoroquinolones prophylaxis [33]. Occurrence of PCP decreased due to the use of trimethoprim-sulphamethoxazole prophylaxis [34].

Viral infections present more frequently between day 31 and 100 post-transplant, the most important are CMV pneumonia and gastrointestinal involvement [35,36,37]. The most common early viral infection, HSV causes gingivostomatitis [38].

The number of fungal infections increases post-HSCT and invasive infections can be a significant cause of morbidity and mortality. The two most common and clinically relevant pathogens are Candida and Aspergillus [39,40]. Fluconazole prophylaxis reduced the incidence of fungal infections [41,42]. IFI is one of the most life-threatening complications following treatment of hematologic malignancies, especially after allogeneic HSCT [43].

The consequence of impaired MBL function would be an enlarged susceptibility to infections [24,44,45]. Low MBL concentration may be a risk factor for infection in patients receiving myelosuppressive chemotherapy [46,47,48]. Microbiologically proved systemic or disseminated infections are more common among patients with malignancy who have MBL deficiency and who received high-dose chemotherapy and autologous HSCT [49]. The duration and deepness of neutropenia influences the frequency and severity of infection [50]. MBL deficients experience longer episodes of febrile neutropenia [46]. Effector functions of MBL are severely compromised during neutropenia, because neutrophils are required for enhanced phagocytosis after MBL-induced complement activation [51].

The normal MBL haplotype is associated with increasing MBL concentrations, whereas most patients with exon 1 mutations are not able to synthesize functional MBL and don’t have elevated serum MBL levels during acute phase response [26,46,52].

According to some studies, that measured the incidence of fever as an end point, did not demonstrate an association with MBL deficiency. Febrile episodes and their duration did not vary on the basis of MBL levels [53,54,55]. Kilpatrick et al [55] found no relationship between MBL levels and chemotherapy-related infection. Rocha et al [56] could not detect an association of mutations in MBL2 gene with the incidence of first infection.

MBL reactive carbohydrate epitopes occur on the surface of several cancer cell lines [15], there might be a general over-representation of MBL deficiency in patients with malignant hematological diseases [47].

Oral mucositis is a common toxic side effect among patients receiving high-dose chemotherapy with autologous HSCT. Mucositis complicates treatment outcome by increasing the risk of infection, necessitating enteric or parenteral nutrition and prolonging hospitalization [57].

Patients and methods

The association between serum MBL level and frequency, severity and occurrence of infections has been studied in 186 patients following autologous HSCT. CRP was measured several times according to clinical decision, and the maximal CRP level during the first 14 days after HSCT was taken in account. Correlation between infections and MBL/CRP ratio were determined.

Subgroups, i.e. multiple myeloma (MM), non-Hodgkin (NHL) and Hodgkin lymphoma (HL) were formed and infectious complications have been compared. Among the examined patients, number of persons with NHL was 63 (female/male: 25/38, age: 52±11), 27 patients' diagnosis was HL (female/male: 12/15, age: 34±9), and 94 patients had MM (female/male: 55/39, age: 56±8). Two patients with other diagnosis were also involved in the trial. The control group consisted of 296 age- and gender-matched healthy individuals (female/male: 156/140, age: 50±16 yrs) selected from consecutive blood donors. Control ones did not have any hematological or liver diseases. The control healthy group was the same as previously published in a large study from our Institute [58]. MBL serum levels and occurrence of MBL deficiency in case of healthy ones and patients with hematological diseases were compared.

Reaching the absolute neutrophil count (ANC) more than 1 G/L was taken in account as neutrophil engraftment and platelet count more than 20 G/L as platelet cell-line engraftment. We examined the distribution of microbiological results according to MBL level. It may be hypothesized that the progression, relapse following transplantation is related to MBL level and susceptibility to infections, among other parameters.

The range of MBL level in healthy population varies between 5 and 5000 ng/ml, <100 ng/ml is defined as MBL deficiency. MBL antigen levels were measured around 100 days after transplantation, in a period without active infection. MBL level is genetically determined and quite stable. There is a small increase during acute phase responses [4]. In a few cases MBL concentration were also measured before and around 100 days after HSCT and were almost equal. Informed consent was signed by the examined patients. After blood samples were taken, native tubes were centrifuged for 15 minutes at 3000 RPM, then sera samples were stored at -70 °C in small aliquots until measuring.

We used a double monoclonal antibody sandwich ELISA system adopted from Minchinton et al to determine MBL levels [23,58]. MBL assay was performed at the Clinical Research Centre of Debrecen University, without prior knowledge of the patients' clinical information.

Continuous variables were summarized as means and standard deviation or as medians and interquartile range and were compared with Mann-Whitney U-test or Student T-test. Kolmogorov-Smirnov and Chi-square tests were used to find out the distribution of variations. Kruskal-Wallis ANOVA by Ranks was used to compare data from more than two groups. Correlation of variables were analysed with Spearman Rank order correlation test. ROC curve analysis was performed to determine the cut-off level of MBL. $P<0,05$ was considered to be significant. Graphpad Prism 5 and MedCalc were used for statistical analysis.

Results

Among the examined 186 patients with malignant hematological diseases, 21 patients were proved to be MBL deficient. 51 infectious episodes (elevated CRP level, fever, other clinical symptoms of infection) were found among MBL deficients, and 372 events were in MBL competent group during the first 360 days after HSCT. The median time of onset of first infection post-HSCT was day +7 [3;8] in MBL deficient and day +6 [4;8] among non-MBL deficient patients (Table 1). The distribution of MBL level and also MBL/CRP ratio were log-normal among the patients, while distribution of CRP was normal with Kolmogorov-Smirnov and Chi-square tests (Figure 1). With Spearman Rank order correlation test, there were strong correlation between logarithmically transformed (log) MBL/CRP ratio and the time of onset of first infection (p=0,04, and after take in account the occurrence of infection as a censoring variation, p=0,0001) (Figure 2), and between log CRP and the time of first infection following transplantation (p<0,05). The time of first infection correlated neither with MBL level nor with log MBL (p=0,35). Correlation between log MBL and log CRP was almost significant (p=0,052), correlation between log MBL and log MBL/CRP ratio was significant (p=0,001) certainly.

Occurrence of infections were similar among MBL deficient and MBL competent ones (2,429 [1,478;3,379] vs 2,248 [1,993;2,516] infectious episodes/patient). Number of infections after HSCT correlated with CRP and MBL/CRP ratio but not with MBL level (Spearman Rank order correlation test, r=0,37, -0,17 and 0,07; p=0,02 and 0,34, respectively). Mann-Whitney U-test showed not significant relationship in case of MBL level and occurrence of first infection following transplantation (p=0,37), and MBL level and first infection in 14 days and 100 days after HSCT. Connections of occurrence of infection in 14 and 100 days and before reaching ANC more than 1,5 G/L and log MBL were not significant with unpaired T-tests.

But relation of occurrence of first infection in 14 and 100 days and before neutrophil engraftment with log CRP and log MBL/CRP ratio were significant.

Cut-off level of MBL according to occurrence of severe infections in posttransplant period, determined by ROC curve analysis was 823 ng/ml. Variables of the two patient-groups separated by MBL cut-off level were compared with Spearman Rank order correlation test. Number of infectious episodes (p=0.0611) and time of onset of first infection after HSCT (p=0.0905) were almost significantly different. Occurrence of infections after HSCT (p=0.0480) and occurrence of infections after the pre-engraftment period in first posttransplant year (during the period from day +14 until day 360) (p=0.0389) were significantly different in patient-groups separated by MBL cut-off level.

Interestingly, MBL serum level was found to be significantly higher in the examined patients with hematological diseases compared to healthy control population (MBL median, 1479 [380,8;2849] vs 1067 [253,5;2121], unpaired t-test, p= 0,005, significantly different). The occurrence of absolute MBL deficiency was not significantly different between hematology patients and healthy controls (11.4% vs 13.9%). The proportion of MBL deficients was the highest among HL patients (Table 2). MBL concentration of the control population and the examined patients according to diagnosis (NHL, HL, MM) were compared. Median MBL level was the highest among patients with NHL. The onset of first infection was the earliest among patients with HL (Table 3). The distribution of infectious episodes according to diagnosis is showed in Table 4.

The most common infections after transplantation are respiratory tract infections and infections with high CRP, fever and severe mucositis.

Time of neutrophil engraftment is related to MBL level significantly in MM group (Spearman Rank order correlation, p=0,024). Strong association was shown between platelet engraftment

time and MBL/CRP ratio among HL patients (p=0,003). Stem cell count and time to engraftment correlated well (p<0,001).

Distribution of Gram positive and negative bacteria species in culture from the patients' central venous catheter and blood is shown in Table 5 and 6. Positive results of central venous catheter culture (n=25) depend on log MBL and MBL/CRP ratio, but the relationship was not significant (t-test, p=0,23 and 0,15).

We examined whether the progression, relapse following transplantation is related to the patients' MBL levels or not. Association between occurrence of relapse and log MBL or log MBL/CRP were not significant (t-test, p=0,9 and 0,76). Among the examined patients, 23 patients have relapsed during the first year following HSCT and other 45 patients later. Time to relapse was not related to MBL and MBL/CRP ratio.

Discussion

Initiation of complement system may occur via classical, alternative and lectin pathway [59]. MBL recognizes carbohydrate patterns [60]. Bacterial infections and autoimmune diseases are frequently associated with complement deficiencies [61]. MBL is a C-type serum lectin [62], the carbohydrate-binding sites allow interaction with the saccharide repeats on microbial surfaces but rarely associated with mammalian high-mannose structures [7]. MBL deficiency is a result of impaired assembly or stability of multimers [63]. MBL functions as a TLR co-receptor that enables the molecule to coordinate and synchronize the innate immune system [64].

The serum levels of functional MBL correlate with MBL2 coding genotypes [58]. MBL concentration is explained by polymorphisms in the promoter region and in exon 1 of the gene [65,66].

According to literature, MBL deficiency is associated with increased susceptibility to infectious diseases, mainly when adaptive immunity is compromised (in early childhood [45,48], or following chemotherapy [46,47,67]). A significant association was shown between low MBL concentrations and serious infections related to chemotherapy [47]. MBL deficients have a greater number of severe infections and experience their first severe infection earlier, compared to non-deficients [54]. The association between low MBL and infections was independent of whether patients received prophylactic antibiotics or GM-CSF or not [68].

The range of MBL level is between 5 and 5000 ng/ml, <100 ng/ml is defined as MBL deficiency. Serum MBL concentration is quite stable, shows small increase during acute phase responses [4]. Among the examined 186 patients 21 ones were MBL deficient. The time of onset of first infection post-HSCT was similar among MBL deficients and non-deficients. There were strong correlation between log MBL/CRP ratio and time of first infection following HSCT, but the onset of first infection was not correlated significantly with log MBL. Occurrence of infections were similar among MBL deficient and MBL competent ones. The number of infections after HSCT correlated with MBL/CRP ratio but not with MBL level. Connections of occurrence of first infection in 14 and 100 days and before neutrophil engraftment and log MBL were not significant, but with log CRP and log MBL/CRP ratio were significant. We could not find strong association between MBL level and incidence, frequency and time of infections. An explanation can be that effector functions of MBL are severely compromised during neutropenia, because neutrophils are required for enhanced phagocytosis after MBL-induced complement activation [51]. Cut-off level of MBL according to occurrence of severe infections in posttransplant period, determined by ROC curve analysis was 823 ng/ml. Number of infections and time of first infection after HSCT were almost significantly different in groups separated by MBL cut-off level. Occurrence of

infections following HSCT and after the pre-engraftment period in first posttransplant year were significantly different in patient-groups separated by MBL cut-off level.

MBL serum level was significantly higher in the examined patients compared to healthy control population. The proportion of MBL deficients was the highest and onset of first infection was the earliest among HL patients.

Hematopoietic recovery and engraftment is related to patient-, disease-, and treatment-related variables [69]. Pre-engraftment phase is characterized by neutropenia, breaks in mucocutaneous barrier and vascular accesses required for patient care, and post-engraftment phase with impaired cell-mediated immunity [70].

Stem cell count and time to engraftment correlated well in the patient-group. Time to neutrophil engraftment is related to MBL level significantly in MM group. Strong association was shown between platelet engraftment time and MBL/CRP ratio in HL patients.

Infections might lead to delay or reduction in chemotherapy and might compromise the effectiveness of therapy [47]. Infections occur frequently and can be serious following high-dose chemotherapy and HSCT. Infections might also compromise the engraftment of stem cells. MBL measurement may be helpful in antibiotic treatment, in case of MBL deficiency earlier and more intensive treatment may be indicated. The most common infections after transplantation are respiratory tract infections and infections with high CRP, fever and severe mucositis. The most of sepsis episodes are associated with infection of the CVC-entry-site [71]. Mostly Gram positive bacteria species were isolated in culture from the examined patients' central venous catheter and blood. Positive results of central venous catheter culture depend on log MBL and MBL/CRP ratio, but not significantly. Infections are cured with appropriate antimicrobial therapy and in some cases with central venous catheter removal [33]. Among the examined patients, relapse and log MBL or log MBL/CRP were not associated significantly.

Extrahepatic transcription of MBL2 gene has been reported in small intestine [72,73]. Transcription of MBL2 is upregulated in inflamed intestinal tissue samples. MBL2 gene is expressed in immune cells infiltrating the inflamed gut [74]. MBL-deficients would be less able to prevent passage of bacteria from the gut to the circulation as compared to MBL competents58 [58]. Oral mucositis grade did not differ significantly between MBL deficient and MBL competent patients in our trial.

MBL2 genotypes were not determined, as individuals with the same genotypes may differ by 10-fold in MBL levels [25]. Measurement of MBL serum levels by ELISA allows reliable quantification of the functional activity of MBL pathway in vivo [75]. Procalcitonin levels were not determined, CRP level is used regularly to monitoring infectious complications in our institution.

The relationship between increased susceptibility to infections and low MBL levels seen in some studies, seems less pronounced in patients with suppression of phagocytic activity due to intensive chemotherapy [1]. We could not find strong association between MBL level and incidence, frequency and time of infections. Log MBL/CRP ratio correlated well with time of first infection following HSCT. Lower MBL concentration may predispose to severe infections in immunocompromised state. Occurrence of infections after the pre-engraftment period in first posttransplant year were significantly different in patient-groups separated by MBL cut-off level.

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Conflict of interest

The authors declare no conflict of interest.

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Table

	Total	MBL <100 ng/ml	MBL >100 ng/ml
number of patients	186	21	165
patients with infections	168	19	149
infected/total (%)	90.3	90.5	90.3
number of infectious episodes	423	51	372
infectious episodes/ one patient	2.274	2.429	2.248
development of first infection (day, median, range)	6 [4;8]	7 [3;8]	6 [4;8]
mean follow-up (day)	331	343	329

bloodstream-infection	32 (7.6%)	3 (5.9%)	29 (7.8%)
fever, high CRP, severe mucositis	106 (25.1%)	15 (29.4%)	91 (24.5%)
upper respiratory tract infection	47 (11.1%)	6 (11.8%)	41 (11.0%)
lower respiratory tract infection	63 (14.9%)	12 (23.5%)	51 (13.7%)
oral mycosis	16 (3.8%)	1 (2.0%)	15 (4.0%)
herpes zoster	14 (3.3%)	1 (2.0%)	13 (3.5%)
HSV	7 (1.7%)	1 (2.0%)	6 (1.6%)
EBV	1 (0.2%)	0	1 (0.2%)
CMV	12 (2.8%)	1 (2.0%)	11 (3.0%)
GI tract disease	56 (13.2%)	7 (13.7%)	49 (13.2%)
elevated CRP level	42 (9.9%)	2 (3.9%)	40 (10.8%)
urogenital and other infection	27 (6.4%)	2 (3.9%)	25 (6.7%)

Table 1. The distribution of infections by MBL levels

Table

	Control	Patients	NHL	HL	MM
case number	296	184	63	27	94
number of MBL-deficients	41	21	7	5	9
median MBL-level (ng/ml)	1067 [253.5;2121]	1479 [380.8;2849]	1623 [406.2;2847]	1365 [322.3;2850]	1338 [324.6;2902]
MBL deficient/ total (%)	13.9	11.4	11.1	18.5	9.6

Table 2. MBL levels of the examined and healthy population

Table

	Total	**NHL**	**HL**	**MM**
number of patients	184	63	27	94
number of infectious episodes	415	186	67	162
infections/one patient	2.27	2.95	2.48	1.72
development of first infection (day, median, range)	6 [3;8]	4 [2.5;6]	4 [0;7]	8 [6;9]
grade of mucositis (mean)	1.44	1.56	1.5	1.34
MBL level (ng/ml) (median, range)	1479 [380.8;2849]	1623 [406.2;2847]	1365 [322.3;2850]	1338 [324.6;2902]
mean follow-up (day)	327	330	324	325

Table 3. Comparison of MBL levels and infections according to diagnosis

Table

	Total	NHL	HL	MM
number of infectious episodes	415 (100%)	186 (100%)	67 (100%)	162 (100%)
bloodstream-infection	30 (7.2%)	10 (5.4%)	7 (10.4%)	13 (8.0%)
fever, high CRP, severe mucositis	95 (22.9%)	47 (25.3%)	16 (23.9%)	32 (19.8%)
upper respiratory tract infection	46 (11.1%)	18 (9.7%)	6 (9.0%)	22 (13.6%)
lower respiratory tract infection	62 (14.9%)	26 (14.0%)	12 (17.9%)	24 (14.8%)
oral mycosis	16 (3.9%)	7 (3.8%)	1 (1.5%)	8 (4.9%)
herpes zoster	13 (3.1%)	5 (2.7%)	3 (4.5%)	5 (3.1%)
HSV, EBV, CMV	20 (4.8%)	10 (5.4%)	1 (1.5%)	9 (5.6%)
GI tract disease	56 (13.5%)	30 (16.1%)	7 (10.4%)	19 (11.7%)
elevated CRP level	51 (12.3%)	21 (11.3%)	10 (14.9%)	20 (12.3%)
urinary tract and other infection	26 (6.3%)	12 (6.5%)	4 (6%)	10 (6.2%)

Table 4. The distribution of infections by diagnosis

Table

culture of central vein catheter	Total	MBL <100	MBL >100
number of patients	100	17	83
positive result of culture	25 (100%)	7 (100%)	18 (100%)
Staphylococcus epidermidis	10 (40%)	3 (42.9%)	7 (38.9%)
Staphylococcus coagulase negative	3 (12%)	1 (14.3%)	2 (11.1%)
Staphylococcus aureus	1 (4%)	0	1 (5.6%)
Enterococcus faecalis	5 (20%)	1 (14.3%)	4 (22.2%)
Streptococcus alpha-hemolising	1 (4%)	1 (14.3%)	0
Klebsiella pneumoniae	1 (4%)	1 (14.3%)	0
Pseudomonas aeruginosa	1 (4%)	0	1 (5.6%)
Acinetobacter baumannii	2 (8%)	0	2 (11.1%)
Bacillus	1 (4%)	0	1 (5.6%)

Table 5. Results of culture from central venous catheter

Table

Blood culture	Total	MBL<100	MBL>100
number of patients	186	21	165
positive result of culture	55 (100%) (43 patient)	5 (100%) (4 patient)	50 (100%) (39 patient)
Staphylococcus epidermidis	17 (30.9%)	1 (20%)	16 (32%)
Staphylococcus hominis	5 (9.1%)	2 (40%)	3 (6%)
Staphylococcus hemolyticus	6 (10.9%)	1 (20%)	5 (10%)
Staphylococcus coagulase negative	9 (16.4%)	0	9 (18%)
Staphylococcus aureus	2 (3.6%)	0	2 (4%)
Enterococcus faecalis	4 (7.3%)	1 (20%)	3 (6%)
Streptococcus	3 (5.5%)	0	3 (6%)
Propionibacterium acnes	5 (9.1%)	0	5 (10%)
Pseudomonas aeruginosa	3 (5.5%)	0	3 (6%)
other Gram negative	1 (1.8%)	0	1 (2%)

Table 6. Results of blood culture according to MBL level

Figure

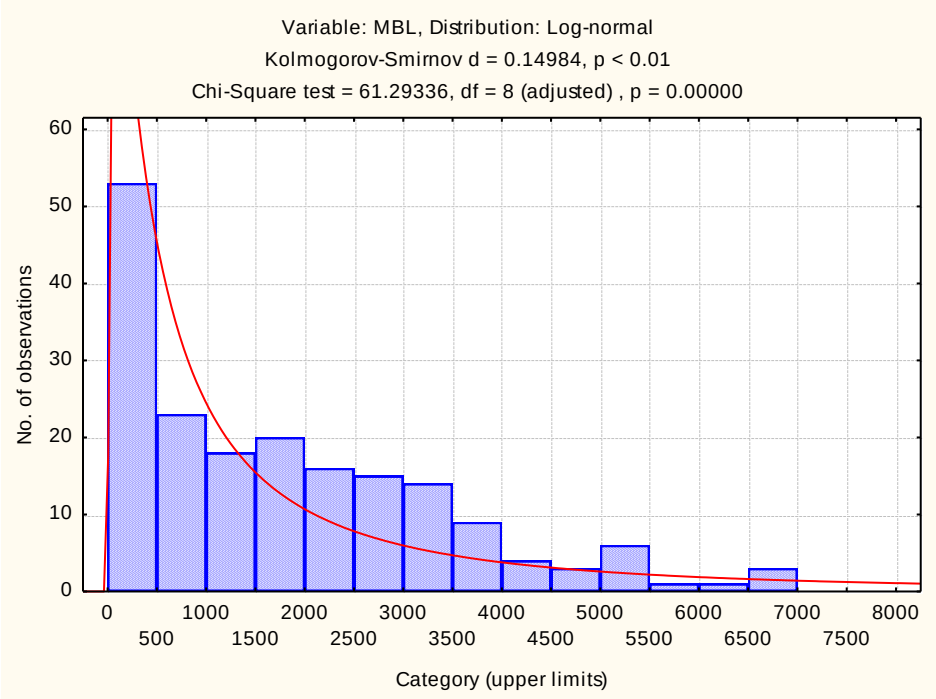

Figure 1. The distribution of MBL level in the examined patient group with hematological malignancies

Figure

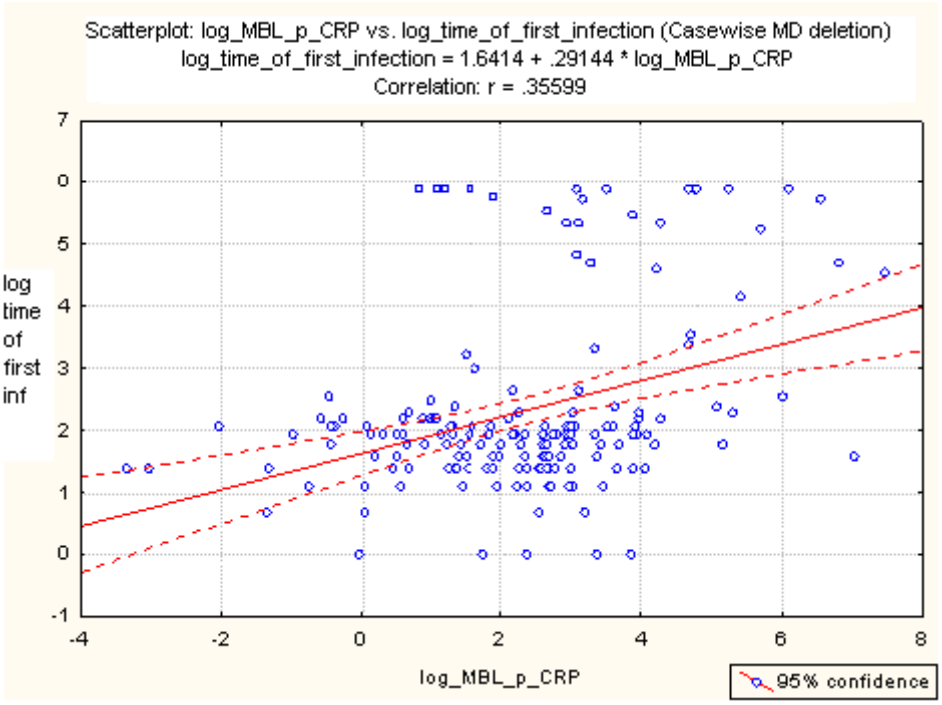

Figure 2. Correlation between log MBL/CRP and log time of first infection

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***Letter to Editor**

Dear Barry D. Kahan, PhD, MD, Editor-in-Chief, Transplantation Proceedings

Thank you for the review of my "Original Works or Clinical Submission" manuscript numbered TransProc2608 entitled "A New Approach to Predict the Chance of Infectious Complications Following Autologous Hematopoietic Stem Cell Transplantation: Mannose-Binding Lectin ELISA" for consideration for publication in Transplantation Proceedings.

Reviewer's comments were:

The authors report a prospective study examining mannose-binding lectin (MBL) levels and risk of autologous hematopoietic stem cell transplantation (HSCT). The results are interesting and provide more evidence about MBL levels as predictors of infection after HSCT.

The title of this manuscript is misleading for the novelty of the study, and should be changed. Mannose-Binding Lectin ELISA, which has been used in other previous studies, is not a new approach at all. The kit is commercial available too.

The changed title of the manuscript would be:

Evaluation of Mannose-Binding Lectin is a Useful Approach to Predict the Risk of Infectious Complications Following Autologous Hematopoietic Stem Cell Transplantation

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Thank you very much for the extensive review and the intend to publish this manuscript as an Original article in the issue containing "Original Works or Clinical Submission" manuscripts in a future publication.

I am very grateful for your kind interest in this manuscript.

Sincerely,

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