

Elsevier Editorial System(tm) for
Transplantation Proceedings
Manuscript Draft

Manuscript Number: TransProc2608R1

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

Article Type: Original Works or Clinical Submission

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

Corresponding Author: Dr. Zita Brigitta Radnay, M.D.

Corresponding Author's Institution: Institute for Internal Medicine, University of Debrecen

First Author: Zita Brigitta Radnay, M.D.

Order of Authors: Zita Brigitta Radnay, M.D.; Miklós Udvardy, Prof., M.D., PhD; Mária Papp, M.D., PhD; Jolán Hársfalvi, PhD; László Rejtő, M.D., PhD; Ildikó Pál, M.D.; Árpád Illés, Prof., M.D., PhD; Attila Kiss, Prof., M.D., PhD

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.

Authors:

Zita Brigitta Radnay M.D.¹, Miklós Udvardy Prof. M.D.¹, Mária Papp M.D.², Jolán Hársfalvi PhD^{3,4}, László Rejtő M.D.¹, Ildikó Pál M.D.¹, Árpád Illés Prof. M.D.¹, Attila Kiss Prof. M.D.¹

Affiliations:

¹Department of Hematology, Institute for Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

²Department of Gastroenterology, Institute for Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

³Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

⁴Clinical Research Center, Faculty of Medicine, University of Debrecen, Hungary

Email addresses of authors:

radnayzita@gmail.com

udvardy.miklosdr@gmail.com

drpappm@yahoo.com

harsfalvi.jolan@med.semmelweis-univ.hu

lrejto@med.unideb.hu

palildiko89@gmail.com

illesarpaddr@gmail.com

akiss@med.unideb.hu

Corresponding author:

Zita Brigitta Radnay MD.

Department of Hematology, Institute for Internal Medicine

Faculty of Medicine, University of Debrecen

Nagyerdei krt. 98.

H-4032 Debrecen, Hungary

Telephone number: +36-20-582-9147

Fax number: +36-52-255-984

Email address: radnayzita@gmail.com

Grant information: The authors declare no conflict of interest.

TÁMOP-4.2.2/B-10/1-2010-0024, PhD student fellowship, Hungary

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

Abbreviations: (in alphabetical order)

Tables: 6

Figures: 2 (color – Yes / No)

Abstract and keywords

Hematopoietic stem cell transplantation (HSCT) associated immunocompromised 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 deficient and non-MBL deficient. Occurrence and number of infections after HSCT correlated with MBL/CRP ratio. Number of severe infections was not higher among MBL deficient. 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

Highlights

- Immunocompromised 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 deficient and non-MBL deficient.
- 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.

1 Introduction

2

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

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

20 MBL is also involved in the recognition of self-targets, such as apoptotic and necrotic cells
21 [13]. The endothelial cells exposed to oxidative stress bind MBL [14]. Neoplastic diseases are
22 often associated with altered glycosylation patterns, so surfaces of malignant cells might be
23 recognised by MBL as non-self [15].

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

26 activity, anti-C4 antibody is needed to determine the amount of C4b bound to the surface
27 [1,16]. The results of this assay correlate well with assay for MBL as antigen, except in case
28 of MASP-2 deficiency [17,18].

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

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

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

42 Gram-positive cocci are responsible for the majority of post-bone-marrow transplant
43 bloodstream infections. The most common Gram-positive species are coagulase-negative
44 *Staphylococcus*, *Streptococcus viridans*, MRSA, enterococci and *Staphylococcus epidermidis*
45 [29,30]. Fluoroquinolones prophylaxis reduced the rate of Gram negative infections but it has
46 a lower efficacy against Gram positive microorganisms [31]. The frequency of resistant Gram
47 negative bacteraemia increases [32]. This may be associated with wider use of intravascular
48 devices and fluoroquinolones prophylaxis [33]. Occurrence of PCP decreased due to the use
49 of trimethoprim-sulphamethoxazole prophylaxis [34].

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

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

58 The consequence of impaired MBL function would be an enlarged susceptibility to infections
59 [24,44,45]. Low MBL concentration may be a risk factor for infection in patients receiving
60 myelosuppressive chemotherapy [46,47,48]. Microbiologically proved systemic or
61 disseminated infections are more common among patients with malignancy who have MBL
62 deficiency and who received high-dose chemotherapy and autologous HSCT [49]. The
63 duration and deepness of neutropenia influences the frequency and severity of infection [50].
64 MBL deficient experience longer episodes of febrile neutropenia [46]. Effector functions of
65 MBL are severely compromised during neutropenia, because neutrophils are required for
66 enhanced phagocytosis after MBL-induced complement activation [51].

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

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

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

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

82

83 Patients and methods

84

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

90 Subgroups, i.e. multiple myeloma (MM), non-Hodgkin (NHL) and Hodgkin lymphoma (HL)
91 were formed and infectious complications have been compared. Among the examined
92 patients, number of persons with NHL was 63 (female/male: 25/38, age: 52±11), 27 patients'
93 diagnosis was HL (female/male: 12/15, age: 34±9), and 94 patients had MM (female/male:
94 55/39, age: 56±8). Two patients with other diagnosis were also involved in the trial. The
95 control group consisted of 296 age- and gender-matched healthy individuals (female/male:
96 156/140, age: 50±16 yrs) selected from consecutive blood donors. Control ones did not have
97 any hematological or liver diseases. The control healthy group was the same as previously
98 published in a large study from our Institute [58]. MBL serum levels and occurrence of MBL
99 deficiency in case of healthy ones and patients with hematological diseases were compared.

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

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

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

116 Continuous variables were summarized as means and standard deviation or as medians and
117 interquartile range and were compared with Mann-Whitney U-test or Student T-test.

118 Kolmogorov-Smirnov and Chi-square tests were used to find out the distribution of variations.

119 Kruskal-Wallis ANOVA by Ranks was used to compare data from more than two groups.

120 Correlation of variables were analysed with Spearman Rank order correlation test. ROC curve
121 analysis was performed to determine the cut-off level of MBL. $P < 0,05$ was considered to be
122 significant. Graphpad Prism 5 and MedCalc were used for statistical analysis.

123

124 Results

125

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

141 Occurrence of infections were similar among MBL deficient and MBL competent ones (2,429
142 [1,478;3,379] vs 2,248 [1,993;2,516] infectious episodes/patient). Number of infections after
143 HSCT correlated with CRP and MBL/CRP ratio but not with MBL level (Spearman Rank
144 order correlation test, $r=0,37$, $-0,17$ and $0,07$; $p=0,02$ and $0,34$, respectively). Mann-Whitney
145 U-test showed not significant relationship in case of MBL level and occurrence of first
146 infection following transplantation ($p=0,37$), and MBL level and first infection in 14 days and
147 100 days after HSCT. Connections of occurrence of infection in 14 and 100 days and before
148 reaching ANC more than 1,5 G/L and log MBL were not significant with unpaired T-tests.

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

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

159 Interestingly, MBL serum level was found to be significantly higher in the examined patients
160 with hematological diseases compared to healthy control population (MBL median, 1479
161 [380,8;2849] vs 1067 [253,5;2121], unpaired t-test, $p=0,005$, significantly different). The
162 occurrence of absolute MBL deficiency was not significantly different between hematology
163 patients and healthy controls (11.4% vs 13.9%). The proportion of MBL deficient was the
164 highest among HL patients (Table 2). MBL concentration of the control population and the
165 examined patients according to diagnosis (NHL, HL, MM) were compared. Median MBL
166 level was the highest among patients with NHL. The onset of first infection was the earliest
167 among patients with HL (Table 3). The distribution of infectious episodes according to
168 diagnosis is showed in Table 4.

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

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

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

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

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

184

185 Discussion

186

187 Initiation of complement system may occur via classical, alternative and lectin pathway [59].
188 MBL recognizes carbohydrate patterns [60]. Bacterial infections and autoimmune diseases are
189 frequently associated with complement deficiencies [61]. MBL is a C-type serum lectin [62],
190 the carbohydrate-binding sites allow interaction with the saccharide repeats on microbial
191 surfaces but rarely associated with mammalian high-mannose structures [7]. MBL deficiency
192 is a result of impaired assembly or stability of multimers [63]. MBL functions as a TLR co-
193 receptor that enables the molecule to coordinate and synchronize the innate immune system
194 [64].

195 The serum levels of functional MBL correlate with MBL2 coding genotypes [58]. MBL
196 concentration is explained by polymorphisms in the promoter region and in exon 1 of the gene
197 [65,66].

198 According to literature, MBL deficiency is associated with increased susceptibility to
199 infectious diseases, mainly when adaptive immunity is compromised (in early childhood
200 [45,48], or following chemotherapy [46,47,67]). A significant association was shown between
201 low MBL concentrations and serious infections related to chemotherapy [47]. MBL deficient
202 have a greater number of severe infections and experience their first severe infection earlier,
203 compared to non-deficients [54]. The association between low MBL and infections was
204 independent of whether patients received prophylactic antibiotics or GM-CSF or not [68].
205 The range of MBL level is between 5 and 5000 ng/ml, <100 ng/ml is defined as MBL
206 deficiency. Serum MBL concentration is quite stable, shows small increase during acute
207 phase responses [4]. Among the examined 186 patients 21 ones were MBL deficient. The
208 time of onset of first infection post-HSCT was similar among MBL deficient and non-
209 deficient. There were strong correlation between log MBL/CRP ratio and time of first
210 infection following HSCT, but the onset of first infection was not correlated significantly with
211 log MBL. Occurrence of infections were similar among MBL deficient and MBL competent
212 ones. The number of infections after HSCT correlated with MBL/CRP ratio but not with MBL
213 level. Connections of occurrence of first infection in 14 and 100 days and before neutrophil
214 engraftment and log MBL were not significant, but with log CRP and log MBL/CRP ratio
215 were significant. We could not find strong association between MBL level and incidence,
216 frequency and time of infections. An explanation can be that effector functions of MBL are
217 severely compromised during neutropenia, because neutrophils are required for enhanced
218 phagocytosis after MBL-induced complement activation [51]. Cut-off level of MBL
219 according to occurrence of severe infections in posttransplant period, determined by ROC
220 curve analysis was 823 ng/ml. Number of infections and time of first infection after HSCT
221 were almost significantly different in groups separated by MBL cut-off level. Occurrence of

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

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

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

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

234 Infections might lead to delay or reduction in chemotherapy and might compromise the
235 effectiveness of therapy [47]. Infections occur frequently and can be serious following high-
236 dose chemotherapy and HSCT. Infections might also compromise the engraftment of stem
237 cells. MBL measurement may be helpful in antibiotic treatment, in case of MBL deficiency
238 earlier and more intensive treatment may be indicated. The most common infections after
239 transplantation are respiratory tract infections and infections with high CRP, fever and severe
240 mucositis. The most of sepsis episodes are associated with infection of the CVC-entry-site
241 [71]. Mostly Gram positive bacteria species were isolated in culture from the examined
242 patients' central venous catheter and blood. Positive results of central venous catheter culture
243 depend on log MBL and MBL/CRP ratio, but not significantly. Infections are cured with
244 appropriate antimicrobial therapy and in some cases with central venous catheter removal
245 [33]. Among the examined patients, relapse and log MBL or log MBL/CRP were not
246 associated significantly.

247 Extrahepatic transcription of MBL2 gene has been reported in small intestine [72,73].
248 Transcription of MBL2 is upregulated in inflamed intestinal tissue samples. MBL2 gene is
249 expressed in immune cells infiltrating the inflamed gut [74]. MBL-deficients would be less
250 able to prevent passage of bacteria from the gut to the circulation as compared to MBL
251 competent⁵⁸ [58]. Oral mucositis grade did not differ significantly between MBL deficient
252 and MBL competent patients in our trial.

253 MBL2 genotypes were not determined, as individuals with the same genotypes may differ by
254 10-fold in MBL levels [25]. Measurement of MBL serum levels by ELISA allows reliable
255 quantification of the functional activity of MBL pathway in vivo [75]. Procalcitonin levels
256 were not determined, CRP level is used regularly to monitoring infectious complications in
257 our institution.

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

266

267 Acknowledgements

268

269 I would like to thank for the supportation and help of my supervisor, Attila Kiss Prof. MD. I
270 performed the clinical examination, data analysis of patients information at Department of
271 Hematology, Institute for Medicine, Clinical Center, University of Debrecen and Stem Cell

272 Transplantation Unit, University of Debrecen. I would like to thank for supportation and
273 advices of Miklós Udvardy Prof. MD, head of the Stem Cell Transplantation Unit, and Árpád
274 Illés Prof. MD, head of the Department of Hematology, Institute for Medicine. MBL assays
275 were performed at the Clinical Research Centre of Debrecen University, according to ELISA
276 methods adopted from Minchinton et al and locally settings performed by Maria Papp MD,
277 Jolán Hársfalvi PhD and their workgroup previously. Zsolt Karányi helped in statistical
278 analysis. I was a PhD student for three years, and at my first year I got supportation by
279 fellowship TÁMOP-4.2.2/B-10/1-2010-0024, the next two years were state-aided. Initial
280 results of this work were presented on a poster in 2011 at EBMT Congress, Paris, France
281 (Radnay Z, Kiss A, Papp M, Rejtő L, Hársfalvi J, Udvardy M. Mannose-binding lectin ELISA
282 is a new approach to predict the chance of infectious complications during autologous
283 haematopoietic stem cell transplantation. *Bone Marrow Transplant* 46 (Suppl. 1), S213-S214,
284 2011.).

285

286 Conflict of interest

287

288 The authors declare no conflict of interest.

289

290 References

291

292 [1] Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin
293 deficiency. *Molecular Immunology*, 2006; 43: 86-96.

294 [2] Eisen DP, Minchinton RM. Impact of Mannose-Binding Lectin on Susceptibility to
295 Infectious Diseases. *Clinical Infectious Diseases*, 2003; 37: 1496-1505.

- 296 [3] Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-
297 binding protein with a common defect of opsonisation. *Lancet*, 1989; 2: 1236-9.
- 298 [4] Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type
299 lectin, mannan-binding protein, in human plasma increases during an acute phase response.
300 *Clin Exp Immunol*, 1992; 90: 31-35.
- 301 [5] Miller ME, Seals J, Kaye R, Levitsky LC. A familial plasma-associated defect of
302 phagocytosis. *Lancet*, 1968; 2: 60-63.
- 303 [6] Thiel S, Vorup-Jensen T, Stover CM, Schwaeble W, Laursen SB, Poulsen K et al. A
304 second serine protease associated with mannan-binding lectin that activates complement.
305 *Nature*, 1997; 386: 506-510.
- 306 [7] Turner MW. Mannose-binding lectin: the pluripotent molecule of the innate immune
307 system. *Immunol Today*, 1996; 17: 532-40.
- 308 [8] Bouwman LH, Roep BO, Roos A. Mannose-Binding Lectin: Clinical Implications for
309 Infection, Transplantation, and Autoimmunity. *Human Immunology*, 2006; 67: 247-256.
- 310 [9] Neth O, Jack DL, Johnson M, Klein NJ, Turner MW. Enhancement of complement
311 activation and opsonophagocytosis by complexes of mannose-binding lectin with mannose-
312 binding lectin-associated serine protease after binding to *Staphylococcus aureus*. *J Immunol*,
313 2002; 169: 4430-4436.
- 314 [10] van Emmerik LC, Kuijper EJ, Fijen CA, Dankert J, Thiel S. Binding of mannan-binding
315 protein to various bacterial pathogens of meningitis. *Clin Exp Immunol*, 1994; 97: 411-416.
- 316 [11] Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding lectin
317 binds to a range of clinically relevant microorganisms and promotes complement deposition.
318 *Infect Immun*, 2000; 68: 688-693.
- 319 [12] Jack DL, Klein NJ, Turner MW. Mannose-binding lectin targeting the microbial world
320 for complement attack and opsonophagocytosis. *Immunol Rev*, 2001; 180: 86-99.

321 [13] Nauta AJ, Raaschou-Jensen N, Roos A, Daha MR, Madsen HO, Borrias-Essers MC et al.
322 Mannose-binding lectin engagement with late apoptotic and necrotic cells. *Eur J Immunol*,
323 2003; 33: 2853.

324 [14] Collard CD, Vakeva A, Morrissey MA, Agah A, Rollins SA, Reenstra WR et al.
325 Complement activation after oxidative stress. Role of the lectin complement pathway. *Am J*
326 *Pathol*, 2000; 156: 1549-1556.

327 [15] Ma Y, Uemura K, Oka S, Kozutsumi Y, Kawasaki N, Kawasaki T. Antitumor activity of
328 mannan-binding protein in vivo as revealed by a virus expression system: mannan-binding
329 protein-dependent cell-mediated cytotoxicity. *Proc Natl Acad Sci USA*, 1999; 96: 371-375.

330 [16] Super M, Levinsky RJ, Turner MW. The level of mannan-binding protein regulates the
331 binding of complement-derived opsonins to mannan and zymosan at low serum
332 concentrations. *Clin. Exp. Immunol*, 1990; 79: 144-150.

333 [17] Thiel S, Moller-Kristensen M, Jensen L, Jensenius JC. Assays for the functional activity
334 of the mannan-binding lectin pathway of complement activation. *Immunobiology*, 2002; 205:
335 446-454.

336 [18] Stengaard-Pedersen K, Thiel S, Gadjeva M, Moller-Kristensen M, Sorensen R, Jensen
337 LT et al. Inherited deficiency of mannan-binding lectin-associated serine protease 2. *N. Engl.*
338 *J. Med*, 2003; 349: 554-560.

339 [19] Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP et al. Interplay
340 between promoter and structural gene variants control basal serum levels of mannan-binding
341 protein. *J Immunol*, 1995; 155: 3013-20.

342 [20] Madsen HO, Garred P, Kurtzhals JA, Lamm LU, Ryder LP, Thiel S et al. A new frequent
343 allele is the missing link in the structural polymorphism of the human mannan-binding
344 protein. *Immunogenetics*, 1994; 40: 37-44.

345 [21] Tacx AN, Groeneveld ABJ, Hart MH, Aarden LA, Hack CE. Mannan binding lectin in
346 febrile adults, no correlation with microbial infection and complement activation. *J. Clin.*
347 *Pathol*, 2003; 56: 956-959.

348 [22] Dahl M, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. A population-based study of
349 morbidity and mortality in mannose-binding lectin deficiency. *J Exp Med*, 2004; 199: 1391-
350 1399.

351 [23] Minchinton RM, Dean MM, Clark TR, Heatley S, Mullighan CG. Analysis of the
352 relationship between mannose-binding lectin (MBL) genotype, MBL levels and function in an
353 Australian blood donor population. *Scand J Immunol*, 2002; 56: 630-41.

354 [24] Garred P, Madsen HO, Hofmann B, Svejgaard A. Increased frequency of homozygosity
355 of abnormal mannan-binding-protein alleles in patients with suspected immunodeficiency.
356 *Lancet*, 1995; 346: 941-3.

357 [25] Steffensen R, Thiel S, Varming K, Jersild C, Jensenius JC. Detection of structural gene
358 mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by
359 polymerase chain reaction with sequence-specific primers. *J Immunol, Methods*, 2000; 349:
360 554-560.

361 [26] Frakking FNJ, van de Wetering MD, Brouwer N, Dolman KM, Geissler J, Lemkes B et
362 al. The role of mannan-binding lectin (MBL) in pediatric oncology patients with febrile
363 neutropenia. *European Journal of Cancer*, 2006; 42: 909-916.

364 [27] Petersen SV, Thiel S, Jensenius JC. The mannan-binding lectin pathway of complement
365 activation: biology and disease association. *Mol Immunol*, 2001; 38: 133-49.

366 [28] Roos A, Garred P, Wildenberg ME, Lynch NJ, Munoz JR, Zuiverloon TC et al.
367 Antibody-mediated activation of the classical pathway of complement may compensate for
368 mannose-binding lectin deficiency. *Eur J Immunol*, 2004; 34: 2589.

369 [29] Pawson H, Jayaweera A, Wigmore T. Intensive care management of patients following
370 haematopoietic stem cell transplantation. *Current Anaest & Critical Care*, 2008; 19: 80-90.

371 [30] Poutsiaka DD, Price LL, Ucuzian A, Chan GW, Miller KB, Snyderman DR. Blood stream
372 infection after hematopoietic stem cell transplantation is associated with increased mortality.
373 *Bone Marrow Transplant*, 2007; 40: 63-70.

374 [31] Cruciani M, Rampazzo R, Malena M, Lazzarini L, Todeschini G, Messori A et al.
375 Prophylaxis with fluoroquinolones for bacterial infections in neutropenic patients: a meta-
376 analysis. *Clin Infect Dis*, 1996; 23(4): 795-805.

377 [32] Cherif H, Kronvall G, Björkholm M, Kalin M. Bacteraemia in hospitalised patients with
378 malignant blood disorders: a retrospective study of causative agents and their resistance
379 profiles during a 14-year period without antibacterial prophylaxis. *The Hematology Journal*,
380 2003; 4: 420-426.

381 [33] Bonadio M, Morelli G, Mori S, Riccioni R, Papineschi F, Petrini M. Fluoroquinolone
382 resistance in hematopoietic stem cell transplant recipients with infectious complications.
383 *Biomedicine & Pharmacotherapy*, 2005; 59: 511-516.

384 [34] Leung AN, Gosselin MV, Napper CH, Braun SG, Hu WW, Wong RM et al. Pulmonary
385 infections after bone marrow transplantation: clinical and radiographic findings. *Radiology*,
386 1999; 210: 699-710.

387 [35] Wah TM, Moss HA, Robertson RJH, Barnard DL. Pulmonary complications following
388 bone marrow transplantation. *Br J Radiol*, 2003; 76: 373-379.

389 [36] Enright H, Haake R, Weisdorf D, Ramsay N, McGlave P, Kersey J et al.
390 Cytomegalovirus pneumonia after bone marrow transplantation. Risk factors and response to
391 therapy. *Transplantation*, 1993; 55(6): 1339-45.

392 [37] Castagnola E, Cappelli B, Erba D, Rabagliati A, Lanino E, Dini G. Cytomegalovirus
393 infection after bone marrow transplantation in children. *Human Immunology*, 2004; 65: 416-
394 422.

395 [38] Soubani AO, Miller KB, Hassoun PM. Pulmonary complications of bone marrow
396 transplantation. *Chest*, 1996; 109: 1066-1077.

397 [39] Raman T, Marik PE. Fungal infections in bone marrow transplant recipients. *Expert*
398 *Opinion Pharmacother*, 2006; 7(3): 307-15.

399 [40] De La Rosa GR, Champlin RE, Kontoyiannis DP. Risk factors for the development of
400 invasive fungal infections in allogeneic blood and marrow transplant recipients. *Transplant*
401 *Infect Dis*, 2002; 4(1): 3-9.

402 [41] Goodman JL, Winston DJ, Greenfield RA, Chandrasekar PH, Fox B, Kaizer H et al.
403 Controlled trial of fluconazole to prevent fungal infections in patients undergoing bone
404 marrow transplantation. *N Engl J Med*, 1992; 326(13): 845-851.

405 [42] Slavin MA, Osborne B, Adams R, Levenstein MJ, Schoch HG, Feldman AR et al.
406 Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow
407 transplantation- a prospective, randomized, double-blind study. *J Infect Dis*, 1995; 171(6):
408 1545-1552.

409 [43] Martino R, Subira M. Invasive fungal infections in haematology: new trends. *Ann*
410 *Hematol*, 2002; 1: 233-243.

411 [44] Summerfield JA, Ryder S, Sumiya M, Thursz M, Gorchein A, Monteil MA et al.
412 Mannose binding protein gene mutations associated with unusual and severe infections in
413 adults. *Lancet*, 1995; 345: 886.

414 [45] Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K et al. Acute
415 respiratory tract infections and mannose-binding lectin insufficiency during early childhood. *J*
416 *Am Med Assoc*, 2001; 285: 1316.

417 [46] Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden
418 of infection in children with malignancy: a prospective study. *Lancet*, 2001; 358: 614-618.

419 [47] Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of
420 mannose-binding lectin and severe infections after chemotherapy. *Lancet*, 2001; 358: 637-
421 638.

422 [48] Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in
423 mannose binding protein gene with childhood infection in consecutive hospital series. *BMJ*,
424 1997; 314: 1229-1232.

425 [49] Horiuchi T, Gondo H, Miyagawa H, Otsuka J, Inaba S, Nagafuji K et al. Association of
426 MBL gene polymorphisms with major bacterial infection in patients treated with high-dose
427 chemotherapy and autologous PBSCT. *Genes Immun*, 2005; 6: 162-166.

428 [50] Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between
429 circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med*, 1966;
430 64: 328-340.

431 [51] Bergmann OJ, Christiansen M, Laursen I, Bang P, Hansen NE, Ellegaard J et al. Low
432 levels of mannose-binding lectin do not affect occurrence of severe infections or duration of
433 fever in acute myeloid leukaemia during remission induction therapy. *Eur J Haematol*, 2003;
434 70: 91-97.

435 [52] Dean M, Minchinton RM, Heatley S, Eisen DP. Mannose binding lectin acute phase
436 activity in patients with severe infection. *J Clin Immunol*, 2005; 25: 346-352.

437 [53] Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP et al. Mannose-
438 binding lectin gene polymorphisms are associated with major infection following allogeneic
439 hemopoietic stem cell transplantation. *Blood*, 2002; 99: 3524-3529.

440 [54] Vekemans M, Robinson J, Georgala A, Heymans C, Muanza F, Paesmans M et al. Low
441 mannose-binding lectin concentration is associated with severe infections in patients with

442 hematological cancer who are undergoing chemotherapy. *Clin Infect Diseases*, 2007; 44:
443 1593-1601.

444 [55] Kilpatrick DC, McIntock LA, Allan EK, Copland M, Fujita T, Jordanides NE et al. No
445 strong relationship between mannan binding lectin or plasma ficolins and chemotherapy-
446 related infections. *Clin Exp Immunol*, 2003; 134: 279-284.

447 [56] Rocha V, Franco RF, Porcher R, Bittencourt H, Silva VA, Latouche A et al. Host defense
448 and inflammatory gene polymorphisms are associated with outcomes after HLA-identical
449 sibling bone marrow transplantation. *Blood*, 2002; 100: 3908-3918.

450 [57] Milstein DMJ, te Boome LCJ, Cheung YW, Lindeboom JAH, van den Akker HP,
451 Biemond BJ et al. Use of sidestream dark-field (SDF) imaging for assessing the effects of
452 high-dose melphalan and autologous stem cell transplantation on oral mucosal
453 microcirculation in myeloma patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*,
454 2010; 109: 91-97.

455 [58] Papp M, Altorjay I, Vitalis Z, Tornai I, Palatka K, Kacska S et al. Mannose-binding
456 lectin deficiency confers risk for bacterial infections in a large Hungarian cohort of patients
457 with liver cirrhosis. *Journal of Hepatology*, 2010; 53: 484-491.

458 [59] Thiel S. Complement activating soluble pattern recognition molecules with collagen-like
459 regions, mannan-binding lectin, ficolins and associated proteins. *Mol Immunol*, 2007; 44:
460 3875-3888.

461 [60] Beinrohr L, Dobo J, Zavodszky P, Gal P. C1, MBL-MASPs and C1-inhibitor: novel
462 approaches for targeting complement-mediated inflammation. *Trends in Molecular Medicine*,
463 2008; 14: 511-521.

464 [61] Botto M, Kirschfink M, Macor P, Pickering MC, Würzner R, Tedesco F. Complement in
465 human diseases: Lessons from complement deficiencies. *Mol Immunol*, 2009; 46: 2774-2783.

466 [62] Kilpatrick DC. Mannan-binding lectin: clinical significance and applications. *Biochimica*
467 *et Biophysica Acta*, 2002; 1572: 401-413.

468 [63] Holmskov U, Thiel S, Jensenius JC. Collectins and ficolins: humoral lectins of the
469 innate immune defense. *Annu Rev Immunol*, 2003; 21: 547-578.

470 [64] Ip WK, Takahashi K, Ezekowitz RA, Stuart LM. Mannose-binding lectin and innate
471 immunity. *Immunol Rev*, 2009; 230: 9-21.

472 [65] Turner MW, Hamvas RM. Mannose-binding lectin: structure, function, genetics, and
473 disease associations. *Rev Immunogenet*, 2000; 2: 305-322.

474 [66] Garred P, Larsen F, Seyfarth J, Fujita R, Madsen HO. Mannose-binding lectin and its
475 genetic variants. *Genes Immun*, 2006; 7: 85-94.

476 [67] Eisen DP, Minchinton RM. Impact of mannose-binding lectin on susceptibility to
477 infectious diseases. *Clin Infect Dis*, 2003; 37: 1496-1505.

478 [68] Vekemans M, Georgala A, Heymans C, Muanza F, Paesmans M, Klustersky J et al.
479 Influence of mannan binding lectin serum levels on the risk of infection during chemotherapy-
480 induced neutropenia in adult haematological cancer patients. *Clin Microbiol Infect*, 2005; 11:
481 20.

482 [69] Kozłowska-Skrzypczak M, Gil L, Komarnicki M. Factors affecting neutrophil recovery
483 after autologous bone marrow-derived stem cell transplantation in patients with acute myeloid
484 leukemia. *Transplantation Proceedings*, 2009; 41: 3868-3872.

485 [70] Dykewicz CA. Summary of the guidelines for preventing opportunistic infections among
486 hematopoietic stem cell transplant recipients. *Clin Infect Dis*, 2001; 33: 139-144.

487 [71] Pearson ML. Guideline for prevention of intravascular device-related infections. Part I.
488 Intravascular device-related infections: an overview. *Am J Infect Control*, 1996; 24: 262-93.

- 489 [72] Wagner S, Lynch NJ, Walter W, Schwaeble WJ, Loos M. Differential expression of the
490 murine mannose-binding lectins A and C in lymphoid and nonlymphoid organs and tissues. *J*
491 *Immunol*, 2003; 170: 1462-1465.
- 492 [73] Seyfarth J, Garred P, Madsen HO. Extrahepatic transcription of the human mannose-
493 binding lectin gene (mbl2) and the MBL-associated serine protease 1-3 genes. *Mol Immunol*
494 2006; 43: 962-971.
- 495 [74] Milanese M, Segat L, Marziliano N, Crovella S. The expression of innate immunity
496 genes
497 in Italian Crohn disease patients. *Eur J Histochem*, 2007; 51: 199-202.
- 498 [75] Petersen SV, Thiel S, Jensen L, Steffensen R, Jensenius JC. An assay for the mannan-
499 binding lectin pathway of complement activation. *J Immunol Methods*, 2001; 257: 107-116.

	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

	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

	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

	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

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

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

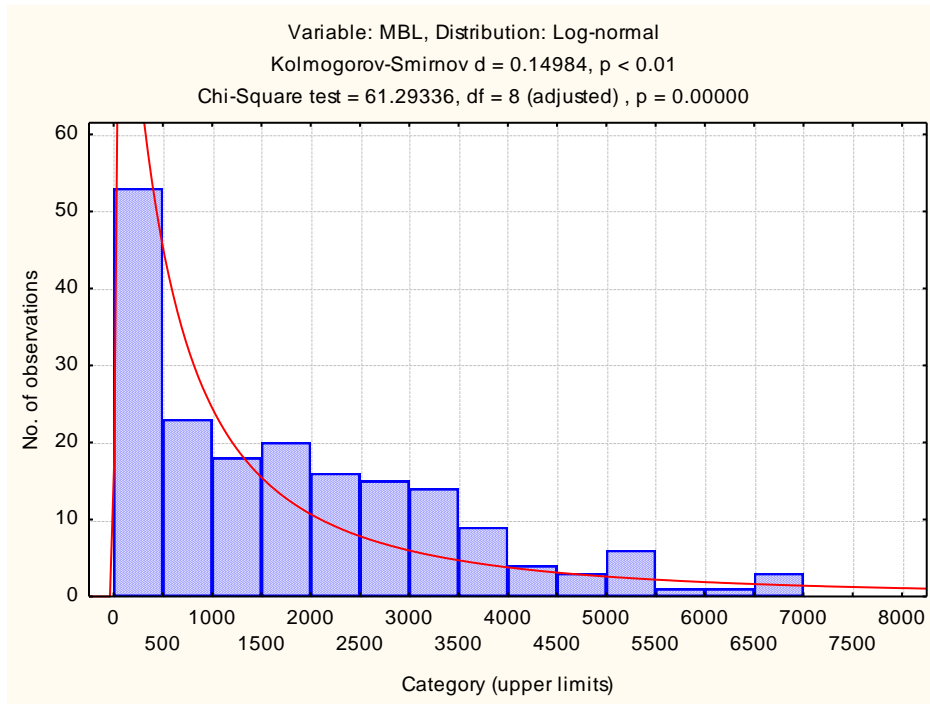


Figure 1. The distribution of MBL level in the examined patient group with hematological malignancies

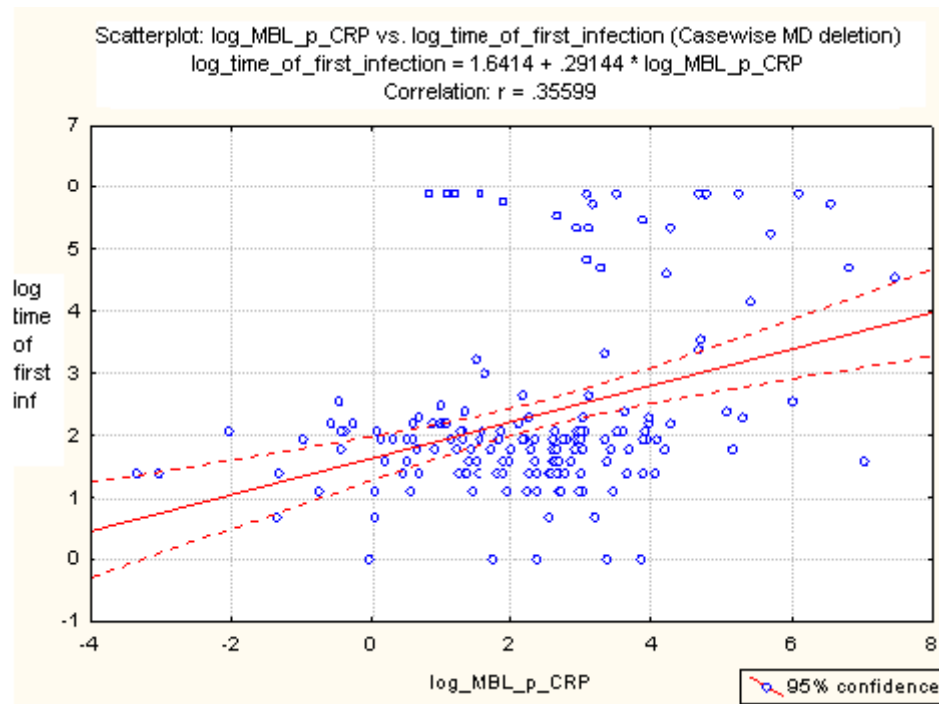


Figure 2. Correlation between log MBL/CRP and log time of first infection

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting http://www.adobe.com/go/reader_download.

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

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

Authors state the willingness and ability to pay all page charges, this document is uploaded again because of the changed Title of the manuscript.

I checked again my manuscript complies with the guidelines to authors. Title Page contain all author email addresses and the designated corresponding author. This Title Page is uploaded in the attached files area, along with this letter. Abstract, Text and References were double spaced, these are not changed. Content and form of the Text of manuscript is not changed.

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,

Zita Brigitta Radnay MD.

Department of Hematology, Institute for Internal Medicine

Faculty of Medicine, University of Debrecen

Nagyerdei krt. 98.

H-4032 Debrecen, Hungary

Telephone number: +36-20-582-9147, Email address: radnayzita@gmail.com

TRANSPLANTATION PROCEEDINGS
BARRY D. KAHAN, PhD, MD, Editor-in-Chief

Editorial Office:
11707 Trudeau Drive
Houston, TX 77065
Telephone: 713-984-0533

Barry D. Kahan, PhD, MD - Editor-in-Chief
Email: bkahan@transplantation-proceedings.org

**THIS SIGNED FORM IS REQUIRED AND MUST BE UPLOADED WITH YOUR
MANUSCRIPT UPON SUBMISSION THROUGH EES. WE WILL NOT PROCEED
WITH YOUR MANUSCRIPT REVIEW UNLESS THIS FORM IS INCLUDED.**

MANUSCRIPT RECEIPT - FINANCIAL AGREEMENT

Title Page With ALL Author Email Addresses: 2
Submitted Text Pages: 21
Submitted Tables: 6 Abstract Included Yes
Submitted Figures: 2
Total Pages Submitted (excluding Title Page and Abstract): 29

Manuscript Title:

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

By submission of this manuscript to *Transplantation Proceedings*, I acknowledge I have read the Guidelines to Authors of Manuscripts Submitted As an Original Work and agree with the contents, and that I have attached a completed and signed Authorship And Conflict Of Interest Statement (ACIS) on behalf of each author listed on this manuscript.

I acknowledge that if accepted, I am responsible for all manuscript page charges, which will be billed to me by Elsevier, the publisher of *Transplantation Proceedings*, at the rate of US\$99.95 per submitted manuscript page, understanding that each Table and Figure will count as one manuscript page each along with the text. I understand that page charges are based on the typed, submitted page, not on the printed page, and that THREE complimentary pages are automatically provided by *Transplantation Proceedings* for manuscripts accepted as an original work to be published in one of our dedicated issues. Authors will be contacted with a tracking number, the number of pages confirmed, and will be informed of the number of pages for which they are responsible. Further, I understand that use of color reproduction of graphics will result in an additional charge. The Abstract and Title page are complimentary by *Transplantation Proceedings*.

Additionally, I agree that this manuscript has not been submitted or published in any other journal, including *Transplantation Proceedings*, and no parts of the manuscript are duplicated. I understand that if the manuscript is accepted for publication, copyright of the manuscript is transferred to Elsevier.

Zita Radnay
Signature of Corresponding Author

Arpad Illes
Signature of Financially Responsible Party

ZITA RADNAY
Printed Name

ARPAD ILLES MD, DSCI
Printed Name