

Low Serum Mannose-Binding Lectin Level Increases the Risk of Death due to Pneumococcal Infection

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(See the editorial commentary by Sprong and van Deuren on pages 517–8)

Background. Previous studies have shown associations between low mannose-binding lectin (MBL) level or variant *MBL2* genotype and sepsis susceptibility. However, MBL deficiency has not been rigorously defined, and associations with sepsis outcomes have not been subjected to multivariable analysis.

Methods. We reanalyzed MBL results in a large cohort with use of individual data from 4 studies involving a total of 1642 healthy control subjects and systematically defined a reliable deficiency cutoff. Subsequently, data were reassessed to extend previous MBL and sepsis associations, with adjustment for known outcome predictors. We reanalyzed individual data from 675 patients from 5 adult studies and 1 pediatric study of MBL and severe bacterial infection.

Results. *XA/O* and *O/O* *MBL2* genotypes had the lowest median MBL concentrations. Receiver operating characteristic analysis revealed that an MBL cutoff value of 0.5 µg/mL was a reliable predictor of low-producing *MBL2* genotypes (sensitivity, 82%; specificity, 82%; negative predictive value, 98%). MBL deficiency was associated with increased likelihood of death among patients with severe bacterial infection (odds ratio, 2.11; 95% confidence interval, 1.30–3.43). In intensive care unit–based studies, there was a trend toward increased risk of death among MBL-deficient patients (odds ratio, 1.58; 95% confidence interval, 0.90–2.77) after adjustment for Acute Physiology and Chronic Health Enquiry II score. The risk of death was increased among MBL-deficient patients with *Streptococcus pneumoniae* infection (odds ratio, 5.62; 95% confidence interval, 1.27–24.92) after adjustment for bacteremia, comorbidities, and age.

Conclusions. We defined a serum level for MBL deficiency that can be used with confidence in future studies of MBL disease associations. The risk of death was increased among MBL-deficient patients with severe pneumococcal infection, highlighting the pathogenic significance of this innate immune defence protein.

Mannose-binding lectin (MBL) has been the subject of intense study, and the biology of this innate immune

molecule in the lectin complement pathway has been scrutinized for association with risks of infectious and inflammatory diseases, ischemic heart disease, and cancer. Serum MBL level and function varies substantially as a function of multiple polymorphisms of the *MBL2* genes. Low MBL levels and impaired function are predominantly seen in the presence of structural gene polymorphism homozygosity and the *LX* promoter haplotype [1]. However, currently, there is no standard for MBL deficiency based on a direct functional assessment

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Table 1. Study populations of healthy control subjects and patients with severe bacterial infection and frequency of mannose-binding lectin (MBL) deficiency, according to serum level or genotype definition.

Country, population	No. of patients	MBL deficiency definition, proportion of patients (%)		P	Study
		MBL level <0.5 µg/mL	Low-producing <i>MBL2</i> genotypes		
Australia					
Blood donors	236	58/236 (25)	35/236 (15)	<.01	[1]
Patients with bloodstream infection and/or pneumonia	195	63/193 (33) ^a	24/171 (14) ^a		[10]
Hong Kong: blood donors and community	1167	257/1167 (22)	85/1167 (7)	<.001	[17]
Gabon: children	93	36/93 (39)	14/93 (15)	<.01	[18]
Iceland: healthy family members of patients with SLE	146	34/146 (23)	14/146 (10)	.002	[19]
United Kingdom					
Patients with pediatric ICU-related infection or SIRS	53	17/53 (32) ^a	10/53 (19) ^a		[12]
Patients with adult ICU-related septic shock	173	44/80 (55) ^a	21/80 (26) ^a		[16]
Spain: patients with pneumococcal infection	99	12/79 (15) ^a	7/77 (9) ^a		[11]
Denmark: patients with pneumococcal bacteremia	72	26/72 (36) ^a	11/72 (15) ^a		[6]
The Netherlands: patients with peritonitis	83	43/81 (53) ^a	10/80 (13) ^a		[20]

NOTE. ICU, intensive care unit; SIRS, systemic inflammatory response syndrome; SLE, systemic lupus erythematosus.

^a Numbers not shown, because not all patients had both MBL level and *MBL2* genotype determined.

of a human cohort or an experimental animal model. Various investigators to date have used either *MBL2* genotypes that typically produce low MBL levels and/or measured low serum MBL levels to define a deficiency state. Various serum MBL level cutoffs of <1.0 µg/mL [2], <0.5 µg/mL [3], and even <0.1 µg/mL [4] have been used without rigorous justification. Similarly, there is little consistency with regard to low-producing *MBL2* genotypes; different studies have used either any structural gene polymorphism (*O*) [5], *LXA/O*, and *O/O* [6] genotypes or *O/O* [7] genotypes alone to define MBL deficiency. Therefore, it is important to introduce more certainty into this field through a systematic assessment of MBL levels in a large, multinational population of subjects.

MBL deficiency, variably defined, has been associated with increased susceptibility to many infectious diseases [8]. Invasive pneumococcal disease appears to be associated with low MBL levels [9, 10], although there have been some conflicting results [6, 11]. Severe bacterial infection and sepsis have also been found to occur more frequently in MBL-deficient patients. The development of systemic inflammatory response syndrome in critically ill patients without infection has been shown to be more common in those with MBL deficiency [12]. Again, these studies have used differing criteria for the assessment of MBL deficiency, including presence of *MBL2* polymorphism alone [6, 13], MBL levels alone [14], and both presence of *MBL2* polymorphism and MBL levels [12, 15]; other studies have included an assessment of MBL function [10]. In some of these studies of sepsis, univariate associations between low MBL levels [14, 16] or low-producing *MBL2* haplotypes [15] and increased risk of death were present.

By analyzing MBL levels in healthy control populations from

multiple studies, we accurately determined the range of MBL levels associated with each of the various *MBL2* genotypes. Using this information, we determined the MBL level that most reliably predicts the presence of low-producing *MBL2* genotypes. Furthermore, we applied this definition of MBL deficiency to a large group of patients with severe bacterial infection to assess the impact of this deficiency state on outcomes in patients with sepsis.

MATERIALS AND METHODS.

Definition of MBL deficiency with use of healthy control subjects. The published literature was searched with use of the PubMed database to identify studies in which MBL levels had been measured by ELISA and in which *MBL2* genotypes (promoter and structural haplotype) had been determined. Investigators identified by this process were invited to participate in a collaborative study. Individual patient data were collated from 1642 healthy subjects from 4 studies (table 1) [1, 17–19]. Three other healthy control populations including 501 subjects with fully characterized *MBL2* genotype and MBL levels are described elsewhere [21–23]. Data from these other studies were not made available for this analysis.

MBL levels were grouped according to *MBL2* genotypes and compared with use of the Kruskal-Wallis test. Receiver operating characteristic analysis was then performed using MBL levels as a predictor of the lowest-producing *MBL2* genotypes. The suggested cutoff value for MBL deficiency was found by maximizing the sensitivity and specificity of the MBL value for predicting low-producing *MBL2* genotypes. Comparison of the frequency of MBL deficiency based on MBL level and *MBL2*

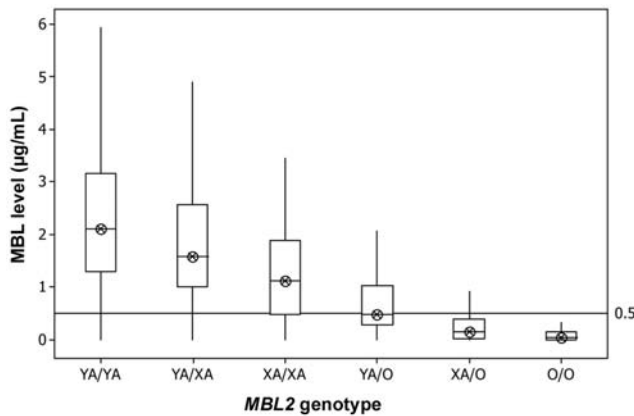


Figure 1. Median, interquartile range, and range of mannose-binding lectin (MBL) level in healthy control subjects, according to *MBL2* genotype. Suggested cutoff for MBL deficiency indicated, 0.5 µg/mL.

genotypes was performed with use of the χ^2 test. Statistical analyses were performed in Minitab, release 14 (Minitab), and Stata, version 9 (StataCorp).

Influence of MBL deficiency on outcome of severe bacterial infection. Published studies of the association between MBL deficiency and bacterial infection or sepsis were identified by search of the PubMed database, and investigators were invited to share individual patient data. These studies [6, 10–12, 16, 20] included 675 patients for whom MBL levels and/or *MBL2* genotypes had been determined. Specifically, MBL levels were measured in 558 patients (table 1). Blood samples for MBL assays were obtained on the day of diagnosis of sepsis [6, 10, 20] or within 48 h [11, 12, 16] after the diagnosis. MBL levels were determined by double-sandwich ELISA in all studies other than the Dutch peritonitis series [20] in which mannan-binding ELISA was used. The patients in this study of combined data were classified as being MBL deficient or replete with use of the MBL serum level cutoff derived from the preceding analysis of healthy control subjects. Data on the specific organism causing sepsis were present for 482 patients; 287 of these patients had bacteremia. Data on the presence or absence of comorbidities were recorded for a total of 326 patients. These included information on the presence of chronic obstructive pulmonary disease, hepatopathy, cancer, diabetes, and HIV infection in 264 patients. An additional 62 patients had comorbidities classified on the basis of the McCabe score [24]. The quality of the genetic associations reported by these studies was assessed on the basis of published criteria [25]. Data for 975 patients included in 3 other studies of MBL and sepsis [13–15] were not made available for this study.

Categorical variables were compared using the χ^2 test. Student's *t* test and the Mann-Whitney log-rank test were used to compare normally and nonnormally distributed variables, re-

spectively. Meta-analysis and binary logistic regression was used for multivariable analysis to determine the association between MBL deficiency and death. Statistical analyses were performed in Minitab, release 14, and Stata, version 9.

RESULTS

Although there were statistically significant differences in MBL serum levels among all of the *MBL2* genotype groupings ($P = .02$, by Kruskal-Wallis log-rank test), the most markedly decreased levels and the least intragroup variation were seen in patients with genotypes *XA/O* and *O/O* (figure 1). The MBL levels in patients with the *YA/O* genotype were clearly intermediate between these obviously low-producing *MBL2* genotypes and the high-producing MBL groups of *YA/YA*, *YA/XA*, and *XA/XA*. The *XA/O* and *O/O* groups were chosen to definitively represent the *MBL2*-deficient genotypes. Receiver operating characteristic analysis revealed that MBL level was a reliable predictor of these *MBL2* genotypes (area under the receiver operating characteristic curve, 0.90) (figure 2). An MBL serum level of 0.50 µg/mL maximized sensitivity and specificity for predicting low-producing *MBL2* genotypes (both were 82%). This cutoff had a positive predictive value of 31% and a negative predictive value of 98%. This level is proposed as a useful indicator of MBL deficiency, particularly for screening purposes, because of its high negative predictive value.

Using this MBL cutoff level, we determined the prevalence of MBL deficiency in the control populations studied and compared this with the prevalence as indicated by low-producing *MBL2* genotypes (table 1). For all of the cohorts of healthy control subjects that were included in this study, the rates of

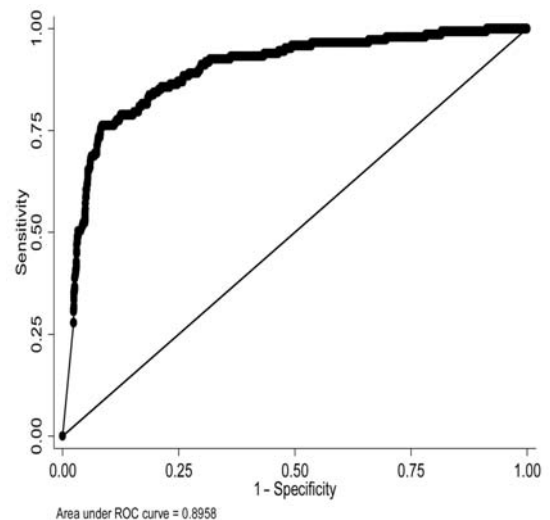


Figure 2. Receiver operator characteristic (ROC) curve of mannose-binding lectin level as a predictor of low-producing *MBL2* genotypes (*XA/O* and *O/O*) in healthy control subjects.

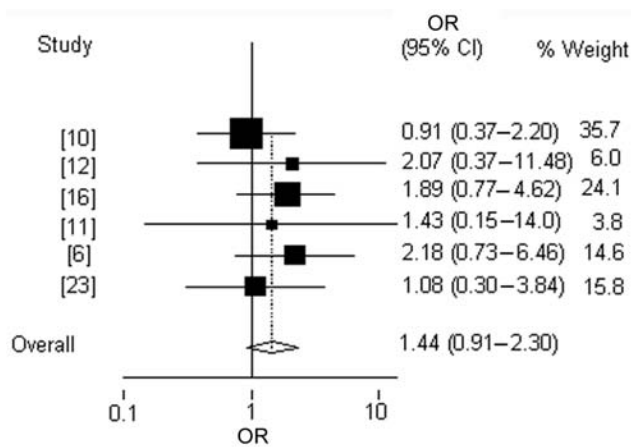


Figure 3. Forest plot of association between mannose-binding lectin (MBL) deficiency (MBL level, $<0.5 \mu\text{g/mL}$) and death.

MBL deficiency defined by an MBL level $<0.5 \mu\text{g/mL}$ were significantly higher than when deficiency was defined by low-producing *MBL2* genotypes (*XA/O* and *O/O*).

Among the studies of MBL and severe bacterial infection that were incorporated in the combined analysis, the median genetic association study quality scores [25] were 7 in 2 case-controlled studies and 6 in the 3 cohort studies; these scores were assessed as being of moderate-to-high quality. These median quality scores are higher than those found in a systematic review of studies of genetic association and sepsis [25]. The most common deficiencies of the studies used in the review were the absence of power calculations and Hardy-Weinberg equilibrium analysis. A power calculation for the present study was performed assuming a 25% mortality rate and a frequency of MBL deficiency of 30%. This mortality rate was chosen because it is midway between the reported mortalities for pneumococcal bacteremia [26] and severe sepsis [27], and the majority of this cohort consists of patient groups with these conditions. Assuming a relative risk of death of 2 among MBL-deficient patients, 168 patients would be required to achieve 80% power to detect a difference between groups at a *P* value of .05. We were able to evaluate survival among 477 patients who had their MBL levels determined; this is substantially more than the number required to power this observation. Overall, there were 123 deaths among 591 patients (mortality, 21%) for whom information on in-hospital survival relating to the episode of severe infection was available.

The association between serum MBL level deficiency and death in infected patients was determined by χ^2 test on crude, pooled data. There was an increase in the rate of MBL deficiency among patients with severe bacterial infection who died (45 of 94 patients who died were MBL deficient), compared with those who survived (116 of 383 patients who survived were MBL

deficient; OR, 2.11; 95% CI, 1.30-3.43; *P* = .001). Meta-analysis, which adjusted for varying prevalences of serum MBL level deficiency and death in the individual studies, showed a similar effect, but the 95% CI did not indicate statistical significance (OR, 1.44; 95% CI, 0.91-2.3; *P* = .12) (figure 3). A funnel plot indicated that there was no reporting bias in the studies of MBL infection outcome (figure 4). This funnel plot involved only the studies included in this reanalysis, because the frequency of serum MBL level deficiency could not be ascertained from the other studies of MBL and sepsis in which individual patient data were not made available [13-15]. Multivariable analysis of defined risk factors was performed for the 454 patients from studies in which APACHE II scores were calculated [10, 16, 20]. After adjustment for APACHE II score, age, and sex, serum MBL level deficiency was associated with a trend toward increased likelihood of death among infected patients (OR, 1.58; 95% CI, 0.90-2.77) (table 2).

Because the largest subset of patients with severe bacterial infection was the subset with pneumococcal disease (186 of 675 patients), this was analyzed separately. The majority of these patients (171 of 186 patients) came from 2 studies that concentrated exclusively on *S. pneumoniae* infection [6, 11] but in which APACHE II scores were not calculated. In addition, although Fine scores [28] were calculated for 99 of the 186 patients with pneumococcal disease, this disease severity index could not be used in the multivariable analysis of factors influencing death, because it was calculated for an insufficient number of patients who died (*n* = 5) to be incorporated. Therefore, the presence of bacteremia and patient comorbidities were used instead to assess independent risk factors for death. Multivariable analysis, adjusted for comorbidity, bacteremia, age, and sex, revealed that, in patients with pneumococcal infection, MBL deficiency increased the risk of death (table 2).

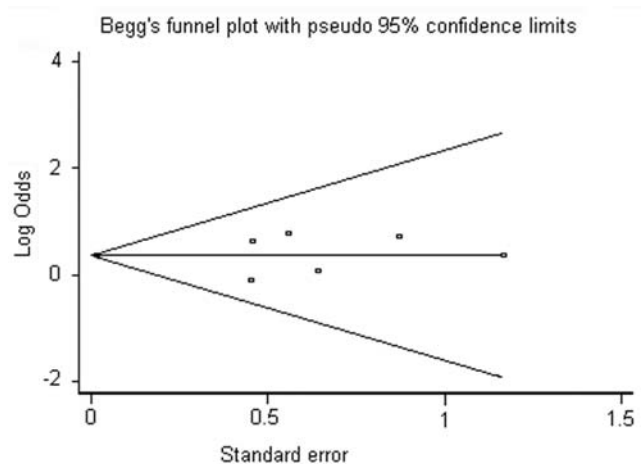


Figure 4. Funnel plot incorporating pseudo 95% CIs of mannose-binding lectin in studies that reported death as an outcome.

Table 2. Factors predicting death in patients with severe bacterial infection.

Patient variable	Patients		Univariate analysis		Multivariable analysis	
	Died	Survived	OR (95% CI)	P	OR (95% CI)	P
Patients with APACHE II scores (n = 454)						
MBL deficiency ^a	38/76	111/281	1.53 (0.89–26.3)	.10	1.58 (0.90–2.77)	.10
Mean APACHE II score	21.7	16		<.001	1.13 (1.09–1.18)	<.001
Age, median years	70	59		<.001	1.04 (1.02–1.05)	<.001
Male sex	60/105	203/347	0.95 (0.59–1.51)	.86	1.32 (0.75–2.33)	.30
Patients with pneumococcal disease (n = 186)						
MBL deficiency	12/26	34/139	2.65 (1.03–6.81)	.02	5.62 (1.27–24.92)	.02
Age, median years	70.5	61		<.01	1.09 (1.03–1.16)	<.01
Bacteremia	21/23	98/126	3 (0.66–27.8)	.10	1.78 (0.27–11.64)	.50
Comorbidities	18/24	84/149	2.32 (0.82–7.53)	.08	1.16 (0.33–4.13)	.80
Male sex	11/17	69/137	0.54 (0.15–1.7)	.24	3.78 (0.71–20.08)	.10

NOTE. Data are proportions of patients, unless otherwise indicated. APACHE, Acute Physiology and Chronic Health Enquiry; MBL, mannose-binding lectin.

^a MBL level, <0.5 µg/mL.

DISCUSSION

We studied a large cohort of healthy subjects with individual data drawn from 4 studies in which both MBL level and promoter and/or structural *MBL2* genotype were characterized. The size of the cohort ($n = 1642$) made it possible to generalize with far greater certainty than with previous studies about the MBL levels to be expected in those with the lowest-producing *MBL2* genotypes. Assessment of the MBL levels found in each of the *MBL2* genotype groups revealed that the interquartile ranges in patients with *XA/O* and *O/O* genotypes are substantially lower than 0.50 µg/mL and that this level coincides with the median in patients with the *YA/O* genotype. Furthermore, the lower quartile (0.28 µg/mL) in patients with the *YA/O* approximates the upper quartile (0.39 µg/mL) in patients with the *XA/O* genotype. The patients with the other *MBL2* genotypes (*YA/YA*, *YA/XA*, and *XA/XA*) had median MBL levels that were clearly higher than this cutoff. On the basis of these findings, we propose that patients with the *XA/O* or *O/O* genotype can be considered to be clearly MBL deficient. It would be inappropriate to use the *O/O* group alone to determine MBL deficiency because of the very substantial overlap with patients with the *XA/O* genotype. Subsequently, receiver operating characteristic analysis confirmed that an MBL level <0.50 µg/mL is a valid indicator of the presence of *MBL2*-deficient genotypes.

It is important to appreciate the large variation in MBL serum levels seen in each *MBL2* genotype group. These non-parametrically distributed values all ranged to 0 µg/mL. This is the obvious explanation for the observation that *MBL2* genotype alone is a poor indicator of the presence of low MBL levels and, therefore, of MBL deficiency. Although our study revealed that a serum MBL level <0.5 µg/mL is a valid indicator of the presence of the low-producing *MBL2* genotypes *XA/O* and *O/O*, such low levels have also been found in a significant

number of persons with alternative genotypes. This accounts for the discrepancy in the frequency of MBL deficiency between the MBL level and genotype definitions explored in this and other studies.

The association between MBL deficiency and severe bacterial infection convincingly demonstrated in this reanalysis of a large group of patients from previous studies confirms previous observations [10, 12, 13, 15, 16]. A trend that approached statistical significance was found between MBL deficiency and death in patients with severe bacterial infection, with use of APACHE II score to adjust for disease severity and with the incorporation of patient risk factors, such as age and sex in multivariable analysis. This extends previous findings of univariate associations between MBL deficiency and death due to sepsis [14–16].

Conflicting reports on the association between invasive pneumococcal disease [6, 9] and MBL deficiency also seem to be resolved by this combined analysis. For the first time (to our knowledge), MBL deficiency was shown to increase the risk of death among patients with pneumococcal infection. Although APACHE II and Fine scores could not be used to adjust for pneumococcal disease severity, bacteremia—which has a major impact on mortality [29]—and comorbidity could be incorporated in the multivariable analysis. This finding suggests that patients with pneumococcal sepsis may be an important group in which to investigate the potential benefits of MBL therapy.

MBL binds to microbial cell surface sugars arranged in dense spatial arrays referred to as pathogen-associated molecular patterns. MBL binds to pneumococcus [30], but this may be abrogated in the presence of a polysaccharide capsule [31]. It must be recognized that the MBL pathway is a minor contributor to complement-mediated defence against *S. pneumoniae*, compared with the classic pathway [32]. MBL does, however,

also contribute to bacterial killing by noncomplement mechanisms, such as opsonophagocytosis. Pathogens other than *S. pneumoniae* were isolated in samples from fewer patients in our study than was *S. pneumoniae*. Specifically, the smaller numbers of patients with *S. aureus* infection may explain the absence of an apparent significant association between MBL deficiency and death in these patients because of insufficient statistical power (multivariable analysis data not shown). MBL appears to play a role in the response to *S. aureus* infection, binding to the organism and precipitating C4 deposition with increased neutrophil phagocytosis [33]. Compelling evidence from a MBL knockout mouse model revealed reduced survival in *S. aureus*-infected MBL-null animals [34]. MBL deficiency appears to predispose to meningococcal sepsis [35], another severe infection caused by an encapsulated organism. This clinical association is also supported by investigations of the ability of MBL to bind to and facilitate killing of *Neisseria meningitidis* [36]. There was only a small number of patients with *N. meningitidis* infection in the current study, because patients from a previous large study did not have MBL levels or *MBL2* promoter haplotypes determined [35].

The most important limitation of the present study is the heterogeneity of both the control and infected populations. Nonetheless, the large sample collection from multiple racial and geographic sources has provided an opportunity to demonstrate robust associations between MBL and predisposition to severe infection. Differences in serum MBL level testing methodologies may interfere with comparison of results. The basic laboratory methodology used for all results from the healthy control population that were collated here was the same methodology used with the double anti-MBL sandwich antibody ELISAs performed. Finally, differences may exist in the nature of the serum standards used to produce standard curves and to derive results.

The nature of the acute-phase MBL response was studied as part of a number of the sepsis studies included here [11, 16, 37]. These studies reveal that, at most, the variation in MBL levels is of a factor of 2–3 fold, with one study showing the same proportions of septic patients having increased, decreased, and stable MBL levels [37]. MBL did not behave as an acute-phase reactant when it was studied in the context of pneumococcal infection [11]. In the context of surgical injury, MBL levels were not shown to alter substantially, in marked comparison to the known acute-phase reactants CRP and IL-6 [38]. It is unlikely, therefore, that a substantial decrease in MBL levels as a result of consumption from sepsis is the cause of the association between low MBL level, sepsis, and poor outcome. This is particularly pertinent to the association demonstrated between MBL deficiency and death in patients with pneumococcal sepsis. Although changes in MBL have been correlated with the type of organism causing sepsis, it has been shown

that MBL is consumed in sepsis due to gram-negative, but not gram-positive, organisms [39].

MBL may be extracted from plasma or produced by recombinant means for clinical use in deficient or septic patients. Phase I studies of MBL infusion have been performed [40]. MBL has a long serum half-life, and there is limited experience of its use for cystic fibrosis [41] and other chronic infections. Recombinant human MBL replacement therapy is now being studied in clinical trials involving patients receiving allogeneic stem cell transplantation and liver transplantation. The data from the large patient cohort that we report suggest a serum level that reliably indicates MBL deficiency. It is clear from this data that low MBL level is a more reliable indicator of deficiency than is the presence of low-producing *MBL2* genotypes. This rationally derived MBL serum level cutoff for deficiency could be used to guide replacement therapy with MBL. Because death in the context of severe bacterial infection, especially when due to pneumococcus, was associated with MBL deficiency after adjustment for bacteremia, age, and comorbidity, severe bacterial pneumonia could be an important future target for study of MBL therapy. Mortality associated with bacteremic pneumococcal infection has remained largely unchanged, despite 50 years of advancements in supportive care. Treatment with an innate, immune-based therapy may provide a new opportunity to reduce this substantial disease burden.

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Potential conflicts of interest. D.P.E. is the inventor on the use patent application “A medical protocol” for hypersupplementation with mannose-binding lectin for the treatment and prevention of sepsis. M.M.D. is listed as an inventor on the patent WO/2003/090774 “Mannose-binding lectin and uses thereof” but has not received any financial interest from this patent. A.C.G. holds stock options in Sirius Genomics. All other authors: no conflicts.

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