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Mannose-Binding Lectin Levels in Critically Ill Children with Severe Infections

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Abstract

Objective—Low mannose-binding lectin (MBL) levels and haplotypes associated with low MBL production have been associated with infection and severe sepsis. We tested the hypothesis that MBL levels would be associated with severe infection in a large cohort of critically ill children.

Design—Prospective cohort study

Setting—Medical and Surgical Pediatric Intensive Care Units (PICUs), Boston Children's Hospital

Patients—Children < 21 years of age admitted to the intensive care units from November 2009 to November 2010.

Interventions—None

Measurements and Main Results—We measured MBL levels in 479/520 (92%) consecutively admitted children with severe or life-threatening illness. We genotyped 213 Caucasian children for MBL haplotype tagging variants and assigned haplotypes. In the univariate analyses of MBL levels with pre-admission characteristics, levels were higher in patients with pre-existing renal disease. Patients who received >100 ml/kg of fluids in the first 24 hours after admission had markedly lower MBL, as did patients post-spinal fusion surgery. MBL levels had no association with infection status on admission, or with progression from systemic inflammatory response syndrome to sepsis or septic shock. Although MBL haplotypes strongly influenced MBL

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levels in the predicted relationship, low MBL-producing haplotypes were not associated with increased risk of infection.

Conclusions—Mannose-binding lectin levels are largely genetically determined. This relationship was preserved in children during critical illness, despite the effect of large-volume fluid administration on MBL levels. Previous literature evaluating an association between MBL levels and severe infection is inconsistent; we found no relationship in our PICU cohort. We found that MBL levels were lower after aggressive fluid resuscitation, and suggest that studies of MBL in critically ill patients should assess MBL haplotypes to reflect pre-illness levels.

Keywords

Mannose-Binding Lectin; MBL; complement activation; innate immunity; child; sepsis; intensive care

INTRODUCTION

The innate immune system is the first line of defense against invasive pathologic organisms and its role is essential in controlling infection in the first 24–48 hours before the adaptive immune system is able to mount an adequate response. One of the primary innate immune processes is activation of the complement system for direct pathogen killing and for opsonization, which marks pathogens for destruction by phagocytes. A critical antimicrobial protein in this pathway is mannose-binding lectin (MBL) which recognizes mannose sugars on the periphery of bacteria, viruses, and fungi, and on damaged human cells. (1, 2) Binding of this protein to cell membranes causes conformational changes that activate directed complement deposition for the invading microbe to be opsonized and killed. (2)

In humans, MBL is produced continuously in the liver; serum levels in healthy individuals have been shown to be influenced by host genetic makeup. (3, 4) The MBL protein is coded by the *MBL2* gene which is comprised of 4 exons located on the long arm of chromosome 10. (5, 6) Within exon 1, there are three common single nucleotide polymorphisms (SNPs) located at codons 52, 54, and 57 (D, B, C; collectively termed O) and a promoter gene polymorphism at - 221 (termed X/Y). (See Figure 1A) (5–9) Heterozygosity for any one of the high producing variants (A/O) produces MBL levels in the near normal or only mildly reduced range; homozygosity (O/O) or compound heterozygosity with promoter X/Y leads to markedly reduced MBL levels. (8, 10, 11)

Low-producing MBL haplotypes remain at a relatively high level in the normal population despite some studies showing that they are associated with worsening severity of illness and increased susceptibility to infection. The level of MBL determining deficiency has not been specifically defined; some use <1000 ng/mL while others hypothesize that clinical consequences begin to occur at levels < 500 ng/mL; homozygous “low-producers” of MBL generally have levels < 50ng/mL. (6, 10, 12–14) It is unclear if there is an advantage to low-producing haplotypes although some have speculated MBL may worsen clinical presentation of infections that cause damage mainly by inflammatory responses.(6, 10, 14–16) Previous studies assessing the relationship between MBL levels and critical illness have varied in cohort size, the population studied, the outcome assessed, and whether/how MBL genotype

was evaluated. We speculate that this may have led to the conflicting reports on whether MBL is associated with severe infection.(7, 16–21) In addition, potentially confounding variables such as fluid administration could dilute MBL levels and influence the association.

Because relative deficiency of MBL has been associated with sepsis in some studies, researchers are evaluating recombinant MBL as a therapy for sepsis and other serious infections. (22–24) To better clarify the association between MBL and infection severity in children, we aimed to examine associations between MBL levels, MBL haplotypes and severe infections in children in the pediatric intensive care unit (PICU). We hypothesized that: a) haplotypes would predict serum MBL levels in the previously described manner and b) critically ill children with severe infections (sepsis and septic shock) would have lower serum MBL levels and higher frequencies of low-producing MBL haplotypes in comparison to those without severe infections.

MATERIALS AND METHODS

We included children admitted to the Medical and Surgical Pediatric Intensive Care Units at Boston Children's Hospital from November 9, 2009 to November 9, 2010. Eligibility criteria were: age < 21 years; estimated PICU stay of ≥ 48 hours (this excluded short-term monitoring patients) or admission due to suspected infection. Patients admitted to the Cardiac ICU were not included in this study due to the alterations in MBL from cardiac bypass as well as the high rate of administration of fresh frozen plasma which contains MBL. Institutional Review Board approval was obtained prior to the beginning of the study. Informed consent was obtained by study coordinators shortly after ICU admission and data were obtained from parent interviews and from the electronic medical record. A second separate consent was obtained for genotyping. Additional details of the study cohort have been published previously. (25)

The Pediatric Risk of Mortality III (PRISM III) score was used to assess illness severity in the first 24 hours. (26) Maximum vasopressor use was scored according to the Sequential Organ Failure Assessment, cardiovascular (CV-SOFA) (27) modified for pediatrics as follows: 0–1: no vasopressors, 2: dopamine < 5 mcg/kg/min, 3: dopamine 5–15 mcg/kg/min or norepinephrine/epinephrine < 0.1 mcg/kg/min, 4: dopamine > 15mcg/kg/min or norepinephrine/epinephrine > 0.1mcg/kg/min.

Suspected infections were those with markers of infection (e.g. cultures sent, antibiotic therapy initiated, chest x-ray findings, etc.) or criteria for community-acquired pneumonia but with negative microbiologic testing. Confirmed infections included 1) bacterial: culture of pathogenic bacteria from blood, CSF, or lung AND treatment with intravenous antibiotics; 2) fungal: positive fungal culture AND antifungal treatment; 3) viral: viral pathogen detected; 4) multiple: more than one of the preceding types. Community-acquired pneumonia was defined as meeting published criteria for pneumonia with bacteria confirmation.(28) Severity of illness categories in the sepsis analysis were as follows: The "SIRS, no infection" category included patients meeting published criteria for systemic inflammatory response syndrome (SIRS) on ICU admission without evidence of infection. (29, 30) Sepsis was defined as suspected or confirmed infection AND SIRS. (29) Severe

sepsis was defined as sepsis with an ICU admission day CV-SOFA score ≥ 2, and includes septic shock.

Laboratory Methods and Genotyping

Blood was obtained as close to admission as possible, either from blood drawn at the time of admission for other purposes, or obtained from previous leftover samples stored in the laboratory refrigerator. All plasma was stored refrigerated, frozen at −80°C within 7 days, then shipped frozen for analysis. MBL measurements were done at the Cytokine Reference Lab using a commercial ELISA Kit from R&D Systems (Minneapolis, MN). Samples were diluted 400-fold to achieve levels within the dynamic range of the assay (0.156–10ng/mL).

Genotyping was reserved for patients of Caucasian race to minimize possible confounding by racial stratification. Single nucleotide polymorphisms were determined for rs1800450 (SNP “B”), rs5030737 (SNP “D”), rs1800451 (SNP “C”), and rs7096206 (Promoter “X/Y”) using TaqMan SNP assays (Applied Biosystems) with proprietary primer/probe combinations (See Fig 1A). SNP rs11003125 (Promoter “H/L”) was not included; this SNP had a much weaker effect on MBL levels in preliminary analysis. (31, 32) Haplotypes were determined as previously described, where Y represents the high producing promoter allele and X the low producing allele. The minor pathogenic alleles B, C, and D were collectively grouped as “O”. (6, 10) We compared both the individual haplotypes listed in Figure 1B as well as diplotypes grouped into 3 categories of High (YA/YA, YA/XA), Mid (YA/YO), and Low (XA/YO, YO/YO, and XA/XA) for improved power.

Statistical Methods

Because MBL levels were not normally distributed, we used nonparametric analyses with Spearman correlations for continuous, Mann-Whitney tests for dichotomous, and Kruskal-Wallis tests for categorical variables. Chi-squared tests were used for the association of haplotype with categorical variables and Kruskal-Wallis for association with continuous or ordinal variables. SPSS statistical package was used for computations (version 19.0.0, IBM Corp.).

RESULTS

We screened 2366 consecutive PICU admissions and enrolled 520/818 (62.5%) eligible children in this study. (25) Of 520 enrolled, 41 did not have an MBL levels measured; this yielded 479 patients with MBL levels included in our study. Baseline characteristics for the study population are shown in Table 1. Most children were admitted via the Emergency Department or the Operating Room, so some MBL levels were obtained from samples just prior to ICU admission. Overall, 94% of samples were drawn within 48-hours of PICU admission.

The univariate analysis of baseline patient characteristics and the effects on MBL levels are shown in Table 1. The median MBL level for the entire sample was 1197 ng/mL (IQR 718–1878 ng/mL; range 110–6154). Of the variables tested, only “Other” race ($p = 0.01$) and renal disease ($p = 0.03$) significantly increased MBL levels.

Of the 520 children enrolled in the study; 213 of the 353 parents who self-identified as white race also gave consent for genotype analysis (60%). Of these, 201/213 (94%) also had sufficient samples to obtain MBL level. The proportion carrying the “O/O” diplotype (3.3%) and the distribution of individual haplotypes (YA 0.535, XA 0.249, and YO 0.216) were comparable to previous studies in white populations. (32) MBL level was correlated with underlying haplotype (See Figure 1B, p = < 0.001, using the promoter X/Y allele and the grouped exonic alleles A/O). Our findings were in agreement with previously described MBL linkage disequilibrium, no novel haplotypes were observed. (31) As expected, homozygosity for an exonic allele (“O”), or compound heterozygosity for “O” and “X” produced the lowest levels. Individual allele frequencies were in Hardy-Weinburg equilibrium.

Univariate analyses were performed on the association of MBL levels with non-infection reasons for PICU admission (emergent vs. elective, post-operative versus not, trauma, status asthmaticus, status epilepticus, and admitted for monitoring). Only admission after operative spinal fusion was associated with a lower MBL level (p = 0.04). We did not identify any association between infection-related admission categories with MBL levels (See Table 2). No specific infectious process was associated with lower or higher MBL levels. “MBL deficiency” defined as MBL level < 1000 ng/mL was also not associated with infection categories (p > 0.05 for both suspected and confirmed infections, including all infections, bacterial infections, and other infections separately, DNS).

The univariate analysis of the association between MBL levels and clinical outcomes is shown in Table 3. We found that subjects receiving very large volumes (> 100cc/kg) of fluid prior to or in the first 12 hours after admission had lower MBL levels (p = 0.007 for Spearman’s correlation, r = 0.12; correlation with non-categorized fluid volume). We also found a significant association between MBL level < 1000 ng/mL and fluid administered prior or within the first 12 hours. Patients with MBL level less than 1000ng/mL received 41cc/kg (median, IQR 17–80) vs. 32.7 cc/kg (median, IQR 7.1–64.5) in patients with MBL greater than 1000 ng/mL (p=0.02 Mann-Whitney U)).

We also analyzed the effect of *MBL2* genotype on outcomes, comparing diplotypes as High (YA/YA, YA/XA), Mid (YA/YO), and Low (XA/YO, YO/YO, and XA/XA) MBL producers (See Figure 2). As shown in Figure 2, the distribution of diplotypes was similar across categories of no SIRS vs. SIRS vs. sepsis vs. severe septic shock (of 213 patients assigned haplotypes, we were unable to assign an infectious category for 1 patient). The outcome variables listed in Table 3 for serum level analysis were tested for genotype association using Chi-square testing and none were significant (all p>0.05). We then further sub-categorized by age (given previous literature has implied there are some instances where younger children may be more affected for low production of MBL or low-producing diplotypes), however, there was also no statistical difference in distribution of diplotypes across the spectrum of non-infected vs. severe sepsis in the youngest age category (children <1 year old, n=43).

To determine whether MBL level and *MBL2* diplotype may have a stronger influence in patients that do not have other, larger infectious risk factors such as neutropenia (oncologic

patients) or chronic respiratory insufficiency, a subpopulation of 107 patients with either no chronic disease or only having asthma was examined. Weak associations between fluid administration ($p = 0.04$, Spearman's correlation, $r = 0.20$) and CV-SOFA score ($p = 0.05$, Spearman's correlation, $r = -0.19$) with lower MBL levels were identified; but no other associations were identified.

DISCUSSION

In contrast to prior reports in adult and pediatric populations, we did not find lower MBL levels in children critically ill from infection compared to those admitted to the PICU for other reasons. (4, 11, 12, 16, 19, 33–38) We did find that MBL levels were diluted in patients that received extremely high volumes of fluid in the first 24 hours; a common occurrence in patients with severe sepsis that could possibly confound the relationship between MBL levels and infection-related critical illness. MBL levels were strongly genetically influenced by the combination of high, intermediate and low producing haplotypes. We found no relationship between carriers of low-producing *MBL2* diplotypes and admission for infection or development of sepsis.

In a study of MBL in critically ill children, Fidler *et al* (4) reported an increasing proportion of low-producing (AO and OO) haplotypes in 50 children with SIRS and sepsis in comparison to children admitted for non-infectious causes. The measured MBL in their population was significantly lower (median MBL level of 100 ng/mL in lowest producing population) than levels in our study. (4) Similarly, Garred *et al* (37) and Gordon *et al* (36) reported MBL polymorphisms increasing risk of sepsis in critically ill adult populations. However, other studies in adults have not found MBL to be associated with risk for sepsis (39–42) or have reported a more nuanced picture that includes a mixture of both pro-inflammatory and anti-infection effects which may be beneficial or detrimental in varying disease states. (33, 34, 43) Specifically in pediatrics, six studies have demonstrated an association between lower MBL levels, or low-MBL producing haplotypes, and increased severity of infection-related disease (4, 5, 15, 16, 44–46) while five studies showed similar findings to ours with a lack of association (12, 46–50) and two studies concluded a possible protective role for low-producing MBL genotypes or levels. (12, 51)

There are likely several etiologies behind the lack of association between MBL levels, low-producing MBL haplotypes, and increased susceptibility to severe infection. First, the redundancy in complement activation within the innate immune system may provide relative resilience. (2) Second, our range of MBL levels (IQR 718–1878) are relatively high in comparison to a previously described cut-off of 500 for clinically important MBL deficiency. (4, 7, 12) Severe MBL deficiency has been described by some as <50 ng/ml, a level not identified in our patients; it has been postulated that patients are relatively protected above this low level due to pathway redundancy. (52, 53) Finally, in pro-inflammatory states which are often seen in critical illness, low-producing MBL may be advantageous if higher MBL production is associated with more severe inflammatory damage via a different mechanism. (42, 54–56) Grouping heterogeneous disease-states together under an umbrella of infection-related critical illness could combine cohorts in which MBL is detrimental due to inflammation with those in which it is beneficial.

Strengths of our study included a relatively large cohort of 520 children compared to prior studies and a study design which allowed for comparison of multiple different subsets of critically ill patients while maintaining an appropriate control group of similar patients. We were able to replicate the previously observed relationship between MBL genotype and MBL level (See Figure 1B). Additionally, we were able to evaluate the association between MBL levels and haplotypes in 284 children with SIRS, sepsis, or severe sepsis.

This study had several limitations. Because we *a priori* limited our population for genetic analysis to whites to prevent racial stratification (6, 32), and because not all patients consented for genotyping, our statistical power for subset group analysis was limited as was our ability to generalize these results to all populations. With limited numbers, we were unable to assess the association of specific types of infections with MBL haplotypes and our findings are limited to the broader category of critically ill patients with infections. Additionally, although patient age may influence the importance of MBL in immune protection (i.e. younger patients lacking humoral immunity may be more dependent on innate immunity), we had relatively few neonates as this study assessed a pediatric ICU population.

CONCLUSIONS

We did not find an association between infection-related causes of illness and MBL levels or *MBL2* haplotypes in children admitted to the PICU. As such, our results do not support testing for MBL deficiency or use of recombinant MBL as a potential immunomodulatory therapy for children with severe and life-threatening infections. Because large-volume fluid resuscitation was often a marker of a higher illness severity, we were not able to determine if fluid dilution of plasma proteins such as MBL may predispose patients to worse outcomes. Our study supports that variability in MBL levels is genetically determined. To control for the risk of MBL serum level dilution with large volumes of fluid, we suggest that future studies in critically ill patients use *MBL2* haplotype carriage to reflect pre-illness MBL levels.

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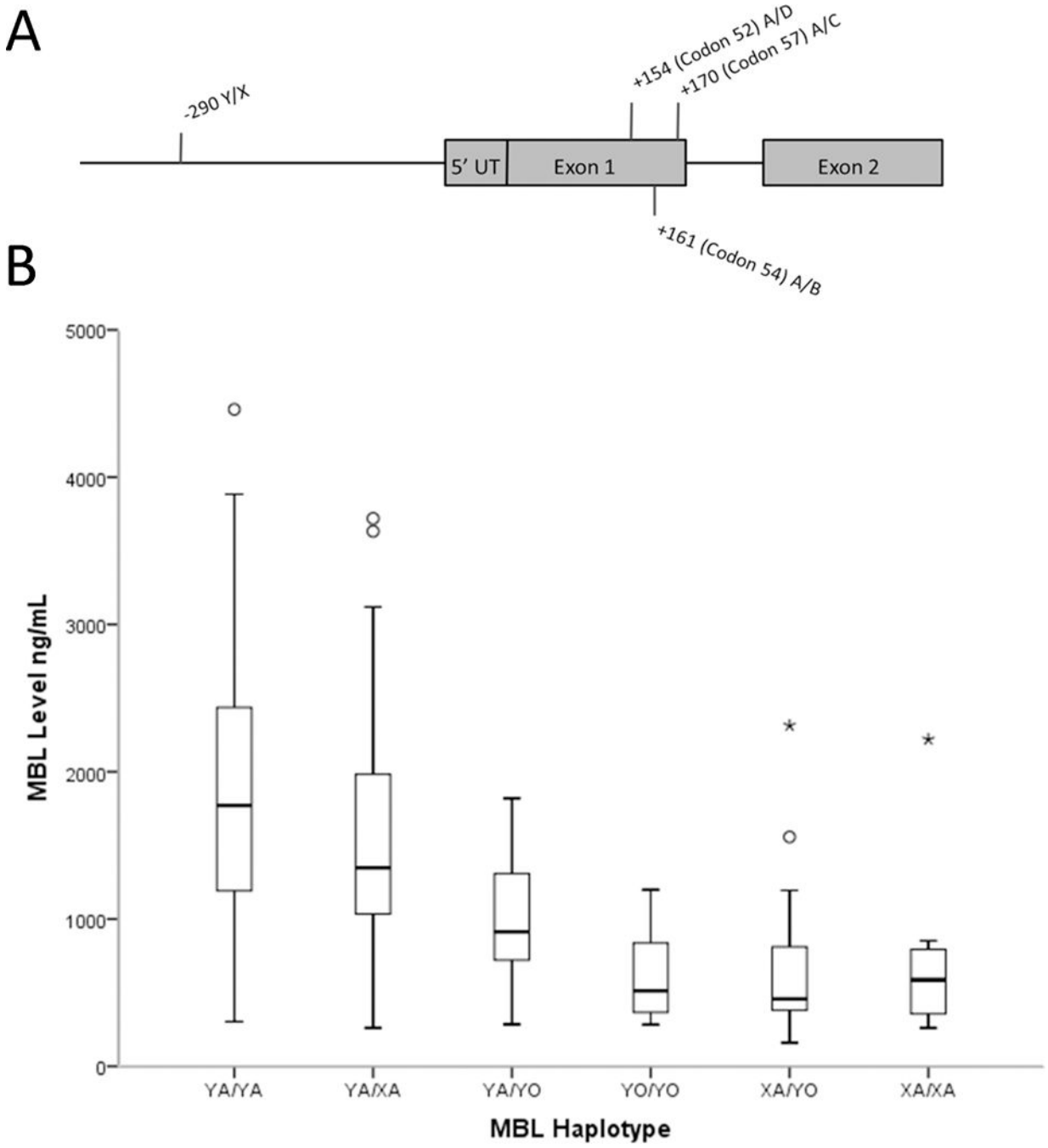

Figure 1.

A) Graphical representation of common MBL allelic variants and their location within the 5′ end of the gene. B) Boxplot demonstrating the effect of haplotype on MBL level in a subset of 201 patients that consented for genotyping. Haplotypes are assigned as previously described and ordered by median MBL level in current study.

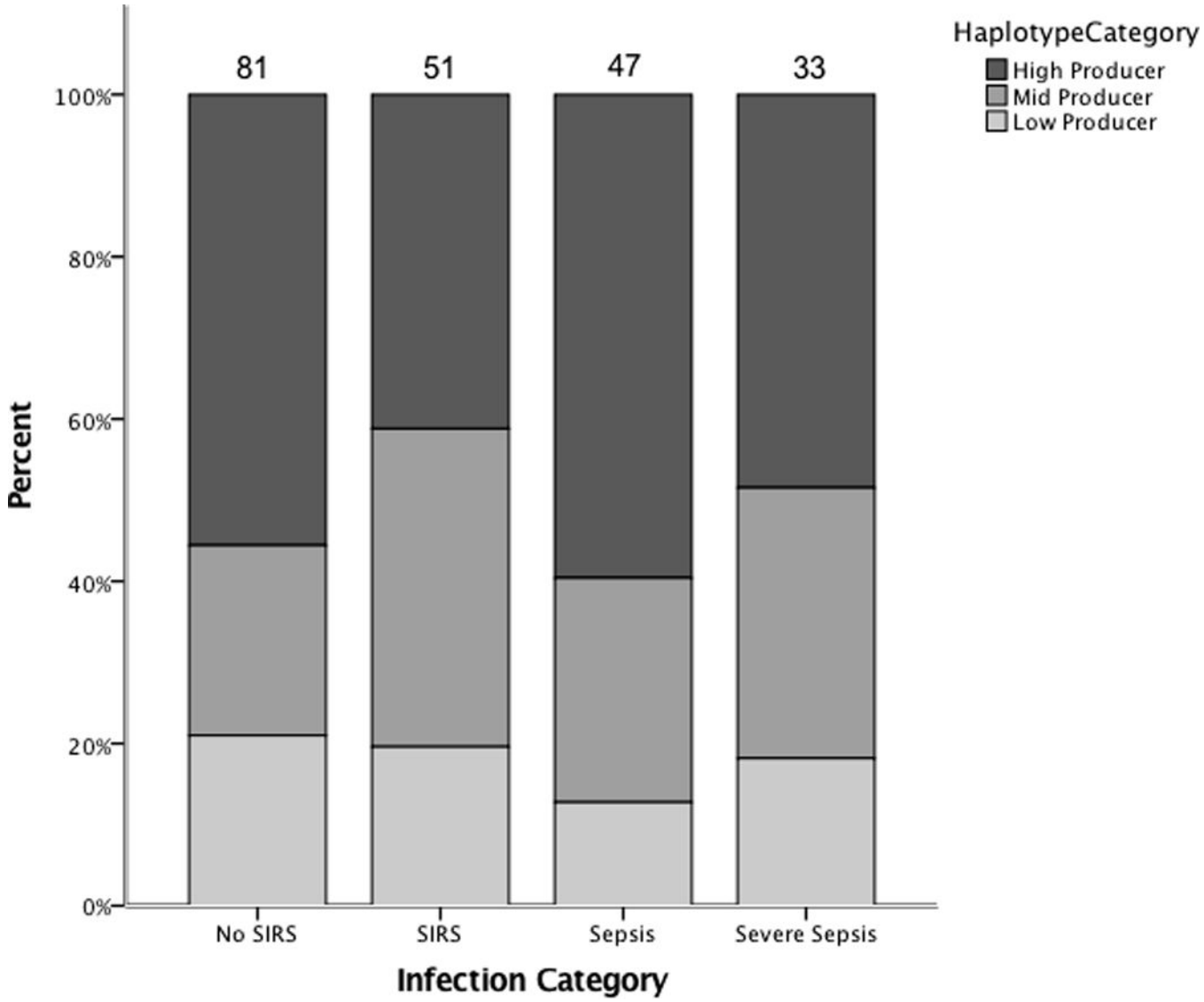

Figure 2.

Bar graph showing relative frequencies of High-producing, Mid-producing, and Low-producing MBL diplotypes in patients grouped by infection status.

TABLE 1

Demographic and Other Characteristics of the Subjects Known Previous to PICU Admission and Association with MBL Level

Characteristic	*N*	%		MBL, ng/mL[a]	*P*Value[b]
Total Sample	479	100.0	1197	(718.9–1878.3)	
Gender					0.84
Male	241	50.3	1169	(767.9–1872.2)	
Female	238	49.7	1205	(662.0–1890.5)	
Age, y					0.73[c]
< 1	95	19.8	1309	(658.6–2052.8)	
1–4	141	29.4	1120	(720.1–1773.6)	
5–12	131	27.3	1154	(754.8–1909.5)	
>13	112	23.4	1220	(730.1–1875.4)	
Race					**0.01**
Caucasian	353	73.7	1129	(694–1786.5)	
Black	42	8.8	1044	(415.2–2386.7)	
Other	84	17.5	1460	(846.6–2443)	
Ethnicity					0.53
Hispanic	76	15.9	1177	(687.9–1943.3)	
Non-Hispanic	396	82.7	1185	(723.1–1854.7)	
Unable to Answer	7	1.5	1590	(448.2–2385.6)	
Insurance					0.54
Private	192	40.1	1165	(665.7–1899.2)	
Government	277	57.8	1202	(752.7–1838.6)	
Underlying Chronic Conditions					0.56
Any	403	84.1	1203	(734.0–1866.6)	
None	76	15.9	1118	(665.7–1896.5)	
Underlying Chronic Conditions[d]					
Respiratory	201	42.0	1235	(711.9–1950.1)	0.95
Asthma	100	20.9	1296	(699.7–1989.7)	0.86
Neurologic	191	39.9	1251	(820.7–1913.5)	0.11
Seizure	103	21.5	1365	(876.7–1913.5)	0.11

Characteristic	*N*	%		MBL, ng/mL[a]	*P*Value[b]
Oncologic	53	11.1	1202	(851.4–1942.6)	0.75
Immunodeficiency	31	6.5	1283	(703.1–1732.0)	0.98
Renal	22	4.6	1619	(1212.0–2119.9)	**0.03**
Liver	10	2.1	1385	(731.9–1853.9)	0.71
Gastrointestinal	43	9.0	1280	(659.8–1980.3)	0.94
Nutritional	49	10.2	1283	(694.9–2119.8)	0.65
Metabolic	39	8.1	1280	(674.5–1753.7)	0.71

[a] Median (Interquartile range)

[b] Testing association with serum MBL level with Mann-Whitney U or Kruskal-Wallis tests

[c] Spearman correlation with age r= 0.016, p=0.73

[d] Compared with patients with other chronic conditions

TABLE 2

Infectious Reasons for Pediatric Intensive Care Unit Admission and Association with MBL Level

Characteristic	*N*	%	MBL, ng/mL[a]	*P* Value[b]
Infection on Intensive Care Unit Admission[c]				0.43
No Infection	238		1112 (581.1–1642.6)	
Suspected Infection, not confirmed	88		1389 (490.6–2287.9)	
Bacterial	90		1151 (533.7–1768.3)	
Viral	45		1301 (792.1–1810.7)	
Multiple	15		1280 (607.9–1952.2)	
Lower Respiratory Tract Infection				0.44
No	434	90.6	1202 (718.5–1913.7)	
Yes	45	9.4	1131 (663.3–1784.7)	
Community Acquired Pneumonia				0.24
No	13		1278 (844.9–1804.6)	
Yes	32		1122 (565.9–1786.3)	
Sepsis[c]				0.6
No SIRS	192	40.1	1117 (739.2–1975.2)	
SIRS, no infection[d]	99	20.7	1115 (768.1–1681.0)	
Sepsis[e]	112	23.4	1219 (662.9–2034.4)	
Severe Sepsis[f]	73	15.2	1308 (723.8–1851.7)	

[a] Median (Interquartile range)

[b] Testing association with serum MBL level with Mann-Whitney U or Kruskal-Wallis tests

[c] Infection specific data unavailable for 3 patients

[d] No viral, bacterial, or fungal infection

[e] Infection with SIRS

[f] Sepsis with CV-SOFA score ≥ 3; includes septic shock

TABLE 3

Treatments and Outcomes of All Patients and Association with MBL Level

Characteristic	*N*	%		MBL, ng/mL[a]	*P* Value[b]
Fluid Administration first 24 hours					***0.007***[c]
0–20cc/kg	173	36.1	1330	(796.7–2020.5)	
20–40cc/kg	91	19.0	1205	(661.2–1984.3)	
40–60cc/kg	72	15.0	1143	(767.9–1897.2)	
60–80cc/kg	48	10.0	1150	(657.2–1849.8)	
80–100cc/kg	31	6.5	1202	(697.4–1732.0)	
> 100cc/kg	64	13.4	899	(584.1–1542.4)	
PRISM III Score					0.16[d]
0	123	25.7	1257	(774.4–1781.5)	
1–3	86	18.0	1148	(677.8–1952.0)	
4–5	85	17.7	983	(531.3–1664.2)	
6–9	86	18.0	1314	(720.6–2074.3)	
10–15	69	14.4	1283	(818.1–2037.2)	
16+	30	6.3	1149	(633.6–1565.5)	
Vasoactive Infusions (CV-SOFA)					0.21
0–1	356	74.3	1186	(751.7–1934.3)	
2	13	2.7	1320	(567.4–2567.8)	
3	71	14.8	1235	(674.2–1819.9)	
4	39	8.1	1167	(607.0–1810.2)	
ARDS/ALI during hospitalization					0.15
Yes	13	2.7	1351	(918.9–2978.5)	
No	466	97.3	1185	(708.8–1869.5)	
ECMO[e]					0.12
Yes	7	1.5	795	(397.9–1480.9)	
No	471	98.3	1198	(721.2–1888.8)	
Discharged From Hospital Alive[f]					0.51
Yes	464	96.9	1169	(717.8–1865.3)	
No	12	2.5	1495	(691.2–1939.0)	

Characteristic	*N*	%		MBL, ng/mL[a]	*P* Value[b]
FFP Administration					0.22
Any	34	7.1	936	(638.7–1537.3)	
None	445	92.9	1202	(725.0–1911.5)	
Respiratory Support					0.5
None or Oxygen	166	34.7	1231	(725.3–1912.9)	
Non-invasive Ventilation	51	10.6	1258	(802.9–1980.5)	
Mechanical Ventilation	243	50.7	1154	(718.9–1801.1)	
High Frequency Ventilation	19	4.0	1038	(502.0–1814.3)	

[a] Median (Interquartile range)

[b] Testing association with serum MBL level with Mann-Whitney U or Kruskal-Wallis tests except where indicated

[c] Spearman Correlation r = 0.123, p = 0.007; correlation with non-categorized fluid volume

[d] Spearman Correlation

[e] Data unavailable for 1 patient

[f] Data unavailable for 3 patients