Association Between Variants of the Mannose-Binding Lectin 2 Gene and Susceptibility to Sepsis in the Hainan Island

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Conflict of interest:  
None declared

Background:  
Sepsis has emerged as a leading cause of death in the intensive care unit. A growing number of studies have shown that genetic variants, especially single nucleotide polymorphisms, are key determinants of inter-individual variation in sepsis response. Therefore, early prediction of the onset and progression of sepsis, along with early intervention in high-risk patients, should be performed to effectively reduce the morbidity and mortality of the disease.

Material/Methods:  
A total of 581 Chinese patients were enrolled in this study, including 271 patients with sepsis and 310 patients without. We measured gene polymorphisms of MBL2 and serum levels of MBL2, tumor necrosis factor (TNF-α), interleukin (IL)-6, IL-4, and IL-10 in all patients. The effects of site mutations on the binding of MBL2 to mannosese-associated serine protease 1 (MASP1) and MASP2 were also analyzed.

Results:  
Of 3 site mutations in the MBL2 gene (rs5030737, rs1800450, and rs1800451), only rs1800450 had a mutant (G/A) genotype. The frequency of the GA genotype and A allele in the sepsis group was higher than that in the non-sepsis group. Furthermore, rs1800450G/A was associated with decreased serum MBL2 and IL-10 levels and decreased MBL2-MASP1 and MBL2-MASP2 interactions. Bioinformatics analysis showed that rs1800450G/A reduced the structural stability of the MBL2 protein and affected its function.

Conclusions:  
MBL2 rs1800450G/A was associated with a higher risk of sepsis, which possibly involved a decreased level of serum MBL2 that broke the balance of inflammation and weakened the binding of MBL2 to MASP1 and MASP2.

Keywords:  
Disease Susceptibility • Genetic Predisposition to Disease • Genetics • Genetics, Medical • Genomic Structural Variation • Sepsis

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Background

Sepsis causes considerable morbidity and is the main cause of death in the intensive care unit (ICU) [1]. Sepsis can change rapidly, often resulting in a poor prognosis due to the lack of a timely diagnosis and treatment. Sepsis is also complicated and progresses rapidly. Without a timely diagnosis and treatment, it is likely to develop as severe sepsis or even septic shock. When more than 3 organs or systems are involved, the case fatality rate is extremely high [2]. Therefore, the occurrence and development of sepsis should be predicted early, while early intervention for high-risk patients can effectively reduce the incidence and mortality of this disease. However, despite advances in diagnosis and treatment, the morbidity and mortality of sepsis remain high, and its mechanism is still unclear [3].

An increasing number of studies have shown that genetic variants, particularly single nucleotide polymorphisms (SNPs), are critical determinants of interindividual differences in responses to sepsis [4,5]. Many scholars have conducted genetic correlation studies on the susceptibility and genetic polymorphisms of sepsis, and a series of susceptibility genotypes of sepsis have been explored [6-9].

Many associated studies have investigated susceptibility to, or mortality from, sepsis for variants of the functionally important immune-related gene, mannose-binding lectin 2 (MBL2). However, the results of these studies are not uniform because of the diversity of ethnic and regional groups [10-13]. Özkan et al found that low MBL levels and the presence of the B allele of the MBL exon 1 gene were important risk factors for the development of neonatal sepsis and pneumonia, especially in premature infants [10]. A study by Liu et al indicated that the variant A allele in the MBL2 gene rs1800450 polymorphism might increase the risk of sepsis by decreasing the MBL serum level [11]. Mills et al [14] genotyped and analyzed 4 important MBL2 SNPs (rs5030737, rs1800450, rs1800451, and rs7096206) in 1839 European patients with sepsis (983 with community-acquired pneumonia [12], 856 with peritonitis sepsis) and 477 controls from the United Kingdom. In this large, well-defined cohort of immune-competent adult patients, the results strongly suggested a lack of significant, clinically meaningful associations between MBL genotypes and either 28-day or 6-month survival from sepsis or susceptibility to community-acquired pneumonia or pneumococcal pneumonia in immune-competent adults.

To date, little is known about the association of MBL2 gene polymorphisms with the risk of sepsis in Chinese populations. To comprehensively assess the association of common genetic variants of the MBL2 gene with sepsis susceptibility, we selected a set of SNPs of the MBL2 gene (rs1800450, rs180045, and rs50307371) and investigated their clinical relevance in relation to the development of sepsis in patients in Chinese Han populations in southern China.

Material and Methods

Study Population and Clinical Evaluation

A total of 581 unrelated patients recruited in this study were of Han Chinese from the island of Hainan in southern China. The inclusion criteria included being between 14 and 85 years of age. The patients enrolled in the sepsis group (n=271, 177 men, 94 women; mean age: 59.2±13.6 years) and the non-sepsis group (n=310, 191 men, 119 women; mean age: 64.3±12.8 years) were in the ICU between January 2017 and December 2018. This study was approved by the Medical Ethics Committee of Hainan Medical University. Approval of the local ethical committee was obtained, as was the written informed consent of patients. A diagnosis of sepsis was based on the latest diagnostic criteria, the Sequential Organ Failure Assessment (SOFA) score (>2) caused by infection [15,16]. Patients were not eligible if they had the following exclusion criteria: (1) age less than 14 years or more than 85 years; (2) were in special groups such as pregnant women and lactating women; or (3) patients who were immunosuppressed before developing sepsis were excluded, including those with known HIV infection, hematologic malignancy, neutropenia (white cell count <1×10⁹/L) or chronic liver failure, according to the APACHE II definition, and patients who had received chemotherapy, immunosuppressants, or systemic steroids in the previous 6 months.

Blood Sample Collection and DNA Extraction

Five milliliters of peripheral venous blood was collected from each patient using ethylenediamine tetra-acetic acid anticoagulant tubes. Blood samples were collected from all patients on the first day of ICU admission. Genomic DNA was extracted from whole blood using a TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) and stored in a freezer at -80°C for long-term storage.

Genotyping

The upstream primer was 5’-CAGCCAGTTCCTCTGGAAG-3, and the downstream primer was 5’-AGTCACGCAGTGTCACAAGG-3. The single base extension (SBE) reaction was performed in a reaction mixture with a final volume of 5 µL that contained 3 µL of purified multiplex PCR product, 1 µL of pooled extension primers, 0.5 µL of SNaPshot Mix, and 0.5 µL of dH₂O. SNaPshot reaction conditions were as follows: 40 SBE cycles of initial denaturation at 95°C for 10 sec, primer annealing at 52°C for 5 sec, and primer extension at 60°C for 30 sec. Shrimp alkaline phosphatase was used for the post-SBE purification of SNaPshot reaction products. For electrophoresis,
purified multiplex SBE reaction products were mixed with 0.9 µL of water, 0.5 µL of shrimp alkaline phosphatase, and 0.6 µL of 10× shrimp alkaline phosphatase buffer. The digestion reaction was incubated at 37°C for 40 min and 75°C for 15 min. A total of 2 µL of product from the minisequencing reaction was mixed with 8 µL formamide containing 0.4% 120 LIZ dye size standard (ThermoFisher Scientific, Waltham, MA, USA). The samples were denatured by incubating them at 95°C for 5 min. After cooling down to -20°C, the fluorescently labeled fragments were resolved by capillary electrophoresis on an ABI 3730XL Genetic Analyzer for allele discrimination. The resulting data were analyzed with GeneMapper v4.0 software.

Serum MBL2, TNF-α, IL-6, IL-4 and IL-10 Measurements

Aliquots of whole blood samples were collected from study participants. Blood samples were collected from sepsis patients within 24 h sepsis diagnosis. After centrifugation, the plasma was aspirated and aliquoted for storage at -80°C. The concentrations of MBL2, TNF-α, IL-6, IL-4, and IL-10 in serum were tested by enzyme-linked immunosorbent assay (ELISA) with an ELISA kit (American R&D Company) according to the manufacturer’s instructions.

MBL2-MASP1 and MBL2-MASP2 Binding Assay

Recombinant human wild-type MBL2 protein and mutant MBL2 protein were expressed in Chinese hamster ovary cells [13,18]. Then, 10 mg/L of purified recombinant human MASP1-N and MASP2-N terminal proteins were added to an ELISA plate and coated overnight at 4°C. Different concentrations of MBL2 protein were added and reacted at 37°C for 1 h. After washing, 1: 1000 mouse anti-human MBL-CRD monoclonal antibody and 1: 5000 horse radish peroxidase (HRP)-labeled sheep anti-rat IgG were added and reacted at 37°C for 30 min. The substrate solution for HRP was tetramethylbenzidine, and the reaction was terminated by the addition of 2 M H₃SO₄. The absorbance at 450 nm (A450) was measured in a microplate reader.

Effects of Site Mutations on the Structure and Function of the MBL2 Protein

The MBL2 proteins of 8 species were aligned using MAFFT v7.38, and the evolutionary tree was calculated using MEGA. Structural domains were analyzed using SMART. iStable software predicted the effect of G54D on protein structure, and Polyphen-2 and SIFT software were used to predict the effect of conservative mutations on protein function.

Statistical Analysis

Allele and genotype frequencies of variants were determined by gene counting. The genotype distribution of each SNP was tested for departure from the Hardy-Weinberg equilibrium using χ² analysis. Additionally, χ² analysis was used to analyze the associations of MBL2 gene polymorphisms with sepsis, and the odds ratio (OR) and 95% confidence interval (CI) were calculated. MBL2, TNF-α, IL-6, IL-4, and IL-10 levels in the sera of patients in the 2 groups were compared by an independent t test. All statistical tests were 2-sided, and a P value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS 24.0.

Results

Overall Clinical Characteristics of Patients

Two independent groups existed, sepsis and non-sepsis, which consisted of 271 and 310 individuals, respectively. The main clinical characteristics of patients are presented in Table 1. The sex and mean age of patients were not significantly different between the 2 groups. The source of infection in patients with sepsis was mainly a respiratory tract infection (31.4%, n=85). Gram-positive and gram-negative infections, fungal infections, and mixed infections accounted for approximately 20.0%, 15.5%, 2.2%, and 7.0% of cases, respectively, in the sepsis group with positive blood cultures. Negative blood cultures were identified in 55.3% of patients in the sepsis group. The common pathogens identified in this study were Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Enterobacter cloacae, and Pseudomonas aeruginosa.

Association of MBL2 Gene Polymorphisms with Sepsis

Only the mutation site of rs1800450 G/A was found in the 3 site mutations of MBL2 gene polymorphisms (rs1800450, rs180045, and rs50307371). The rs1800450 genotype distributions were consistent with the Hardy-Weinberg equilibrium (χ²=1.128, P=0.288; Table 2).

The frequency of a GA genotype was significantly higher in the sepsis group than in the non-sepsis group (P=0.010). Allele frequency analysis also showed that the frequency of the A allele in the sepsis group was much higher than that in the non-sepsis group (P=0.029; Table 3). Further, χ² analysis showed that GA genotype patients were 1.682 times more likely to develop sepsis than GG genotype patients (OR=1.682, 95% CI=1.340-2.505). Patients who carried the A allele were more likely to acquire sepsis than G allele carriers (OR=1.465, 95% CI=1.041-2.061), which means that the variation of rs1800450, G→A increased the incidence of sepsis (Table 3).
We found the rs1800450 variant had a certain effect on the MBL2 serum levels in the sepsis and non-sepsis groups, and was more obvious in the sepsis group. But there was no statistical difference among patients with GG, GA, and AA genotypes in the non-sepsis group. In the sepsis group, the MBL2 serum levels of patients with GA and AA genotypes were markedly lower than those in patients with the GG genotype ($P \leq 0.001$ and $P=0.001$, respectively; Figure 1).

Two independent groups existed, sepsis and non-sepsis, which consisted of 271 and 310 individuals, respectively. The main clinical characteristics of patients are presented in Table 1. The mean age and gender of patients were not significantly different between the two groups. The source of infection in patients with sepsis was mainly a respiratory tract infection (31.4%, n=85). Gram-positive and Gram-negative infections, fungal infections, and mixed infections accounted for approximately 20.0%, 15.5%, 2.2%, and 7.0%, respectively, of cases in the sepsis group. The common pathogens identified in this study were *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. Negative blood cultures were identified in 55.3% of patients in the sepsis group.

Table 1. Overall clinical characteristics of patients in the sepsis group and non-sepsis group.

|                          | Sepsis group | Non sepsis group | P value |
|--------------------------|--------------|------------------|---------|
| Age (years)              | 59.2±13.6    | 64.3±12.8        | 0.58    |
| Gender                   |              |                  | 0.81    |
| Female                   | 94 (34.7%)   | 119 (38.4%)      |         |
| Male                     | 177 (65.3%)  | 191 (61.6%)      |         |
| Source of infection      |              |                  |         |
| Respiratory tract infection | 85 (31.4%)    |                  |         |
| Primary bloodstream infection | 63 (23.2%)    |                  |         |
| Urinary tract infection  | 47 (17.3%)   |                  |         |
| Catheter associated infection | 37 (13.7%)    |                  |         |
| Wound infection          | 34 (12.5%)   |                  |         |
| Others                   | 33 (12.2%)   |                  |         |
| Pathogens (positive blood cultures) |          |                  |         |
| Gram-positive            | 54 (20.0%)   |                  |         |
| Gram-negative            | 42 (15.5%)   |                  |         |
| Fungi                    | 6 (2.2%)     |                  |         |
| Mixed Gram-negative and Gram-positive | 19 (7.0%) |                  |         |
| Negative blood cultures  |              |                  |         |
| Number of organs with dysfunction, n (%) | 150 (55.3%) |                  |         |
| 1                        | 98 (36.2%)   |                  |         |
| 2                        | 75 (27.7%)   |                  |         |
| 3 or more                | 12 (4.4%)    |                  |         |

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Table 2. The rs1800450 genotype distributions of Hardy-Weinberg equilibrium in total (n%).

| SNPs   | N | GG  | GA  | AA  | $\chi^2$ | P     |
|--------|---|-----|-----|-----|----------|-------|
| rs1800450 | 581 | 441 (75.9) | 127 (21.9) | 13 (2.2) | 1.128    | 0.288 |

Only the mutation site of rs1800450 G/A was found in the three site mutations of MBL2 gene polymorphisms (rs1800450, rs180045, and rs50307371). The rs1800450 genotype distributions were consistent with the Hardy-Weinberg equilibrium ($\chi^2=1.128, P=0.288$; Table 2).

**MBL2, TNF-α, IL-6, IL-4, and IL-10 Levels in Serum**

We found the rs1800450 variant had a certain effect on the MBL2 serum levels in the sepsis and non-sepsis groups, and was more obvious in the sepsis group. But there was no statistical difference among patients with GG, GA, and AA genotypes in the non-sepsis group. In the sepsis group, the MBL2 serum levels of patients with GA and AA genotypes were markedly lower than those in patients with the GG genotype ($P \leq 0.001$ and $P=0.001$, respectively; Figure 1).

Serum TNF-α, IL-6, IL-4, and IL-10 levels in patients with GG, GA, and AA genotypes in the non-sepsis group had no significant differences. We found that serum TNF-α levels in septic...
patients with GA and AA genotypes were markedly higher than those in septic patients with the GG genotype (P=0.008 and P=0.005, respectively). IL-10 levels in septic patients with GA and AA genotypes were lower than those in patients with the GG genotype. However, a significant difference in IL-6 and IL-4 levels between groups was not noted (Figure 2A-2D).

Effect of Site Mutations on the Binding of MBL2 to MBL-Associated Serine Proteases 1 and 2

To further examine the effect of the amino acid substitution G54D on MBL2-MASP1 and MBL2-MASP2 interactions, we also investigated the binding of wild-type and mutated MBL2 proteins to MASP1 and MASP2. As expected, the binding of mutated MBL2 protein to MASP1 and MASP2 was weaker than that of the wild-type MBL2 protein (Figure 3A, 3B).

Effects of Site Mutations on MBL2 Protein Structure and Function

Sequence analysis of the human MBL2 protein showed that the corresponding mutation of rs1800450G/A was G54D. Multisequence alignment of MBL2 proteins from 8 species indicated that the 54th amino acid G of the human MBL2 protein was a highly conserved site in species that was located in the middle of a highly conserved motif and might have important functions (Figures 4, 5). From domain analysis, MBL2 has a known conserved domain (127-245), a low complexity domain (61-107), and 2 unknown conserved domains (14-61 and 108-126). The G54D mutation is located in the unknown conserved region of the N-terminal segment (Figure 6).

Analysis with iStable predicted that the G54D mutation in the MBL2 protein would change the stability of the protein: The result was “decrease”, which means that the mutation was expected to reduce the structural stability of the protein and might affect the structure of conserved N-terminal regions.

Table 3. Genotypic and allelic frequency distribution of rs1800450 in the sepsis group and non-sepsis group. P=0.014 for dominant association (GA+AA vs GG). P=0.971 for recessive association (AA vs GA+GG).

| Genotype | Sepsis group n/% | Non-sepsis group n/% | P   | χ²   | OR     | 95% CI  |
|----------|------------------|----------------------|-----|------|--------|---------|
| GG       | 193 (71.2)       | 248 (80.0)           |     |      |        |         |
| GA       | 72 (26.6)        | 55 (17.7)            | 0.010 | 6.622 | 1.682  | 1.130-2.505 |
| AA       | 6 (2.2)          | 7 (2.3)              |     |      |        |         |
| G        | 458 (84.5)       | 551 (87.9)           |     |      |        |         |
| A        | 84 (15.5)        | 69 (12.1)            | 0.028 | 4.828 | 1.465  | 1.041-2.061 |
| GA+AA    | 78 (28.8)        | 62 (20.0)            | 0.014 | 6.098 | 0.619  | 0.422-0.907 |
| GA+GG    | 265 (97.8)       | 303 (97.7)           | 0.971 | 0.01  | 1.020  | 0.339-3.074 |

P=0.014 for dominant association (GA+AA vs GG). P=0.971 for recessive association (AA vs GA+GG). The frequency of a GA genotype was significantly higher in the sepsis group than in the non-sepsis group (P=0.014). Allele frequency analysis also showed that the frequency of the A allele in the sepsis group was much higher than that in the non-sepsis group (P=0.029; Table 3). χ² analysis showed that GA genotype patients were 1.682 times more likely to develop sepsis than GG genotype patients (OR=1.682, 95% CI=1.340-2.505). Patients who carried the A allele were more likely to acquire sepsis than G allele carriers (OR=1.465, 95% CI=1.041-2.061), which means that the variation of rs1800450, G→A increased the incidence of sepsis (Table 3).
Analysis with Polyphen-2 predicted that this mutation would be “probably damaging” with a score of 1.00. The predicted SIFT score was 0.02, and the result was “affect protein function”, suggesting that the mutation might have an impact on protein function.

**Discussion**

As an important ingredient within the complement system, MBL2 plays an essential role in innate immunity and is a first-line anti-infection immune molecule in a host’s nonspecific immunity [16]. Humans have 2 types of MBL genes: a functional MBL gene (MBL2) and a pseudogene (MBL1). Three SNPs of MBL2 are located on codons 52 (rs5030737C/T, termed A/D), 54 (rs1800450G/A, termed A/B), and 57 (rs1800451G/A, termed A/C) of exon 1; the 3 variant alleles are collectively termed O and the wild-type allele is termed A [16]. The polymorphisms of the MBL2 gene might affect its opsonic action in the body and correlate with susceptibility to infectious diseases [16].

We found that only mutations of codon 54 (rs1800450G/A, termed A/B) of exon 1 were found in the Chinese patients. Allele frequency analysis showed that the frequency of the A
**Figure 3.** (A) The binding between the wild-type and the mutated MBL2 proteins to MASP1. (B) The binding between the wild-type and the mutated MBL2 proteins to MASP2. Origin v8.0, United States OriginLab Corporation.

**Figure 4.** Multi-sequence alignment of MBL2 proteins from eight species. MAFFT v7.0 (http://mafft.cbrc.jp/alignment/server/).
allele in the sepsis group was significantly higher than that in the non-sepsis group, and that patients who carried the A allele were more likely to acquire sepsis than the G allele carriers. Therefore, rs1800450G/A, among the MBL2 gene polymorphisms, was closely related to susceptibility to sepsis.

The maintenance of a protective function of MBL2 in the body is closely related to the level of MBL2 in plasma: The lower the MBL2 level is, the more susceptible patients are to infectious diseases. Serum levels of MBL2 were mainly determined by polymorphisms of the promoter and exon 1; a study found that a single nucleotide mutation of the first exon codon 54 (GGC-GAC) of exon 1 decreased MBL2 serum levels and affected the complement activation of our immune system, resulting in infectious and immune-diversified diseases [16]. In the present study, the rs1800450 variant had a certain effect on the MBL2 serum levels in the sepsis and non-sepsis groups. However, there were no statistical differences among patients with GG, GA, and AA genotypes in the non-sepsis group. MBL2 serum levels in septic patients with the GA and AA genotype were markedly lower those in patients with the GG genotype. It was suggested that the single nucleotide mutations reduced the MBL2 polymer and weakened ligand-binding capacity. In addition, it might make the MBL2 polymer more easily degraded by metalloprotease to regulate the concentration of MBL2 in plasma [16].

The level of MBL2 is closely related to the inflammatory state of the body and the level of inflammatory secretions. MBL2 can reduce the expression of the pro-inflammatory factors IL-1 alpha and IL-1 beta and increase the release of IL-10 and IL-6 in monocytes after stimulation with lipopolysaccharide [16]. Elevating the level of MBL2 could promote the release of TNF and IL-6 in monocytes, thus controlling the further aggravation of infection [17]. Zhang et al found that the miR-608 rs4919510 polymorphism was significantly associated with a higher production of proinflammatory cytokines (TNF-α, IL-6, and IL-1β) and lower production of anti-inflammatory cytokines (IL-10,
IL-4) in patients with sepsis [18]. The results further indicated that the G→C variant of the rs4919510G/C polymorphism might enhance the production of proinflammatory cytokines and weaken the production of anti-inflammatory cytokines. Therefore, a single variant may impact the release of these inflammatory markers and increase the risk of sepsis. To verify that the MBL2 rs1800450G/A mutation can regulate the release of inflammatory factors and the occurrence of sepsis, the present study examined whether this mutation changed the secretion levels of pro- (TNF-α and IL-6) and anti-inflammatory factors (IL-4 and IL-10). In our study, the serum levels of TNF-α and IL-10 in patients with GA and AA genotypes were significantly higher than those in patients with the GG genotype. We found that the rs1800450G/A mutation site had no significant effect on the expression of IL-6 and IL-4. This suggested that rs1800450G/A mediated the occurrence of sepsis and might by affecting the secretion of inflammatory cytokines and disrupting the balance of inflammation in the body.

Binding of the MBL2 protein to serine protease (MASP-1 and MASP-2) is required to activate the lectin pathway, trigger the complement system, and resist infection [19,20]. Based on the significant clinical relevance of the rs1800450 polymorphism, we further hypothesized that amino acid substitutions might affect MBL2-MASP1 and MBL2-MASP2 interactions. As expected, the variant MBL2 protein also shows a weaker binding interaction with MASP1 and MASP2. Therefore, one can deduce that the rs1800450 polymorphism is not only a useful clinical biomarker for the prognostic assessment of sepsis but also a functional SNP, which affects MBL2 protein activities and binding interactions with MASP1 and MASP2.

Through bioinformatics analysis, we also found that the 54th amino acid G of the human MBL2 protein was a highly conserved site in species that was located in the middle of the highly conserved motif, and which may have important functions. However, site mutations can reduce the stability of the MBL2 protein structure and affect protein function, thus affecting the efficiency of MBL2 in vivo, making the body susceptible to infection.

Several potential limitations should be taken into consideration in this study. First, the sample size of patients we recruited was small, and a control group with healthy individuals was lacking. Furthermore, we predicted that the rs1800450G/A polymorphism possibly reduced the stability of the MBL2 protein structure and affected protein function according to bioinformatics analysis; more relevant experiments are needed for further verification. Finally, the exact mechanisms by which the rs1800450G/A polymorphism is involved in the pathogenesis of sepsis are unknown, and further mechanistic research must be carried out.

Conclusions

This study demonstrated that MBL2 rs1800450G/A was associated with a higher risk of sepsis, which possibly involved a decreased level of MBL2 in serum, a break in the balance of inflammation, weakened binding of MBL2 to MASP1 and MASP2, and reduced stability of protein structure and function. Future studies in large-scale populations and different ethnic groups might be needed to validate any clinical relevance and to explore the molecular mechanisms involved. This would be conducive to the rapid diagnosis and development of individualized targeted treatments for sepsis.

Declaration of Figures’ Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.
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