GENOTYPES CODING FOR LOW SERUM LEVELS OF MANNOSE-BINDING LECTIN ARE UNDERREPRESENTED AMONG INDIVIDUALS SUFFERING OF NON-INFECTIONOUS SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

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Running title: MBL2 and MASP2 gene polymorphisms in SIRS.

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ABSTRACT

Gene polymorphisms, giving rise to low serum levels of mannose binding lectin (MBL) or MBL-associated protease (MASP) 2, have been associated with an increased risk of infections. The objective of this study was to assess the outcome of intensive care unit (ICU) patients with systemic inflammatory response syndrome (SIRS) regarding the existence of functionally relevant MBL2 and MASP2 gene polymorphisms. **Methods:** The study included 243 ICU patients with SIRS admitted to our hospital, as well as 104 healthy control subjects. MBL2 and MASP2 single nucleotide polymorphism’s were genotyped using a sequence-based typing technique. **Results:** No differences were observed regarding the frequencies of low-MBL genotypes (O/O and XA/O) and MASP2 polymorphisms between patients with SIRS and healthy controls. Interestingly, ICU patients with a non-infectious SIRS had a lower frequency for low-MBL genotypes and a higher frequency for high-MBL genotypes (A/A, A/XA) than either ICU patients with an infectious SIRS or healthy controls. The existence of low/high-MBL genotypes or MASP2 polymorphism had no impact in the mortality rates of the included patients. **Conclusions:** the presence of high-MBL producing genotypes in patients with a non-infectious insult is a risk factor for SIRS and ICU admission.
INTRODUCTION

Sepsis is the main cause of death in intensive care units (ICU), with mortality rates above 50% in patients with septic shock (54). Increasing evidence suggest that variations in genes encoding for different components of the immune system influence the individual capacity to respond adequately to infections. Genetic polymorphisms of several molecules of the innate immune system, such as the tumor necrosis factor-α (TNF-α) (35), the interleukin-1 receptor antagonist (IL-1RA) (2) and more recently in the plasminogen activator inhibitor 1 (PAI-1) (10), have been associated with an increased mortality in patients with severe sepsis and septic shock.

The mannose-binding lectin (MBL) is an important element of the innate immune defense system. The MBL is a circulating C-type plasma lectin, mainly produced by the liver, which binds to the specific carbohydrates present on the surface of different microorganisms (21, 38). In serum, MBL is present as oligomers (mainly trimers and tetramers) bound to the MBL-associated serine proteases (MASPs) also produced in the liver, mainly, the MASP-2. Once bound to the carbohydrate residues, the MBL/MASP-2 complex acts as an opsonin for phagocytosis for numerous pathogens and activates the complement (21, 34, 38, 56). MBL is not only involved in complement activation but is also a potent modulator of pro-inflammatory cytokine production (27). Additionally, MBL is capable of increasing the clearance of the endotoxin by Kupffer cells (40) and the turnover of fibrinogen by cleavage of prothrombin, generating thrombin (29).

Three missense single-nucleotide polymorphisms (SNP) have been reported within the exon 1 of the MBL2 gene introducing amino acid replacements at codon 52 (allele D), 54 (allele B), or 57 (allele C) which cause a reduction of the MBL levels due to impaired assembly of MBL monomers into functional oligomers (14).
addition to these structural variant alleles, three SNP in the promoter region of the MBL2 gene at positions –550 (H/L), +4 (P/Q) and, particularly, –221 (Y/X), influence the rate of transcription and are also associated with low concentrations of serum MBL (32, 47). Genetically-defined MBL-deficiency is common and appears to predispose to serious infections (9), particularly during early childhood (28), in patients undergoing chemotherapy (41) as well as in adults with concomitant diseases (15, 16). In the ICU setting, although several studies have suggested the existence of a relationship between low MBL serum levels and an increased risk of infections, the association with death has yielded conflicting results (17, 19, 24, 25, 50).

In addition to the MBL2 polymorphisms, an inherited deficiency of MASP-2 has also been reported. This deficiency is due to a homozygous mutation in the exon 3 of the MASP2 gene, resulting in a change of aspartic acid to glycine at position 105 (Asp105Gly) of the CUB1 protein domain, which is an essential region for the formation of functional MBL/MASP-2 complexes. This mutation renders the MASP-2 incapable of binding to the MBL and therefore interrupts the MBL pathway of complement activation and also reduces the plasmatic concentration of MASP-2 (48). Heterozygous patients for the MASP2 Asp 105Gly SNP have no impairment in the lectin complement pathway (11, 12, 51). Several additional variants have been identified in the exon 3 of the MASP2 gene without causing a reduction of the levels or activity of the protein (31, 52).

The possible association between MASP-2 deficiency and susceptibility to infections remains largely unknown. Schlapbach et al have recently demonstrated that MASP-2 deficiency increases the risk of neutropenic fever in pediatric cancer patients (43). Granell et al. found an increased risk of invasive aspergillosis following allogeneic stem cell transplantation in adult patients heterozygous for the
Asp105Gly SNP (20). On the other hand, three recent studies were unable to demonstrate an increased frequency for the Asp105Gly SNP in adult patients with community-acquired pneumonia, pneumococcal bacteremia in HIV-infected patients and among renal transplant recipients with infectious complications (5, 12, 26). The aim of the present study was to investigate the implications of MBL2 and MASP2 polymorphisms in the outcome of ICU patients with systemic inflammatory response syndrome (SIRS).

MATERIALS AND METHODS

Study population

We prospectively collected blood samples from 243 Caucasian patients admitted to the medical ICU of the Hospital Clinic of Barcelona, between January 2003 and January 2004. Inclusion criteria for patients were age > 18, with a minimum ICU stay of 24 h and to meet the criteria for SIRS (see below). The patients were included and followed until hospital discharge. For further comparison 104 healthy Caucasian blood donors from the geographic area of Barcelona were also included in the study. The present study was conducted with the approval of the hospital Ethics Committee and the informed consent from the patients or their relatives within 24 h after admission.

Definitions for community or nosocomial acquired infection in ICU patients were established according to the Sepsis Forum Consensus Conference and the CDC guidelines (4, 13). The criteria for SIRS, sepsis, severe sepsis and septic shock were defined according to SCCM/ESICM/ACCP/ATS/SIS consensus conference (30). Clinical data, including demographic details and the severity indexes (Acute Physiology and Chronic Health Evaluation (APACHE) II, Simplified Acute Physiology Score (SAPS) II, and Sequential Organ Failure Assessment (SOFA)) were recorded for each patient at ICU admission and thereafter on a daily basis.
Multi-organ failure was considered in case of acute progressive dysfunction of two or more **organ systems**, with a minimum failure score of 3 points for each organ.

**MBL2 and MASP2 genotyping**

Genomic DNA was extracted from 1.5 mL of EDTA-treated whole blood samples by using the QIAamp DNA blood mini kit according to the manufacturer’s instructions (QIAGEN GmbH, Hilden, Germany) and then stored at -80°C until used. Genotyping of the **MBL2** and the **MASP2** gene was performed by using a sequencing-based typing method according to the published sequences (GenBank accession number AF360991 for the **MBL2** gene and NG_007289 for the **MASP2** gene). Six SNPs in the **MBL2** gene (-550 G/C (**rs11003125**), -221 C/G (**rs7096206**), +4 C/T (**rs7095891**), codon 52 CGT/TGT (**rs5030737**), codon 54 GGC/GAC (**rs1800450**) and codon 57 GGA/GAA (**rs1800451**)) within the promoter and the exon 1 of the **MBL2** gene were analyzed as previously reported (31). Briefly, a 969 bp fragment encompassing from the promoter to the end of exon 1 of the **MBL2** gene was obtained by polymerase chain reaction (PCR) amplification using the sense 5’-GGGGAATTCCTGCCAGAA GT-3’ and anti-sense 5’-CATATCCCAGGCA GTTTCTC-3’ primers and the Expand 20Kb PLUS PCR System (Roche Diagnostics GmbH, Manheim, Germany). Five SNPs in the **MASP2** gene (Pro111Leu (**rs56392418**), Asp105Gly (**rs72550870**), Arg84Gln (**rs61735600**), Thr73Met (**rs61735601**) and Arg103Cys (**rs id not submitted**)) within the exon 3 of the **MASP2** gene were analyzed as previously reported (31). Similarly, a 354-bp fragment from the exon 3 of the **MASP2** gene was PCR-amplified by using the sense 5’-GCGAGTACGACTTCGTCAAGG-3’ and antisense 5’-CTCGGCTGCATAGAAGGCCTC-3’ oligonucleotides and the Expand High Fidelity PCR System (Roche Diagnostics GmbH, Manheim, Germany). The cycling conditions for amplification were 94°C for 8 min; 35 cycles at 94°C for 45 seconds,
58°C for 30 seconds, 72°C for 90 seconds and finally 72°C for 10 min. Five microliters of the resulting PCR reaction were treated with ExoSAP-IT® (USB Corporation, Cleveland, Ohio, USA) and then subjected to direct sequencing with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) following manufacturer’s instructions with the sense and antisense gene-specific primers mentioned above.

**Genetic groups**

The SNPs at exon 1 of the *MBL2* gene are in strong linkage disequilibrium with those at the promoter and give rise to seven common haplotypes, (HYPA, LYQA, LYPA, LXPA, LYPB, LYQC and HYPD). The HY haplotype induces high MBL concentrations, whereas exon 1 mutations (O variants) and the LX haplotypes cause reduced MBL concentrations (47). Therefore, the analysis of the exon 1 and the promoter region of the *MBL2* gene allowed the categorization of individuals into three groups according to their *MBL2* genotype. Group I included genotypes responsible for high MBL levels (A/A, A/XA), group II genotypes responsible for intermediate MBL levels (A/O, XA/XA), and, finally, group III included genotypes responsible for low MBL levels (O/O, XA/O) according to the previously published results (32). The *MASP2* genotype was divided into wild-type or mutant (Asp105Gly).

**Statistical analysis**

Continuous variables were compared with the Student’s t test or Mann-Whitney U test when the distribution departed from normality, and described as mean (+standard deviation) or median (range of values), respectively. Categorical data were compared by the Chi-square or the Fisher’s exact test as appropriate. Deviations from Hardy-Weinberg expectations were tested using the Chi-square test for comparing observed and expected values. Statistical significance was
defined as a two-tailed $P$-value $<0.05$. Statistical analysis was carried out by the program SPSS (version 15.0; SPSS, Inc., Chicago, IL, USA).

RESULTS

The study population consisted of 243 consecutive adult patients (mean age 62.8 years $\pm$ 15.7, range 18-85; 63% men). The mean APACHE II, SAPS II and SOFA scores at ICU entry were 17.4 $\pm$ 6.4, 37 $\pm$ 11.9 and 8.7 $\pm$ 3.1, respectively. Within 24 h of ICU admission 101 patients (41.5%) met the criteria for septic shock and 28 (11.5%) for severe sepsis. For the remaining patients, 54 (22.2%) met the criteria for sepsis and 60 (24.7%) for a non-infectious SIRS. The main clinical characteristics of the patients included in the study are showed in table 1. Most of the patients had a medical indication for ICU admission, mainly a community acquired pneumonia. In the group of septic shock patients, nosocomial pneumonia was the focus of infection in nearly 10% of ICU admissions. Additionally, 13% of the patients developed nosocomial pneumonia during ICU hospitalization. 

$MBL2$ was genotyped in 216 patients (88.8% of the included patients) and $MASP2$ in 240 patients (98.7% of the included patients). $MBL2$ and $MASP2$ were also genotyped in the 104 healthy blood donor volunteers. Genotype frequencies found for patients and control groups did not differ from those predicted by the Hardy-Weinberg expectations ($P = 0.43$ and $P = 0.072$ for healthy controls and ICU patients with SIRS respectively regarding $MBL2$ genotypes; $P = 0.88$ and $P = 0.66$ for healthy controls and ICU patients with SIRS respectively regarding $MASP2$ genotypes).

Table 2 shows the frequencies for the $MBL2$ and $MASP2$ genotypes found among the ICU patients and the healthy control group. No significant differences in the frequencies for the different $MBL2$ haplotypes were found among the ICU patients with SIRS, and the healthy controls (data not shown). The LYPB was the predominant $MBL2$ variant haplotype in both the ICU patients with SIRS and in the
healthy controls. No overall statistical significant differences were observed for the frequencies of high- (48.6% vs. 54.8%; \( P = 0.29 \)), intermediate- (38.4 % vs. 29.8 %; \( P = 0.13 \)) and low- (12.9 % vs 15.3 %; \( P = 0.55 \)) MBL2 genotypes among the patients with SIRS and the healthy controls, respectively (table 2). The group of patients with an infectious SIRS, the analysis of which always included patients with sepsis, severe sepsis and septic shock, had an increased frequency for low-MBL2 genotypes (15.9%) when compared with the frequency found among patients with a non-infectious SIRS (3.8%) (\( P = 0.025 \)). When comparisons were established with the healthy control group no differences could be found (15.9% vs 15.3%; \( P = 0.91 \)). In view of the results we decided to evaluate the frequencies for high-MBL2 genotypes and observed a higher prevalence of these genotypes among ICU patients with a non-infectious SIRS compared to the frequencies found in ICU patients with an infectious SIRS (63.4% vs 43.9%; \( P = 0.014 \)). A higher frequency for high-MBL2 genotypes was also found when comparisons were established between ICU patients with a non-infectious SIRS and the healthy control group, although these differences did not reach statistical significance (63.4% vs 54.8%; \( P = 0.3 \)). We also analyzed the severity indexes at ICU entry among patients with a non-infectious SIRS and observed higher APACHE II (19.7 \( \pm \) 5.5 vs 10 \( \pm \) 1.4; \( P = 0.02 \)), SAPS II (34 \( \pm \) 8.7 vs 32 \( \pm \) 8.5; \( P = 0.75 \)) and SOFA scores (8.8 \( \pm \) 2.8 vs 5 \( \pm \) 0; \( P = 0.04 \)) in patients with high-MBL2 genotypes than in patients with low-MBL2 genotypes. Although infectious SIRS patients with high-MBL2 genotypes also had higher APACHE II (16 \( \pm \) 6.8 vs 16 \( \pm \) 7.3; \( P = 0.9 \)), SAPS II (37.7 \( \pm \) 12.2 vs 35.6 \( \pm \) 13.7; \( P = 0.54 \)) and SOFA (9.1 \( \pm \) 3.4 vs 8.9 \( \pm \) 3.4; \( P = 0.84 \)) scores compared to patients with low-MBL2 genotypes, differences were not statistically significant.
The analysis of the exon 3 of the *MASP2*, revealed no homozygous Asp105Gly carriers among patients or controls. As shown in table 2, no significant differences were found for the frequency of the heterozygous *MASP2* Asp105Gly SNP between the ICU patients with SIRS (5%) and the healthy controls (2.8%) (*P* = 0.56). One ICU patient, included in the sepsis group, was heterozygous for the Pro111Leu polymorphism while none of the healthy controls had it. No differences were found in the frequency for the *MASP2* Asp105Gly SNP between the group of ICU patients with an infection (5%) and those with a non infectious SIRS (5%).

Table 3 shows the outcomes of the patients during ICU stay according to the existence of normal or low-*MBL2* producing genotypes and the presence of the wild type *MASP2* or the heterozygous Asp105Gly SNP. No differences in the length of hospital or ICU stay were seen between the different groups. The frequencies and the total days of invasive mechanical ventilation or for renal replacement techniques were also similar among the groups. Fifty-three (21.8%) patients acquired a nosocomial infection while hospitalized in the ICU, 32 (13%) of which was a pneumonia. No differences in the global incidence of nosocomial infection nor in the frequency of hospital acquired pneumonia was seen between the group of ICU patients with low-*MBL2* genotypes or with the heterozygous Asp105Gly SNP and the rest of the patients. We also compared the clinical outcomes of ICU patients with high-*MBL2* genotypes and those with low-*MBL2* genotypes, excluding from the analysis those with intermediate-*MBL2* genotypes, regarding the clinical variables exposed in table 3 and found non significant differences between them (data not shown).

Regarding the mortality rate, 73 (30%) of the patients died during hospital admission, 54 (22.2%) of which during ICU stay. Multi-organ failure was the main cause of death (68.5%), although its frequency was different among ICU patients
with a non-infectious SIRS and in those with an infectious cause of SIRS (38.8% vs 76.3%; \( P = 0.003 \)). Again the existence of low-producing \textit{MBL2} genotypes or the heterozygous \textit{MASP2} Asp105Gly SNP had no influence in the mortality rate. When mortality was analyzed taking in consideration the existence of high, intermediate or low \textit{MBL2} producing genotypes, no significant differences were found (21.4%, 33.7% and 29.5% respectively).

Microbial samples obtained at ICU entry were positive in 93 (50.8%) of the patients with an infectious SIRS. No differences were found regarding the frequencies for Gram-positive, Gram-negative or fungi among patients with low-\textit{MBL2} genotypes or heterozygous for the Asp105Gly SNP (Table 3).

**DISCUSSION**

Despite the intense efforts that have been made to reduce the mortality related to sepsis, mainly driven through the implementation of the “Surviving Sepsis Campaign” guidelines (6), mortality rates continue to be high, around 40 to 70% in patients with septic shock (42). It has been estimated that each year in the United States 750,000 new cases of sepsis are diagnosed and its frequency is rising due to an increasing aging population, the use of invasive diagnostic procedures and aggressive therapies together with the high prevalence of chronic diseases (33).

Although both innate and adaptive immune systems are involved in the pathogenesis of sepsis, the innate immune system plays a pivotal role. Moreover, it has been suggested that susceptibility and response to infectious disease might be inheritable (3, 45). Numerous studies have evaluated different components of the innate immune system, including the MBL, searching for SNP associated with an increased risk for developing more severe forms of sepsis, organ dysfunction or death in patients with sepsis (2, 17, 19, 24, 25, 35, 50).

The serum concentration and functional activity of the MBL is determined by SNPs
at the promoter and the exon 1 of the **MBL2** gene, while SNP at the exon 3 of the **MASP2** gene, mainly the Asp105Gly, causes reduction in the serum levels of MASP-2. In the present study non significant differences for the frequencies of **MBL2** haplotypes and genotypes were observed between ICU patients with SIRS and healthy controls. The prevalence of low-**MBL2** genotypes was similar to the frequency observed by Garcia-Laorden *et al.* in Spain (12) and others in previous studies (17, 18). The LYPB, as previously reported in other Caucasian populations (1, 46), was the predominant variant type haplotype both in the group of patients with SIRS and in the healthy blood donors. Regarding the **MASP2** gene SNP, neither patients nor healthy controls were homozygous for the Asp105Gly SNP. No significant differences in the frequency for the heterozygous **MASP2** Asp105Gly was seen between ICU patients with SIRS and the healthy controls, the prevalence of which was in turn similar to the one found in previous studies (12, 48). One Caucasian patient carried the **MASP2** Pro111Leu SNP, primarily described by Lozano *et al.* in North-African individuals, which is not capable of causing reductions in the serum levels or activity of the MASP-2 (31, 52).

Previous studies have demonstrated a high prevalence of **MBL2** deficient genotypes among patients with sepsis admitted to ICU (17, 19, 50). Accordingly, our data show that patients with an infectious SIRS had a higher frequency for low-**MBL2** genotypes compared to patients with a non-infectious SIRS. This was due to the higher prevalence of high-**MBL2** genotypes observed in ICU patients with a non-infectious SIRS. The observation of higher frequency for high-**MBL2** genotypes among ICU patients with a non-infectious SIRS is coincidental with that previously reported by Garred *et al.* (17).

The SIRS describes physiological and laboratory abnormalities that accompany inflammation independently from the original cause (30). In a recent study, Dulhunty
et al have demonstrated that patients with a non-infectious SIRS present clinical differences from patients with sepsis (7). According to this study patients with sepsis died more frequently from multi-organ failure than patients with non-septic SIRS, which was also observed in our study. We hypothesize that some of the clinical differences observed between non-infectious and infectious SIRS patients might reflect, at least in part, the existence of different subjacent physiological mechanisms.

The results of our study suggest that patients with a non-infectious insult and high-\textit{MBL2} genotypes are at risk of developing SIRS and require ICU admission without having significant infectious complications. High levels of functional MBL could directly be associated with the pro-inflammatory adverse effects following uncontrolled complement activation, which has been previously demonstrated in ischemic-reperfusion experimental mice models (22, 36, 55). According to these models, an excess of production of MBL or the administration of exogenous MBL following induced ischemia causes organ (myocardial, kidney, intestine) reperfusion injury due to complement activation while low MBL levels result protective. One of the hallmarks of reperfusion to ischemic tissues is the severe oxidative stress that occurs at the level of the endothelium, which causes vascular injury. It has been suggested that the lectin pathway initiates complement activation following oxidative stress, particularly after myocardial, intestine and skeletal muscle ischemic-reperfusion induced injury, at least in experimental models (23). Additionally, MBL has been reported to recognize apoptotic and necrotic cells (37, 39), which could also trigger activation of the complement through the lectin pathway in patients with high-\textit{MBL2} genotypes and a non-infectious SIRS. In fact a defective apoptotic clearance has been found in MBL defective mice (49). Unfortunately we did not
measure the level of inflammation or complement activation in our patients and therefore we could not demonstrate a higher degree of activation, as we would have expected from our results, in patients with a non-infectious SIRS and high-\textit{MBL2} genotypes. However this group of patients had higher severity indexes particularly higher SOFA scores, which measures organ failure due to endothelium injury.

The analysis of the outcomes of the ICU patients with SIRS, particularly mortality, revealed no differences regarding the existence of low-\textit{MBL2} genotypes. No differences were either seen between low and high-\textit{MBL2} genotypes (data not shown). The relationship between mortality and the presence of low-\textit{MBL2} genotypes continues to be a controversial issue with studies supporting this association (17, 19, 24) and others like the present one finding no relationship (25, 50). In a recent meta-analysis by Eisen \textit{et al.} (8), only a trend towards an increased mortality among patients with low-\textit{MBL2} genotypes and bacterial infections was found. We could not observe a better outcome in patients with intermediate-\textit{MBL2} genotypes as demonstrated by Helleman \textit{et al.} (24). It should be noted that one the limitations of our study is the short follow up period of the patients included.

Nowadays, with the application of increasingly effective organ support treatments, withdraw of life support therapy is the most common terminal event in ICU patients and thus longer term mortality analyses are recommended (53).

Regarding the genotyping of the exon 3 of the \textit{MASP2}, no significant differences were found in the frequency for the heterozygous \textit{MASP2} Asp105Gly SNP between patients with an infectious and a non-infectious SIRS. The \textit{MASP2} Asp105Gly SNP was not associated with any of the clinical variables associated with an increased severity of the ICU admission including mortality. Our results were similar to those reported by Garcia-Laorden \textit{et al.} in patients with community acquired pneumonia.
(12), but not to the ones from the study of Henckaets et al. which observed an association between the existence of the heterozygous MASP2 Asp105Gly SNP and higher mortality rates in ICU patients (25).

Our study was not capable of finding an increased prevalence of any microorganism or between Gram positive or negative bacteria among patients with low-MLBL2 genotypes. While some of the published studies have found an association between Gram positive infections and MBL deficiency (8, 24), others have observed a link between Gram negative infections and MBL deficiency (44).

The present study has important design limitations that have to be taken into consideration. First, our control group was composed by healthy unmatched blood donors. Although the use of healthy blood donors as a control group has commonly been used in genetic studies, it has the potential risk of misclassification of individuals particularly when evaluating a condition with a high penetrance, such as the MBL deficiency. Second, due to the retrospective design of the study, no power calculation was performed which could seriously affect its capacity to detect small differences, particularly when analyzing the different subgroups of SIRS patients. Therefore additional independent studies are required to replicate our observations.

In conclusion, this study shows that low-MLBL2 genotypes were significantly underrepresented among patients with a non-infectious SIRS and were not associated with more severe forms of sepsis or to death. The presence of MASP2 polymorphism’s were not related to infectious SIRS and had no impact on the prognosis of ICU patients with SIRS. Thus, high-MBL producers seem to be at risk of developing a non-infectious SIRS, while low-MBL producers are not likely to be at higher risk of developing severe forms of infectious SIRS.
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# FIGURE LEGENDS AND TABLE FOOTNOTES

## TABLE 1. Baseline characteristics of the ICU patients included in the study

| Characteristics | Septic shock (n=101) | Severe sepsis (n=28) | Sepsis (n=54) | Non-infectious SIRS (n=60) |
|-----------------|----------------------|----------------------|---------------|---------------------------|
| Age (years)     | 64.2±14.9            | 60.6±17.9            | 57.8±16.4     | 66±14.5                   |
| Sex: M/F        | 65/36                | 14/14                | 33/21         | 41/19                     |
| APACHE II score | 19.1+5.8             | 16+6.7               | 14.2+6.5      | 18.1+5.8                  |
| SAPS II score   | 38.5+13.1            | 39+12.3              | 33.9+10.1     | 35.5+11                   |
| SOFA score      | 10.45+2.7            | 8.1+3.4              | 6.9+2.7       | 8+2.8                     |
| Invasive mechanical ventilation | 72 (71.3) | 12 (42.8) | 27 (50) | 44 (73.3) |
| Type of admission |                      |                      |               |                           |
| Acute surgery   | 24 (23.7)            | 3 (10.7)             | 5 (9.2)       | 14 (23.3)                 |
| Elective surgery| 6 (5.9)              | 1 (3.5)              | 2 (3.7)       | 10 (16.6)                 |
| Medical         | 71 (70.3)            | 24 (85.7)            | 47 (87)       | 36 (60)                   |
| Chronic disease |                      |                      |               |                           |
| Chronic respiratory disease | 27 (26.7) | 7 (25) | 19 (35.1) | 14 (23.3) |
| Heart failure NYHA IV | 3 (2.9)    | 2 (7.1) | 3 (5.5) | 8 (13.3)   |
| Chronic kidney failure | 5 (4.9)    | 1 (3.5) | 0 | 3 (5) |
| Cirrhosis       | 6 (5.9)             | 0                    | 0             | 4 (6.6)                   |
| Diabetes mellitus (type 1 and 2) | 24 (23.7) | 3 (10.7) | 15 (27.7) | 11 (18.3) |
| Active cancer   | 3 (2.9)             | 1 (3.5)              | 1 (1.8)       | 2 (3.3)                   |
| Hematological malignancies | 11 (10.9) | 0 | 3 (5.5) | 3 (5) |
| AIDS            | 0                   | 2 (7.1)              | 0             | 0                         |
| Immunosuppressive or corticosteroid treatment | 21 (20.8) | 0 | 9 (16.6) | 7 (11.6) |
| Focus of infection at admission |                      |                      |               |                           |
| Non-pneumonic lower respiratory tract infections | 7 (6.9) | 1 (3.5) | 14 (25.9) | NA |
| Community-acquired pneumonia | 40 (39.6) | 14 (50) | 28 (51.8) | NA |
| Hospital-acquired pneumonia | 10 (9.9) | 1 (3.5) | 2 (3.7) | NA |
| Urinary tract infection | 10 (9.9) | 5 (17.8) | 0 | NA |
| Bacterial meningitis | 3 (2.9) | 1 (3.5) | 2 (3.7) | NA |
| Peritonitis      | 18 (17.8)           | 2 (7.1)              | 5 (9.2)       | NA                        |
| Other infections | 13 (12.8)           | 4 (14.3)             | 3 (5.5)       | NA                        |

*Data are presented as the mean ± standard deviation or number (%). *NA*: not applicable
TABLE 2. *MBL2* and *MASP2* genotype frequency of the ICU patients with SIRS and the healthy control group

| Expression type | Patients | Healthy controls | P | Non infectious SIRS | Infectious SIRS | P | Septic shock | Severe sepsis | Sepsis |
|----------------|---------|------------------|---|--------------------|----------------|---|--------------|--------------|--------|
| **MBL2 genotypes** |         |                  |   |                    |                |   |              |              |        |
| Group 1 (high-MBL) | 105 (48.6) | 57 (54.8) | 0.29 | 33 (63.4) | 72 (43.9) | 0.014 | 41 (43.6) | 13 (61.9) | 18 (36.7) |
| A/A | 68 (31.5) | 33 (31.8) | 21 (40.4) | 47 (28.7) | 23 (24.4) | 11 (52.4) | 13 (26.5) |
| A/XA | 37 (17.1) | 24 (23) | 12 (23) | 25 (15.2) | 18 (19.2) | 2 (9.5) | 5 (10.2) | 23 (46.9) |
| Group 2 (intermediate-MBL) | 83 (38.4) | 31 (29.8) | 0.13 | 17 (32.7) | 66 (40.2) | 0.33 | 38 (40.4) | 5 (23.8) | 23 (46.9) |
| XA/XA | 10 (4.6) | 3 (2.8) | 2 (3.8) | 8 (4.9) | 4 (4.2) | 1 (4.8) | 3 (6.1) |
| A/O | 73 (33.8) | 28 (27) | 15 (28.9) | 58 (35.3) | 34 (36.2) | 4 (19) | 20 (40.8) |
| Group 3 (low-MBL) | 28 (12.9) | 16 (15.4) | 0.55 | 2 (3.8) | 26 (15.9) | 0.025 | 15 (15.9) | 3 (14.2) | 8 (16.3) |
| XA/O | 19 (8.8) | 8 (7.7) | 1 (1.9) | 18 (11) | 10 (10.6) | 2 (9.5) | 6 (12.2) |
| O/O | 9 (4.1) | 8 (7.7) | 1 (1.9) | 8 (4.9) | 5 (5.3) | 1 (4.7) | 2 (4.1) |
| Total | 216 | 104 | 52 | 164 | 94 | 21 | 49 |
| **MASP2 genotype** |         |                  |   |                    |                |   |              |              |        |
| Wild type | 227 (94.6) | 101 (97.1) | 0.41 | 57 (95) | 170 (94.4) | 1 | 96 (96) | 28 (100) | 46 (88.4) |
| Asp105Gly\(^c\) | 12 (5) | 3 (2.8) | 0.56 | 3 (5) | 9 (5) | 1 | 4 (4) | 0 | 5 (9.6) |
| Pro111Leu\(^c\) | 1 (0.4) | 0 | 1 | 0 | 1 (0.5) | 1 | 0 | 0 | 1 |
| Total | 240 | 104 | 60 | 180 | 100 | 28 | 52 |

\(^a\)Y and X indicate base exchanges at codon -221. A, normal structural allele; O, variant alleles (B, codon 54, C, codon 57; and D, codon 52). \(^b\)Data are presented as number (%). \(^c\)Correspond to the heterozygous genotypes
TABLE 3. Type of microorganism isolated and outcomes of the patients according to the existence of a deficient *MBL2* genotype or the heterozygous *MASP2* Asp105Gly genotype

| Variable                        | Non deficient MBL2 genotypes | Deficient MBL2 genotypes | P    | Non deficient MASP2 genotypes | Heterozygous MASP2 Asp105Gly genotypes | P   |
|---------------------------------|-----------------------------|--------------------------|------|-------------------------------|----------------------------------------|-----|
| Gram-positive                   | 28 (20.3)                   | 6 (23.1)                 | 0.748| 40 (23.4)                     | 2 (22.2)                              | 1   |
| Gram-negative                   | 36 (26.1)                   | 6 (23.1)                 | 0.747| 43 (25.1)                     | 2 (22.2)                              | 1   |
| Mixed                           | 2 (1.4)                     | 0                        | 1    | 3 (1.8)                       | 0                                       | 1   |
| Fungi                           | 6 (4.3)                     | 2 (7.7)                  | 0.614| 9 (5.3)                       | 1 (11.1)                              | 0.41|
| Length of ICU Stay (days)       | 10.2±10.7                   | 10.4±12.1                | 0.925| 10.4±10.9                     | 8.9±5.4                               | 0.638|
| Length of Hospital Stay (days)  | 23.2±19.5                   | 21.1±15.9                | 0.618| 23.2±19.1                     | 21.0±13.7                             | 0.7 |
| Highest APACHE II score         | 20.4±7.1                    | 19.2±7.2                 | 0.458| 20.8±7.0                      | 17.7±7.8                              | 0.159|
| Highest SAPS II score           | 45.4±16.6                   | 46.0±16.0                | 0.881| 45.3±16.3                     | 52.3±17.4                             | 0.168|
| Highest SOFA score              | 10.3±3.4                    | 10.1±3.9                 | 0.794| 10.3±3.4                      | 9.0±2.3                               | 0.201|
| Invasive mechanical ventilation  | 143 (76.1)                  | 20 (71.4)                | 0.595| 171 (75)                      | 11 (91.7)                             | 0.303|
| Invasive mechanical ventilation (days) | 6.7±9.3                  | 6.0±9.3                  | 0.74 | 6.8±9.6                       | 6.4±5.6                               | 0.89 |
| Renal replacement               | 25 (13.3)                   | 6 (21.4)                 | 0.253| 32 (14)                       | 0                                       | 0.377|
| Renal replacement (days)        | 1.1±4.5                     | 0.4±1.3                  | 0.47 | 1.0±4.1                       | 0                                       | 0.413|
| Parenteral nutrition            | 38 (20.2)                   | 6 (21.4)                 | 0.882| 43 (18.9)                     | 2 (16.7)                              | 1   |
| Nosocomial infection            | 39 (20.7)                   | 6 (21.4)                 | 0.934| 50 (21.9)                     | 3 (25)                                | 0.73 |
| Hospital acquired pneumonia     | 22 (11.7)                   | 4 (14.3)                 | 0.755| 30 (13.1)                     | 2 (16.7)                              | 0.665|
| Overall Hospital mortality      | 59 (31.4)                   | 6 (21.4)                 | 0.284| 70 (30.7)                     | 3 (25)                                | 0.676|
| ICU Mortality                   | 43 (22.9)                   | 5 (17.9)                 | 0.464| 53 (23.2)                     | 1 (8.3)                               | 0.457|
| Multi-organ failure             | 39 (20.7)                   | 5 (17.9)                 | 0.714| 48 (21.1)                     | 1 (9.1)                               | 0.469|

a Data are presented as the mean ± standard deviation or number (%). b Includes patients with mechanical ventilation on admission to ICU and those requiring mechanical ventilation later during ICU stay. c Excluding patients in which indication for ICU admission was a nosocomial infection.