

Mannose-binding lectin and mannose-binding lectin-associated serine protease 2 in susceptibility, severity, and outcome of pneumonia in adults

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Background: Community-acquired pneumonia (CAP) is the leading cause of death from infection in developed countries. Mannose-binding lectin (MBL) and MBL-associated serine protease 2 (MASP-2) deficiencies are common primary immunodeficiencies the clinical penetrance of which remains controversial. MBL is a serum lectin that mediates phagocytosis and activates the lectin pathway of complement involving MASP-2.

Objective: We sought to evaluate the significance of MBL deficiency (*O/O* genotypes) and insufficiency (*O/O* plus *XA/O* genotypes), as well as MASP-2 deficiency (*D105G* mutation), in the susceptibility to and severity and outcome of CAP in adults. **Methods:** MBL and MASP-2 serum levels, as well as lectin pathway activity with regard to *MBL2* and *MASP2* genotypes, were measured in healthy control subjects. For susceptibility, 848 patients with CAP, 1447 healthy control subjects, and a control group of 519 patients without relevant infectious diseases were studied in a case-control study. Severity and outcome were evaluated in a prospective study of the 848 patients.

Results: We found similar frequencies of *MBL2* and *MASP2* alleles and genotypes among patients and control subjects. However, in a multivariate analysis MBL insufficiency was associated with the development of the most severe forms of

sepsis ($P = .007$), acute respiratory failure ($P = .009$), multiorgan dysfunction syndrome ($P = .036$), intensive care unit admission ($P = .020$), and death ($P = .003$).

Conclusion: Our large study suggests that MBL plays a redundant role in human defenses against primary infection, at least in adults with CAP, and provides, for the first time, evidence that MBL insufficiency predisposes to higher severity and fatal outcome in patients with CAP, irrespective of the causal microorganisms. (J Allergy Clin Immunol 2008;121:121-129.)

Key words: Community-acquired pneumonia, sepsis, mannose-binding lectin, mannose-binding lectin-associated serine protease 2, primary immunodeficiency

Community-acquired pneumonia (CAP) remains the leading cause of death from infection in developed countries. The incidence of CAP is higher in those less than 5 years of age and in the elderly.¹ Several microorganisms can be causative agents of CAP, with *Streptococcus pneumoniae* being the most common.¹ CAP severity varies widely from almost asymptomatic to septic shock (SSh), multiple organ dysfunction syndrome (MODS), and eventually death, and despite effective antimicrobial agents, the mortality rate in hospitalized patients ranges from 2% to 30%.¹ Genetic variability affecting the host response might influence the susceptibility to and the severity and outcome of infection. Primary immunodeficiencies are the more obvious example.^{2,3}

Mannose-binding lectin (MBL) is a serum lectin secreted as an acute-phase protein. MBL binds several carbohydrate structures present on the surfaces of diverse microorganisms, triggering the lectin pathway (LP) of complement activation. Thereafter, LP activation involves several serine proteases, in particular MBL-associated serine protease 2 (MASP-2), to generate the C3 convertase. The LP can also be activated by H-, M-, and L-ficolins.⁴ MBL acts as opsonin either directly or by virtue of its ability to activate complement, also mediating an antimicrobial effect through the membrane attack complex.^{5,6} Deficient and low MBL serum levels are mainly due to the presence of 3 common point mutations in exon 1 of the *MBL2* gene (alleles *B*, *C*, and *D*, termed *O* alleles, with *A* being the wild-type allele). Serum MBL levels are very low or absent in patients homozygous for *O* alleles. In addition, the presence of the promoter allele *X*, instead of the *Y* allele, has an important downregulating effect on MBL serum levels. As a result, individuals with *XA* alleles at one chromosome and *O* alleles at the other (*XA/O*) have very

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Abbreviations used

ARDS:	Acute respiratory distress syndrome
ARF:	Acute respiratory failure
CAP:	Community-acquired pneumonia
ICU:	Intensive care unit
LP:	Lectin pathway
MASP-2:	Mannose-binding lectin serine protease 2
MBL:	Mannose-binding lectin
MODS:	Multiple organ dysfunction syndrome
OR:	Odds ratio
PGN:	Peptidoglycan
SS:	Severe sepsis
SSh:	Septic shock

low serum MBL levels.^{5,6} On the basis of a well-described correlation between genotypes and MBL serum levels, *O/O* together with *XA/O* genotypes are considered MBL-insufficient genotypes, being common in most populations.⁵⁻⁷ MASP-2 deficiency caused by the *D105G* mutation in the *MASP2* gene was reported in 2003.⁸ *D105G* allelic frequency is 0.024 in Spaniards.⁹

MBL and MASP-2 deficiencies are common primary immunodeficiencies of the complement system.² MBL deficiency is not a primary immunodeficiency in the classical sense of the term because its clinical penetrance is extremely low. Epidemiologic studies have suggested that MBL deficiency is a weak susceptibility factor for infectious diseases, mainly in children between 6 and 18 months of age, with its role still being debated, particularly in adults with underlying diseases.^{6,10-12} Likewise, the clinical penetrance of MASP-2 deficiency remains controversial.⁹

We have analyzed the significance of *MBL2* and *MASP2* variability in the susceptibility to and the severity and outcome of CAP in a large group of adults.

METHODS**Patients and control subjects**

Eight hundred forty-eight white Spanish patients with CAP hospitalized at 3 hospitals in Spain were prospectively included. The diagnosis of pneumonia was assumed in the presence of acute onset of signs and symptoms, suggesting lower respiratory tract infection and radiographic evidence of a new pulmonary infiltrate that had no other known cause. Patients with defined severe immunosuppression and patients with tuberculosis, obstructive pneumonia caused by neoplasia, or pneumonia as the terminal event of chronic and progressive disease were excluded from the study. Five hundred nineteen unrelated age-matched (61.41 ± 14.12 years) and sex-matched (32.95% women) white Spanish patients from the same 3 hospitals without a previous history of relevant infectious diseases served as the control group (control patients). In addition, 1447 (40.30 ± 14.72 years and 51.2% women) unrelated healthy volunteers served as the healthy control group. Sepsis, severe sepsis (SS), SSh, and MODS were defined by using the American College of Chest Physicians/Society of Critical Care Medicine criteria.¹³ The pneumonia severity index was measured by using the Fine scale.¹⁴ Acute respiratory failure (ARF) was defined as an oxygen saturation of less than 90% on room air or a P_{aO_2} of less than 60 mm Hg. Acute respiratory distress syndrome (ARDS) was defined by using the American European Consensus Conference Definition.¹⁵

Measurement of MBL and MASP-2 serum levels and LP activity

MBL and MASP-2 serum levels, as well as MBL-dependent LP activation, were measured in serum samples from a group of healthy control subjects by using an MBL oligomer ELISA kit (Antibodyshop,

Gentofte, Denmark), a MASP-2 ELISA kit (Hycult Biotechnology b.v., Uden, The Netherlands), and a WIELISA-kit (Wieslab, Lund, Sweden), respectively.

Analysis of *MBL2* and *MASP2* polymorphisms

MBL2 polymorphisms were analyzed as described elsewhere.¹⁶ Ninety-two DNAs were genotyped by using an INNO-LiPA MBL2 kit (Innogenetics, Gent, Belgium). Haplotypes were simplified as *YA*, *XA*, and *O*.

Genetic analysis of the *MASP2 D105G* variant was carried out by means of PCR-RFLP. Genotyping was confirmed by means of direct sequencing. Primers and conditions are available on request.

Informed consent was obtained from the patients or their relatives. The protocol was approved by the local ethics committees of the 3 hospitals.

Statistical analysis

Quantitative variables are presented as arithmetic means $\pm$ SEMs. The differences in MBL serum levels and LP activity with regard to the analyzed genotypes were compared with the Mann-Whitney *U* test or the Kruskal-Wallis H test when needed. Comparison of the distribution of *MBL2* and *MASP2* genotypes based on susceptibility, severity, and outcome was performed with the χ^2 test or the Fisher exact test when needed, and odds ratios (ORs) with 95% CIs were calculated. The relation between severity or outcome and genotype was evaluated by using binary logistic regression models, and age, sex, hospital of origin, and comorbidities were included as independent variables. The causative agent of CAP was included as an independent variable in some analyses. Survival rates were estimated by using the Kaplan-Meier method, and their comparison related to genotypes was performed with the long-rank test. Multivariate analysis adjusted by age, sex, hospital of origin, and comorbidities was carried out with the Cox proportional hazard model. Statistical analysis was performed with SPSS 10.0 (SPSS, Inc, Chicago, Ill).

RESULTS

Between January 2002 and March 2006, patients admitted to 3 different university hospitals were evaluated for the diagnosis of CAP. After excluding those patients without informed consent, those patients with ethnicity other than white Spanish, and those who fulfilled the exclusion criteria, a total of 848 patients were finally included. The main demographic and clinical variables of the patients are available in Table E1 in the Online Repository at www.jacionline.org. *S pneumoniae* was the most common causative agent (195 [22.99%]). Ninety-five percent of the patients received initial antibiotic treatment according to American Thoracic Society guidelines.¹⁷ No differences in antibiotic use were observed when stratifying patients based on severity or outcome: SS and SSh, 92.6% and 93.8%, respectively; ARF, 95.1%; ARDS, 97.3%; and 90-day mortality, 97%.

We first evaluated the functional significance of the *MBL2* and *MASP2* variants in healthy control subjects. A marked decrease in MBL serum levels was observed in individuals with MBL-insufficient genotypes (*XA/O* + *O/O*) when compared with those with MBL-sufficient genotypes (*A/A* + *YA/O*; 61.93 ± 8.68 and 1145.12 ± 93.98 ng/mL, respectively; $P < .001$; Fig 1, A). These results are in agreement with our previous results and those of others.^{6,9,12} The distribution of MASP-2 serum levels also tightly correlated with *MASP2* genotypes (Fig 1, B): *D105G*-heterozygous subjects showed lower MASP-2 serum levels than subjects with the wild-type genotype (188.45 ± 31.77 vs 438.60 ± 25.32 ng/mL; $P = .002$), whereas MASP-2 levels were previously shown to be severely reduced in *D105G*-homozygous subjects.^{8,9} When the MBL-dependent LP activity was measured among

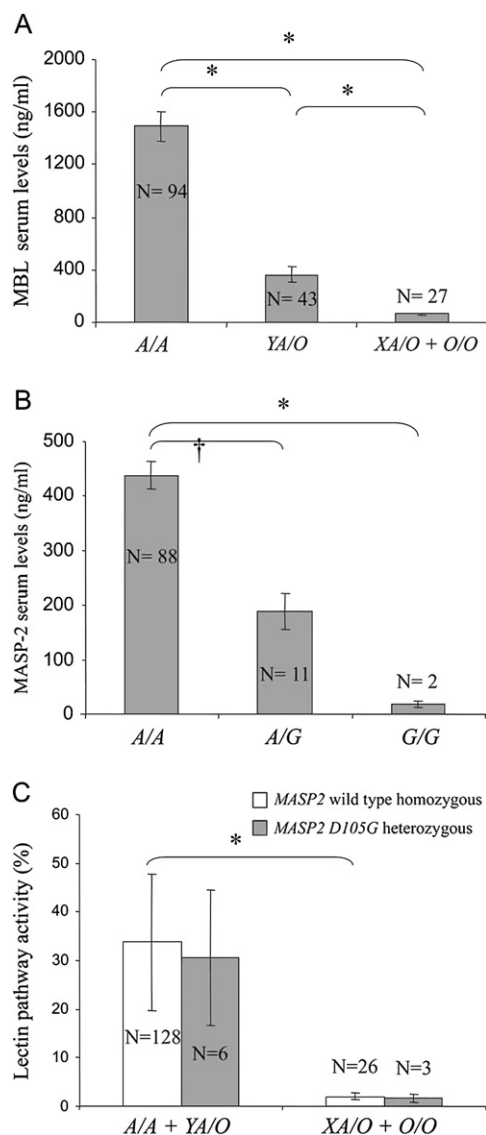


FIG 1. MBL and MASP-2 serum levels (as nanograms per milliliter) and MBL-dependent lectin pathway activity (as a percentage) in healthy control subjects related to their *MBL2* and *MASP2* genotypes. **A**, MBL serum levels. * $P < .001$. **B**, MASP-2 serum levels: comparison of the 3 groups: $P < .001$. * $P = .033$, † $P = .002$. **C**, MBL-dependent lectin pathway activity. * $P < .001$. All graphics show the arithmetic means and SEMs. A/A + YA/O, MBL-sufficient genotypes; XA/O + O/O, MBL-insufficient genotypes.

subjects with wild-type *MASP2* with regard to *MBL2* genotypes, a markedly decreased LP activity was observed in subjects with *MBL2*-insufficient genotypes than in those bearing *MBL2*-sufficient genotypes ($2.04\% \pm 0.81\%$ vs $33.75\% \pm 7.13\%$, respectively; $P < .001$; Fig 1, C). Patients homozygous for the *MASP2* D105G allele were previously shown to present an almost null LP activity.^{8,9} However, heterozygosity for the D105G allele did not show a significant effect on LP activity (Fig 1, C).

No differences in allelic or genotypic frequencies were observed when *MBL2* exon 1 wild-type (A) and mutated (O) alleles were analyzed (Table I). A weak significance was observed when the allelic frequency of the promoter X allele was compared between patients with CAP and control subjects ($P = .041$; OR, 0.82; 95% CI, 0.67-0.99), although no differences were observed when patients and healthy control subjects were compared. Nor

TABLE I. *MBL2* and *MASP2* alleles and genotypes in patients with CAP and control subjects

	CAP	Control subjects	Healthy control subjects
<i>MBL2</i> alleles			
Structural alleles	n = 1696	n = 1038	n = 2894
Total A	1311 (77.30)	815 (78.52)	2229 (77.02)
Total O	385 (22.70)	223 (21.48)	665 (22.98)
B	269 (15.86)	144 (13.87)	412 (14.24)
C	37 (2.18)	32 (3.08)	83 (2.87)
D	79 (4.66)	47 (4.53)	170 (5.87)
Promoter alleles	n = 1696	n = 1038	n = 2894
X	329 (19.40)	236 (22.74)	596 (20.59)
Y	1367 (80.60)	802 (77.26)	2298 (79.41)
<i>MBL2</i> genotypes			
Structural genotypes	n = 848	n = 519	n = 1447
A/A	510 (60.14)	319 (61.46)	861 (59.50)
A/O	291 (34.32)	177 (34.10)	506 (34.97)
O/O	47 (5.54)	23 (4.43)	80 (5.53)
Structural and promoter genotypes	n = 848	n = 519	n = 1447
A/A + YA/O	732 (86.32)	439 (84.59)	1224 (84.59)
XA/O + O/O	116 (13.68)	80 (15.41)	223 (15.41)
<i>MASP2</i> alleles			
A	n = 1682	n = 1028	n = 1610
A	1636 (97.27)	993 (96.60)	1573 (97.70)
G	46 (2.73)	35 (3.40)	37 (2.30)
<i>MASP2</i> genotypes			
A/A	n = 841	n = 514	n = 805
A/A	795 (94.53)	479 (93.19)	770 (95.65)
A/G	46 (5.47)	35 (6.81)	33 (4.10)
G/G	0	0	2 (0.25)

A/A + YA/O, MBL-sufficient genotypes; XA/O + O/O, MBL-insufficient genotypes. Values are presented as the number of chromosomes (percentage) for the alleles and the number of individuals (percentage) for genotypes. O is the designation for the variant alleles B, C, and D of the *MBL2* gene. The A allele of the *MASP2* gene is the wild-type allele, whereas the G allele designates the D105G mutation. $P > .1$ for all comparisons, except for analysis of X alleles in patients with CAP versus control patients ($P = .041$; OR, 0.82; 95% CI, 0.67-0.99).

were differences found for the *MASP2* D105G mutation among patients and control subjects, although 2 D105G-homozygous healthy control subjects were identified (Table I). When patients with CAP caused by pneumococcus or gram-negative bacteria or those without causative confirmation were compared with control groups, no differences were observed (see Table E2 in the Online Repository at www.jacionline.org). No significant differences in *MASP2* genotypes were observed when the causative agent was taken into account (data not shown).

The relevance of *MBL2* and *MASP2* variants in the severity of CAP was evaluated by the analysis of the development of the most severe forms of sepsis as the primary variable (Table II). Those patients without sepsis or with a nonsevere form of sepsis presented with similar genotypic frequencies and were grouped as having “non-SS.” The frequency of MBL-insufficient genotypes was higher in patients with SS (21.19%) than in those with SS (16.79%) or non-SS (11.47%; χ^2 test for lineal trend, 9.21; $P = .01$). Likewise, patients with more severe forms of sepsis had higher frequencies of MBL-insufficient genotypes (18.82%) than those with non-SS ($P = .006$; OR, 1.79; 95% CI, 1.17-2.73). In addition, MBL insufficiency was also associated with need of intensive care unit (ICU) admission ($P = .008$; OR, 1.82; 95% CI, 1.15-2.84) and development of ARF ($P = .041$; OR, 1.68; 95% CI, 1.03-2.83), ARDS ($P = .047$; OR, 2.29; 95% CI, 0.96-4.99), and MODS ($P = .030$; OR, 1.91;

TABLE II. Severity and outcome of patients with CAP related to *MBL2* genotypes

Characteristic	A/A+YA/O, no. (%)	XA/O+O/O, no. (%)	P value* OR (95% CI)	Multivariate P value† OR (95% CI)	Multivariate P value‡ OR (95% CI)
NSS	525 (88.53)	68 (11.47)	.006§ 1.79 (1.17-2.73)	.007§ 1.86 (1.19-2.9)	.005 2.00 (1.24-3.25)
SS	114 (83.21)	23 (16.79)			
SSh	93 (78.81)	25 (21.19)			
General ward	582 (88.05)	79 (11.95)	.008 1.82 (1.15-2.84)	.020 1.78 (1.10-2.90)	.024 1.82 (1.08-3.06)
ICU	150 (80.81)	37 (19.79)			
No bacteremia	677 (86.68)	104 (13.32)	.531 1.33 (0.61-2.67)	.640 1.19 (0.57-2.47)	.292 1.53 (0.69-3.40)
Bacteremia	54 (83.08)	11 (16.92)			
No ARF	222 (90.24)	24 (9.76)	.041 1.68 (1.03-2.83)	.009 1.93 (1.18-3.18)	.005 2.17 (1.27-3.72)
ARF	506 (84.62)	92 (15.38)			
No ARDS	703 (86.90)	106 (13.10)	.047 2.29 (0.96-4.99)	.205 1.70 (0.75-3.89)	.152 1.92 (0.79-4.67)
ARDS	29 (74.36)	10 (25.64)			
No MODS	663 (87.24)	97 (12.76)	.030 1.91 (1.04-3.38)	.036 1.90 (1.04-3.45)	.137 1.64 (0.86-3.14)
MODS	68 (78.16)	19 (21.84)			
PSI I-III	298 (88.43)	39 (11.57)	.185 1.35 (0.88-2.11)	.166 1.35 (0.88-2.05)	.26 1.29 (0.83-2.02)
PSI IV-V	412 (84.95)	73 (15.05)			
No 90-d exitus	678 (87.37)	98 (12.63)	.006 2.31 (1.22-4.19)	.003 2.34 (1.32-4.14)	.008 2.30 (1.25-4.23)
90-d exitus	54 (75.0)	18 (25.0)			

NSS, Nonsevere sepsis includes patients without either severe sepsis or septic shock; MODS, multiorgan dysfunction syndrome; PSI, pneumonia severity index; A/A+YA/O, MBL-sufficient genotypes; XA/O+O/O, MBL-insufficient genotypes.

*P value for the bivariate comparison.

†P value for the multivariate analysis, including the variables age, sex, hospital of origin, and comorbidities, except for the analysis of the pneumonia severity index, which only includes hospital of origin.

‡P value for the multivariate analysis, including the variables age, sex, hospital of origin, comorbidities, and pathogen (*S pneumoniae*, gram-negative bacteria, and no pathogen identified), except for the analysis of the pneumonia severity index, which only includes hospital of origin, and the analysis of the bacteremia, which does not include the variable no pathogen identified.

§NSS versus SS plus SSh analysis.

||NSS versus SS versus SSh: $P = .010$, $\chi^2 = 9.2$.

95% CI, 1.04-3.38; Table II) but not with bacteremia or the pneumonia severity index.

Interestingly, when the hospital of origin together with age, sex, and comorbidities were included as independent variables in a multivariate analysis, MBL insufficiency was associated with the development of the most severe forms of sepsis ($P = .007$; OR, 1.86; 95% CI, 1.19-2.9) but also with the development of ARF and MODS and a higher rate of ICU admission (Table II).

No significant differences were observed when the risk of fatal outcome at 28 days was analyzed. However, when 90-day mortality was evaluated, we observed that patients who died had a higher frequency of MBL-insufficient genotypes, and this association was independent of age, sex, hospital of origin, and comorbidities ($P = .003$; OR, 2.34; 95% CI, 1.32-4.14; Table II). Kaplan-Meier analysis (Fig 2) showed a significantly different survival for MBL-insufficient and MBL-sufficient patients (long-rank χ^2 test, 5.65; $P = .017$). Cox Regression for 90-day survival, adjusted for the independent variables of age, sex, hospital of origin, and comorbidities, was also significant ($P = .007$; hazard ratio, 2.19; 95% CI, 1.23-3.87).

Interestingly, a nonsignificant trend toward an increased frequency of MBL-insufficient genotypes among patients with greater severity of disease and poor mortality was also found when patients were studied on the basis of the causative microorganism (CAP caused by *S pneumoniae*, gram-negative bacteria, or no pathogen identified; data not shown). A multivariate analysis showed that MBL insufficiency predisposed to severe forms of sepsis ($P = .005$; OR, 2.00; 95% CI, 1.24-3.25), need for ICU admission, development of ARF, and increased 90-day mortality, irrespective of the causative microorganism, age, sex, hospital of origin, and comorbidity (Table II).

MASP2 alleles and genotypes did not associate with any measurement of severity or outcome.

DISCUSSION

Our prospective study provides, for the first time, evidence that MBL insufficiency predisposes to more severe forms of sepsis and to fatal outcome in patients with CAP. However, the case-control study does not support a role of MBL insufficiency in the susceptibility to CAP.

We did not observe differences in the frequencies of *MBL2*-insufficient genotypes among 2 large groups of control individuals and 848 adults with CAP, even when CAP caused by gram-positive bacteria (mostly *S pneumoniae*) or gram-negative bacteria was evaluated. Our results differ from those of Roy et al,¹⁸ who found a significant increase of the *O/O MBL2* genotype in patients with invasive pneumococcal infection compared with that seen in healthy control subjects. Two studies did not find any association,^{19,20} although a meta-analysis of these 3 studies yielded a significantly increased prevalence of the *O/O* genotype in patients with invasive pneumococcal disease than seen in healthy control subjects.²⁰ We did not find significant differences in the frequencies of MBL-insufficient or *O/O* genotypes between bacteremic and nonbacteremic patients, even when pneumococcal CAP was analyzed. However, we studied patients with CAP, whereas the 3 aforementioned reports analyzed patients with invasive pneumococcal disease. On the other hand, even a nonsignificant trend toward lower frequencies of MBL-insufficient genotypes in patients with gram-positive CAP (mostly pneumococci) than in healthy control subjects was observed in our survey. In agreement with our results, Pérez-Castellano et al,²¹ in the only other study of MBL and CAP ($n = 100$), did

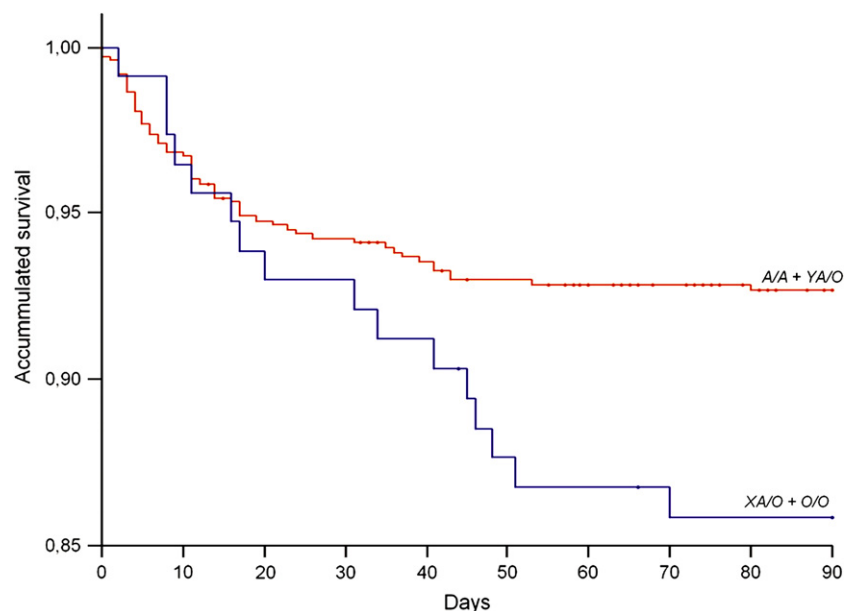


FIG 2. Kaplan-Meier estimation of survival at 90 days in the 848 patients with CAP, according to *MBL2* genotypes conferring MBL sufficiency (A/A + YA/O) or MBL insufficiency (XA/O + O/O). Long-rank χ^2 , 5.65; $P = .017$. There was a hazard ratio of 2.19 (95% CI, 1.23-3.87; $P = .007$) in the Cox proportional hazards model correcting for age, sex, hospital of origin, and comorbidities.

not observe any association between MBL deficiency and pneumococcal CAP, and even an increased risk of bacteremia was observed in patients (88%) with the wild-type *MBL2* genotype.

The absence of association between MBL insufficiency and pneumococcal CAP is not surprising because no binding of *S pneumoniae* to MBL could be demonstrated *in vitro*.²² Several other mechanisms of the innate immune system can provide sufficient protection against pneumococci and other bacteria in the absence of MBL.²³⁻²⁵ In fact, the classical pathway and, to a much lesser extent, the alternative pathway are the dominant complement pathways during innate immunity against *S pneumoniae* in mice, and loss of the MBL pathway did not affect, or only minimally affected, first-line host defense.²⁶⁻²⁸

MBL2-insufficient genotypes were, nonetheless, significantly more prevalent among patients with more severe forms of the disease when primary and most secondary variables were analyzed. Association remained significant in a multivariate analysis corrected not only for age, sex, and comorbidities but also for hospital of origin, therefore adjusting any differences in admission policies and in therapeutic management among hospitals and ICUs, as well as in putative genetic differences, among the 3 populations. Interestingly, multivariate analysis also confirmed that MBL insufficiency predisposes to more severe forms of the disease and to a poor outcome, irrespective of the causative microorganisms.

No differences were observed at 28-day all-cause mortality. However, MBL-insufficient genotypes were associated with decreased 90-day survival independent of age, sex, comorbidities, and hospital of origin. With increasingly effective and widely available organ support treatments, withdrawal of life-sustaining therapy is now the most common terminal event in ICU patients.²⁹ That is why longer-term mortality analyses, even 1-year analyses, are advocated. In our study Kaplan-Meier survival analysis during a 3-month follow-up period showed a lower survival of

patients with MBL insufficiency, with a progressive separation of the 2 curves at 15 days.

An association between *MBL2* variant alleles, promoter alleles, or both with a poor prognosis of critically ill patients requiring intensive care management was previously reported in some,³⁰⁻³² but not all,^{19,33} studies. In a study of ICU patients with ARDS, a significant association between the codon 54 *BB* genotype with increased severity and 60-day mortality was found.³⁴ However, these results should be taken with caution because when *O* alleles (*B*, *C*, and *D*) were examined together, no associations were found. Recently, Hellemann et al³⁵ reported that *O/O* homozygotes had a poor outcome compared with individuals with the *A/A* genotype, whereas patients with the *A/O* genotype had the most favorable outcome. However, they found that *MBL2* alleles were associated with mortality only after ICU discharge. Conversely, we found a poor outcome in both *O/O* and *XA/O* patients; 68% of our patients died in the ICU, with no significant differences in *MBL2* genotypes with patients who died in the general ward. Several important differences might account for the observed discrepancies with our study. Hellemann et al³⁵ studied a heterogeneous population of ICU patients, whereas we only assessed patients with CAP. In addition, 23.3% of their patients had systemic inflammatory response syndrome without infection, and survival was higher among patients with sepsis than in those without it. Lastly, strong differences existed in the microbial species identified.

The reasons for the observed effects of MBL on severity and poor outcome in CAP remains unclear, but several nonmutually exclusive MBL-mediated mechanisms might be involved. First, MBL might be able to limit the systemic spread of infecting pathogens. Experimental models in mice suggest that only when other concomitant infection-predisposing factors, such as neutropenia or severe burns, are present are MBL-null mice infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa* more

prone to systemic bacterial infection and death than wild-type mice.^{28,36} However, the MBL complement pathway was nonredundant in the same experimental model against blood-borne infection. MBL was found to be present in bronchoalveolar lavage fluids from patients with pneumonia but not in fluids from healthy individuals, indicating that MBL might leak from the systemic circulation at the site of inflammation in the lungs to contain the spread of the infection.³⁷

Second, MBL has been shown to contribute signals to human PBMCs, leading to the suppression of LPS-induced proinflammatory cytokines.³⁸ In addition, MBL modulates the production of proinflammatory cytokines to *Neisseria meningitidis* in human MBL-deficient whole-blood assays in a dose-dependent fashion. An altered pattern of proinflammatory cytokine production was also observed in experimental models of sepsis with MBL-null mice.^{28,36}

Third, MBL binds both soluble and insoluble peptidoglycan (PGN), the major cell-wall component of gram-positive bacteria, with high affinity.³⁹ During the bacterial lifecycle, PGN is shed, and cell wall-active antibiotics cause a rapid and significant release of PGN.⁴⁰ It was shown that MBL binding to PGN inhibits PGN-induced proinflammatory cytokine production by macrophages.⁴¹

Fourth, experiments *in vitro* and in MBL-null mice have demonstrated that MBL recognizes altered self-structures in necrotizing and apoptotic cells, facilitating their clearance by macrophages.⁴² This mechanism could theoretically become clinically relevant for the progression of the sepsis syndrome.⁴³ Interestingly, the 2 latter mechanisms would bring into line our observation of more severe forms of sepsis in MBL-insufficient patients, particularly in those with CAP caused by capsulated gram-positive bacteria, with the absence of MBL binding to capsulated *S pneumoniae* and *S aureus*.²⁶

We did not observe any effect of MASP-2 deficiency in the susceptibility to and severity of CAP. Homozygous MASP-2 deficiency could theoretically play a greater role in the susceptibility and severity of CAP because L-ficolin was shown to bind *S aureus*, *S pneumoniae*, and lipoteichoic acids from gram-positive bacteria,²³ and L- and H-ficolin participate in the clearance of apoptotic cells.⁴⁴ However, we have previously reported 2 healthy adults with MASP-2 deficiency,⁹ and a third MASP-2-deficient healthy donor was identified in the present study, showing that the clinical penetrance of MASP-2 deficiency might be also very low. Because of the low frequency of *D105G* homozygosity (approximately 6 in 10,000 individuals), thousands of patients and control subjects would be needed to detect any significant association between MASP-2 deficiency and CAP.

Several strengths of our study minimize limitations of genetic association studies. First, our study of a large cohort of patients reduces type I error. Second, we included only white subjects of Spanish origin, thus limiting the likelihood of population stratification caused by ethnic heterogeneity. Third, we analyzed patients with CAP as the only infection at admission, whereas past studies dealing with genetic *MBL2* variability and sepsis were limited to heterogeneous ICU populations, frequently including patients with systemic inflammatory response syndrome without infection. Fourth, the increased risk to severe forms of sepsis and poor outcome of MBL-insufficient individuals was found to be independent of age, sex, comorbidities, causative agent, and hospital of origin.

We acknowledge several limitations of our study. Clearly other factors complicate the interpretation of association studies in infectious diseases. The specific cause of the infectious process and virulence of the microorganism, as well as the delay in diagnosis and treatment, can undoubtedly act as confounders. Although current recommendations for treatment of sepsis and SSH were applied, a common protocol was not specifically used. On the other hand, we have established a role of MBL in the severity of CAP, but a role of other lectins, such as ficolins, cannot be excluded.

Finally, identifying unique host factors that predispose certain individuals to more severe manifestations of infectious diseases will undoubtedly remain an important goal, particularly in the context of immunomodulatory therapy. Recombinant human MBL is in development as a novel therapeutic approach. Infusions of MBL were shown to have a good safety profile in phase I trials.^{45,46} Additional knowledge of the proinflammatory and anti-inflammatory roles of the MBL-dependent complement pathway in sepsis is needed before the potential of this intervention might merit assessment.

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Clinical implications: We show that MBL and MASP-2 insufficiency, 2 primary immunodeficiencies, have no role in susceptibility to CAP, but MBL insufficiency predisposes to higher severity and fatal outcome in patients with CAP.

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TABLE E1. Demographic and clinical characteristics of patients with CAP

Characteristics*	No. (%)
Age (y; n = 848)	65.16 ± 17.55†
Sex (n = 848)	
Male	556 (65.57)
Female	292 (34.43)
ICU admission (n = 848)	
No	661 (77.95)
Yes	187 (22.05)
Sepsis (n = 848)	
Nonsevere sepsis	593 (69.93)
Severe sepsis	137 (16.16)
SSh	118 (13.92)
MODS (n = 848)	
No	761 (89.74)
Yes	87 (10.26)
Acute respiratory failure (n = 848)	
No	247 (29.13)
Yes	601 (70.87)
ARDS (n = 848)	
No	809 (95.40)
Yes	39 (4.60)
Bacteremia (n = 848)	
No	783 (92.31)
Yes	65 (7.69)
90-d Exitus (n = 848)	
No	776 (91.51)
Yes	72 (8.49)
Comorbidity (n = 844)‡	
No	250 (29.62)
COPD	249 (29.50)
Asthma	49 (5.81)
Neoplasia	95 (11.26)
Ischemic cardiopathy	97 (11.49)
Diabetes	198 (23.46)
Renal insufficiency	68 (8.06)
Hepatic insufficiency	55 (6.52)
Neurological pathology	135 (16.00)
Autoimmune pathology	19 (2.25)
Psychiatric pathology	6 (0.71)
Pneumonia severity index (n = 822)	
I-III (low)	337 (41.00)
IV-V (moderate-high)	485 (59.00)
Causative agent identified (n = 848)	
No	516 (60.85)
Yes	332 (39.15)
<i>Streptococcus pneumoniae</i>	195 (22.99)
<i>Pseudomonas aeruginosa</i>	24 (2.83)
<i>Legionella pneumophila</i>	16 (1.89)
<i>Haemophilus influenzae</i>	16 (1.89)
<i>Chlamydophila pneumoniae</i>	12 (1.42)
Virus	9 (1.06)
Others	60 (7.08)
Antibiotic adherence to ATS guidelines (n = 848)	807 (95.16)

Nonsevere sepsis, Patients without either severe sepsis or SSh; COPD, chronic obstructive pulmonary disease; ATS, American Thoracic Society.

*The number of patients with available data is shown in parentheses.

†For age, the value is presented as mean ± SD.

‡Some patients had more than 1 comorbidity.

TABLE E2. *MBL2* genotypes in patients with CAP related to the causative agent

	<i>Streptococcus pneumoniae</i> (n = 195)	Gram-positive bacteria (n = 216)	Gram-negative bacteria (n = 96)	No pathogen (n = 516)	Healthy control subjects (n = 1447)
<i>A/A + YA/O</i>	173 (88.72)	193 (89.35)	78 (81.25)	444 (86.05)	1224 (84.59)
<i>XA/O + O/O</i>	22 (11.28)	23 (10.65)	18 (18.75)	72 (13.95)	223 (15.41)

A/A + YA/O, MBL-sufficient genotypes; *XA/O + O/O*, MBL-insufficient genotypes.

Values are presented as numbers (percentages). Subjects infected by both gram-positive and gram-negative pathogens have been excluded.