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The MBL2 genotype relates to COVID-19 severity and may help to select the optimal therapy

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Abstract

Objectives: Sars-CoV-2 acute infection is clinically heterogeneous, ranging from asymptomatic cases to patients with a severe, systemic clinical course. Among the involved factors age and preexisting morbidities play a major role; genetic host susceptibility contributes to modulating the clinical expression and outcome of the disease. Mannose-binding lectin is an acute-phase protein that activates the lectin-complement pathway, promotes opsonophagocytosis and modulates inflammation, and is involved in several bacterial and viral infections in humans. Understanding its role in Sars-CoV-2 infection could help select a better therapy.

Methods: We studied MBL2 haplotypes in 419 patients with acute COVID-19 in comparison to the general population and related the haplotypes to clinical and laboratory markers of severity.

Results: We recorded an enhanced frequency of MBL2 null alleles in patients with severe acute COVID-19. The homozygous null genotypes were significantly more frequent in patients with advanced WHO score 4–7 (OR of about 4) and related to more severe inflammation, neutrophilia, and lymphopenia.

Conclusions: Subjects with a defective MBL2 genotype (i.e., 0/0) are predisposed to a more severe acute Sars-CoV-2 infection; they may benefit from early replacement therapy with recombinant MBL. Furthermore, a subset of subjects with the A/A MBL genotype develop a relevant increase of serum MBL during the early phases of the disease and develop a more severe pulmonary disease; in these patients, the targeting of the complement may help. Therefore, COVID-19 patients should be tested at hospitalization with serum MBL analysis and MBL2 genotype, to define the optimal therapy.

Keywords: COVID-19; MBL2; Sars-CoV-2

Introduction

Sars-CoV-2 infection appeared in 2019 as a novel, clinically heterogeneous disease, ranging from asymptomatic cases to patients with a severe, systemic clinical course. In fact, severe respiratory involvement appears in 5–20 % of cases with pneumonia that may progress to acute respiratory distress syndrome (ARDS) and multiorgan failure. Mortality is about 5 % [1]. A better understanding of the relationships between pathophysiology and the clinical phenotypes of COVID-19 will be a key to developing personalized management [2]. Among the involved factors the age, also due to the different expression of virus receptors in children and adults [3, 4] and preexisting morbidities [5, 6] have a role in conditioning the severity and the prognosis of Sars-CoV-2 acute infection. In addition, the improvement of diagnostic approaches [7] and of the management of the disease [8], as well as the changes in the virus pathogenicity [9] mitigated the last waves of the disease, with less severe inflammation [10, 11], immunological alterations [12] and clinical severity of COVID patients.

Genetic host susceptibility has a relevant role in conditioning the clinical expression and the outcome of the disease [13, 14], and a multitude of genes related to the viral entry in the cell, and to the host immunity was investigated [15]. The functionality of many proteins of innate immunity is regulated by gene polymorphisms that modulate their synthesis and activity. Mannose-binding lectin (MBL) 2 is a prototype of such proteins. It is an acute-phase protein produced in...
monomeric form by the liver that polymerizes up to octamers in serum. Mannose-binding lectin activates the lectin-complement pathway, promotes opsonophagocytosis, and modulates inflammation; such activities are closely related to the level of polymerization. Three promoter variants modulate MBL2 gene expression and subsequently serum levels of the protein, i.e., H/L variant (−550), X/Y variant (−221), and P/Q (+4 in the 5′ UTR). Three other variants within intron 1 impair protein polymerization thus reducing protein activity, i.e., codon 52 (D variant), codon 54 (B variant), and codon 57 (C variant). The six variants are in linkage disequilibrium giving rise to haplotypes with different levels of activity ranging from high (HYA) to intermediate (LYA, LXA haplotypes), or null (HYP, LYC, HYB haplotypes) activity [16]. MBL is a typical example of an innate protein with a dual activity. The impaired activity of MBL enhances the risk of severe infectious diseases [17, 18]; furthermore, it negatively modulates the cystic fibrosis (CF) phenotype [19] and increases the risk to develop gastric cancer in patients with Helicobacter pylori colonization [20]. But, a large study in a centenarian population demonstrated that the hyperactivity of the protein would enhance the risk of autoimmune diseases [21].

More recently, a role of MBL2 toward COVID-19 emerged and the interaction of MBL2 with Sars-CoV-2 spike protein with the subsequent lectin activation of complement was demonstrated [22]. Various studies reported that MBL2 polymorphisms that cause a reduced activity of the protein were related to a more severe Sars-CoV-2 infection [22–24], and these observations could open the way to therapies that could restore the activity of the protein [16, 25]. Other studies focused on the enhanced activation of the complement induced by MBL2 and severe pulmonary inflammation in patients with COVID-19, suggesting the use of therapies that could reduce complement activation [26–29].

Therefore, we studied MBL2 haplotypes in a large group of patients with acute COVID-19 in comparison to the general population and related the haplotypes to clinical and laboratory markers of severity.

**Materials and methods**

**MBL2 polymorphism analysis**

DNA was extracted by circulating cells using NucleoSpin Blood Kit (Macherey-Nagel) in accordance with manufacturer instructions. The analysis of MBL2 promoter polymorphisms was carried out by PCR (primers and conditions available on request) followed by restriction fragment length polymorphism analysis. The analysis of MBL2 exon 1 polymorphisms was analyzed by double digestion with MboII/BanI and then with MwoI. Thus, 14 % polyacrylamide gel was used to screen the digestion products.

**Statistical analysis**

Data were reported as median (interquartile range). Comparisons between groups were evaluated by the Wilcoxon test. Statistical analysis was performed by SPSS. In order to assess the association between Sars-Cov-2 infection and MBL genotype, bivariate analyses were performed with a significance level of 5 % (p<0.05), using Pearson's chi-square test and odds ratio (OR) with a 95 % confidence interval (95 % CI).

**Patients**

We studied 419 adult patients with the diagnosis of COVID-19 admitted to one of our hospitals. The study was approved by the Ethical Committee of the University Federico II of Naples; the lone exclusion criterion was the refusal or the impossibility to undersign the informed consent. The diagnosis of Sars-CoV-2 infection was confirmed by molecular analysis on a nasopharyngeal swab [7]. The patients were classified on the basis of the seven ordinal scale made by the World Health Organization (WHO)-Research and Development Blueprint expert group and used in previous influenza studies [30, 31]. The data obtained from COVID-19 patients were compared to those of 672 subjects from the general population (GP) of Southern Italy previously studied by our group [18, 20].

**Results**

As shown in Figure 1A, the frequency of the MBL haplotypes HYA (high functionality) and LYA or LXA (intermediate functionality) was significantly lower in COVID-19 patients as compared to the GP (p<0.0001 and p<0.001, respectively), while the frequency of the null haplotypes, i.e., LYB, LYC or HYD was significantly higher (p<0.0001). As shown in Figure 1B, the frequency of the HYA haplotype was significantly lower in COVID-19 patients of WHO 5–7 subgroup (n=122) as compared either to the GP (p<0.0001) and to COVID-19 patients of WHO 3 (n=115) and 4 (n=182) subgroups (p<0.0001 in both cases), while the frequency of the null haplotypes was significantly lower in COVID-19 patients of the WHO subgroup 5–7 either in comparison to the GP and to COVID-19 patients of the subgroup 4. Finally, the frequency of the LYA or LXA haplotypes was significantly lower in COVID-19 patients of both subgroups 4 and 5–7 vs. the GP, while no differences were recorded between the three WHO subgroups.

Therefore, we evaluated the frequency of the three MBL genotypes i.e., A/A, A0 (where 0 means B+C+D), and 0/0 in COVID-19 patients in comparison to the GP. As shown in Figure 1B, the frequency of the A/A genotype resulted significantly lower (p<0.0001) in COVID-19 patients, while the frequency of either the A/0 and the 0/0 genotypes resulted significantly higher in patients with COVID-19 (p<0.001 and p<0.0001, respectively).
As shown in Figure 2B, the frequency of the A/A genotype was significantly lower in COVID-19 patients of the subgroup 5–7 as compared either to the GP (p<0.0001), and to patients of the subgroup 3 (p<0.0001) and 4 (p<0.001), while the frequency of the 0/0 genotype showed the opposite trend with a significant increase in COVID-19 patients of the subgroup 5–7 as compared either to the GP (p<0.0001), and to patients of the subgroup 3 (p<0.0001) and 4 (p<0.001). The frequency of the A/0 genotype was significantly lower in patients of all three subgroups, each compared to the GP (p<0.001), with no differences between the three subgroups.

Figure 3 shows the correlations between MBL genotypes, and several prognostic biomarkers of COVID-19 tested at hospital admission, i.e., serum IL-6, C-Reactive Protein (CRP), blood neutrophil, total lymphocyte, and T-lymphocyte number within the three subgroups of COVID-19 patients. The values of serum IL-6 resulted significantly higher in COVID-19 patients with the 0/0 MBL genotype as compared either to patients with the A/0 and to those with the A/A genotype (p<0.0001 in both cases). The number of circulating neutrophils was significantly higher in COVID-19 patients with the MBL 0/0 genotype in comparison to either those with the A/0 and those with the A/A genotype (p<0.001 and p<0.0001, respectively). Either the number of total lymphocytes or the number of T-lymphocytes was significantly lower in patients of the WHO subgroup 5–7 in comparison to those of the subgroups 4 (p<0.001) and 3 (p<0.0001). The values of serum CRP were not significant among groups although there was an increasing trend in patients with the MBL 0/0 genotype in comparison to either those with the A/0 and those with the A/A genotype.

Finally, Table 1 shows the OR for severe COVID-19 associated with the MBL alleles and genotypes. The 0 allele (i.e., B, C or D alleles) confers an OR of 1.9 (p<0.0001) to develop a severe COVID-19 (i.e., WHO 5,6,7); the 0/0 genotype confers a 4.1 OR (p<0.0001) to develop a severe COVID-19 as compared to...
The A/A genotype. The A/0 genotype causes a slight increase in the OR for severe COVID-19 (i.e., 1.6, p=0.043).

Discussion

Our study describes the enhanced frequency of MBL2 null alleles in patients with severe acute COVID-19. The homozygous null genotypes are significantly more frequent in patients with advanced WHO scores 4–7 with an OR of about 4 and are related to enhanced inflammation, i.e., higher levels of serum IL-6 [32, 33] and more severe neutrophilia and lymphopenia that in turn are negative prognostic factors in patients with COVID-19 [34–36]. We could also see an increasing trend of serum CRP levels in patients with the MBL 0/0 genotype in comparison to either those with the A/0 and those with the A/A genotype (Figure 3).

Recent studies have demonstrated that the MBL protein interacts with the spike protein of Sars-CoV-2 and activates the complement. Furthermore, MBL inhibits virus entry in the cells [22]. The null alleles impair the polymerization of the protein reducing its activity [16] and this would enhance the infectivity and reduce the clearance of the virus rendering the disease more severe. These data confirm previous observations that reported a protective effect of the MBL A allele toward severe COVID-19 [22] and a higher
frequency of the MBL B null allele in patients with COVID-19 as compared to healthy controls [37, 38] or in severe COVID-19 patients as compared to less severe infected patients [23, 24]. This data has been also recently confirmed in children, where the B allele was found to be associated with COVID-19 severity [39]. Our data indicate that either the B and D null alleles are more frequent in patients with severe COVID-19 (data not shown). Interestingly, in a recent study no association was found between MBL2 polymorphic genotypes, and a condition named “long covid”. A term used to identify patients that although recovered from COVID-19 present clinical symptoms not justified by other pathologies [40].

MBL deficiency is frequent and causes enhanced susceptibility to several diseases that include respiratory tract infections [17], recurrent bacterial infections, particularly during chemotherapy, neonatal sepsis, post-transplantation infections [16], and a more severe pulmonary and liver expression in patients with cystic fibrosis [19]. Despite such a myriad of clinical conditions that now include severe COVID-19, few studies explored the substitutive treatments with the protein [16]. In fact, since the observation of Miller et al. in 1968, which demonstrated that fresh plasma restored the opsonization defect in a young child with recurrent infections [41], studies in mice, and later in humans were performed each on a limited number of patients with psoriasis, recurrent infections, cystic fibrosis, neutropenia. Such studies demonstrated that MBL substitution with plasma-derived protein was safe and would restore normal levels of the serum protein, but the clinical results were discordant [16]. Later, a phase I trial in patients with protein deficiency demonstrated that the recombinant MBL was safe and tolerated, does not cause immunogenicity, and restored normal levels of serum protein [41]. A study demonstrated that mice treated with recombinant MBL survived otherwise fatal Ebola, becoming immune to virus rechallenge [42]. It is known that MBL interacts with mannose-associated serine protease 1 (MASP) to activate the complement and that the interaction requires that MBL would reach a satisfactory

Figure 3: Correlations between MBL genotypes, and several prognostic biomarkers of COVID-19.

Table 1: Contingency tables and odds-ratio for risk factors of severe COVID-19 infection.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Mild COVID-19 (WHO 3–4)</th>
<th>Severe COVID-19 (WHO 5–7)</th>
<th>OR (95 % CI)</th>
<th>p-Value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/0 genotype</td>
<td>27</td>
<td>30</td>
<td>4.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A/A genotype</td>
<td>159</td>
<td>43</td>
<td>2.2–7.6</td>
<td></td>
</tr>
<tr>
<td>A/0 genotype</td>
<td>111</td>
<td>49</td>
<td>1.6</td>
<td>0.043</td>
</tr>
<tr>
<td>A/A genotype</td>
<td>159</td>
<td>43</td>
<td>1.0–2.6</td>
<td></td>
</tr>
<tr>
<td>0 allele</td>
<td>167</td>
<td>104</td>
<td>1.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A allele</td>
<td>427</td>
<td>140</td>
<td>1.4–2.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Pearson’s chi-square test.
level of polymerization. The plasma-derived MBL is poorly polymerized and is early degraded, while the recombinant MBL better interacts with MASP and is more stable [25]. Our data encourage a trial with recombinant MBL in hospitalized COVID-19 patients with low levels of serum MBL due to null genotypes. Interestingly, during Sars epidemic infection in 2003, lower serum levels of MBL were found in affected patients, although no relationships between MBL2 genotypes and the disease were observed, and MBL inhibited the entry of the virus in fetal rhesus kidney cells [43].

Furthermore, MBL has a dual activity. For example, too much active protein relates to the enhanced risk of autoimmune diseases, as we demonstrated in a large population of centenarians [21]. Similarly, in several COVID-19 patients serum levels of MBL and MASP strongly enhance during the acute phase, and such increase hyperactivates the complement causing a more severe pulmonary disease [26, 43]. Of course, such a latter trend is typical of subjects with the wild-type HYA MBL2 genotype. For this reason, the targeting of the complement [27] or of the MASP protein [29] that links MBL to the complement was suggested.

To conclude: our study indicates that subjects with a defective MBL2 genotype (i.e., 0/0) are predisposed to a more severe acute Sars-CoV-2 infection; they might benefit from early replacement therapy with recombinant MBL. In addition, a subset of subjects with the A/A MBL genotype may develop an increase of serum MBL and MASP during the first phases of the disease with the risk to develop more severe pulmonary disease, and in these patients, the targeting of MASP or of the complement may help to prevent hyperinflammation. Thus, we suggest that all COVID-19 patients should be tested at hospitalization with serial serum MBL analysis and MBL2 genotype to define the optimal therapy.

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Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all subjects involved in the study.

Ethical approval: This prospective multicenter study was approved by the Ethical Committee of the University Federico II, Naples.

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