Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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MBL deficiency-causing B allele (rs1800450) as a risk factor for severe COVID-19

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ABSTRACT

The COVID-19 pandemic represents one of the greatest challenges in modern medicine. The disease is characterized by a variable clinical phenotype, ranging from asymptomatic carriage to severe and/or critical disease, which bears poor prognosis and outcome because of the development of severe acute respiratory distress syndrome (SARS) requiring ICU hospitalization, multi-organ failure and death. Therefore, the determination of risk factors predisposing to disease phenotype is of outmost importance. The aim of our study was to evaluate which predisposing factors, including *MBL2* genotyping, affected clinical phenotype in 264 COVID-19 patients. We demonstrated that older age along with underlying comorbidities, primarily obesity, chronic inflammatory disorders and diabetes mellitus, represent the most important risk factors related to hospitalization, the development of pneumonia and SARS. Moreover, we found that the presence of the MBL deficiency-causing B allele (rs1800450) was significantly associated with almost 2-fold increased risk for developing pneumonia and requiring hospitalization, suggesting its usage as a molecular predictor of severe disease in SARS-CoV-2 infected individuals.

1. Introduction

The COVID-19 pandemic is caused by the novel SARS-CoV-2 affecting all the countries globally, and therefore, it represents one of the greatest challenges in modern medicine. It is worth noting that among infected patients, only 20% exhibit a severe respiratory illness requiring hospitalization, and one quarter of these patients progress to SARS requiring ICU care (Emami et al., 2020; Li et al., 2020).' Clinical and epidemiological data show that older patients with or without previous medical history, comprise the population group most vulnerable for the development of serious sequelae of the disease and poor outcome (Emami et al., 2020; Rydyznski Moderbacher et al., 2020). However, recent data suggest that younger individuals, although on a smaller scale, can also have serious sequelae with an increase in rates of morbidity and fatality (Li et al., 2020; Van Der Made et al., 2020; Zhu et al., 2020).

A multidisciplinary approach is being implemented worldwide to tackle the various obstacles involved in the process of identifying which individuals are candidates of a poor prognosis. This is currently the focus of multiple approaches, with research in molecular immunology being

Abbreviations: COVID-19, coronavirus disease 2019; ICU, Intensive care unit; MBL2, Mannose binding lectin 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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the most promising area to deliver valuable answers.

Among the mediators of the first-line host defense against pathogens is mannose-binding-lectin (MBL). MBL is a pattern recognition molecule of hepatic origin, which binds to mannose or sugar motifs on pathogen surfaces (bacteria, fungi, viruses, protozoa) activating the complement system, but it can also interact directly with collectin receptors promoting opsono-phagocytosis through a complement-independent manner (Turner, 2003).

Although MBL serum concentrations show important changes with age (Sallenbach et al., 2011), they are primarily determined by single nucleotide polymorphisms (SNPs) in both exon 1 and promoter of the *MBL2* gene (Speletas et al., 2015; Turner, 2003). SNPs in the promoter region at positions −550 and −221, known as variants H/L (rs11003125) and X/Y (rs7096206) respectively, also influence *MBL2* expression, although only the X variant significantly reduces MBL serum levels. Additionally, three SNPs at codons 52, 54 and 57 of exon 1 are referred to as variants D (rs5030737), B (rs1800450) and C (rs1800451) respectively, while the wild-type allele is referred to as allele A; the O allele represents the variant alleles D, B or C (Speletas et al., 2015; Turner, 2003). The presence of the O allele impairs the oligomerization of MBL protein, resulting in reduced levels of functional protein circulating in the serum (Madsen et al., 1995). Therefore, the combination of genetic alterations into both exon 1 and promoter result in 3 MBL genotype expression groups which are associated with high (YA/YA, YA/XA), medium (XA/XA, YA/O) and low (XA/O, O/O) MBL serum levels; the latter genotype group is also referred to as genotypic MBL deficiency (Speletas et al., 2015; Turner, 2003).

Individuals with MBL deficiency display an increased susceptibility to several infections, including those triggered by HIV and *Neisseria meningitidis*, while MBL has been shown to opsonize and inhibit the infectivity of influenza A virus (Garred et al., 1997; Hartshorn et al., 1993; Hibberd et al., 1999). Interestingly, previous studies have demonstrated that MBL also contributes to the immune response against SARS-CoV and MBL deficiency is a susceptibility factor for acquisition of SARS (Ip et al., 2005; Zhang et al., 2005). Therefore, taking into consideration the variable clinical presentation of SARS-CoV-2 infection, the aim of our study was to evaluate which predisposing factors, including *MBL2* genotyping, affect COVID-19 clinical phenotype.

2. Material and methods

2.1. Subjects

Two hundred and sixty-four (264) patients (male/female: 180/84, mean age ± SD: 42.8 ± 18.4 years) were enrolled in the study from March to October 2020. The ethnicity of patients included 152 Greeks, 62 Turks, 16 Ukrainians, 15 Indonesians, 6 Uzbeks, 3 Moldovans, 3 Americans, 2 Cubans and 1 patient from each of the following countries: Albania, Belarus, Bulgaria, Germany, and Kyrgyzstan. Most patients of non-Greek origin were passengers and crew members on a cruise ferry who became infected with COVID-19 in March 2020.

Patients were initially divided into five (5) groups according to the severity of their disease: (a) asymptomatic (66, 25.0%), (b) mild (84, 31.8%), (c) moderate requiring hospitalization (15, 5.7%), (d) severe with pneumonia (77, 29.2%), and (e) severe respiratory failure necessitating intubation and mechanical ventilation (22, 8.3%). Four patients (1.5%) died while hospitalized. Demographic and clinical data of the enrolled patients are presented in Table 1.

Written informed consent was obtained from all participants. The study was carried out in accordance with the principles of the Helsinki Declaration and was approved by the ethical committee of the Faculty of Medicine, University of Thessaly (No. 2115).

2.2. Molecular analyses

DNA was extracted from peripheral blood, with the detection of *MBL2* genetic alterations performed by allele-specific polymerase chain reaction, followed by restriction fragment length polymorphism (PCR-RFLP) analysis as described (Speletas et al., 2015).

2.3. Statistical analysis

Categorical variables are described with the use of frequency and relative frequency. Continuous variables are described using means and

Table 1
Demographic and clinical characteristics of the patients of the study. The patients divided in five (5) Groups according to the severity of the disease: Group 1, asymptomatic patients; Group 2, mild disease; Group 3, moderate disease with hospitalization; Group 4, severe disease; Group 5, critical disease necessitating intubation and mechanical ventilation.

Parameter	Total	Group 1 (asymptomatic)	Group 2 (mild)	Group 3 (moderate)	Group 4 (severe)	Group 5 (critical)	p #
No	264	65	85	15	77	22	0
Sex (male/female)	180/84	44/21	58/27	9/6	55/22	14/8	0.902 (C)
Age (mean ± SD), y	42.8 ± 18.4	35.1 ± 13.7	30.9 ± 11.0	45.1 ± 16.9	56.4 ± 16.2	62.7 ± 13.4	**<0.001** (K-W)
Comorbidity (n, %)	94, 35.6	6, 9.2	8, 9.4	5, 33.3	56, 72.7	19, 86.4	**<0.001** (C)
Obesity (n, %)	31, 11.7	0, 0	2, 2.4	2, 13.3	22, 28.6	5, 22.7	**<0.001** (C)
Hypertension (n, %)	49, 18.6	1 1.5	1, 1.2	2, 13.3	33, 42.8	12, 54.5	**<0.001** (C)
CHD (n, %)	11, 4.2	0, 0	0, 0	0, 0	7, 9.1	4, 18.2	**<0.001** (C)
CVD (%)	8, 3.0	0, 0	0, 0	1, 6.7	3, 3.9	4, 18.2	**<0.001** (C)
CRD (n, %)	20, 7.6	1, 1.5	1, 1.2	0, 0	11, 14.3	7, 31.8	**<0.001** (C)
Dyslipidemia (n, %)	19, 7.2	1, 1.5	0, 0	1, 6.7	14, 18.2	3, 13.6	**<0.001** (C)
Diabetes mellitus (n, %)	21, 8.0	0, 0	2, 2.4	0, 0	14, 18.2	5, 22.7	**<0.001** (C)
Hypothyroidism (n, %)	12, 4.5	0, 0	2, 2.4	2, 13.3	6, 7.8	2, 9.1	**0.047** (C)
Malignancies (n, %)	6, 2.3	0, 0	2, 2.4	0, 0	2, 2.6	2, 9.1	0.163 (C)
Chronic liver/renal diseases (n, %)	6, 2.3	1, 1.5	0, 0	0, 0	5, 6.5	0, 0	0.057 (C)
Chronic immune and/or hematologic diseases ^ (n, %)	6, 2.3	1, 1.5	0, 0	0, 0	3, 3.9	2, 9.1	0.092 (C)
Others * (n, %)	16 (6.1)	3, 4.6	0, 0	1, 6.7	11, 14.3	1, 4.5	**0.005(C)**

Abbreviations: CHD, chronic heart disease (atrial fibrillation, heart failure, arrhythmias, prosthetic valve); CRD, chronic respiratory diseases (chronic obstructive pulmonary disease, asthma, sleep apnea); CVD, cerebrovascular disease (including also chronic venous disease); SD, standard deviation.

^ Chronic immune and/or hematologic diseases included patients with systemic lupus erythematosus (1), rheumatoid arthritis (1), IgA deficiency (1), beta-thalassemia (1), sickle-cell disease (1), autoinflammatory disease (1).

* Comorbidity-others included patients with history of transplantation (liver or kidney), history of severe infections (meningitis, flu due to H1N1, recurrent urinary infections), history of major surgery (partial gastrectomy, nephrectomy, partial colectomy), dementia, depression.

Statistical analysis: C, Chi-square; F. Fisher's exact test; K-W = Kruskal Wallis test; M−W, Mann-Whitney *U* test.

standard deviation. The analysis of continuous variables was conducted using the Mann-Whitney *U* test, since the assumption of normal distribution was violated. Data were checked for deviation from normal distribution using the Shapiro-Wilk normality test. Categorical data were analyzed with the use of Chi-square test or Fisher's exact test. Multivariate analysis was performed in the form of binary logistic regression for all parameters, with a statistical significance of $p > 0.2$ in univariate analysis. For all the analyses, a 5% significance level was set. Analysis was carried out with SPSS (version 25.0).

3. Results

3.1. Associations of demographic and clinical characteristics with COVID-19 phenotype

As presented in Tables 1 and 2 as well as in Supplementary Table 1, the older age and underlying comorbidities substantially affected the phenotype of COVID-19 patients. Interestingly, among comorbidity factors the presence of obesity, hypertension, chronic respiratory disease, diabetes mellitus and thyroid disease were significantly associated with a worse COVID-19 phenotype (Table 1). Thus, elderly patients with obesity, hypertension, chronic heart or respiratory disease, dyslipidemia, diabetes mellitus and thyroid disease more frequently displayed symptoms leading to hospitalization (Table 2). In multivariate analysis however, the most important risk factors identified which predisposed patients to display severe symptoms requiring hospitalization included older age, the presence of obesity and a history of chronic autoimmune or hematologic disorder (Table 2).

In addition, older age and comorbidity (including most of underlying diseases) significantly predisposed patients to the development of pneumonia. However, in multivariate analysis, the most important risk factors were similar to those leading to hospitalization (Table 3). Conversely, obese and diabetic patients displayed a 5.8-fold and 5.5-fold increased risk, respectively, for development of SARS (Supplementary Table 1). In this context males more frequently developed SARS compared to females, although this impact was lost in multivariate analysis (Supplementary Table 1).

3.2. MBL deficiency and COVID-19 phenotype

Six patients were homozygotes and 72 were heterozygotes for the B allele, eight patients were heterozygotes for the C allele, with three patients were homozygotes and 42 heterozygotes for the D allele (allele frequencies 15.9%, 1.5% and 9.1%, respectively). In addition, 17 patients were homozygotes and 88 were heterozygotes for the X allele (allele frequency: 23.1%). Table 4 presents an overview of *MBL2* genotypes/haplotypes and the genetic MBL deficiency in the patients of the study. As also indicated in Table 4, genetic MBL deficiency was displayed in 47 out of the 264 patients (17.8%).

Interestingly, the presence of the B allele (rs1800450) was significantly associated with a worse phenotype, as carriers more frequently displayed symptoms and signs of the disease resulting in both the requirement for hospitalization and development of pneumonia. In particular, the presence of the B allele was significantly associated with an approximately 2-fold increased risk for hospitalization and 1.5-fold increased risk for pneumonia (Tables 2 and 3, Fig. 1). It is worth noting that the impact of B allele to pneumonia development and the risk of hospitalization was weaker than other comorbidities (Tables 2 and 3), however the B allele retained its impact on pneumonia development in multivariate analysis, although statistical significance was marginally lost ($p = 0.052$, odds ratio: 2.14 (0.99–4.62, Table 2). On the other hand, neither the presence of other *MBL2* polymorphisms nor the genotyping MBL deficiency was significantly associated with the COVID-19 phenotype (Tables 2-4, Supplementary Table 1).

4. Discussion

Similar to previous studies (Chakinala et al., 2021; Dalekos et al.,

Table 2
Association of clinical parameters and *MBL2* genotyping with the need of hospitalization in the patients of the study.

	Hospitalization		Univariate analysis			Multivariate analysis	
	Yes ^ (n 114)	No (n 150)	*p* #	Odds Ratio (OR)	Relative Risk (RR)	*p*	Odds Ratio (OR)
Sex (male/female)	78/36	102/48	0.942 (C)	1.02 (0.60–1.72)	1.01 (0.75–1.36)		
Age (mean ± SD), y	56.2 ± 16.4	32.7 ± 12.4	**<0.001** (M-W)			**<0.001**	**1.08 (1.05–1.11)**
Comorbidity (n, %)	80 (70.2)	14 (9.3)	**<0.001 (C)**	**22.86 (11.57–45.16)**	**4.26 (3.11–5.82)**		
Obesity (n, %)	29 (25.4)	1 (1.3)	**<0.001 (F)**	**25.25 (5.88–108.44)**	**2.56 (2.11–3.11)**	**0.011**	**9.04 (1.64–49.81)**
Hypertension (n, %)	47 (41.2)	2 (1.3)	**<0.001 (F)**	**51.91 (12.25–220.0)**	**3.08 (2.50–3.79)**	0.250	2.69 (0.50–14.52)
CHD (n, %)	11 (9.6)	0 (0)	**0.001 (F)**		**2.46 (2.12–2.85)**	0.999	–
CVD (%)	8 (7.0)	0 (0)	**<0.001 (F)**		**2.42 (2.09–2.79)**	0.999	–
CRD (n, %)	18 (15.8)	2 (1.3)	**<0.001 (F)**	**13.86 (3.15–61.15)**	**2.29 (1.85–2.83)**	0.658	1.51 (0.24–9.34)
Dyslipidemia (n, %)	18 (15.8)	1 (0.6)	**<0.001 (F)**	**27.94 (3.67–212.71)**	**2.42 (2.00–2.92)**	0.072	11.68 (0.80–170.05)
Diabetes mellitus (n, %)	19 (16.7)	2 (1.3)	**<0.001 (F)**	**14.80 (3.37–64.99)**	**2.31 (1.88–2.85)**	0.107	5.57 (0.69–44.85)
Thyroid disease (n, %)	10 (8.8)	2 (1.3)	**0.006 (F)**	**7.12 (1.53–33.15)**	**2.02 (1.51–2.71)**	0.061	7.07 (0.92–54.58)
Malignancies (n, %)	4 (3.5)	2 (1.3)	0.408 (F)	2.69 (0.48–14.96)	1.56 (0.87–2.80)		
Chronic liver/renal diseases (n, %)	5 (4.4)	1 (0.6)	0.088 (F)	6.84 (0.79–59.34)	1.97 (1.34–2.90)	0.828	0.75 (0.05–10.46)
Chronic immune and/or hematologic diseases (n, %)	5 (4.4)	1 (0.6)	0.088 (F)	6.84 (0.79–59.34)	1.97 (1.34–2.90)	**0.033**	**15.37 (1.25–189.02)**
Others (n, %)	13 (11.4)	3 (2.0)	**0.003 (F)**	**6.31 (1.75–22.70)**	**2.00 (1.51–2.64)**	0.798	1.25 (0.23–6.68)
B allele (rs1800450) (frequency, %)	45 (19.7)	39 (13.0)	**0.036 (C)**	**1.99 (1.17–3.40)**	**1.44 (1.10–1.89)**	0.052	2.14 (0.99–4.62)
C allele (rs1800451) (frequency, %)	2 (0.8)	6 (2.0)	0.295 (F)	0.43 (0.09–2.16)	0.57 (0.17–1.91)		
D allele (rs5030737) (frequency, %)	17 (7.4)	31 (10.3)	0.254 (C)	0.76 (0.40–1.18)	0.85 (0.57–1.28)		
X allele (rs7096206) (frequency, %)	50 (21.9)	72 (24.0)	0.497 (C)	0.92 (0.56–1.51)	0.95 (0.72–1.27)		
MBL deficiency (n, %)	22 (19.3)	25 (16.7)	0.579 (C)	1.20 (0.64–2.25)	1.10 (0.78–1.55)		

Abbreviations: SARS, severe acute respiratory distress syndrome; the others are similar with those in Table 1.
^ The term "Yes" of Hospitalization includes patients with moderate disease, severe disease and critical disease necessitating intubation and mechanical ventilation.
# Statistical analysis: C, Chi-square; F. Fisher's exact test; M−W, Mann-Whitney *U* test; T, Student's *T*-test.

Table 3
Association of pneumonia risk with clinical parameters and *MBL2* genotyping in the patients of the study.

	Pneumonia		Univariate analysis			Multivariate analysis	
	Yes (n 106)	No (n 158)	*p* #	Odds Ratio (OR)	Relative Risk (RR)	*p*	Odds Ratio (OR)
Sex (male/female)	73/33	107/51	0.818 (C)	1.06 (0.63–1.81)	1.04 (0.75–1.43)		
Age (mean ± SD), y	57.5 ± 15.7	32.9 ± 12.5	**<0.001** (M-W)			**<0.001**	**1.09 (1.06–1.12)**
Comorbidity (n, %)	78 (73.6)	16 (10.1)	**<0.001** (C)	**24.52 (12.52–48.15)**	**5.01 (3.53–7.11)**		
Obesity (n, %)	29 (27.4)	2 (1.3)	**<0.001** (F)	**29.19 (6.79–125.52)**	**2.82 (2.30–3.46)**	**0.002**	**15.06 (2.78–81.52)**
Hypertension (n, %)	45 (42.5)	4 (2.5)	**<0.001** (F)	**28.22 (9.73–81.84)**	**3.22 (2.57–4.05)**	0.952	1.05 (0.25–4.36)
CHD (n, %)	11 (10.4)	0 (0)	**<0.001** (F)		**2.65 (2.26–3.11)**	0.999	–
CVD (%)	8 (7.5)	0 (0)	**<0.001** (F)		**2.60 (2.23–3.04)**	0.999	–
CRD (n, %)	18 (17.0)	2 (1.3)	**<0.001** (F)	**15.85 (3.59–69.93)**	**2.49 (1.99–3.10)**	0.572	1.68 (0.28–10.26)
Dyslipidemia (n, %)	17 (16.0)	2 (1.3)	**<0.001** (F)	**14.80 (3.34–65.56)**	**2.45 (1.96–3.08)**	0.138	4.74 (0.61–37.13)
Diabetes mellitus (n, %)	19 (17.9)	2 (1.3)	**<0.001** (F)	**16.93 (3.85–74.39)**	**2.52 (2.02–3.13)**	0.055	7.72 (0.96–62.21)
Thyroid disease (n, %)	9 (8.5)	3 (1.9)	**0.016** (F)	**4.76 (1.26–18.03)**	**1.94 (1.35–2.79)**	0.153	4.00 (0.60–26.70)
Malignancies (n, %)	4 (3.8)	2 (1.3)	0.224 (F)	3.04 (0.55–16.90)	1.68 (0.94–3.02)		
Chronic liver/renal diseases (n, %)	5 (4.7)	1 (0.6)	**0.041** (F)	**7.72 (0.89–67.07)**	**2.12 (1.44–3.13)**	0.859	0.80 (0.06–10.13)
Chronic immune and/or hematologic diseases (n, %)	5 (4.7)	1 (0.6)	**0.041** (F)	**7.72 (0.89–67.07)**	**2.12 (1.44–3.13)**	**0.013**	**24.48 (1.99–301.81)**
Others (n, %)	13 (12.2)	3 (1.9)	**0.001** (F)	**7.18 (1.99–25.85)**	**2.16 (1.62–2.87)**	0.562	1.63 (0.31–8.56)
B allele frequency (rs1800450) (%)	41 (19.3)	43 (13.6)	**0.037** (C)	**1.76 (1.03–3.01)**	**1.38 (1.03–1.85)**	0.229	1.64 (0.73–3.69)
C allele frequency (rs1800451) (%)	2 (0.9)	6 (1.8)	0.481 (F)	0.48 (0.10–2.45)	0.61 (0.18–2.05)		
D allele frequency (rs5030737) (%)	17 (8.0)	31 (9.8)	0.704 (C)	0.88 (0.46–1.70)	0.93 (0.62–1.39)		
X allele frequency (rs7096206) (%)	46 (21.7)	76 (24.1)	0.453 (C)	0.82 (0.50–1.37)	0.89 (0.65–1.21)		
MBL deficiency (n, %)	20 (18.9)	27 (17.1)	0.729 (C)	1.12 (0.59–2.12)	1.07 (0.73–1.25)		

Abbreviations: Similar with those in Table 1.
Statistical analysis: C, Chi-square; F. Fisher's exact test; M−W, Mann-Whitney *U* test; T, Student's *T*-test.

Table 4
Overview of the frequency of *MBL2* polymorphisms, along with the genotypes/haplotypes and MBL deficiency in the patients of the study. The grouping was performed according to the severity of COVID-19 (see Material and Methods).

	Total (No, %)	Group 1 (asymptomatic) (No, %)	Group 2 (mild) (No, %)	Group 3 (moderate) (No, %)	Group 4 (severe) (No, %)	Group 5 (critical) (No, %)	*p1*	*p2*
No patients	264	65	85	15	77	22		
B allele (rs1800450)	84 (15.9)	17 (13.1)	22 (12.9)	7 (23.3)	28 (18.2)	10 (22.7)	0.262	**0.036**
C allele (rs1800451)	8 (1.5)	0 (0)	6 (3.5)	0 (0)	1 (0.6)	1 (2.3)	0.289	0.295
D allele (rs5030737)	48 (9.1)	11 (8.5)	20 (11.8)	2 (6.7)	10 (6.5)	5 (11.4)	0.512	0.254
X allele (rs7096206)	122 (23.1)	37 (28.5)	35 (20.6)	5 (16.7)	37 (24.0)	8 (18.2)	0.395	0.576
	MBL2 genotypes							
YA/YA	67 (25.4)	17 (26.1)	22 (25.9)	5 (33.3)	19 (24.7)	4 (18.2)	0.882	0.790
(related to high mbl levels) YA/XA (related to high mbl levels)	59 (22.3)	17 (26.1)	19 (22.3)	3 (20.0)	16 (20.8)	4 (18.2)	0.922	0.460
XA/XA (related to medium mbl levels)	17 (6.5)	8 (12.3)	3 (3.5)	0 (0)	6 (7.8)	0 (0)	0.339	0.382
YA/O (related to medium mbl levels)	74 (28.0)	15 (23.1)	24 (28.2)	3 (20.0)	24 (31.2)	8 (36.4)	0.662	0.400
XA/O (related to low mbl levels)	28 (10.6)	3 (4.6)	10 (11.8)	2 (13.3)	9 (11.7)	4 (18.2)	0.389	0.240
O/O (related to low mbl levels)	19 (7.2)	5 (7.7)	7 (8.2)	2 (13.3)	3 (3.9)	2 (9.0)	0.666	0.563
	MBL2 genotype groups							
High MBL (YA/YA, YA/XA)	126 (47.7)	34 (52.3)	41 (48.2)	8 (53.3)	35 (45.5)	8 (36.4)	0.728	0.396
Medium MBL (XA/XA, YA/O)	91 (34.5)	23 (35.4)	27 (31.8)	3 (20.0)	30 (38.9)	8 (36.4)	0.660	0.656
Low MBL (XA/O, O/O) *	47 (17.8)	8 (12.3)	17 (20.0)	4 (26.7)	12 (15.6)	6 (27.3)	0.401	0.580

* Low MBL genotypes refer also as genotypic MBL deficiency. ^*p1* refers to the comparison of all (5) groups; *p2* refers to the comparison of groups (a) and (b) vs (c), (d) and (e), namely the comparison of hospitalized vs non-hospitalized patients.

2021; Emami et al., 2020; Jordan et al., 2020; Kyriazopoulou et al., 2021; Li et al., 2021; Rydyznski Moderbacher et al., 2020), we demonstrated that older age and underlying comorbidities - including severe obesity, chronic heart and respiratory disease and additional chronic disorders - significantly affected the phenotype of SARS-CoV-2 infection. Furthermore, we observed that patients carrying the B allele (rs1800450) of the *MBL2* gene also displayed worsened clinical presentation, as these patients more frequently required hospitalization because of pneumonia necessitating supplemental oxygen administration.

As presented, elderly patients and those with underlying comorbidities including obesity, diabetes and chronic inflammatory conditions can predict an elevated risk of severe illness as a result of COVID-19 (Jordan et al., 2020; Li et al., 2021; Longmore et al., 2021; Tiruneh et al., 2021). Therefore, the impact of immunosenescence in elderly patients, along with impairing macrophage and lymphocyte function in

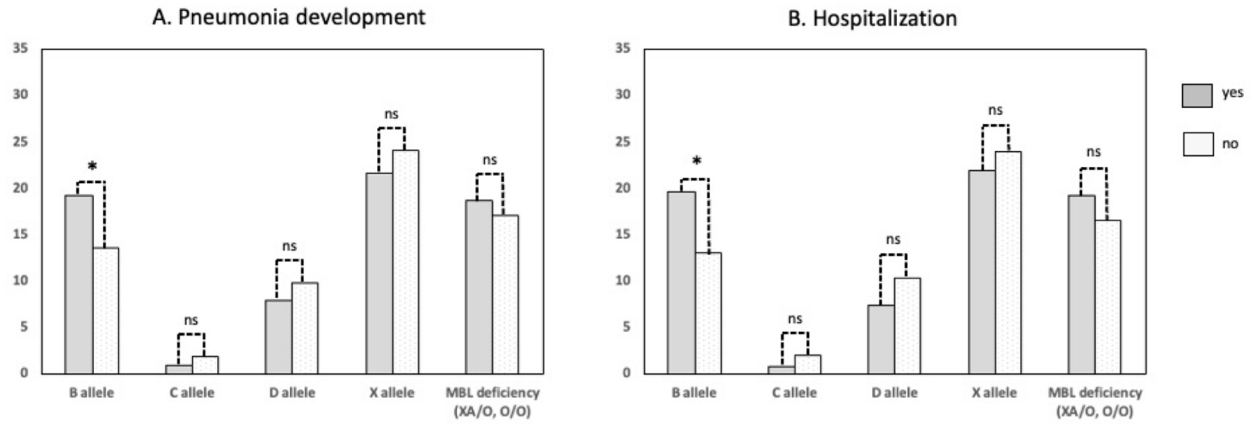

Fig. 1. MBL genotypes and the risk of pneumonia development and hospitalization. Bars demonstrate allele frequencies of B, C, D and X alleles and the frequency of MBL deficiency; (*) indicates a statistical significance < 0.05; ns, non-significant.

patients with chronic disorders, results in an inability of the immune system to respond satisfactorily to harmful foreign invaders (such as SARS-CoV-2), which may increase patients' susceptibility to disease complications (Cheng et al., 2021).

In our study, we demonstrated that the presence of the B allele (rs1800450) of the *MBL2* gene was significantly associated with a worse clinical phenotype in COVID-19 patients, and particularly with pneumonia development. The contribution of MBL deficiency in human infectious and non-infectious diseases has been established in several studies (reviewed by Gupta & Gupta, 2020) (Gupta and Gupta, 2021). Thus, Liu et al. reported that low levels of MBL are associated with an increased risk of community-acquired pneumonia and a greater risk of death (Liu et al., 2014). Moreover, it has been established that collectins, including MBL, play a fundamental role in recognizing microbial and viral substrates in lungs, including the spike-protein (S-protein) of coronaviruses (Leth-Larsen et al., 2007), regulating pulmonary and systemic inflammation. In this context, Tu et al. reported that the presence of the B allele of the *MBL2* gene was significantly associated with an increased risk of SARS (Tu et al., 2015), although in another study from Hong-Kong with 180 SARS patients, such an association was not confirmed (Yuan et al., 2005). Considering COVID-19, Medetalibeyoglu et al. recently reported that the presence of the B allele was significantly associated with a higher risk for severe disease and the need of ICU care (Medetalibeyoglu et al., 2021).

Of note, we found no association of genetic MBL deficiency and disease phenotype. This seems a paradox, and we cannot exclude the possibility that our results may be affected by the small size and a possible diverse composition of our cohort. However, it should be emphasized that serum MBL levels are also affected by several factors including age, oxidative stress, smoking, second-hand smoke emission, exposure to fuel emissions, vitamin D levels and diet (with acidic diets reducing and alkaline diets increasing MBL levels) (Chen et al., 2014; Gupta and Gupta, 2021; Sallenbach et al., 2011; Tran et al., 2014). These factors could be the cause of no association between MBL deficiency and clinical presentation of the disease, as in our study most of the above-mentioned data was not available.

Conversely, the B allele of the *MBL2* gene is the more common polymorphism affecting MBL serum levels in humans, leading several studies to focus solely in its study (Medetalibeyoglu et al., 2021; Tu et al., 2015). For example, while genetic MBL deficiency was not considered a major risk factor for chronic obstructive pulmonary disease (COPD) (Dahl and Nordestgaard, 2009), the presence of the deficiency-causing B allele has been associated with 4.9 times increased odds of hospital admission due to infection-induced COPD exacerbations bearing a worse outcome (Lin et al., 2011; Yang et al., 2003). Nevertheless, in our study we investigated all polymorphisms affecting the genetic MBL deficiency, albeit no correlations of other *MBL2* polymorphisms with COVID-19 phenotype were found.

Our findings may have important implications such as the prioritization of individuals with B variants during vaccination strategies (Gupta and Gupta, 2021), or the encouragement of the affected individuals to receive mannose-specific plant lectins in an attempt to prevent a poor outcome after SARS-CoV-2 infection, as also suggested for other coronaviruses infections (Medetalibeyoglu et al., 2021).

In conclusion, our study further confirms the contribution of age and underlying comorbidity in the clinical phenotype of SARS-CoV-2 infection. Furthermore, we demonstrated a possible association between the MBL deficiency-causing B allele with pneumonia development in COVID-19 patients, suggesting its usage as a molecular predictor of severe disease in patients infected by SARS-CoV-2.

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CRediT authorship contribution statement

Matthaios Speletas: Conceptualization, Investigation, Data curation, Writing - original draft, Supervision. **Katerina Dadouli:** Data curation, Validation. **Argyro Syrakouli:** Investigation, Validation. **Nikolaos Gatselis:** Data curation. **Georgios Germanidis:** Data curation, Validation. **Varvara A. Mouchtouri:** Data curation, Validation. **Ioannis Koulas:** Investigation. **Anna Samakidou:** Data curation. **Anastasia Nikolaidou:** Data curation. **Aggelos Stefos:** Data curation. **Iordanis Mimtsoudis:** Data curation. **Sophia Hatzianastasiou:** Data curation. **Michalis Koureas:** Data curation. **Lemonia Anagnostopoulos:** Data curation. **Maria Tseroni:** Data curation. **Gerasimina Tsinti:** Investigation. **Symeon Metallidis:** Data curation, Validation. **George Dalekos:** Data curation, Validation. **Christos Hadjichristodoulou:** Conceptualization, Data curation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imbio.2021.152136.

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