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Original article

Reduced COVID-19 mortality linked with early antibodies against SARS-CoV-2, irrespective of age

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ABSTRACT

Background: COVID-19 pandemic has generated a million deaths worldwide. The efficiency of the immune system can modulate individual vulnerability with variable outcomes. However, the relationships between disease severity and the titer of antibodies produced against SARS-CoV-2 in non-vaccinated, recently infected subjects need to be fully elucidated.

Methods: A total of 99 patients admitted to a COVID-unit underwent clinical assessment and measurement of serum levels of anti-spike protein (S1) IgM, and anti-nucleocapsid protein IgG. Patients were stratified according to the clinical outcome (i.e., discharged at home or in-hospital death).

Results: Following hospitalization, 18 died during the hospital stay. They were older, had lymphopenia, a higher co-morbidity rate, and longer hospital stay than 81 patients who were discharged after healing. Patients in this latter group had, at hospital admittance, 7.9-fold higher serum concentration of IgM, and 2.4-fold higher IgG levels. Multivariate Cox regression models indicated age and anti-nucleocapsid protein IgG concentration at admission as independently associated with the risk of in-hospital death.

Conclusions: An efficient immunological response during the early phase of COVID-19 protects from mortality, irrespective of age. Advanced age is a critical risk factor for poor outcome in infected subjects. Further studies must explore potential therapeutic strategies able to restore a valid functional humoral immunity in elderly patients with poor antibody response during the early stage of COVID-19 infection.

1. Introduction

COVID-19 pandemic has generated, to date, over 4.6 million deaths worldwide [1]. The high infection rate, however, is counterbalanced by variable clinical outcomes at the individual level. SARS-CoV-2 infection can lead to asymptomatic disease in a large number of subjects (about 40–45% of infections [2]) or to severe clinical presentations with systemic involvement, possibly causing death in the most vulnerable subjects.

Previous studies identified a series of predictors of disease severity and mortality including elderly, the presence of multiple comorbidities

[3,4], hypoxia, radiologic evidence of extensive lung involvement, biomarkers of end-organ dysfunction, and abnormal bio-humoral tests as the presence of coagulation defects, elevated aminotransferases, indices of renal dysfunction [4].

However, comprehensive knowledge of factors causing the worst clinical outcome in infected patients is still under evaluation.

In this respect, a major role seems to be played by an altered immune function. In particular, COVID-19 patients frequently show lymphopenia that, when present, has been linked with increased disease severity [5,6]. On the other hand, how the titer of antibodies against SARS-CoV-2 can modulate the severity of disease in infected, non-vaccinated subjects

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is still unclear. Serum IgM and IgG can be detected 5–14 days after the onset of symptoms [7], and the concentration of these antibodies has been correlated with the viral load, in particular in older subjects [8]. However, the relationship between the antibody response to SARS-CoV-2 and the risk of death in COVID-19 patients is controversial, since negative clinical outcomes have been linked with increased [9], or reduced [10–12] antibody titer following a SARS-CoV-2 infection.

The present study is aimed at evaluating, as the primary outcome, the role of anti-spike IgM and anti-nucleocapsid IgG against SARS-CoV-2 on in-hospital mortality, in a cohort of COVID-19 patients.

2. Subjects and methods

2.1. Subjects

Enrolled in the study were 99 SARS-CoV-2 infected patients (mean age 68.2 ± 1.6 years, age range 30–93 years, 57 males) admitted to a dedicated internal medicine COVID-unit in the large regional hospital Policlinico of Bari, Apulia, from January 12 to April 25, 2021. Patients entered the unit few hours after admission in the emergency unit, following a positive real-time RT-PCR for SARS-CoV-2 obtained from nasopharyngeal swab. The overall hospital stay was calculated from the day of hospital admittance to that of the final outcome, i.e., discharge at home or death. All patients underwent blood sampling on the day of hospital admission, and a full clinical evaluation including the analysis of comorbidities. None of the patients had previously received COVID-19 vaccination.

Patients transferred to intensive care units were excluded from enrolment, since information about the final clinical outcome in different wards was not available at the time of analysis. Other exclusion criteria were previous therapy with immunomodulating drugs or known blood diseases. The study protocol was approved by the local Ethics Committee (study No. 6362, authorization No. 0,034,675).

2.2. Antibodies assessment

The total antibody (Ab), IgM antibody and IgG antibody against SARS-CoV-2 in plasma samples were tested using Abbott qualitative chemiluminescent immunoassays (CMIA, Abbott Laboratories, USA) according to the manufacturer's instructions. The Abbott anti-SARS-CoV-2 IgG assay detects antibodies to the nucleocapsid protein of SARS-CoV-2, while the Abbott anti-SARS-CoV-2 IgM assay detects antibodies to the receptor-binding domain (RBD) of the spike protein (S1).

Briefly, sample, SARS-CoV-2 antigen coated paramagnetic microparticles, and assay diluent are combined and incubated, the IgG and IgM antibodies to SARS-CoV-2 present in the sample bind to the SARS-CoV-2 antigen coated microparticles. Anti-human IgG/IgM acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as a relative light unit (RLU) on the Abbott Architect i2000sr Platform (Abbott Laboratories, Illinois, USA). There is a direct relationship between the amount of IgG/IgM antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optics.

2.3. Statistical analysis

According to the Shapiro-Wilk test, the distribution of continuous variables was not Gaussian. Data were therefore presented as median and interquartile range. Categorical variables were expressed as counts and percentages. The Chi-squared test (proportions) or the Mann-Whitney U test were employed to evaluate differences.

Significant variables were used as independent variables in univariate and multivariate Cox regression models, to identify risk factors for survival. Time was considered as days to the event, i.e., discharge or death. The primary outcome was the dependent variable and was in-

hospital death during the hospital stay. The hazard ratio (HR) along with the 95% CI were reported. Models were fitted using R software version 4.1.1 (R Project for Statistical Computing, available from <https://www.r-project.org/>).

3. Results

Following hospitalization, 81 patients were discharged after healing, and 18 patients died during hospital stay. The general features of the two groups are depicted in Table 1. Patients who died were older, had a higher co-morbidity rate, and a longer hospital stay than discharged subjects. When specific comorbidities were examined, only cerebrovascular and chronic renal diseases were more frequent in patients who underwent in-hospital death, than in discharged subjects. The rates of all other specific comorbidities recorded at admittance were similar in the two subgroups of patients, as also gender distribution, time interval occurring from symptom appearance to hospital admission, blood pressure, prevalence of active smokers, and symptoms (Table 1).

Patients were admitted, on average, 6.1 (0.25–10) days after the onset of symptoms. In the whole group, at the day of admission, IgM and IgG antibodies were present in 65.7% and in 31.3% of patients, respectively.

The features of white blood cells count, CRP and IgM/IgG serum concentrations in the two groups of patients are depicted in Table 2. Patients who died had absolute lymphopenia and a trend for increased CRP serum concentration, as compared with discharged subjects. In this last subgroup, patients were more frequently IgM (but not IgG) positive, than those who underwent in-hospital death. Furthermore, patients who were discharged showed, the day of hospital admission, 7.9-fold higher serum concentration of IgM, and 2.4-fold higher IgG levels, as compared with patients who underwent in-hospital death.

Table 1

Clinical characteristics on hospitalization of 99 patients with symptomatic SARS-CoV-2 infection.

	Hospital discharge	Death	<i>p</i> -value
Subjects (n.)	81	18	
Age (years)	66.0 (55.5–77.5)	83.5 (7.5–91.0)	0.000001
Comorbidities n. (%)	59 (74.7%)	18 (100%)	0.03
COPD	9 (11.1%)	3 (16.7%)	ns
Type 2 diabetes	22 (27.2%)	8 (44.4%)	ns
Arterial hypertension	45 (55.6%)	8 (44.4%)	ns
Cardiovascular diseases	26 (32.1%)	10 (55.6%)	ns
Cerebrovascular diseases	11 (13.6%)	7 (38.9%)	0.01
Chronic liver diseases	5 (6.2%)	0	ns
Cancer	6 (7.4%)	3 (16.7%)	ns
Chronic renal diseases	8 (9.9%)	7 (38.9%)	0.001
Hospital stay (days)	8 (4.5–14.5)	13.5 (8.0–18.5)	0.01
Males:Females	47:34	10:8	ns
Symptoms appearance to hospital admission (days)	6 (0–10)	4 (0.5–7)	ns
Active smokers n. (%)	2 (2.5%)	2 (11.1%)	ns
Arterial pressure (mmHg)			
Systolic	130 (124.8–145.8)	140 (116.5–152.5)	ns
Diastolic	80 (70–88.5)	80 (65–86.3)	ns
Symptoms n. (%)			
Headache	11 (13.6%)	1 (5.6%)	ns
Cough	25 (30.9%)	4 (22.2%)	ns
Fatigue	21 (25.9%)	5 (27.8%)	ns
Dyspnea	35 (43.2%)	9 (50%)	ns
Nausea	6 (7.4%)	1 (5.6%)	ns
Vomiting	3 (3.7%)	1 (5.6%)	ns
Arthralgia	3 (3.7%)	0	ns
Myalgia	2 (2.5%)	1 (5.6%)	ns
Ageusia	2 (2.5%)	0	ns
Anosmia	1 (1.2%)	0	ns

Values are median and interquartile range, or frequencies (%); ns = not significant.

Table 2

White blood cells count, CRP and IgM/IgG serum concentrations in 99 patients admitted for symptomatic SARS-CoV-2 infection, according to outcome.

	Hospital discharge	Death	p-value
Subjects (n.)	81	18	
Leukocytes (x10 ³ cells/μL)	7.7 (5.1–11.5)	6.0 (3.37.9)	ns
Neutrophils (%)	76.8 (68.3–84.9)	79.6 (75.2–88.0)	ns
Neutrophils (x10 ³ cells/μL)	6.1 (3.8–9.1)	4.8 (2.5–6.4)	ns
Lymphocytes (%)	14.6 (9.7–23.4)	13.2 (5.9–15.7)	ns
Lymphocytes (x10 ³ cells/μL)	1.0 (0.7–1.6)	0.7 (0.3–1.1)	0.01
Neutrophils/lymphocytes ratio	5.1 (2.9–8.3)	6.1 (5.0–15.0)	ns
CRP (mg/dL)	41.8 (16–103)	74.8 (29.7–132.8)	ns
IgM positive patients n.	59 (72.8%)	6 (33.3%)	0.001
IgM (AU/mL)	4.7 (0.9–13.8)	0.1 (0.07–1.6)	0.0008
IgG positive patients n.	25 (30.9%)	6 (33.3%)	ns
IgG (AU/mL)	2.5 (0.4–5.5)	0.1 (0.04–1.9)	0.004

Values are median and interquartile range, or frequencies (%); ns = not significant.

To examine the confounding effect of age, we separately examined subjects aged 75 or more years. In this subset (Table 3), patients who were discharged at home and those who died showed similar age, leukocytes, neutrophils and lymphocytes levels, and CRP serum concentration. A nonsignificant trend towards an increased rate of IgM- and IgG-positive patients was present among patients discharged at home. In this subset of older subjects IgM and IgG serum levels were increased in patients who were discharged, compared to patients undergoing in-hospital death.

In the whole population, we performed univariate analyses considering the outcome of death during the hospital stay as the dependent variable. Variables showing significant differences between groups, that is age, rate of cerebrovascular and chronic renal diseases, lymphocyte count, IgM and IgG serum levels were the independent covariates (Table 4). Variables emerging from univariate analysis with p values of < 0.05 such as age, serum concentration of IgG were included in the multivariate Cox regression model. In this final model, age (i.e., increasing risk with age) and serum IgG levels measured at admission (i.e., protective effect proportional to concentration) resulted independently associated with the risk of in-hospital death (Fig. 1).

Kaplan-Meier curves were drawn for IgG positive and negative patients and compared using log-rank tests. The analysis showed a significantly higher survival probability in patients who were IgG-

Table 3

White blood cells count, CRP and IgM/IgG serum concentrations in a subgroup of patients aged 75 or more year and admitted for symptomatic SARS-CoV-2 infection, selected according to outcome.

	Hospital discharge	Death	p-value
Subjects (n.)	22	16	
Age (years)	84.5 (78.8–87.5)	86.5 (79.2–91)	ns
Leukocytes (x10 ³ cells/μL)	8.7 (5.7–11.9)	6.3 (4.2–8)	ns
Neutrophils (%)	79 (65.5–89.8)	80.2 (76.0–90.4)	ns
Neutrophils (x10 ³ cells/μL)	6.6 (3.9–10.5)	5.3 (3.3–6.5)	ns
Lymphocytes (%)	13.3 (5.9–25.9)	12.9 (5.6–14.9)	ns
Lymphocytes (x10 ³ cells/μL)	0.9 (0.6–1.5)	0.7 (0.3–1.1)	ns
Neutrophils/lymphocytes ratio	6.0 (2.5–15.1)	6.2 (5.3–16.4)	ns
CRP (mg/dL)	53.4 (14.5–108.8)	74.8 (23.0–109.5)	ns
IgM positive patients n.	15 (68.2%)	6 (37.5%)	ns
IgM (AU/mL)	7.2 (0.4–15.2)	0.4 (0.07–1.8)	0.02
IgG positive patients n.	12 (54.5%)	6 (37.5%)	ns
IgG (AU/mL)	5.3 (0.9–6.8)	0.09 (0.03–2.0)	0.01

Values are median and interquartile range, or frequencies (%); ns = not significant.

Table 4

Unadjusted Cox proportional hazards regression models for death in 99 patients hospitalized for symptomatic SARS-CoV-2 infection.

Variable	Univariate HR (95% CI)	p-value
Age (years)	1.054 (1.008–1.102)	0.02
Cerebrovascular diseases	2.61 (0.99–6.86)	0.051
Chronic renal diseases	2.49 (0.95–6.5)	0.06
Lymphocytes (x10 ³ cells/μL)	0.335 (0.096–1.168)	0.08
IgM (AU/mL)	0.8676 (0.7418–1.015)	0.07
IgG (AU/mL)	0.786 (0.6191–0.99)	0.04

positive the day of hospital admission (Fig. 2).

4. Discussion

Results from the present study confirm, in subjects hospitalized for COVID-19, the negative prognostic value of elderly, and identified the levels of nucleocapsid protein-oriented IgG against SARS-CoV-2 recorded at admission as protective against mortality, irrespective of age.

In symptomatic patients, clinical manifestations usually develop within 14 days after exposure to SARS-CoV-2. However, it is still unclear whether the extent of antibody responses at the clinical presentation can modulate disease severity.

Previous studies reported higher concentrations of serum antibodies in immunocompetent COVID-19 patients with severe clinical presentation of disease [13,14], and the presence of high IgM titer at day 25 post-illness onset has been linked with higher in-hospital mortality [9].

Thus, according to the cited papers, having a high level of serum antibodies should not protect against a severe presentation of disease, and should not reduce the mortality risk.

Evidence about this topic, however, is controversial.

A recent study found no difference in the antibody titer at baseline and in the peak antibody level between those who survived and those who underwent in-hospital death, although these results were limited by a low number of enrolled and deceased patients [15].

Further evidence, however, reported attenuated IgG response in non-survivors [10], and low IgM titers in patients with severe COVID-19 presentation [11,12]. Results from these studies were similar to findings from our series of patients, in whom higher concentrations of serum IgM and IgG were detected in survivors, as compared with those with the worst outcome.

Antibody response to SARS-CoV-2 is not homogeneous, since infected subjects can differently develop antibodies against the spike (S) and nucleocapsid (N) proteins. These proteins are usually used as antigens in clinical serology assays, but available data indicate that differential antibody response towards these two different viral structures can lead to divergent clinical outcomes, with the worst outcome linked with a low value of spike-targeting responses [13,16,17].

In particular, a scarce response, in terms of neutralizing antibodies, during the early stage of disease might be responsible for a poor clinical outcome in infected patients [17]. The S protein contains the receptor-binding domain, which has a major role in binding the human ACE2 receptors [18,19] and, in turn, in determining the entry of the virus into host cells [20].

Results from the present study confirm, in survivors, the presence of higher levels of IgM against spike glycoprotein, and of nucleocapsid protein-oriented IgG, as compared with those who underwent in-hospital death.

A prevalent spike-oriented IgM response has been previously described in infected patients who recovered, as compared to deceased subjects, who produced an elevated antibody response to the nucleocapsid [16]. In another series of COVID-19 patients, infected subjects with the mildest course of disease showed higher ratios of IgG antibodies targeting S1 or receptor binding domains of the spike, compared to nucleocapsid antigen [13]. Additionally, the lack of anti-spike neutralizing antibodies within the first weeks from the onset of symptoms has

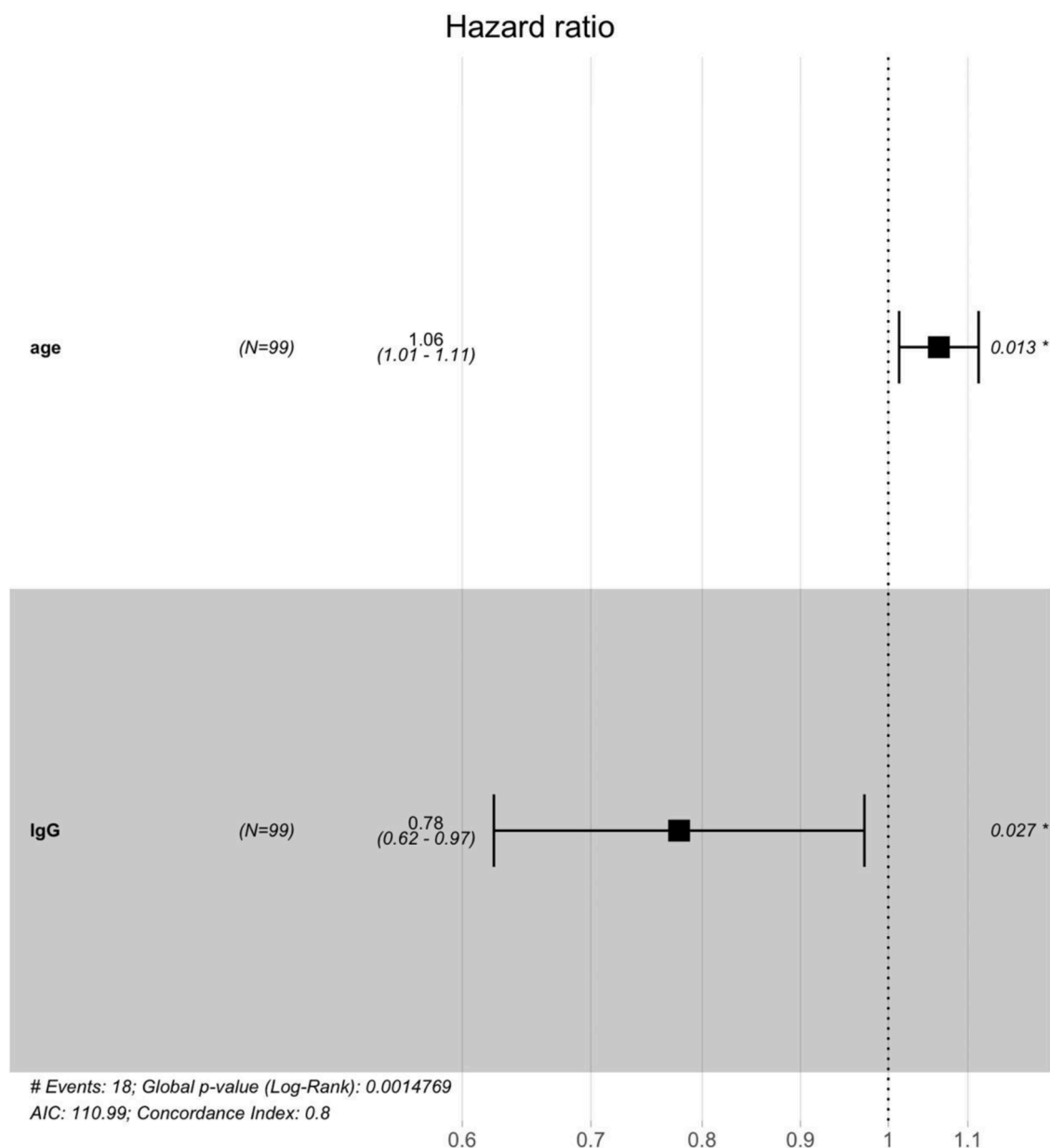


Fig. 1. Results from multivariate Cox regression model considering death during the hospital stay as the dependent variable, age and serum concentrations of IgG at admission as independent covariates. Entered the analysis 99 patients hospitalized for COVID-19. Data are hazard ratios, 95% confidence intervals and p-values.

been correlated with increased mortality risk [17].

Taken together, these data should point to the early appearance of specific functional antibodies against S as possibly involved in a more favorable outcome following SARS-CoV-2 infection in symptomatic patients. In fact, in the present series, the rate of anti-spike IgM positive subjects was higher in the subset of subjects with the best clinical outcome, who also had a shorter hospital stay.

Results from multivariate analysis also indicate a favorable effect of nucleocapsid protein-oriented IgG levels on survival. The nucleocapsid protein is essential for SARS-CoV-2 replication and RNA packaging into new virions [21]. This protein has been considered as a potentially useful target for antiviral drugs [22], and data for the present study suggest that the early appearance of IgG against N-protein should be protective against mortality, irrespective of age.

All subjects were admitted, on average, during the first week after the appearance of symptoms. Thus, the protective effect linked with the early presence of neutralizing antibodies is likely due to the role of these antibodies in limiting the viral spread, the entry in cells, and, therefore,

the progression and severity of the disease.

A bias might derive, in the present study, from different timing of sampling for antibodies assessment in enrolled subjects since infection, with possible increased variability in individual humoral profiles. In our series, however, all serum samples were taken on the same day of hospital admission, and the time interval between symptom appearance and hospital admission was comparable between patients discharged at home and those who underwent in-hospital death. Furthermore, according to previous evidence, the duration to induce high levels of IgG and IgM following a SARS-CoV-2 infection is, respectively, 14 days and 10 days from the onset of the disease [23]. In our series, IgM and IgG were measured before these time intervals, i.e., following a median duration of disease of 6.1 days, IQR 0.25–10 days. Thus, IgM and IgG values recorded in the present study can be considered as early antibody serum concentration.

Another limitation of the present study is the presence of a demographic unbalance, due to the prevalence of aged subjects among those who underwent in-hospital death. This kind of age distribution,

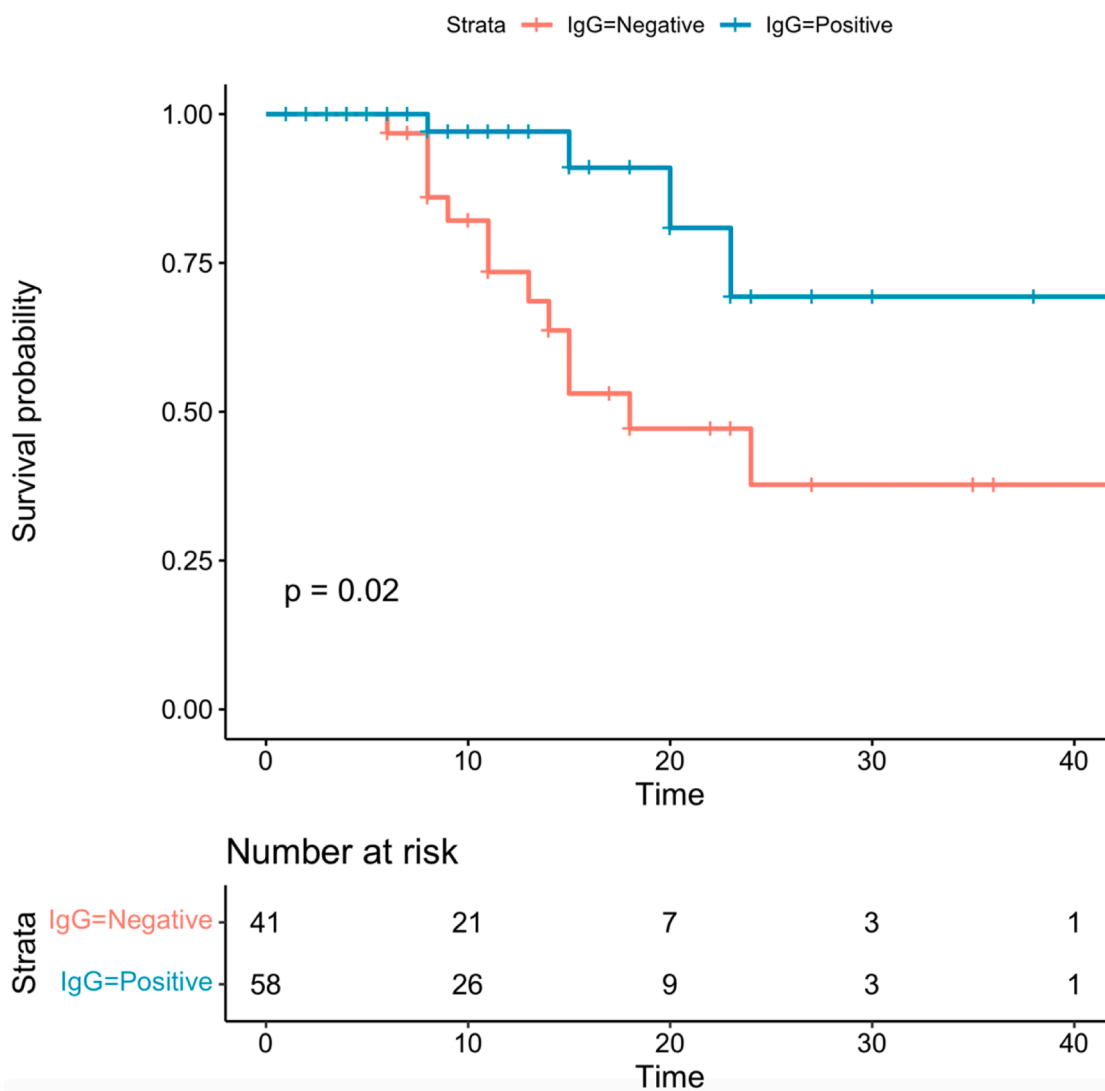


Fig. 2. Kaplan-Meier curves exploring survival probability during the hospital stay in 99 patients admitted for COVID-19, who were IgG positive or negative at admission. Curves have been compared using log-rank tests.

however, is comparable to that reported in previous studies [24–27], and confirms the well-known role of advanced age in increasing the mortality risk in COVID-19 patients [28].

The scarce number of deaths observed in the present series might affect the statistical power in Cox regression models. In fact, the power of survival models is a function of the number of events experienced, rather than the total sample size, and power declines with fewer events [29]. However, the risk of unbiased results can be reduced by a low number of covariates [30]. In the present series, the multivariate analysis has been limited to two covariates. Although generalizability is low, age and IgG serum concentrations were significantly different between discharged and death patients, and regression models confirmed their role as significant predictors of death. These findings, however, should be confirmed in a larger cohort, possibly considering a wider panel of covariates.

Finally, the possibility exists that the survival of enrolled patients has been affected by medications used to treat COVID-19 during the hospital stay. The present study was not designed to assess the efficacy of specific medications on the explored outcome such as discharge or death. However, the therapeutic approach was standardized according to local and national guidelines, and all patients received comparable clinical management. The reported results seem to be therefore linked to factors

independent by the individual medical management. In this respect, although elderly can certainly affect the immune function in infected patients, data indicate the presence of a different vulnerability, among aged subjects, linked with the extent of the antibody response against the virus. Of note, our results revealed a high prevalence (68.2%) of anti-spike IgM positivity among enrolled patients aged 75 or more years discharged at home. These subjects presented higher anti-spike IgM and anti-nucleocapsid protein IgG serum concentrations than deceased patients with comparable age. Thus, a valid immune response to the S and N proteins is also possible in elderly patients, leading to a more favourable course of disease.

In conclusion, results from the present study confirm the role of an efficient immunological response in the early phase of COVID-19 as a protective factor against mortality, irrespective of age. Although advanced age is a critical risk factor for a poor outcome in infected subjects, further studies are needed to explore possible therapeutic interventions able to restore a valid functional humoral immunity in elderly patients with scarce antibody response during the early stage of COVID-19 infection.

Appendix A. Appendix Title

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