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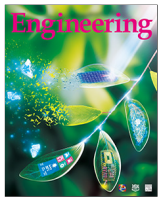

Article

Temporal Profiles of Antibody Responses, Cytokines, and Survival of COVID-19 Patients: A Retrospective Cohort in Wuhan, China

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ABSTRACT

The longitudinal immunologic status of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients and its association with the clinical outcome are barely known. Thus, we sought to analyze the temporal profiles of specific antibodies, as well as the associations between the antibodies, proinflammatory cytokines, and survival of patients with coronavirus disease 2019 (COVID-19). A total of 1830 laboratory-confirmed COVID-19 cases were recruited. The temporal profiles of the virus, antibodies, and cytokines of the patients until 12 weeks since illness onset were fitted by the locally weighted scatter plot smoothing method. The mediation effect of cytokines on the associations between antibody responses and survival were explored by mediation analysis. Of the 1830 patients, 1435 were detectable for SARS-CoV-2, while 395 were positive in specific antibodies only. Of the 1435 patients, 2.4% presented seroconversion for neither immunoglobulin G (IgG) nor immunoglobulin M (IgM) during hospitalization. The seropositive rates of IgG and IgM were 29.6% and 48.1%, respectively, in the first week, and plateaued within five weeks. For the patients discharged from the hospital, the IgM decreased slowly, while high levels of IgG were maintained at around 188 AU·mL^{-1} for the 12 weeks since illness onset. In contrast, in the patients who subsequently died, IgM declined rapidly and IgG dropped to 87 AU·mL^{-1} at the twelfth week. Elevated interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-1β (IL-1β), interleukin-2R (IL-2R), and tumor necrosis factor-α (TNF-α) levels were observed in the deceased patients in comparison with the discharged patients, and 12.5% of the association between IgG level and mortality risk was mediated by these cytokines. Our study deciphers the temporal profiles of SARS-CoV-2-specific antibodies within the 12 weeks since illness onset and indicates the protective effect of antibody response on survival, which may help to guide prognosis estimation.

1. Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a once-in-a-century pandemic. As of 4 September 2020, there were over 26 million confirmed cases, with over 860 000 deaths worldwide [1]. The clinical features of patients with COVID-19 have now been widely acknowledged [2–5]. In addition, an increasing number of studies have indicated that virus load measurements are indicative of active replication; thus, virus load measurements are routinely used for the monitoring of progression, treatment response, and relapse [6–9]. The viral antigen-specific antibody response among COVID-19 patients has started to emerge, revealing the early and synchronous seroconversion of immunoglobulin G (IgG) and immunoglobulin M (IgM) and the short-term dynamics of these antibodies within one month of symptoms onset [10,11]. However, the longitudinal profiles of the viral load and immune responses to SARS-CoV-2 after one month are barely known. Furthermore, the associations between

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antibody responses and the clinical outcomes of COVID-19 patients are still under investigation.

Approximately 20% of COVID-19 cases develop severe disease manifestations [12]. Cytokine release syndrome (CRS) is common in severe COVID-19 cases. Elevated serum interleukin-6 (IL-6) and C-reactive protein (CRP, a protein driven by IL-6) correlate with acute respiratory distress syndrome (ARDS) and adverse clinical outcomes [5,13]. Cytokine levels have been associated with a viral load of SARS-CoV-2 [14]. More fundamentally, it remains to be determined whether a robust antibody response corresponds with the suppression of cytokine storm in COVID-19 patients, and therefore contributes to recovery from the disease.

Considering the urgent need for reliable data on the longitudinal profiles of serum antibodies and their impact on recovery in order to guide clinical treatment and prognosis estimation, we systematically assessed the virus dynamics and the profiles of virus-specific antibodies in a large cohort of patients with COVID-19. Associations between the antibodies, proinflammatory cytokines, and survival of COVID-19 patients were further investigated.

2. Methods

2.1. Patients

Patients presenting detectable SARS-CoV-2 or seroconversion of the specific IgM and/or IgG were enrolled at Tongji Hospital, Wuhan, China, from 27 January to 24 March 2020. Health professionals asked patients for the onset date of symptoms. The demographics and admission date of all patients were extracted from clinical records. Cases were categorized into mild, moderate, severe, and critical types according to) *Diagnosis and treatment protocol for novel coronavirus pneumonia* (*trial version 7*) released by the National Health Commission of China [15]. Mild and moderate patients with COVID-19 were classified as non-severe cases in the analyses, while severe and critical cases were classified as severe cases. The standard of care for mild/moderate cases of COVID-19 included antiviral, symptomatic, and supportive therapies; additional oxygen therapy, hormone therapy, immunotherapy, mechanical ventilation, and/or extracorporeal membrane oxygenation were added to treat severe/critical cases whenever necessary. The participants were followed until 11 May 2020, when the clinical outcomes (i.e., discharge or death) had all been ascertained during hospitalization. A further confirmation of the survival status of patients after discharge was conducted by inquiring from the Notifiable Disease Report System. This study was approved by the Institutional Review Board of Tongji Hospital and conformed to the principles outlined in the Declaration of Helsinki. Written informed consent was waived by the Ethics Commission of the designated hospital for the investigation of emerging infectious diseases using electronic medical records.

2.2. Detection of SARS-CoV-2 nucleic acid and its specific antibodies and cytokines

Oropharyngeal, nasopharyngeal, and venous blood specimens were collected consecutively during the patients' hospitalization whenever necessary. Laboratory tests were done using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) for the nucleotide protein (*N*) and open reading frame 1ab (*ORF1ab*) genes with commercial kits for SARS-CoV-2 detection (DaAn Gene Co., Ltd., Guangzhou, China) [16]. In this study, the recombinant SARS-CoV-2 antigen for IgM and IgG immunoassays was constructed by expressing and purifying a fusion protein including both the spike and the nucleocapsid proteins based on the published SARS-CoV-2 nucleic acid sequence on Genbank (MN908947.3). Serum was isolated from blood for the assays of IgM and IgG specific to SARS-CoV-2 using chemiluminescent immunoassay kits (Yhlo Biotech Co., Ltd., Shenzhen, China). A concentration of greater than or equal to 10 $AU{\cdot}mL^{-1}$ was classified as positive for each antibody. The criteria for the positive detection of nucleic acids and the specificity of antibody tests are provided in Appendix A supplementary information. Serum inflammatory cytokines were detected, including IL-6, interleukin-1β (IL-1β), interleukin-2R (IL-2R), interleukin-8 (IL-8), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α).

2.3. Statistical methods

The main analyses were restricted to patients with detectable SARS-CoV-2; those with undetectable SARS-CoV-2 and seroconversion of the specific IgM and/or IgG were added as a sensitivity analyses. The median (interquartile range (IQR)) was used to describe the distribution of continuous variables, and the frequency (percentage) was used to present the distribution of categorical variables. The weekly positive detection rates of the collected serological samples for a specific week during patient hospitalization and the concentrations of antibodies to SARS-CoV-2 were calculated and compared using the χ^2 test, the Fisher exact test, the Kruskal–Wallis test, or the Mann–Whitney U test, when appropriate. The daily viral load (cycle threshold (Ct) values), positive detection rates of antibodies, and logarithmically converted concentrations of antibodies and cytokines were plotted. Next, smooth lines were fitted using the locally weighted scatter plot smoothing (LOWESS) method—an outlier-resistant method based on local polynomial fits to avoid distortion of the results by a small fraction of outliers. LOWESS does not require the specification of a function to fit a model to all the data in the sample; rather, it requires large, densely sampled datasets to produce good models [17]. Given the higher detection rate of the *N* gene than that of the *ORF1ab* gene, we used the Ct values of the *N* gene to represent the Ct values of SARS-CoV-2. To capture the dynamics of the viral load, a Ct value of 40 was assigned to samples with undetectable SARS-CoV-2 when drawing the trend curves. A cytokine score was created by calculating the number of proinflammatory cytokines in the upper quartile [18]. Patients with 3–6 cytokine scores were classified as having a high inflammation status, while those with 0–2 scores were classified as having a low inflammation status. Mediation analysis [19] was conducted to explore whether the cytokine score mediated the association between virus-specific antibodies and the survival of COVID-19 patients. All statistical analyses were two-sided, and a *P* value of less than 0.05 was considered to be statistically significant. Stata MP 16.0 software (StataCorp, College Station, USA) was used for statistical analyses.

3. Results

3.1. Seroconversion of COVID19 patients

In this study, 1830 patients were recruited; among these, 1435 (78.4%) exhibited detectable SARS-CoV-2, while 395 (21.6%) were negative for nucleic acids but positive for the specific antibodies. The median age of the 1435 patients with detectable SARS-CoV-2 was 61 years (IQR: 50–69 years), and 683 (47.6%) were males. In this group, there were 334 (23.3%) severe and 1101 (76.7%) non-severe cases. During their hospitalization, 49 (3.4%) of the patients died. The median duration of the hospitalization for these patients (referred to herein as "the deceased patients") was 23 days (IQR: 17–40 days). Among the 1435 patients with detectable SARS-CoV-2, 35 (2.4%) did not present seroconversion for IgM or IgG during their hospitalization. No differential seroconversion rates of IgM

Table 1
Antibody response of patients with detectable SARS-CoV-2.

Variables	Antibody response			
	IgM(+) IgG(+)	IgG(+) IgM(−)	IgG(−) IgM(−)	*P* value
Cases (n = 1435)[a]	1175 (81.9%)	225 (15.7%)	35 (2.4%)	–
Samples (n = 3456)[b]	2661 (77.0%)	715 (20.7%)	80 (2.3%)	–
Duration from symptoms onset to hospitalization (day) [c], median (IQR)	15 (10–28)	17 (11–31)	11 (3–22)	0.006
Hospitalization time (day), median (IQR)	28 (17–40)	22 (12–37)	13 (8–21)	< 0.001
Age (year)				0.884
< 60 (n = 638)	521 (81.7%)	100 (15.7%)	17 (2.7%)	–
≥ 60 (n = 797)	654 (82.1%)	125 (15.7%)	18 (2.3%)	–
Sex				0.330
Male (n = 683)	556 (81.4%)	106 (15.5%)	21 (3.1%)	–
Female (n = 752)	619 (82.3%)	119 (15.8%)	14 (1.9%)	–
Clinical condition				0.117
Non-severe (n = 1101)	891 (80.9%)	179 (16.3%)	31 (2.8%)	–
Severe (n = 334)	284 (85.0%)	46 (13.8%)	4 (1.2%)	–
Outcome				0.153
Discharge (n = 1386)	1140 (82.3%)	213 (15.4%)	33 (2.4%)	–
Death (n = 49)	35 (71.4%)	12 (24.5%)	2 (4.1%)	–

Data are presented as median (IQR) or n (%). P values are from χ^2 or the Kruskal–Wallis test. Percentages do not add to 100% because of rounding. n is the number of COVID-19 patients.

[a] Patients with at least one positive detection were identified as positive for IgM/IgG.

[b] Antibody test result was determined based on each measurement.

[c] Two patients were missing information on the date of symptoms onset.

or IgG were observed according to age, sex, or disease severity . The seroconversion rate of IgM was slightly lower in the deceased patients than in the discharged cases (71.4% versus 82.3%, P = 0.053) (Table 1). Of the severe/critical patients, 65.8% and 5.9% received hormone therapy and monoclonal antibody therapy, respectively. Similar overall antibody responses in the 1830 patients with detectable or undetectable SARS-CoV-2 were observed (Table S1 of Appendix A). Compared with the patients with detectable SARS-CoV-2, those with undetectable nucleic acids but detectable antibodies were less likely to die (2.0% versus 3.4%).

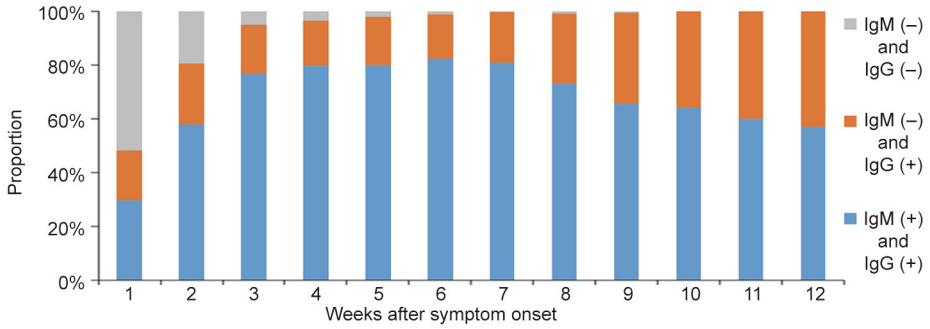
3.2. Temporal profiles of specific antibodies to SARS-CoV-2

For the specific antibody tests, 3456 blood samples from 1435 patients with COVID-19 were collected. Of these, 505, 402, 236, 158, and 134 patients underwent one, two, three, four, and greater than or equal to five serological tests, respectively. Five blood samples from two patients reporting an unclear date of symptom onset were excluded from the temporal profile analyses. In addition, seven serum specimens from four patients that were collected for antibody tests later than 84 days since illness onset were excluded to avoid the outlier effect. Of the serum samples collected in the first week, 51.9% did not present seroconversion for IgM or IgG, followed by a rapid decline to 5.0% in the third week (Fig. 1(a)). The weekly positive detection rates of IgM and IgG since illness onset are separately presented in Fig. 1(b) and Table S2 in Appendix A. The weekly positive detection rates of IgM and IgG were comparable between groups with different age, sex, or disease severity (Figs. 1(c)–(e)). The positive detection rate of IgM declined starting in week 2 and was eliminated in week 11 in the deceased patients. However, the seropositive rate of IgM among the discharged patients increased within the first three weeks, peaked at around 80% in weeks 4–6, and then gradually decreased to approximately 60% in week 12 (Fig. 1(f)). The differential antibody profiles between the discharged and deceased patients were maintained in the severe cases (Fig. 1(g)). Similar seroconversion patterns were observed in all 1830 patients with detectable or undetectable SARS-CoV-2 (Fig. S1 of Appendix A).

Compared with the deceased patients, patients who were discharged from the hospital had significantly higher levels of IgM and IgG (P < 0.001, Table S3 in Appendix A). The concentrations of virus-specific antibodies increased along with the decline of the viral load within the first five weeks. Among all cases with positive SARS-CoV-2, IgG was maintained at approximately 180 AU·mL^{-1} from weeks 5–12. However, the average IgM concentration approached the cutoff of 10 AU·mL^{-1} in week 12 (Table S4 in Appendix A and Fig. 2(a)). No obviously differential antibody profiles were observed by age group, sex, or disease severity (Figs. 2(b)–(d)). In the discharged patients, IgM decreased slowly to approximately 18.4 AU·mL^{-1}, and a high level of IgG was maintained at around 187.8 AU·mL^{-1} in week 12 since illness onset. In contrast, in the deceased patients, IgM reached the cutoff of 10 AU·mL^{-1} at week 7, and IgG declined to around 87 AU·mL^{-1} at week 12 (Fig. 2(e) and Table 2). Even in severe cases, the differential profiles of IgM and IgG were maintained in patients with different clinical outcomes (Fig. 2(f)). Similar temporal profiles of SARS-CoV-2 and its specific antibodies were observed in all 1830 patients (Fig. S2 in Appendix A). Compared with the patients with positive SASR-CoV-2, those with undetectable nucleic acids presented higher IgG levels (Fig. S2(b) in Appendix A).

3.3. The mediation effect of cytokines on the association between virus-specific antibodies and clinical outcomes

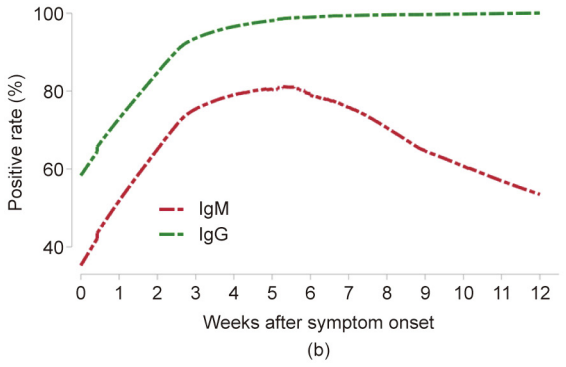
The distribution of the weekly detection numbers of each cytokine is listed in Table S5 in Appendix A. Compared with the discharged patients, the deceased patients possessed much higher levels of IL-6, IL-8, IL-10, IL-1β, IL-2R, and TNF-α (all P < 0.001, Figs. 3(a)–(f)). Similar differential distributions of cytokines by clinical outcomes were also observed in severe patients (Fig. S3 in Appendix A). Among the 1131 cases with available cytokine data, 251 were classified as having high inflammation status (cytokine score ≥ 3). No statistically significant mediation was observed between IgM (per standard deviation (SD) increase) and survival by high inflammation status (Fig. 3(g)). However, high inflammation status mediated 12.5% of the effect of IgG (per SD increase) on survival (total effect: odds ratio (OR), 0.43; 95% confidence interval (CI): 0.28–0.67; indirect effect: OR, 0.90; 95% CI: 0.87–0.95) (Fig. 3(h)).



Distribution of weekly detection rates of antibody response status (n = 3444).

Variable	Week											
	1	2	3	4	5	6	7	8	9	10	11	12
IgM (+) and IgG (+)	8	48	169	333	510	604	442	303	141	61	21	12
IgM (–) and IgG (+)	5	19	40	70	115	121	103	109	73	34	14	9
IgM (–) and IgG (–)	14	16	11	14	12	8	1	3	1	0	0	0
Total	27	83	220	417	637	733	546	415	215	95	35	21

(a)



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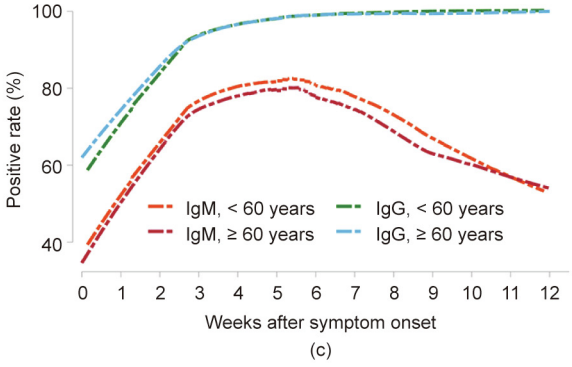

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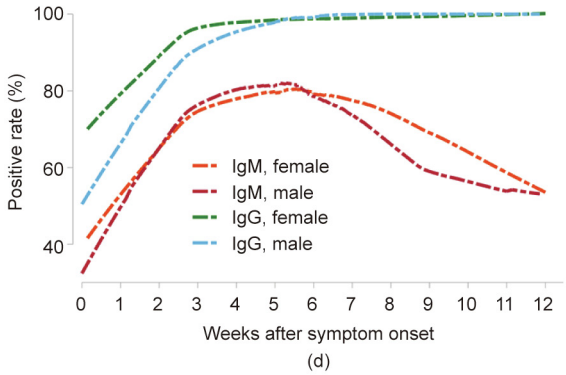
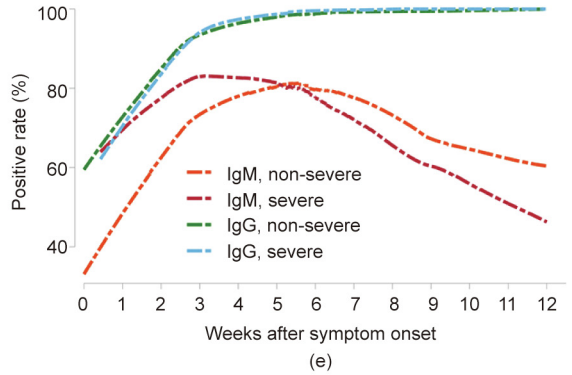


(d)



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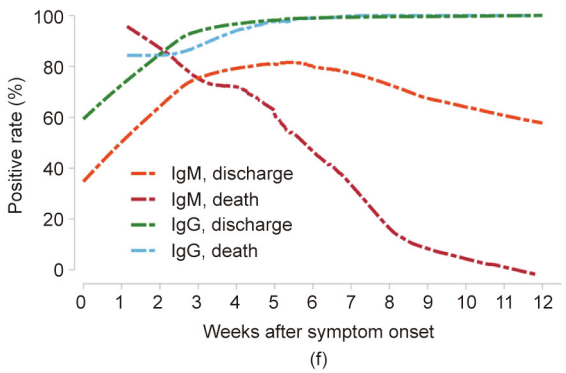
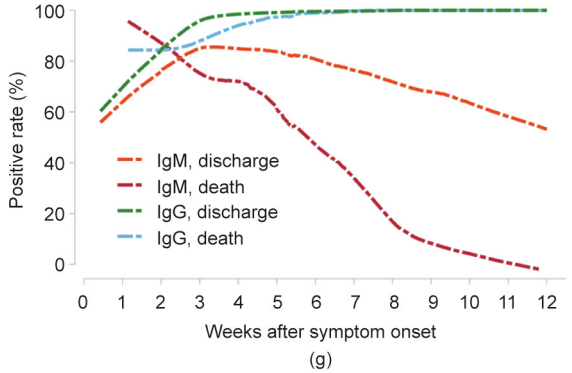


(f)



(g)

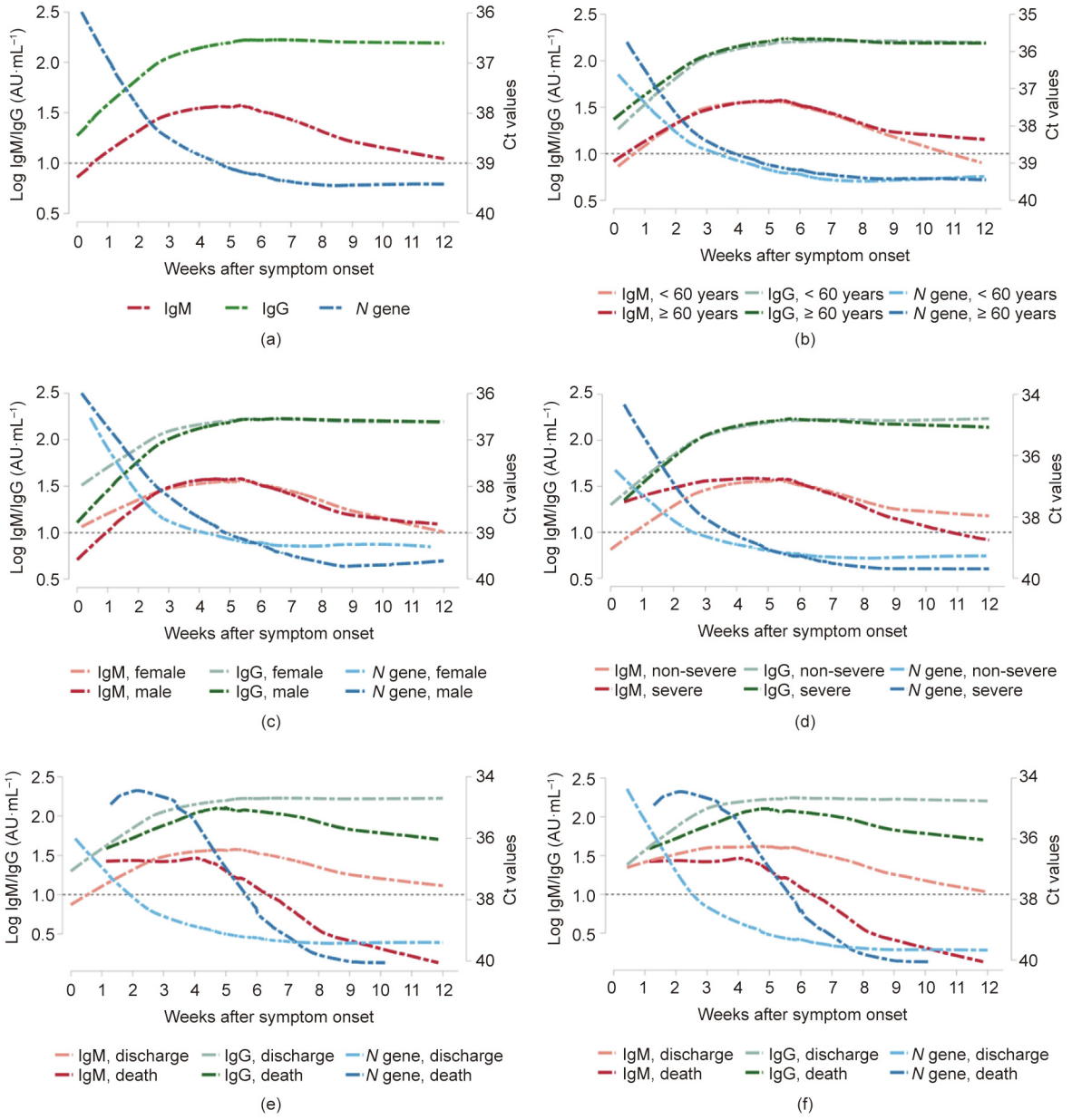


Fig. 2. Temporal profiles of IgM, IgG, and viral load among patients with detectable SARS-CoV-2. 3444 serology samples and 4062 oropharyngeal/nasopharyngeal samples were included. (a) Dynamic profiles of IgM/IgG levels and viral load after symptoms onset; (b–e) dynamic profiles of IgM/IgG levels and viral load after symptoms onset, stratified by age, sex, disease severity, and clinical outcome; (f) dynamic profiles of IgM/IgG levels and viral load among severe COVID-19 patients after symptoms onset, stratified by clinical outcome. The horizontal line represents the cutoff value for IgG/IgM ($\geq$ 1, positive; < 1, negative).

4. Discussion

In the current study, approximately half of the patients presented seroconversion of SARS-CoV-2 within the first week since illness onset, which is comparable with previously reported seroconversion rates of 37%–60% [11,20–22]. Seroconversion then reached 95% in the third week. The finding is consistent with the results of a nationwide prevalence survey of SARS-CoV-2 in Spain, which revealed a seroprevalence close to 90% after 14 days since a positive polymerase chain reaction (PCR) test [23]. In addition, we found that the titers of IgM and IgG reached their maximum at week 5 and decreased thereafter; this finding is supported by a previous study revealing the maximum positivity rates of IgM and IgG at weeks 4 and 6, respectively [22]. The decline of

Fig. 1. Temporal trend in the detection rates of virus-specific IgM and IgG among patients with detectable SARS-CoV-2. (a) Distribution of weekly detection rates of antibody response status; (b) dynamic changes in IgM/IgG positive rates after symptoms onset; (c–f) dynamic changes in IgM/IgG positive rates after symptoms onset stratified by age, sex, disease severity, and clinical outcome; (g) dynamic changes in IgM/IgG positive rates among severe COVID-19 patients after symptoms onset stratified by clinical outcome. A total of 3456 serology samples were collected from 1435 COVID-19 patients. Five blood samples from two patients who reported an unclear date of symptom onset were excluded from the temporal trend analyses. Seven serum specimens from four patients that were collected for antibody tests later than 84 days since illness onset were also excluded to avoid the outlier effect. Therefore, 3444 serology samples were included in the temporal trend analyses.

Table 2
Comparison of weekly concentrations of IgM and IgG among patients with detectable SARS-CoV-2 according to the clinical outcome (n = 3444)[a].

Week	IgM				*P* value	IgG				*P* value
	Discharge		Death			Discharge		Death		
	Number of samples	median (IQR, AU·mL^{-1})	Number of samples	median (IQR, AU·mL^{-1})		Number of samples	median (IQR, AU·mL^{-1})	Number of samples	median (IQR, AU·mL^{-1})	
1	27	5.1 (1.7–12.7)	0	—	—	27	9.0 (1.9–141.4)	0	—	—
2	79	15.3 (3.4–71.6)	4	15.8 (7.4–117.1)	0.915	79	98.1 (26.3–184.3)	4	45.0 (17.2–111.9)	0.339
3	207	42.2 (10.5–100.2)	13	40.0 (18.9–117.6)	0.966	207	153.1 (100.9–189.2)	13	110.0 (81.0–164.2)	0.079
4	396	38.1 (12.6–101.7)	21	28.0 (6.2–53.4)	0.237	396	163.7 (125.3–192.6)	21	118.9 (81.3–179.2)	0.057
5	612	40.4 (13.7–114.0)	25	30.1 (6.2–105.1)	0.450	612	174.5 (141.4–204.7)	25	149.9 (101.0–199.3)	0.063
6	719	41.1 (16.0–100.8)	14	61.4 (24.2–127.3)	0.448	719	183.0 (153.4–213.2)	14	164.6 (145.2–231.0)	0.605
7	531	37.7 (15.7–79.2)	15	2.7 (2.0–39.9)	0.001	531	181.2 (152.3–207.3)	15	139.4 (92.2–245.7)	0.086
8	400	27.2 (10.1–56.6)	15	2.2 (1.6–3.0)	< 0.001	400	179.1 (150.8–200.7)	15	72.1 (44.3–102.7)	< 0.001
9	208	19.6 (7.5–49.7)	7	3.7 (2.3–4.0)	0.001	208	183.3 (147.4–209.3)	7	86.7 (27.7–98.5)	0.002
10	90	17.5 (7.6–46.9)	5	1.1 (1.0–1.1)	0.007	90	188.3 (156.3–207.7)	5	123.6 (95.4–146.1)	0.125
11	32	26.9 (5.9–48.8)	3	3.5 (0.8–5.5)	0.022	32	191.0 (155.9–216.4)	3	26.7 (20.3–70.2)	0.007
12	20	18.4 (4.1–32.2)	1	0.8 (0.8–0.8)	0.099	20	187.8 (130.1–217.4)	1	86.9 (86.9–86.9)	0.186

Data are presented as median (IQR). *P* values are from the Mann–Whitney U test.
[a] Five blood samples from two patients who reported an unclear date of symptom onset were excluded from the temporal profile analyses. Seven serum specimens from four patients that were collected for antibody tests later than 84 days since illness onset were also excluded to avoid the outlier effect.

SARS-CoV-2-specific antibodies has also been observed in a population-based survey [24]. Two successive nationwide serological household surveys in Brazil found that the SARS-CoV-2 antibody decreased from the highest observed prevalence in the first survey by almost 50% in the second survey, which was conducted one month later in Breves. It was inferred that the serum titers in previously positive individuals might have fallen below the detection threshold for the test between the first and second surveys. This decline was prevalent among asymptomatic patients with COVID-19, 40% of whom became seronegative after an 8-week period [25]. Since the participants in the current study were symptomatic patients, it is reasonable to observe a relatively slower decline of antibodies in our study. Of the patients with detectable SARS-CoV-2, 35 patients (2.4%) presented no seroconversion during hospitalization. A recent study also observed that two out of 63 patients maintained IgG- and IgM-negative status during hospitalization [11]. Therefore, it is reasonable to infer that a tiny minority of COVID-19 cases might have immunological unresponsiveness, which implies that a virus-eliminating immune response to SARS-CoV-2 might be difficult to induce in some people [26].

Previous studies have shown that the initial viral load of SARS-CoV-2 is associated with the prognosis [27]. In the current study, deceased COVD-19 patients presented a higher initial viral load than the discharged patients. Compared with the slow decrease in IgM from week 5 to week 12 in the discharged patients, the concentration of IgM declined rapidly in the deceased patients. Moreover, unlike the high IgG level that was maintained in the discharged patients, the IgG concentration in the patients who later died began to decline starting in week 5. Considering that IgM provides the first line of defense during a viral infection and that IgG represents a primary humoral immune response to protect patients against COVID-19 [28], it is inferred that the rapid elimination of antibodies results in adverse outcomes. Worryingly, it has been shown that a neutralizing monoclonal antibody targeting the receptor-binding domain of the Middle East respiratory syndrome (MERS) coronavirus spike can enhance viral entry [29]. A suggestive possibility of antibody-dependent enhancement (ADE) of SARS-CoV-2 infection has been proposed [30].

Based on the current cohort, compared with the deceased patients, significantly high concentrations and seropositive rates of IgG were observed in the discharged patients, even in those with severe manifestations. This finding implies that the ADE of SARS-CoV-2 might not be a predominant determinant of clinical outcome, and that maintainable high levels of IgM and IgG might be the indicators of rapid recovery.

High levels of IL-6 have been observed in patients with severe disease and adverse survival outcomes of COVID-19 [12,31], suggesting that disease progression might be due to virally driven hyperinflammation. Moreover, IL-10 and TNF-α have been reported to be higher in intensive care unit (ICU) COVID-19 patients than in non-ICU COVID-19 patients [32]. The current study revealed the mediation effect of these cytokines in the association between IgG and the prognosis. Although no previous epidemiological studies have explored the mediation effects of cytokines on the association of prognosis with antibodies, recent studies have supportively indicated that the administration of convalescent plasma with a high titer of SARS-CoV-2-specific antibody (IgG) contributed to increased concentrations of IgG, declined CRP and IL-6 levels, and improved clinical status in COVID-19 patients [33–36].

The strengths of the present study are as follows: First, based on a large cohort with more than 1800 COVID-19 patients, we revealed the temporal profiles of antibody responses during the 12-week period after illness onset. Compared with previous studies, the current study had a much larger sample size and longer follow-up time, ensuring the validity of our conclusions. Second, for the first time, our study revealed that a stronger antibody response to SARS-CoV-2 contributed to improved survival of patients with COVID-19, and that this protective effect was

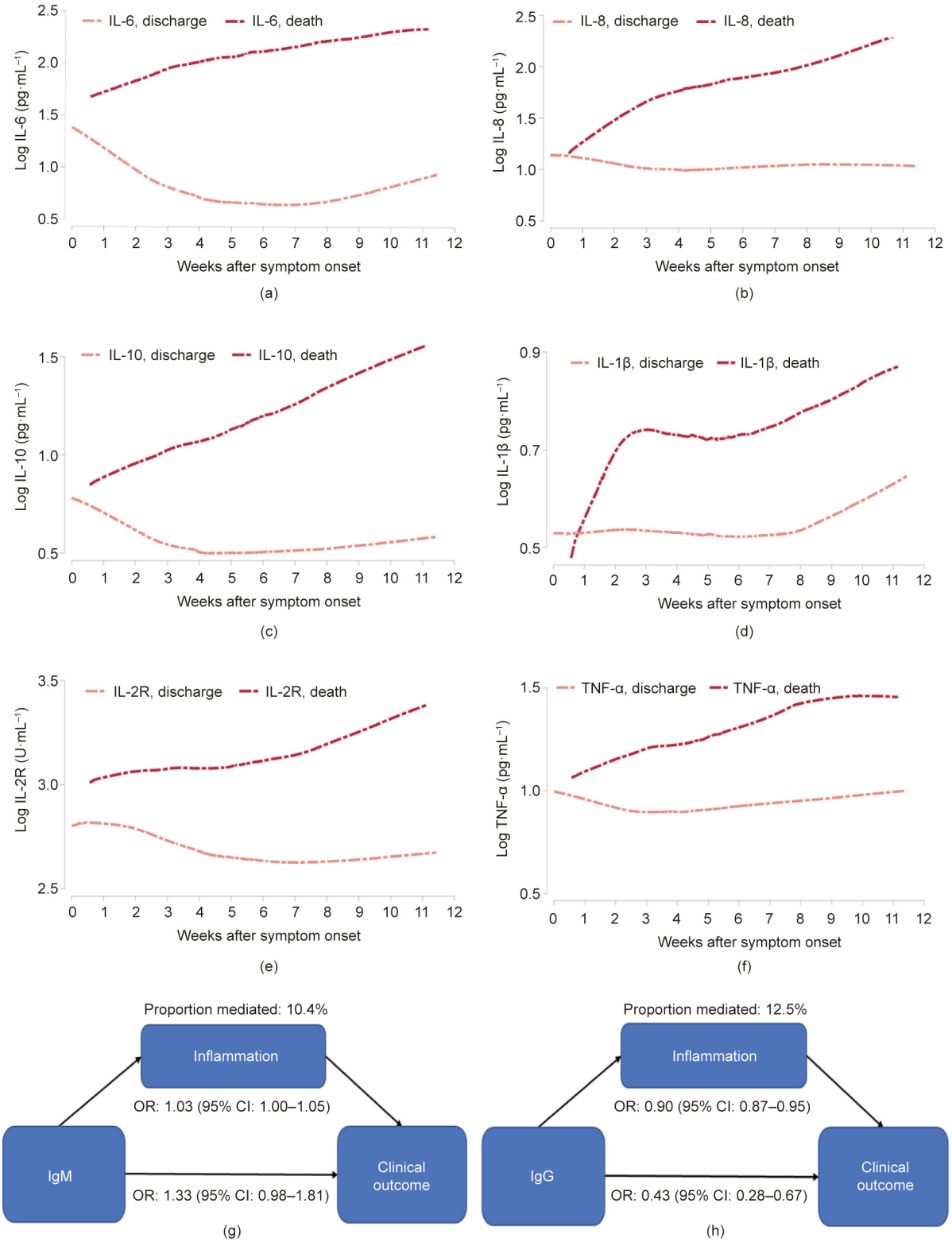


Fig. 3. Temporal profiles of cytokines and the mediation effects of cytokines on the association between prognosis and IgM/IgG. (a–f) Temporal profiles of IL-10, IL-1β, IL-2R, IL-6, IL-8, and TNF-α among patients with detectable SARS-CoV-2 according to clinical outcome. (g) Mediation analysis of the association between immunoglobulin M (IgM) and survival. OR: odds ratio; CI: confidence interval. (h) Mediation analysis of the association between IgG and survival. A cytokine score was constructed by summing the number of inflammatory cytokines (IL-10, IL-1β, IL-2R, IL-6, IL-8, and TNF-α) in each upper quartile. The mediator (binary) was classified by the cytokine score as having a high inflammation status if the cytokine score was ≥ 3 or a low inflammation status if the cytokine score was < 3. The proportion mediated was calculated by log(Indirect effect)/log (Total effect). All models were adjusted for age, sex, hormone therapy, and monoclonal antibody therapy.

independent of disease severity. This finding indicates the importance of antibody surveillance in patients with COVID-19, especially in severe cases. In addition, this finding partially eliminates the warning of ADE of SARS-CoV-2 infection during clinical convalescent plasma therapy. Third, for the first time, we reported the mediation effect of cytokines on the association between low levels of IgG antibody and a worse survival outcomes, which might partially explain the protective effect of the immune response to SARS-CoV-2.

The study also has limitations. First, a limited number of specimens were collected in the very early and late stages of the observation period. Therefore, the figures in the very early and late stages of the observation period should be interpreted with caution. Second, a cytokine mediation effect on the association between antibody response and clinical outcome was proposed based on epidemiological data, and further functional studies are needed to reveal the potential mechanisms. Third, although patients without seroconversion had shorter hospitalization stays, the average sampling numbers during hospitalization were comparable between those with and without seroconversion (average 2.4 versus 2.3 samples per patient). Therefore, no observation of seroconversion might not be biased by shorter hospitalization. Fourth, interferon (IFN) systems play a pivotal role in antiviral defense and may serve as a key determinant for the outcome of COVID-19 infection [37]; however, relevant data (e.g., IFN-α or -β) were not available for the current study, and should be considered in future research.

5. Conclusions

As an emerging infectious disease, COVID-19 is far less thoroughly known than other widespread diseases. This study sheds light on the temporal profiles of antibodies in patients with COVID-19 and on the protective effect of antibody response on survival, which may help to guide prognosis monitoring.

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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Compliance with ethics guidelines

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2021.04.015.

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