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Distinct durability of IgM/IgG antibody responses in COVID-19 patients with differing severity

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Dear Editor,

The coronavirus disease 2019 (COVID-19) pandemic is generating a demand for simultaneous vaccine development, which is expected to prevent future outbreaks by eliciting sufficient and protective immunity. The speed of vaccine development has been remarkable; however, key insights into natural infection-induced immunization are still urgently needed for appropriate vaccine-induced immunization. A number of studies have recently investigated the simultaneous or sequential seroconversion of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific immunoglobulin (Ig) M and IgG in COVID-19 patients using enzyme-linked immunosorbent assay (ELISA)-based methods (Long et al., 2020) and have presented functional evidence for virus-specific humoral immunity with neutralization tests.

Magnitude and durability are two critical properties of humoral immunity in providing adequate and sustained immune protection. SARS-CoV-2 causes a spectrum of clinical manifestations, from asymptomatic to mild/moderate (MM) symptoms and even severe/critical (SC) complications. Recent studies have revealed a stronger antibody response in SC patients and have independently associated higher antibody titers with a more severe clinical classification. A re-

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cent study showed that the humoral response of COVID-19 patients at 1 and 6 months after infection was evidenced by the significantly decreased receptor-binding domain (RBD)-IgG and RBD-IgM antibody titers (as measured by ELISA) (Gaebler et al., 2021). However, it remains unclear whether naturally acquired infection immunity is durable in COVID-19 patients with differing severity and whether higher antibody titers are correlated with a more rapid viral clearance.

To investigate the kinetics of antibody responses, we recruited 92 individuals with quantitative reverse transcription polymerase chain reaction (qRT-PCR)-confirmed SARS-CoV-2 infection, 49 with MM symptoms and 43 with SC symptoms, with 338 sequential serum/plasma samples. The patients were followed up from January 25 to May 21, 2020, and their characteristics were summarized in Table S1 in Supporting Information. SARS-CoV-2 spike RBD (S-RBD) IgM and IgG and nucleocapsid protein (NP) IgM and IgG were detected using ELISA kits (Figure 1A). Within 30 d after symptom onset (dao), NP-IgM, NP-IgG, S-RBD-IgM and S-RBD-IgG became seropositive in all SC patients, and the seroconversion curves of the two groups (SC and MM) were almost identical (Figure 1A, Figure S1 in Supporting Information), while the median peak titers for S-RBD-IgM and NP-IgM were approximately two-fold higher in the SC patients than in the MM patients (Figure 1B). More strikingly, the SC patients had peak titers for S-RBD-IgG and NP-IgG 4-fold and 8-fold higher, respectively, than the MM patients. Compared with the asymptomatic patients, the MM patients had higher peak titers for NP-IgM, and the SC patients had higher peak titers for S-RBD-IgG, NP-IgM, and NP-IgG (Figure 1B). We also observed a dramatic difference in the durability of S-RBD-IgG between the SC and MM patients (Figure 1A). After reaching a plateau, the S-RBD-IgM and S-RBD-IgG titers rapidly declined in all MM patients, and both became seronegative by 100 dao. By contrast, all SC patients remained S-RBD-IgG seropositive with an approximate median titer of 1:160 by 100 dao (Figure 1A and C). Although the NP-IgG titers slightly declined after reaching their peaks, the titers stayed at a stable level of approximately 1:160 in the MM patients and 1:1,280 in the SC patients (Figure 1A and D).

To explore the effects of age and sex on the antibody response, we further analyzed the kinetics of antibody responses and peak titers with differing ages and sex. In the SC patients, the peak NP-IgM and NP-IgG titers in the patients older than 65 years (>65 group) were clearly higher than those for the patients aged 18–65 years (≤65 group) (Figure S2A and B in Supporting Information). In addition, the >65 group presented a trend toward higher mean NP-IgG titers than the ≤65 group throughout the disease course and higher mean S-RBD-IgG and NP-IgM titers after the plateau (Figure S2A in Supporting Information). There was no significant difference among the peak antibody titers between the male and female patients (Figure S3A–D in Supporting Information). However, the female patients presented a tendency toward higher mean S-RBD-IgM, S-RBD-IgG, and NP-IgG titers after reaching the peaks in the MM group (Figure S3A in Supporting Information), as well as a trend toward higher mean S-RBD-IgG titers in the SC group (Figure S3C in Supporting Information).

To further confirm the correlation between S-RBD-IgM and S-RBD-IgG titers and sera/plasma protection efficacy against SARS-CoV-2 infection, we tested the neutralization capacity *in vitro* using a single-round of HIV-1-based pseudovirus infection of 293 T/ACE2+TMPRSS2 cells (Materials and Methods in Supporting Information). Notably, sera/plasma with negative or lower titers of S-RBD-IgG and S-RBD-IgM exhibited inferior 50% inhibitory concentrations (IC_{50}) (Figure 1E). In line with the decreased S-RBD-IgM/IgG titers, the sera/plasma from the MM patients displayed lower IC_{50} than that from the SC patients (Figure 1F).

Next, we tested whether the magnitude of S-RBD-specific antibodies is correlated with viral shedding periods. We assigned 45 of the 92 patients to a short viral shedding period (SVS, ≤30 dao) group and 47 to a long viral shedding period (LVS, >30 dao) group; Table S2 in Supporting Information summarizes the patients' characteristics. The number and proportion of patients in the SVS and LVS groups who were asymptomatic, MM or SC were comparable (Table S3 in Supporting Information). The SVS and LVS groups exhibited comparable S-RBD-IgM and S-RBD-IgG seropositive rates (Figure S4A in Supporting Information) and peak titers of the tested antibodies (Figure S4B in Supporting Information). The patients with differing peak S-RBD-IgM or S-RBD-IgG titers also had comparable viral shedding periods (Figure S4C in Supporting Information). There was no correlation between the viral shedding periods and peak S-RBD-IgG or S-RBD-IgM titers (Figure 1G). More importantly, the SVS and LVS groups had a similar dynamic curve for neutralizing titers (Figure 1H).

Our study sheds further light on how SARS-CoV-2-specific antibodies vary and wane with time and the varying durability in COVID-19 patients with differing disease severity. In the MM patients, we observed a waning of antibody titers and neutralizing activity of sera/plasma over time. The SC patients presented comparatively stronger and longer sustained S-RBD-specific and NP-specific antibody responses and higher levels of neutralizing antibodies until 114 dao. In patients with the Middle East respiratory syndrome (MERS)-CoV or SARS-CoV, viral-specific antibodies could last more than a year and up to 34 months. However, antibody protection only lasts 6–12 months in patients with several coronavirus infections (Edridge et al., 2020). Given the fact that most individuals with SARS-CoV-2 infection had mild to moderate clinical manifestations or were even asymptomatic, the short duration of natural infection-induced antibody response raises a serious concern about

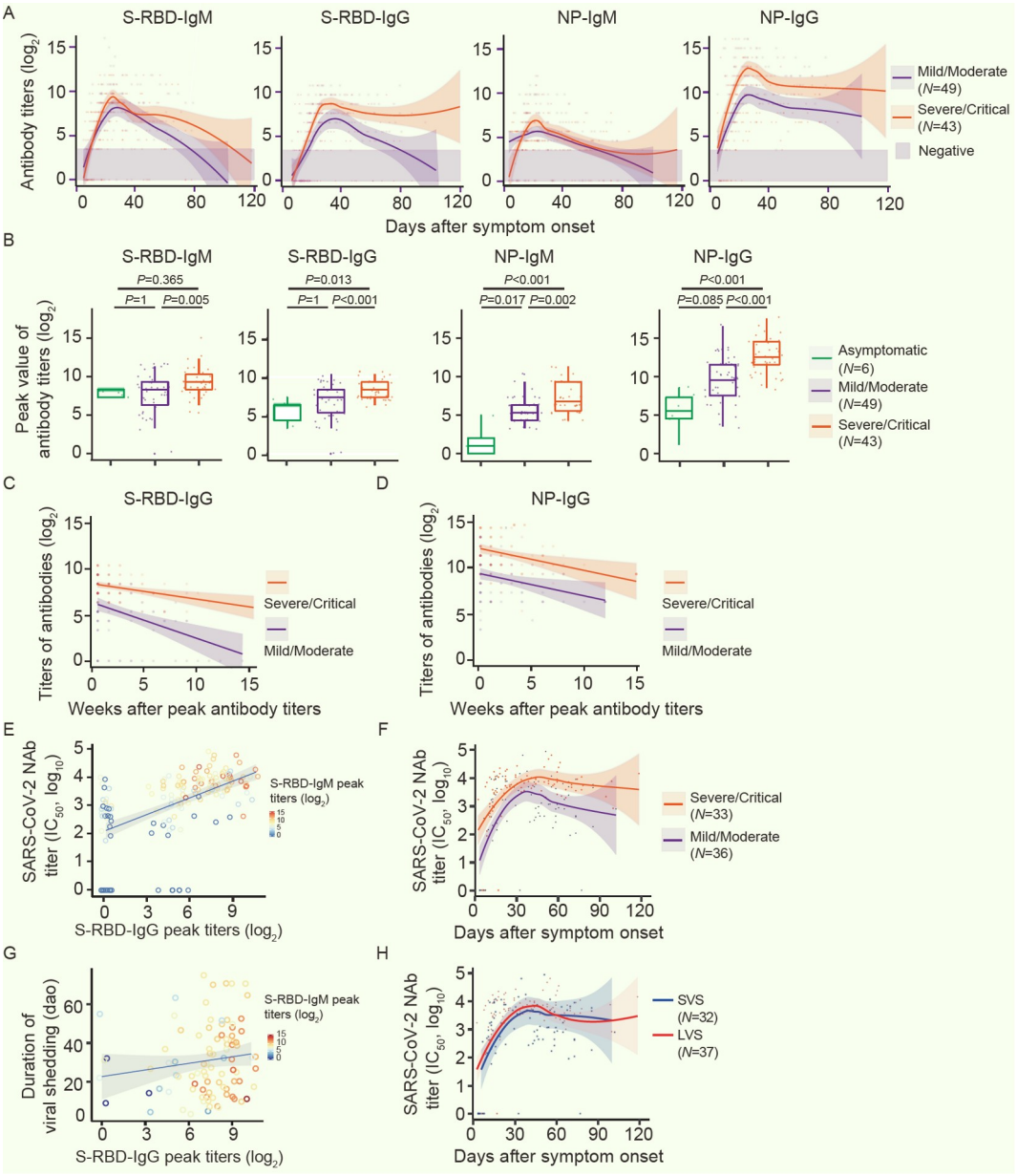

Figure 1 The dynamic trend profiling of antibody titers between mild/moderate and severe/critical COVID-19 patients, with short and long viral shedding periods. According to disease severity and clinical classification, 92 COVID-19 patients were classified into MM (N=49) and SC (N=43) groups. A, Line plot demonstrating the dynamic trend profiling of S-RBD-IgM, S-RBD-IgG, NP-IgM, and NP-IgG antibody titers (loess smoothed normalized counts±standard error (SE)) over time after symptom onset in 49 MM and 43 SC patients. B, The peak antibody titers (the highest antibody titer) for S-RBD-IgM, S-RBD-IgG, NP-IgM, and NP-IgG in 6 asymptomatic patients, 49 MM and 43 SC patients. We applied the Wilcoxon test to compare the antibody titers among the asymptomatic, MM and SC patients. C, Linear regression demonstrating the trend in S-RBD-IgG antibody titers over time after the peak antibody titers in 49 MM and 43 SC patients. D, Linear regression demonstrating the trend in NP-IgG antibody titers over time after the peak antibody titers in 49 MM and 43 SC patients. E, The correlation analysis among the titers of neutralizing antibodies (NAbs), S-RBD-IgG, and S-RBD-IgM ELISA titers from the sera/plasma of COVID-19 patients. X represents S-RBD-IgG ELISA titers ($\log_2$), and Y represents SARS-CoV-2 NAbs ($\log_{10}$). The S-RBD-IgM ELISA titers ($\log_2$) are displayed as colors ranging from blue to red as shown in the key. The NAb titers against SARS-CoV-2 pseudovirus were detected by luciferase assay system and calculated as IC_{50}. F, Line plot demonstrating the dynamic trend profiling of SARS-CoV-2 NAb titers (IC_{50}, loess smoothed normalized counts±SE, $\log_{10}$) from 2 to 114 dao in 36 MM and 33 SC patients. G, The correlation analysis among the viral shedding periods, peak S-RBD-IgG ELISA titers and peak S-RBD-IgM ELISA titers. X represents peak S-RBD-IgG ELISA titers ($\log_2$), and Y represents viral shedding periods (days after symptom onset, dao). The S-RBD-IgM ELISA titers ($\log_2$) are displayed as colors ranging from blue to red as shown in the key. H, Line plot demonstrating the dynamic trend profiling of SARS-CoV-2 NAb titers (loess smoothed normalized counts±SE) over time after symptom onset (dao, day 2 to day 114) in 32 SVS and 37 LVS patients.

achieving herd immunity through the mass exposure of the population to the virus. Our results are consistent with the rapid decay of SARS-CoV-2-specific antibodies in patients with mild COVID-19 (Ibarrondo et al., 2020).

Fortunately, the stronger and longer (at least 114 dao) sustained antibody response in the SC patients might hint at the

probability of success in developing effective vaccines, based on the notion that only S protein induces a high titer of neutralizing antibodies that prevent the virus from attaching to host cells and infection. However, viral shedding duration was not associated with the titers of S-RBD-IgM/IgG and neutralizing antibodies, which indicates that antibody protection alone might be insufficient for achieving virus clearance in COVID-19 patients. Similarly, the previous study found that the antibodies were seroconverted and were not followed by a rapid decline in viral load. The SC patients had a good level of neutralizing antibodies (that is, the antibodies required for protection) until 114 dao; while, the MM patients might be left with less protective antibody levels at 3 months post-infection. However, the vanished antibodies did not mean without protective immunity, given that memory immune cells might rapidly start a new immune response when the virus is re-encountered. Other immune components (T cells, innate immune cells, and mucosal immunity) and other vaccine antigens (e.g., NP and M protein) should also be taken into consideration for optimizing vaccine design.

Our results also question the clinical use of passive antibody therapy by the transfusion of convalescent plasma/sera into critically ill patients. First, the rapid decline in neutralization activities might reduce the effectiveness of convalescent plasma/sera. Second, SC patients usually have high levels of neutralizing antibodies. Third, the neutralizing titers were not associated with the viral shedding duration. Thus, further analysis is needed of the benefits of convalescent plasma/sera transfusion in future studies.

In addition to IgG and IgM, several studies have suggested that IgA antibody detection could be a complementary aid in diagnosing COVID-19. For IgM seronegative patients, an IgA antibody test provides better diagnostic outcomes in the early stages and might help close the serological gap of COVID-19. IgA could mediate virus neutralization, mostly in the upper respiratory tract. As with IgM and IgG, IgA exhibits neutralization but at a lower potency. Due to the limited availability of samples and commercial IgA kits at the time, however, we did not measure secretory IgA antibody levels in the present study, a factor we will explore in a future study.

This study has several limitations, including the small sample size, variable time interval for each patient from admission to symptom onset, variable sampling interval for each patient, and the lack of non-survivors in the cohort. A recent study showed that levels of IgG to the spike protein were relatively stable over 6 months (Dan et al., 2021); whether the antibody titers will remain steady for longer periods needs to be explored in future studies. In addition, our previous study found a longer duration and a higher mean number of RNA copies of SARS-CoV-2 in fecal samples compared with samples from the upper respiratory tract (Zhang et al., 2021). However, we did not test stool samples in the present study, and it is unclear whether there was a difference in viral shedding durations between the nasopharyngeal swabs and stool samples. These findings are important for vaccine development and passive transfusion in controlling the COVID-19 epidemic.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

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