CORRESPONDENCE

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Temporal antibody responses to SARS-CoV-2 in patients of coronavirus disease 2019

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Dear Editor,

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a pandemic within a few months. Up to 31st July 2020, it had affected over 17,000,000 individuals worldwide causing over 670,000 deaths[1]. In most cases, COVID-19 is associated with mild symptoms, while some patients develop severe disease[2,3]. Previous investigations of COVID-19 patients have demonstrated that SARS-CoV-2 IgM antibodies are usually detectable a week after illness onset and can persist for one month after infection[4]. IgG antibody can be detected 10 days from illness onset, which may last for a longer period[4]. It was also shown that antibody titers are higher and longer-lived in more severely ill patients than in mildly ill patients[5], some of the latter do not develop a detectable antibody response[6]. However, data on the simultaneous evaluation of cellular immune response, cytokine production, and specific antibody were lacking[7].

Here, we assessed the longitudinal clinical, laboratory, viral, and immunological data from 366 COVID patients. Severity of the COVID-19 patients was defined according to the "Diagnosis and Treatment Scheme of New Coronavirus Infected Pneumonia"[8]. An IgM and IgG antibody detection kit was employed to detect the antibody responses in COVID-19 patient serum. A total of 366 RT-PCR confirmed COVID-19 patients were enrolled. The median age was 62 years and 177 (48.4%) were male (Supplementary Table S1). The delay from symptom

onset to the hospital admission was 20 days (interquartile range (IQR) 10–29). Among these, 65 patients had documented diabetes and 136 had hypertension. 144 patients were diagnosed as having severe disease and 222 having mild disease.

The anti-SARS-COV-2 IgM antibody profile was illustrated (Fig. 1a). Based on the calculation of every 10 days, both the IgM positive rates (82.1%) and the antibody level (geometric mean reciprocal titer (GMRT) 39.1, 95% confidence interval (CI) 8.1–190.6) peaked at 20–30 days and decreased thereafter with 50% tested positive and the GMRT evaluated to be 9.2 (95% CI: 2.2–39.6) at 60-day after symptom onset, which is the last observation point of the study. The anti-SARS-COV-2 IgG response profile was also calculated every 10 days (Fig. 1b). The positive rates peaked at 60–70 days (100%). Based on the GMRT estimation, the IgG antibody level peaked at 20–30 days of illness (GMRT 138.0, 95% CI 66.0–288.6) and decreased slowly thereafter (GMRT 94.3, 95% CI 61.6–145.5).

The magnitude of IgM antibody was delineated in relation with five variables (age, gender, the delay from symptom onset to admission, comorbidities, and disease severity) (Fig. 1c–h and Supplementary Table S2). Their effects on the antibody responses were estimated by performing generalized estimating equation (GEE) analysis. Before the peaking point, the patients of younger age (Fig. 1c), female (Fig. 1d), and those with mild disease (Fig. 1e) produced lower IgM GMRT (all $P < 0.05$).

After the peak point, a lower GMRT of IgM antibody was also observed for younger age, and additionally for patients with hypertension or patients with longer days from symptom onset to admission (Fig. 1c, f, h and Supplementary Table S2) ($P < 0.05$, $P < 0.001$, and $P < 0.05$), respectively. By applying GEE analysis, significant effects on antibody level of IgM were determined from age, days from symptom onset to admission and disease

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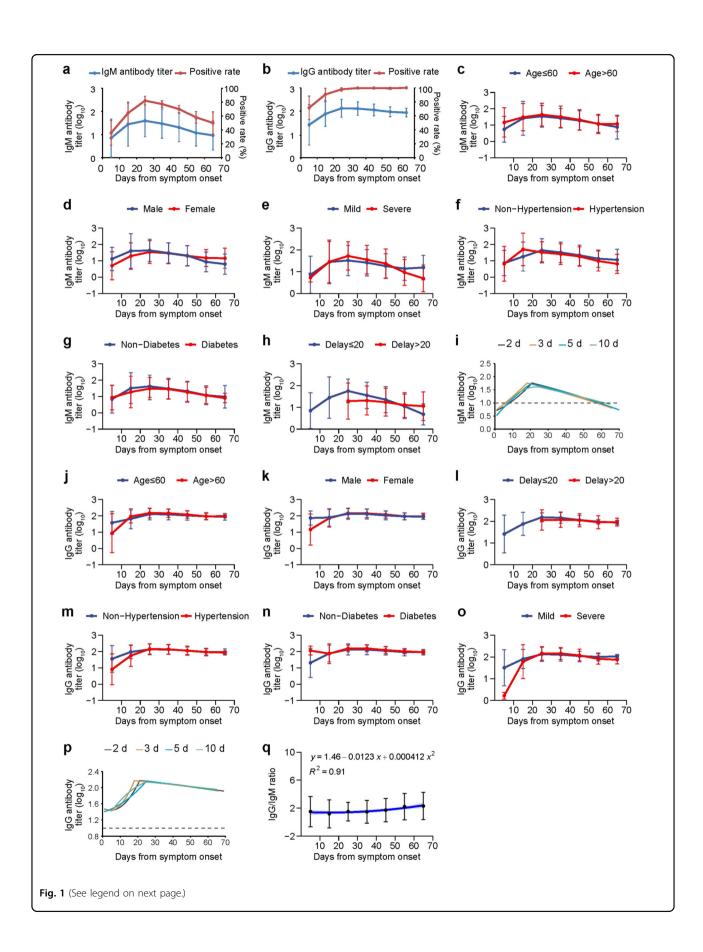[1]Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, HUST, Wuhan, Hubei 430030, China
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Fig. 1 (See legend on next page.)

severity ($P < 0.05$, $P < 0.001$, and $P < 0.05$), respectively. The maximum of IgM antibody titer for each patient was compared, and only the patients with >20 days from symptom onset to admission had a lower peaking level of IgM antibody titer ($P < 0.05$, Supplementary Table S3). The quadratic fitting curves were performed on titers calculated every 2-day, 3-day, 5-day, and 10-day points, respectively, with the curve based on 10-day point attaining the highest R^2. Accordingly, the half-life of IgM was estimated as ~51 days and the diminish time was estimated to be around 62 days post symptom onset (Fig. 1i and Supplementary Table S4).

The IgG antibody response was similarly delineated by the days from disease onset to detection (Fig. 1j–o). Different from IgM antibody, a higher GMRT of IgG antibody was observed in patients with diabetes ($P < 0.05$) before the peak point (Fig. 1n), while significant higher GMRT of IgG was observed in patients with older age ($P < 0.01$, Fig. 1j), shorter days from symptom onset to admission ($P < 0.001$, Fig. 1l) or diabetes ($P < 0.001$, Fig. 1n) after the peak point. By applying GEE analysis, significant effects on GMRT of IgG were determined from age, diabetes, and disease severity ($P < 0.01$, $P < 0.001$, and $P < 0.05$). The maximum of IgG antibody titer for each patient was compared, and only patients with younger age and >20 days from symptom onset to admission had a lower peak level of IgG ($P < 0.05$ and $P < 0.001$, Supplementary Table S3). The quadratic fitting curves were performed on titers calculated every ten-day points, which showed the highest R^2. Accordingly, the half-life of IgG was estimated as ~53 days and the diminish time was estimated at about 211 days post symptom onset (Fig. 1p and Supplementary Table S4).

The IgG/IgM ratio was calculated as $\log 10(1/IgG)$ divided by $\log 10(1/IgM)$. The dynamic change of IgG/IgM ratio was simulated as a line along the clinical course, which rose slowly till 50 days after symptom onset and then rapidly increased. A quadratic curve fitted the points with an R^2 of 0.91, indicating a decent goodness of fit (Fig. 1q). Moreover, no factor was found to be related with the IgG/IgM ratio by ordinal logistic regression model, indicating a stable profile that weas devoid of influence from host-related factors (Supplementary Table S5).

Next, the antibody production change over the course was determined. The study period was divided into three periods: 1–28 January, 29 January–6 February, and 7 February–17 March 2020. The IgG and IgM antibody from 125, 140, and 101 COVID-19 patients were evaluated at serial time points following infection and were plotted and fitted to a linear regression. A decrease of the GMRT of the IgM and IgG was observed for the samples collected at the late period than those taken at the early epidemic and the differences of IgM and IgG were significant during 32–42 days after symptom onset among the three periods (all $P < 0.05$) (Supplementary Fig. S1 and Table S6). The effect of age, gender, days from symptom onset to admission, hypertension, diabetes, and disease severity were adjusted. The significant differences still existed after adjusting the above variables.

Altogether 267 and 363 patients were evaluated for the serum cytokines and peripheral lymphocyte subsets, respectively. Sequential evaluation disclosed a significant decrease of the evaluated lymphocyte subsets (Supplementary Fig. S2). The longitudinal profile of the serum cytokines showed an increase of IL-6 during the first week of infection (Supplementary Fig. S3). The severe patients had higher levels of IL-2R, IL-6, IL-8, and TNF-α, and lower counts of NK, B-lymphocytes, T helper cells (Th), suppressor T cell (Ts), and T lymphocytes on admission compared to the mild patients (all $P < 0.05$, Supplementary Table S7).

Finally, we performed a comprehensive correlation analysis of COVID-19-specific antibodies and all the other

evaluated immunological indices, including 4 types of cytokines and 14 lymphocyte subsets. According to the spearman correlation analysis, the magnitude of IgG antibody titer was well correlated with tumor necrosis factor α (TNF-α) ($r = 0.536$), and T lymphocyte count ($r = -0.679$) (Supplementary Fig. S2). There was a negative correlation between IgM antibody titer and T lymphocyte count ($r = -0.750$). Only low correlations were observed between IgG or IgM antibody titer with the other cytokine and lymphocyte parameters (Supplementary Figs. S3 and S4).

According to our results, SARS-CoV-2-specific IgM antibody titer can reach the peak levels by the early 3–4 weeks and was predicted to last for about two months. IgG titer can also reach the peak levels at 3–4 weeks and the diminish time was estimated to be 7 months after symptom onset. Patients with older age or severe disease had higher IgM antibody level during the disease, but with delayed IgG antibody production during early infection. In contrast, patients with older age or severe disease achieved a higher IgG level than patients of younger age and mild disease. This study has revealed the magnitude and longitude of the antibody response, which could be used as a useful indicator for diagnosis and for evaluation of reinfection in case of re-exposure to the virus. Future investigation integrating the quantification of virus copy number and the titer of neutralizing antibody might further enhance our understanding of the interplay between viral replication, antibody responses, and disease progression.

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