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Original Article

Impact of corticosteroids on initiation and half-year durability of humoral response in COVID-19 survivors

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ABSTRACT

Background: The impact of corticosteroids on humoral responses in coronavirus disease 2019 (COVID-19) survivors during the acute phase and subsequent 6-month period remains unknown. This study aimed to determine how the use of corticosteroids influences the initiation and duration of humoral responses in COVID-19 survivors 6 months after infection onset.

Methods: We used kinetic antibody data from the lopinavir–ritonavir trial conducted at Jin Yin-Tan Hospital in January 2020, which involved adults hospitalized with severe COVID-19 (LOTUS, ChiCTR2000029308). Antibody samples were collected from 192 patients during hospitalization, and kinetic antibodies were monitored at all available time points after recruitment. Additionally, plasma samples were collected from 101 COVID-19 survivors for comprehensive humoral immune measurement at the half-year follow-up visit. The main focus was comparing the humoral responses between patients treated with systemic corticosteroid therapy and the non-corticosteroid group.

Results: From illness onset to day 30, the median antibody titre areas under the receiver operating characteristic curve (AUCs) of nucleoprotein (N), spike protein (S), and receptor-binding domain (RBD) immunoglobulin G (IgG) were significantly lower in the corticosteroids group. The AUCs of N-, S-, and RBD-IgM as well as neutralizing antibodies (NAbs) were numerically lower in the corticosteroids group compared with the non-corticosteroid

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group. However, peak titres of N, S, RBD-IgM and -IgG and NAbs were not influenced by corticosteroids. During 6-month follow-up, we observed a delayed decline for most binding antibodies, except N-IgM (β −0.05, 95% CI [−0.10, 0.00]) in the corticosteroids group, though not reaching statistical significance. No significant difference was observed for NAbs. However, for the half-year seropositive rate, corticosteroids significantly accelerated the decay of IgA and IgM but made no difference to N-, S-, and RBD-IgG or NAbs. Additionally, corticosteroids group showed a trend towards delayed viral clearance compared with the non-corticosteroid group, but the results were not statistically significant (adjusted hazard ratio 0.71, 95% CI 0.50–1.00; $P = 0.0508$).

Conclusion: Our findings suggested that corticosteroid therapy was associated with impaired initiation of the antibody response but this did not compromise the peak titres of binding and neutralizing antibodies. Throughout the decay phase, from the acute phase to the half-year follow-up visit, short-term and low-dose corticosteroids did not significantly affect humoral responses, except for accelerating the waning of short-lived antibodies.

Introduction

Although corticosteroids have become standard care for patients with severe or critical coronavirus disease 2019 (COVID-19),[1-3] their initial use during the early stages of the pandemic was met with uncertainty.[4] The controversy arose owing to potential limited clinical benefit in managing respiratory viral infections and increased risk of post-viral infection in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) infections.[5-7] Understanding the impact of corticosteroid therapy on the initiation and duration of humoral responses in COVID-19 survivors remains elusive. Prior reports have indicated that corticosteroids might impair antigen-presenting cells,[8] suppress developmental germinal centers,[9] and curtail humoral immune responses during antiviral immune reactions.[10,11] Furthermore, corticosteroids may delay viral RNA clearance,[12] leading to differing antibody-level fluctuations. In this study, we hypothesized that corticosteroid therapy during the acute phase of SARS-CoV-2 infection might impede the initiation of adaptive immune responses,[13,14] resulting in a decline in subsequent humoral responses among COVID-19 survivors. Over the past 3 years, we have compiled databases of early anti-SARS-CoV-2 antibody responses and memory immune responses among patients with severe COVID-19 in Wuhan, China.[15-18] Here, we reanalyzed this comprehensive dataset to determine how the use of corticosteroids influences the initiation and duration of humoral responses in COVID-19 survivors 6 months after infection onset.

Methods

Ethical approval

The study was approved by the Research Ethics Commission of Jin Yin-tan Hospital (No. KY-2020–78.01). Written informed consent was obtained from all study participants.

Study participants and study design

All patients enrolled in this study had microbiologically confirmed SARS-CoV-2 infection; no participants experienced reinfection or received any COVID-19 vaccinations before assessment of immunogenicity. To assess the impact of corticosteroid therapy on the acute humoral immune response during hospitalization, we analyzed kinetic antibody data from the LOTUS trial (ChiCTR2000029308),[19] conducted at Jin Yin-tan Hospital in January 2020. Kinetic antibody level measurement was preformed at days 1, 5, 10, 14, 21, and 28 after recruitment, and continued until either death or discharge, whichever came first. Data on the kinetics of antibodies during the acute phase of infection were analyzed using robust locally weighted regression, with the *x*-axis representing days from illness onset to the time of sample collection. Additionally, survivors underwent face-to-face quality of life assessment and humoral immune measurement 6 months after infection. The median antibody titre was calculated using the area under the receiver operating characteristic curve (AUC). Therefore, we used data from the lopinavir–ritonavir (LPV-RTV) cohort in the LOTUS trial to assess the antibody response from the initial infection up to 6 months post infection.

Immunogenicity

Within the LPV-RTV cohort, we compared titres and seropositivity of SARS-CoV-2 antigen-specific antibodies between the corticosteroid and non-corticosteroid groups at all available time points. Additionally, we examined the kinetic changes in SARS-CoV-2-specific antibodies from hospitalization to the half-year follow-up visit after illness onset. The detailed protocol for these assessments has been previously described.[16]

Measurement of antibodies against SARS-CoV-2 antigens in participants' blood samples was performed using standardized enzyme-linked immunosorbent assay (ELISA) and microneutralization assays at the National Health Commission Key Laboratory of Systems Biology of Pathogens and Christophe Mérieux Laboratory, Beijing, China. These assays are used to detect titres of immunoglobulin A (IgA), IgM, IgG, and neutralizing antibodies (NAbs) against the nucleoprotein (N), spike protein (S), and receptor-binding domain (RBD) of SARS-CoV-2. Specifically, NAbs against the original SARS-CoV-2 strain were titrated on Vero cells using a microneutralization assay.

Corticosteroid therapy

The primary exposure in this study was systemic corticosteroid therapy. Inhalational corticosteroid therapy was not administered to any participants. All corticosteroid preparations, including methylprednisolone, prednisolone, dexamethasone, and/or hydrocortisone, were standardized and converted into methylprednisolone for analysis. Patients who did not receive corticosteroid treatment were classified as the control group.

The impact of therapeutic corticosteroids on the humoral immune response mainly depends on the dosage and duration of therapy, as well as the timing of treatment. To investigate the specific contribution of corticosteroid therapy parameters to the immune response, we assessed the immune response among patients based on the number of days from illness onset to the start of corticosteroid treatment (≤15 and >15 days, stratified according to the median value), the duration of corticosteroid therapy (<7, 7–10, and >10 days, according to World Health Organization management guidelines for COVID-19), and the accumulated dose (≤560 and >560 mg methylprednisolone or equivalent), in comparison with participants who did not receive corticosteroid therapy.

Statistical analysis

Continuous variables with skewed distribution are summarized as median (Q_1, Q_3). For categorical variables, the percentage of patients in each category was calculated. Clinical characteristics were compared between patients who received systemic corticosteroids and those who did not using the Mann–Whitney *U* test and chi-squared test, as appropriate.

To visualize the trend of changes in antibody titre over time (from baseline to day 28) in response to corticosteroid treatment, a locally weighted scatterplot smoothing method was applied. To compare the antibody response efficacy in the LPV-RTV inpatient cohort, the AUC for each patient was calculated as the antibody titre multiplied by time.

To compare the kinetics of antibody titres over time and the absolute antibody titres between participants with and without corticosteroids therapy, we used a log-linear classification model. The duration of antibody seropositivity was assessed using a logistic regression model. Generalized estimating equations (GEE) were used to study changes in seropositivity over time, with the significance level set at 0.05. Unadjusted and adjusted Cox models were applied to determine whether the use of corticosteroids affected viral shedding of SARS-CoV-2 in the LPV-RTV inpatient cohort. Statistical tests were used to confirm that the proportional hazards assumption was met.

To ascertain the impact of corticosteroids on humoral response and viral clearance, we used multivariable models, including log-linear classification, logistic regression, and Cox regression models, to confirm all corticosteroid-related findings while adjusting for important confounding variables. These covariates included age at infection, sex, comorbidities (hypertension, diabetes, cerebrovascular disease, chronic kidney disease, and malignancies), SARS-CoV-2 viral load, and disease severity upon admission (seven-category scale). Concomitant therapy with additional immunosuppressants or drugs that could potentially influence the antibody response and viral clearance was not administered. Statistical significance was set at $P < 0.05$ for all two-tailed tests. SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

Results

Patients and corticosteroid therapy

The blood sample collection protocol is presented in Fig. 1. Using antibody titre data from different time points among adults hospitalized with severe COVID-19 from the LPV-RTV trial,[18] we investigated the influence of corticosteroid therapy on initiation of the humoral immune response during hospitalization and the duration of antibody seropositivity up to 6 months after infection. During hospitalization, antibody samples were available from 192 patients. At the half-year follow-up visit after the initial infection, plasma samples were collected from 101 COVID-19 survivors to detect antibodies. Among the above 192 patients, 67 (34.9%) were in the corticosteroid group and 125 (65.1%) were in the non-corticosteroid group. The clinical and demographic characteristics of the two groups are presented in Table 1.

Impact of corticosteroid therapy on antibody kinetics during the acute phase of SARS-CoV-2 infection

We analyzed data from the LPV-RTV cohort to characterize the kinetics of antibody responses during hospitalization in both the corticosteroid and non-corticosteroid groups. To visualize the temporal changes in each antibody, we used a locally weighted scatterplot smoothing method to fit a smoothing curve. As depicted in Fig. 2, the titres of each antibody showed a significant increase over time, and the magnitude of this increase was similar between the corticosteroid and non-corticosteroid groups.

Subsequently, we sought to investigate whether corticosteroid therapy influenced the peak of antibodies against SARS-CoV-2 during hospitalization. For this analysis, we used a log-linear classification model, adjusting for age, gender, diabetes, malignancy and seven-category scale at day 1. Our results showed no significant impact of corticosteroid therapy on any antibodies against SARS-CoV-2, as summarized in Supplementary Table 1.

To assess the potential impact of corticosteroids on initiation of the immune response, we calculated the AUC of antibody titres against SARS-CoV-2-specific antigens from illness onset (baseline) to day 28. This AUC reflects the overall condition of immune response initiation following infection. Overall, the median AUCs of N-, S-, and RBD-IgG antibodies were significantly lower in the corticosteroid group than those in the non-corticosteroid group (median values: 20.5 *vs*. 23.5, 25.0 *vs*. 30.3, and 22.3 *vs*. 28.3, respectively; $P < 0.05$). Additionally, the AUCs of N-, S-, and RBD-IgM as well as of NAbs were numerically lower in the corticosteroid group (6.4 *vs*. 7.8, 8.3 *vs*. 10.2, 11.1 *vs*. 11.6, and 78.0 *vs*. 91.8, respectively) than those in the non-corticosteroid group. The dose, starting time, and duration of therapy were found to influence the chronological evolution of antibody titres in the acute phase. Specifically, for subgroup analysis, significant differences in AUCs for NAb titres were observed only in individuals initiating corticosteroid therapy within 15 days after illness onset (median 64.0, Q_1–Q_3 14.0–134.6, $P < 0.05$), those with a therapy duration of less than 7 days (median 67.4, Q_1–Q_3 19.0–137.0, $P < 0.05$) and those with an accumulated corticosteroid dose of less than 560 mg (median 67.8, Q_1–Q_3 24.5–134.9,

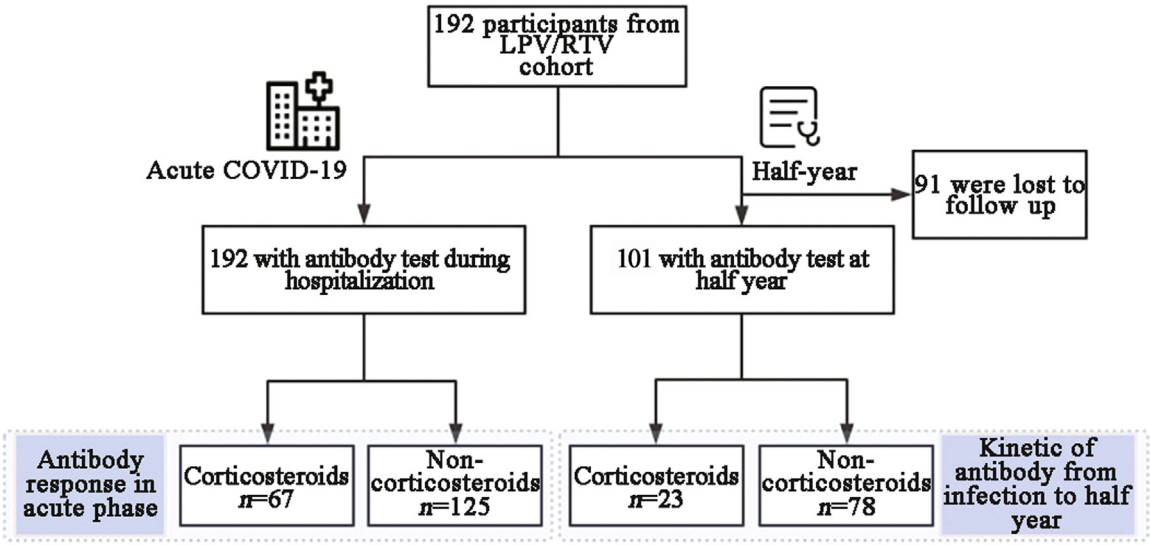

Fig. 1. Schematic of blood sampling and flowchart of analysis. Samples for immunogenicity measurement in the lopinavir–ritonavir (LPV-RTV) cohort were collected during hospitalization and at the half-year follow-up visit. The LPV-RTV cohort (excluding those lost to follow-up) was divided into corticosteroid and non-corticosteroid groups. COVID-19: Coronavirus disease 2019.

Table 1
Characteristics of participants with or without corticosteroid in lopinavir–ritonavir cohort.

Characteristics	Total ($n = 192$)	Non-corticosteroids ($n = 125$)	Corticosteroids ($n = 67$)	U/χ^2	P values
Age (years)	58.0 (49.0, 68.0)	57.0 (46.5, 68.0)	62.0 (52.0, 68.0)	6919.0000*	0.1815
Male	114/191 (59.7)	69/124 (55.6)	45/67 (67.2)	2.3986†	0.1214
Current smoking	14/191 (7.3)	6/124 (4.8)	8/67 (11.9)	3.6956†	0.0956
Comorbidity					
Hypertension	65/154 (42.2)	40/93 (43.0)	25/61 (41.0)	0.0621†	0.8033
Diabetes	23/191 (12.0)	13/124 (10.5)	10/67 (14.9)	0.8101†	0.3681
Cerebrovascular disease	13/191 (6.8)	8/124 (6.5)	5/67 (7.5)	0.0692†	0.7925
Chronic kidney disease	6/152 (3.9)	5/93 (5.4)	1/59 (1.7)	1.0372†	0.2286
Malignancies	6/191 (3.1)	5/124 (4.0)	1/67 (1.5)	1.4496†	0.3085
Seven-category scale at day 1				16.2978†	0.0003
3 Hospitalization, not requiring supplemental oxygen	27/191 (14.1)	17/124 (13.7)	10/67 (14.9)		
4 Hospitalization, requiring supplemental oxygen	136/191 (71.2)	98/124 (79.0)	38/67 (56.7)		
5 Hospitalization, requiring nasal high-flow oxygen therapy and/or non-invasive mechanical ventilation	28/191 (14.7)	9/124 (7.3)	19/67 (28.4)		
Illness onset to admission (days)	2.5 (0, 5.0)	3.0 (0, 5.0)	2.0 (0, 5.0)	6009.0000*	0.6393
Duration of corticosteroid therapy (days)	NA	NA	11.0 (4.0, 30.0)	NA	
Corticosteroids dose (equivalent methylprednisolone, mg/day)	NA	NA	560.0 (240.0, 903.8)	NA	
Days from illness onset to corticosteroids therapy (days)	NA	NA	13.0 (11.0, 17.0)	NA	
Hospital length of stay (days)	15.0 (12.0, 17.4)	15.0 (12.0, 16.0)	17.0 (12.0, 23.0)	6559.5000*	0.0095

Information is missing in some cases and the denominator is indicated. Disease severity was characterized using a seven-category scale during the hospital stay, which comprised the following categories: 1, not admitted to hospital with resumption of normal activities; 2, not admitted to hospital but unable to resume normal activities; 3, admitted to hospital but not requiring supplemental oxygen; 4, admitted to hospital and requiring supplemental oxygen; 5, admitted to hospital and requiring high-flow nasal cannula (HFNC), non-invasive mechanical ventilation (NIV), or both; 6, admitted to hospital and requiring extracorporeal membrane oxygenation, invasive mechanical ventilation (IMV), or both; and 7, death. Data are presented as median (Q_1, Q_3), or n/N (%) when data are missing. To correct for multiple comparisons between two groups of study participants with different severity scales, a Bonferroni corrected α-threshold of 0.0167 was used. *U value. †χ^2 value. NA: Not applicable.

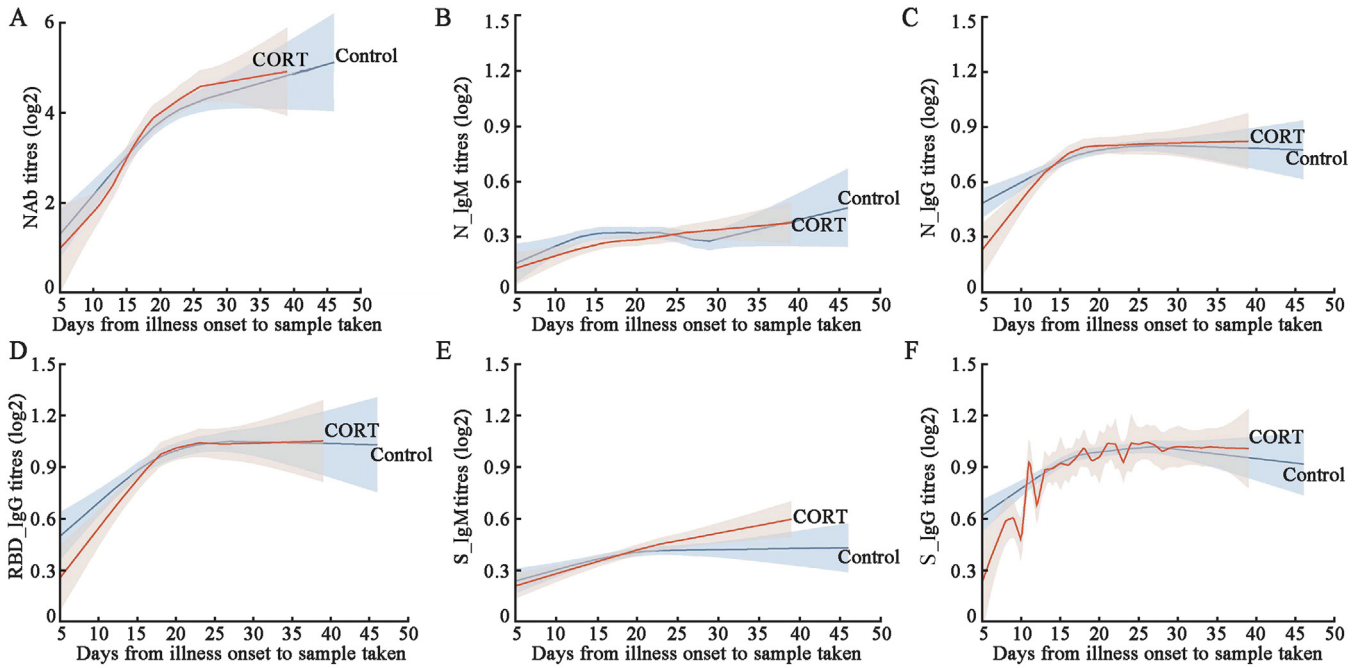

Fig. 2. Kinetics of titres of NAb (A), N_IgM (B), N_IgG (C), RBD_IgG (D), S_IgM (E) and S_IgG (F) against SARS-CoV-2 in the acute phase during hospitalization. The *y*-axis indicates antibody titres (log2). The *x*-axis indicates days from illness onset to sample taken. The red line refers to corticosteroids group, while the blue line refers to non-corticosteroids group. Immunoglobin (Ig) levels are shown as mean optical density (log2). CORT: Corticosteroid; Ig: Immunoglobulin; N: Nucleocapsid; NAb: Neutralizing antibody; RBD: Receptor-binding domain; S: Spike; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

P <0.05) compared with the non-corticosteroid group (Supplementary Table 2). Similar patterns were observed for other IgG and IgM antibodies against SARS-CoV-2 antigens in participants receiving corticosteroids within 15 days of illness onset, those with a therapy duration of less than 7 days, and those with an accumulated dose of less than 560 mg. However, for those who initiated therapy more than 15 days after illness onset, those with a duration of therapy longer than 7 days, and those with an accumulated corticosteroid dose of more than 560 mg, there were no significant differences when compared with the non-corticosteroid group (Table 2, Supplementary Table 2).

Table 2
Impact of corticosteroids on the AUC of antibodies during hospitalization adjusting for age, gender, diabetes, malignancy and seven-category scale at day 1 in the lopinavir–ritonavir cohort (N = 192).

Items	Neutralizing antibody	N_IgM	N_IgG	S_IgM	S_IgG	RBD_IgM	RBD_IgG
Corticosteroids (n = 67, 34.9%)	−32.64 (−70.22, 4.93)	−3.89 (−7.82, 0.04)	−5.92 (−11.48, −0.36)	−1.66 (−6.26, 2.95)	−8.52 (−16.02, −1.02)	−1.40 (−6.31, 3.52)	−9.80 (−18.00, −1.60)
Initiation from illness onset							
≤15 d (n = 42, 21.9%)	−65.51 (−108.45, −22.57)	−7.01 (−11.52, −2.50)	−10.19 (−16.58, −3.81)	−5.03 (−10.32, 0.27)	−13.57 (−22.22, −4.92)	−4.68 (−10.35, 0.99)	−15.31 (−24.77, −5.85)
>15 d (n = 25, 13.0%)	24.23 (−28.82, 77.29)	1.51 (−4.06, 7.09)	1.47 (−6.42, 9.36)	4.18 (−2.37, 10.72)	0.22 (−10.47, 10.91)	4.29 (−2.71, 11.30)	−0.26 (−11.95, 11.43)
Duration of therapy							
<7 d (n = 25, 13.0%)	−82.18 (−134.66, −29.71)	−5.36 (−10.94, 0.22)	−13.38 (−21.14, −5.62)	−6.65 (−13.11, −0.18)	−17.12 (−27.60, −6.64)	−9.05 (−15.85, −2.25)	−17.18 (−28.67, −5.69)
7–10 d (n = 8, 4.2%)	−58.49 (−146.38, 29.41)	−9.10 (−18.44, 0.25)	−8.93 (−21.93, 4.07)	−5.55 (−16.38, 5.28)	−17.73 (−35.29, −0.18)	−5.07 (−16.46, 6.32)	−23.40 (−42.65, −4.15)
>10 d (n = 34, 17.7%)	9.97 (−36.76, 56.70)	−1.63 (−6.60, 3.34)	0.30 (−6.61, 7.22)	2.92 (−2.83, 8.68)	−0.06 (−9.39, 9.27)	5.11 (−0.94, 11.17)	−1.27 (−11.51, 8.96)
Accumulated dose							
≤ 560 mg (n = 35, 18.2%)	−77.54 (−123.94, −31.13)	−5.96 (−10.92, −1.01)	−13.02 (−19.85, −6.18)	−6.69 (−12.40, −0.98)	−18.41 (−27.62, −9.21)	−8.08 (−14.10, −2.06)	−19.20 (−29.35, −9.04)
> 560 mg (n = 32, 16.7%)	14.14 (−33.01, 61.29)	−1.72 (−6.76, 3.31)	1.48 (−5.47, 8.42)	3.59 (−2.21, 9.39)	1.80 (−7.56, 11.15)	5.57 (−0.55, 11.69)	−0.01 (−10.32, 10.31)

Data are presented as β and 95% CI with non-corticosteroid group (n = 125, 65.1%) as reference. CIs have been adjusted, but the sample size was small. Generalized estimating equations were used to analyze repeated measurement data across two time points in each category. AUC: Area under the receiver operating characteristic curve; CIs: Confidence intervals; Ig: Immunoglobulin; N: Nucleocapsid; RBD: Receptor-binding domain; S: Spike.

Changes in the seropositive rate and antibody kinetics 6 month after infection influenced by corticosteroid therapy

At the half-year follow-up visit for the LPV-RTV cohort, more than 90% of individuals still tested positive for IgG against SARS-CoV-2 antigens (Supplementary Fig. 1, Supplementary Table 3). However, the proportion of individuals testing positive for IgM and IgA (short-lived antibodies) decreased significantly from the acute phase to the half-year follow-up visit (Fig. 3, Supplementary Table 3). Furthermore, although most antibodies showed a delayed decline in the corticosteroid group compared with the non-corticosteroid group, statistical significance was not reached for most cases (Supplementary Table 4). Notably, only the waning of N-IgM antibodies reached significance with corticosteroid therapy (β −0.05, 95% CI −0.10, 0.00). Upon closer examination of corticosteroid treatment details, an accumulated corticosteroid dose of less than 560 mg was associated with a delayed decline in N-IgM (β −0.08, 95% CI −0.14, −0.01), which reached statistical significance. Although corticosteroid therapy did not show a significant impact on the decline of S-IgM levels (β −0.03, 95% CI −0.08, 0.01), subgroup analysis revealed a delayed decline in S-IgM antibodies in the cortosteroid group compared with the non-corticosteroid group when the therapy was initiated within 15 days from illness onset (β −0.06, 95% CI −0.11, −0.01) after adjusting for age, gender, diabetes, malignancy and seven-category scale at day 1 (Supplementary Table 4).

For the seropositive rate at the half-year follow-up visit, corticosteroid therapy accelerated the decay of N-, RBD-, and S-IgM and of N-, RBD-, and S-IgA (odds ratio [OR] 0.37 [95% CI 0.20–0.70], OR 0.50 [95% CI 0.26–0.96], and OR 0.46 [95% CI 0.26–0.81]; OR 0.41 [95% CI 0.21–0.81], OR 0.27 [95% CI 0.11–0.69], and OR 0.24 [95% CI 0.10–0.60], respectively) (Table 3). Corticosteroid group showed no significant impact on N-, RBD-, and S-IgG antibodies compared with the non-corticosteroid group (1.10 [95% CI 0.27–4.42], 1.00 [95% CI 0.25–3.94], and 0.16 [95% CI 0.01–2.10], respectively).

NAbs against SARS-CoV-2 were generally positive in 62 of 65 (95.4%) individuals in the non-corticosteroid group and 21 of 23 (91.3%) in the corticosteroid group over the 6 months following SARS-CoV-2 infection (Supplementary Table 3). There was no significant difference in the seropositivity rate at the half-year follow-up visit between individuals with and without corticosteroid therapy (Table 3). Furthermore, the decline in NAbs between the peak titres during hospitalization and the half-year follow-up visit in the corticosteroid group did not show significant differences compared with the non-corticosteroid group (Fig. 4, Supplementary Table 4).

Effect of corticosteroids on viral clearance

The main concern hindering the use of steroids in COVID-19 treatment is their potential effect on delayed viral clearance. To investigate this, we used a Cox regression model, analyzing longitudinal viral titre data from nasopharyngeal swabs collected during the LPV-RTV trial. Among the 192 patients in the LPV-RTV cohort, corticosteroid administration was associated with a delay in SARS-CoV-2 viral clearance (hazard ratio [HR] 0.69, 95% CI 0.49–0.98). However, after adjusting for age, gender, diabetes, malignancy and seven-category scale at day 1, corticosteroids did not show a significant impact on viral clearance (HR 0.71, 95% CI 0.50–1.00) (Supplementary Table 5), although a trend in this direction was still observed.

Discussion

Despite several randomized controlled trials (RCTs) demonstrating the benefits of corticosteroids in acute respiratory distress syndrome, clinical practice in this respect remains variable. Debate regarding the impact of steroids on antibody kinetics in COVID-19 has been ongoing. In the present study, we found that corticosteroid therapy had a

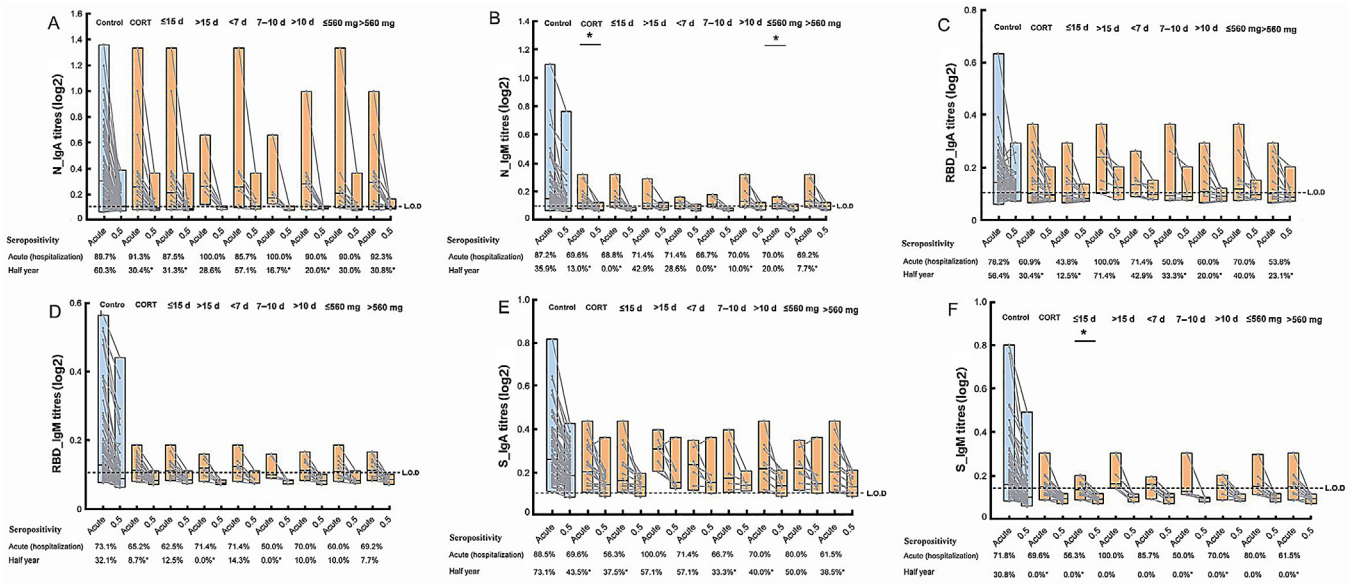

Fig. 3. Kinetics of antigen-specific antibody titres (short-lived) from acute infection to 6-month follow-up in participants with and without corticosteroids. SARS-CoV-2 antigen-specific antibody titres at peak immunity (peak titres during hospitalization) and at the half-year follow-up visit in the lopinavir–ritonavir cohort are shown. Bars in each column indicate medians, each dot indicates a single participant, and gray lines denote individual neutralizing antibody titres between two time points. Panels A and B (N_IgA and N_IgM titres), C and D (RBD_IgA and RBD_IgM titres), and E and F (S_IgA and S_IgM titres) show titres and seropositivity changes in the antibody assay from acute infection to 6 months. All assays were performed using the original SARS-CoV-2 strain. Samples were measured using enzyme-linked immunosorbent assay. The two rows of percentages beneath the chart denote the seropositive rates during the acute phase and at the 6 month, respectively. Acute=acute infection (hospitalization); 0.5=0.5 years after SARS-CoV-2 infection; Control=non-corticosteroids; CORT=corticosteroids; ≤15 d and >15 d=initiation of corticosteroids from illness onset ≤15 days and >15 days; <7 d, 7–10 d, and >10 d=duration of corticosteroid therapy <7 days, 7–10 days, and >10 days; ≤560 mg and >560 mg=accumulated corticosteroid dose ≤560 mg and >560 mg. *indicates statistical significance compared with the non-corticosteroid group, which was estimated using a log-linear classification estimating model. Ig: Immunoglobulin; L.O.D: Limit of detection; N: Nucleocapsid; RBD: Receptor-binding domain; S: Spike; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

notable effect on impairing initiation of the antibody response among hospitalized patients with COVID-19 during the acute phase, leading to reduced levels of N-, S-, and RBD-IgM and NAbs in a numerical manner and significantly lower N-, S-, and RBD-IgG antibodies. However, peak titres of antibodies (IgM, IgG, and NAbs) during the acute phase were not influenced by corticosteroids. Subsequently, corticosteroids induced a delayed decline in SARS-CoV-2-specific binding antibodies (IgM, IgA, and IgG) from the acute phase to the half-year follow-up visit. In terms of the 6-month seropositivity rate, corticosteroid therapy accelerated the decay of N-, S-, and RBD-IgM and -IgA antibodies. However, the seropositivity rates of N-, S-, and RBD-IgG, and NAbs over a half-year period were comparable between the corticosteroid group and non-corticosteroid group, as were the kinetics of NAbs during the declining period.

We identified the peak time of the acute phase as a reference point to divide the post-infection period into two phases: the pre-peak phase and the declining phase. A previous study did not observe a delay in the primary immune response with steroid therapy.[20] However, we observed an impaired antibody titre escalation rate among COVID-19 survivors treated with corticosteroids during the acute phase. The impaired rate of antibody titre elevation may be attributed to impaired generation and function of dendritic cells in participants receiving corticosteroid therapy.[21-23] Further investigation is required to fully elucidate the underlying mechanism. Notably, however, the peak antibody titres were not significantly affected by corticosteroids, consistent with previous research suggesting that individuals receiving corticosteroids can still mount an adequate antibody response after vaccination.[24] Therefore, we can conclude that corticosteroids weaken the mobilization time and response speed of antibodies against SARS-CoV-2 without affecting the ultimate peak and acute-phase response capacity ceiling.

In the decay phase (from the acute phase to half-year follow-up), corticosteroids had an accelerated effect on decay of short-lived antibodies at 6 months. However, corticosteroids use did not significantly affect protective immunity within the first 6 months. This observation is consistent with previous research indicating that dexamethasone does not lead to changes in the antibody response during the first few months among immunocompetent patients hospitalized with severe COVID-19.[25] Furthermore, corticosteroids did not show a statistically significant effect on viral clearance, although a trend was observed. This finding is in line with previous research indicating that low-dose steroids do not impede viral clearance in COVID-19.[25,26] In summary, the non-significant results for virus clearance and antibody response complemented each other, and the implementation of corticosteroids did not harm long-term protective immunity in the convalescence phase.

We acknowledge several limitations in this study, including a relatively small number of participants in the LPV-RTV cohort. Despite this, our current data consistently revealed a discernible trend in the impact of corticosteroids across different types of antibody, strongly suggesting credibility regarding the corticosteroid effect. However, it is important to emphasize that the estimated effect values derived from the present small sample warrant cautious interpretation. Further studies with larger sample sizes are necessary to validate and strengthen these findings. Moreover, the distribution of the population across different subgroups exhibited heterogeneity. In the pre-peak phase, corticosteroids significantly impaired the AUCs of binding antibody and NAb titres only in subgroups that included patients initiating therapy within 15 days after illness onset, those with a therapy duration of less than 7 days (overlapping with the 15-day period after illness onset), and those receiving an accumulated corticosteroid dose of less than 560 mg. This phenomenon can be explained by the large proportion of the population with early timing of corticosteroid management, along with the rela-

Table 3
Impact of corticosteroids on duration of antibody seropositivity against SARS-CoV-2 antigen at the half-year follow-up visit (N = 101).

Items	Neutralizing antibody	N_IgM	N_IgA	N_IgG	S_IgM	S_IgA	S_IgG	RBD_IgM	RBD_IgA	RBD_IgG
Corticosteroids (n = 23, 22.8%)	0.68 (0.12–3.96)	0.37 (0.20–0.70)	0.41 (0.21–0.81)	1.10 (0.27–4.42)	0.46 (0.26–0.81)	0.24 (0.10–0.60)	0.16 (0.01–2.10)	0.50 (0.26–0.96)	0.27 (0.11–0.69)	1.00 (0.25–3.94)
Initiation from illness onset										
≤15 days (n = 16, 15.8%)	NA	0.25 (0.13–0.49)	0.37 (0.17–0.80)	NA	0.31 (0.15–0.67)	0.12 (0.04–0.36)	NA	0.53 (0.24–1.19)	0.09 (0.03–0.25)	NA
>15 days (n = 7, 6.9%)	NA	0.82 (0.29–2.26)	0.53 (0.21–1.35)	NA	0.96 (0.66–1.40)	0.93 (0.26–3.28)	NA	0.45 (0.21–0.98)	3.04 (0.65–14.34)	NA
Duration of therapy										
<7 d (n = 7, 6.9%)	NA	0.55 (0.20–1.48)	0.71 (0.17–3.00)	NA	0.65 (0.34–1.27)	0.35 (0.06–1.92)	NA	0.66 (0.25–1.75)	0.49 (0.08–2.96)	NA
7–10 d (n = 6, 5.9%)	NA	0.24 (0.09–0.62)	0.31 (0.13–0.71)	NA	0.27 (0.08–0.87)	0.15 (0.04–0.64)	NA	0.27 (0.08–0.86)	0.18 (0.04–0.82)	NA
>10 d (n = 10, 9.9%%)	NA	0.37 (0.16–0.82)	0.34 (0.16–0.70)	NA	0.47 (0.22–1.00)	0.24 (0.08–0.72)	NA	0.57 (0.24–1.39)	0.23 (0.08–0.63)	NA
Accumulated corticosteroid dose										
≤ 560 mg (n = 10, 9.9%)	0.59 (0.06–5.61)	0.43 (0.18–1.03)	0.39 (0.15–1.05)	1.52 (0.25–9.21)	0.57 (0.30–1.10)	0.34 (0.09–1.25)	NA	0.46 (0.18–1.18)	0.40 (0.09–1.67)	1.39 (0.13–14.76)
> 560 mg (n = 13, 12.9%)	0.76 (0.08–7.14)	0.33 (0.16–0.70)	0.43 (0.20–0.89)	0.89 (0.16–4.87)	0.39 (0.18–0.82)	0.19 (0.06–0.54)	NA	0.54 (0.25–1.16)	0.20 (0.07–0.55)	0.81 (0.19–3.46)

Data are presented as OR and 95% CI with non-corticosteroid group (n = 78, 77.2%) as reference after adjusting for age, gender, diabetes, malignancy and seven-category scale at day 1 (7-category ordinal scale that ranges from 1 [discharged with normal activity] to 7 [death]) in hospital. Generalized estimating equations were used to analyze repeated measurement data across two time points in each category. OR >1 indicates delayed effect on negative conversion of antibody, and OR <1 indicates promoted effect on negative conversion. CI: Confidence interval; Ig: Immunoglobulin; N: Nucleocapsid; NA: Not applicable; OR: Odds ratio; RBD: Receptor-binding domain; S: Spike; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2..

Fig. 4. Kinetics of neutralizing antibody titres influenced by the corticosteroid category compared with the non-corticosteroid group from acute infection to the half-year visit. Neutralizing antibody titres against the original SARS-CoV-2 strain at peak immunity (peak titres during hospitalization) and at the half-year follow-up visit are shown. Bars in each column indicate medians, each dot indicates a single participant, and gray lines denote individual neutralizing antibody titres between two time points. Figure shows titres change in the live-virus neutralizing antibody assay from acute infection to 6 months in the lopinavir–ritonavir (LPV-RTV) cohort. All assays were performed using the original SARS-CoV-2 strain. The results of neutralizing antibody titres are reported as logarithmic values. Acute=acute infection (hospitalization); 0.5=0.5 years after SARS-CoV-2 infection; Control=non-corticosteroids; CORT=corticosteroids; ≤15 d and >15 d=initiation of corticosteroids from illness onset ≤15 days and >15 days; <7 d, 7–10 d, and >10 d=duration of therapy of corticosteroids <7 days, 7–10 days, and >10 days; ≤560 mg and >560 mg=accumulated corticosteroid dose ≤ 560 mg and > 560 mg. ELISA: Enzyme-linked immunosorbent assay; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

tionship between the early usage of corticosteroids and impaired AUC values.

Knowledge of the specific antibody kinetics after infection can play a crucial role in diagnosing and predicting disease progression. Subsequent subgroup analyses from the Recovery trial[27] revealed that patients testing positive for COVID-19 antibodies upon hospital admission have a better prognosis. Considering the global prevalence of COVID-19 for more than 3 years at the time of writing, it can be inferred that each country has developed herd immunity to some degree, leading to the ability to detect antibodies against SARS-CoV-2 in a substantial portion of the population. Notably, however, detection of viral antibodies may not be possible among individuals with compromised immune systems. Therefore, the use of corticosteroids requires careful consideration of the pros and cons in this subset of the population. There is a growing need for randomized controlled clinical trials that specifically target this subgroup to determine the potential benefits. Additionally, the detection of IgA, IgM, IgG, and NAbs against SARS-CoV-2 antigens could serve as an alternative diagnostic tool with diminished sensitivity of reverse transcription-polymerase chain reaction (RT–PCR). Moreover, the antibody profile has the potential to assist in serologic surveillance and serve as a biomarker for evaluating the risk of reinfection. These insights have important implications for clinical practice and public health strategies in managing COVID-19 cases. Further investigation is warranted to fully understand the implications of corticosteroid therapy in humoral immunity and its long-term effects on COVID-19 survivors.

In this study, we newly observed the divergent effects of corticosteroid therapy on the initiation of antibody responses during the acute phase of SARS-CoV-2 infection and at 6 months. Specifically, corticosteroid therapy was associated with an impaired rate of mounting an antibody response without influencing the maximum value. Importantly, protective immunity over the 6-month period following SARS-CoV-2 infection was not affected by corticosteroids. These significant findings

warrant validation in future studies and call for re-evaluation in patients requiring short-term corticosteroid use.

Availability of data and materials

Data will be made available on request.

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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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pccm.2024.02.005.

References

1. Agarwal A, Hunt B, Stegemann M, et al. A living WHO guideline on drugs for covid-19. *BMJ*. 2020;370:m3379. doi:10.1136/bmj.m3379.
2. Bhimraj A, Morgan RL, Shumaker AH, et al. Infectious Diseases Society of America guidelines on the treatment and management of patients with COVID-19. *Clin Infect Dis*. 2020:ciaa478. doi:10.1093/cid/ciaa478.
3. Corticosteroids. *COVID-19 Treatment Guidelines*; 2023. Updated April 20, 2023. Available from: https://www.covid19treatmentguidelines.nih.gov/therapies/cimmunomodulators/orticosteroids/[Last Accessed on April 9, 2023].
4. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet*. 2020;395:473–475. doi:10.1016/S0140-6736(20)30317-2.
5. Li H, Chen C, Hu F, et al. Impact of corticosteroid therapy on outcomes of persons with SARS-CoV-2, SARS-CoV, or MERS-CoV infection: a systematic review and meta-analysis. *Leukemia*. 2020;34:1503–1511. doi:10.1038/s41375-020-0848-3.
6. Arabi YM, Mandourah Y, Al-Hameed F, et al. Corticosteroid therapy for critically ill patients with middle east respiratory syndrome. *Am J Respir Crit Care Med*. 2018;197:757–767. doi:10.1164/rccm.201706-1172OC.
7. Lee N, Allen Chan KC, Hui DS, et al. Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. *J Clin Virol*. 2004;31:304–309. doi:10.1016/j.jcv.2004.07.006.
8. Fauci AS, Pratt KR, Whalen G. Activation of human B lymphocytes. IV. Regulatory effects of corticosteroids on the triggering signal in the plaque-forming cell response of human peripheral blood B lymphocytes to polyclonal activation. *J Immunol*. 1977;119:598–603.
9. Cooper MD, Weller EM. Developmental suppression of germinal center and IgG production by prednisolone and 6-mercaptopurine. In: Fiore-Donati L, Hanna MG, eds. *Lymphatic Tissue and Germinal Centers in Immune Response: Proceedings of the Second International Conference on Germinal Centers of Lymphatic Tissue, held in Padua, Italy, June 26–28, 1968*. New York: Springer New York; 1969:277–284.
10. Ashwell JD, Lu FW, Vacchio MS. Glucocorticoids in T cell development and function*. *Annu Rev Immunol*. 2000;18:309–345. doi:10.1146/annurev.immunol.18.1.309.
11. Strehl C, Ehlers L, Gaber T, Buttgereit F. Glucocorticoids-all-rounders tackling the versatile players of the immune system. *Front Immunol*. 2019;10:1744. doi:10.3389/fimmu.2019.01744.
12. Hui DS. Systemic corticosteroid therapy may delay viral clearance in patients with middle east respiratory syndrome coronavirus infection. *Am J Respir Crit Care Med*. 2018;197:700–701. doi:10.1164/rccm.201712-2371ED.
13. Akondy RS, Fitch M, Edupuganti S, et al. Origin and differentiation of human memory CD8 T cells after vaccination. *Nature*. 2017;552:362–367. doi:10.1038/nature24633.
14. Hartley GE, Edwards E, Aui PM, et al. Rapid generation of durable B cell memory to SARS-CoV-2 spike and nucleocapsid proteins in COVID-19 and convalescence. *Sci Immunol*. 2020;5:eabf8891. doi:10.1126/sciimmunol.abf8891.
15. Ren L, Fan G, Wu W, et al. Antibody responses and clinical outcomes in adults hospitalized with severe Coronavirus disease 2019 (COVID-19): a post hoc analysis of LOTUS China trial. *Clin Infect Dis*. 2021;72:e545–e551. doi:10.1093/cid/ciaa1247.
16. Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet*. 2021;397:220–232. doi:10.1016/S0140-6736(20)32656-8.
17. Guo L, Wang G, Wang Y, et al. SARS-CoV-2-specific antibody and T-cell responses 1 year after infection in people recovered from COVID-19: a longitudinal cohort study. *Lancet Microbe*. 2022;3:e348–e356. doi:10.1016/S2666-5247(22)00036-2.
18. Huang L, Yao Q, Gu X, et al. 1-year outcomes in hospital survivors with COVID-19: a longitudinal cohort study. *Lancet*. 2021;398:747–758. doi:10.1016/S0140-6736(21)01755-4.
19. Cao B, Wang Y, Wen D, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. *N Engl J Med*. 2020;382:1787–1799. doi:10.1056/NEJMoa2001282.
20. Hsueh PR, Huang LM, Chen PJ, Kao CL, Yang PC. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. *Clin Microbiol Infect*. 2004;10:1062–1066. doi:10.1111/j.1469-0691.2004.01009.x.
21. Piemonti L, Monti P, Allavena P, et al. Glucocorticoids affect human dendritic cell differentiation and maturation. *J Immunol*. 1999;162:6473–6481.
22. Rozkova D, Horvath R, Bartunkova J, Spisek R. Glucocorticoids severely impair differentiation and antigen presenting function of dendritic cells despite upregulation of toll-like receptors. *Clin Immunol*. 2006;120:260–271. doi:10.1016/j.clim.2006.04.567.
23. Matyszak MK, Citterio S, Rescigno M, Ricciardi-Castagnoli P. Differential effects of corticosteroids during different stages of dendritic cell maturation. *Eur J Immunol*. 2000;30:1233–1242. doi:10.1002/(SICI)1521-4141(200004)30:4<1233::AID-IMMU1233>3.0.CO;2-F.
24. Kubiet MA, Gonzalez-Rothi RJ, Cottey R, Bender BS. Serum antibody response to influenza vaccine in pulmonary patients receiving corticosteroids. *Chest*. 1996;110:367–370. doi:10.1378/chest.110.2.367.
25. Mühlemann B, Thibeault C, Hillus D, et al. Impact of dexamethasone on SARS-CoV-2 concentration kinetics and antibody response in hospitalized COVID-19 patients: results from a prospective observational study. *Clin Microbiol Infect*. 2021;27:1520 e7-1520.e10. doi:10.1016/j.cmi.2021.06.008.
26. Fang X, Mei Q, Yang T, et al. Low-dose corticosteroid therapy does not delay viral clearance in patients with COVID-19. *J Infect*. 2020;81:147–178. doi:10.1016/j.jinf.2020.03.039.
27. Casirivimab and imdevimab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. 2022;399:665–676. doi:10.1016/S0140-6736(22)00163-5.