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Effect of Corticosteroids on Long-Term Humoral and Memory T-Cell Responses in Follow-Up Visit of Hospitalized Patients With COVID-19

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BACKGROUND: Corticosteroids have beneficial effects in improving outcomes in hospitalized patients with severe COVID-19 by suppressing excessive immune responses. However, the effect of corticosteroids on the humoral and T-cell responses of survivors of COVID-19 1 year after infection remains uncertain, as it relates to the extent of immediate, antigen-specific defense provided by protective memory.

RESEARCH QUESTION: What is the effect of corticosteroids on long-term humoral and T-cell immune responses?

STUDY DESIGN AND METHODS: In this retrospective cohort study conducted at a single center, we analyzed data from a cohort who had survived COVID-19 to compare the 1-year seropositivity and titer changes in neutralizing antibodies (NAbs) and SARS-CoV-2-specific antibodies. Additionally, we evaluated the magnitude and rate of SARS-CoV-2-specific T-cell response in individuals who received corticosteroids during hospitalization and those who did not.

RESULTS: Our findings indicated that corticosteroids do not statistically influence the kinetics or seropositive rate of NAbs against the Wuhan strain of SARS-CoV-2 from 6 months to 1 year. However, subgroup analysis revealed a numerical increase of NAbs titers, from 20.0 to 28.2, in categories where long-term (> 15 days) and high-dose (> 560 mg) corticosteroids were administered. Similarly, corticosteroids showed no significant effect on nucleoprotein and receptor-binding domain IgG at 1 year, except for spike protein IgG (β, 0.08; 95% CI, 0.04-0.12), which demonstrated a delayed decline of titers. Regarding T-cell immunity, corticosteroids did not affect the rate or magnitude of T-cell responses significantly. However, functional assessment of memory T cells revealed higher interferon-γ responses in CD4 (β, 0.61; 95% CI, 0.10-1.12) and CD8 (β, 0.63; 95% CI, 0.11-1.15) memory T cells in the corticosteroids group at 1 year.

INTERPRETATION: Based on our findings, short-term and low-dose corticosteroid therapy during hospitalization does not appear to have a significant effect on long-term humoral kinetics or the magnitude and rate of memory T-cell responses to SARS-CoV-2 antigens. However, the potential harmful effects of long-term and high-dose corticosteroid use on memory immune responses require further investigation. CHEST 2024; 166(2):281-293

KEY WORDS: antibody; corticosteroid; COVID-19; immunological memory; protective immune; T cell

Take-home Points

Study Question: What is the impact of corticosteroids on long-term humoral and T-cell immune responses?

Results: Short-term and low-dose corticosteroid therapy during hospitalization does not have a significant effect on long-term humoral kinetics, nor on the magnitude and rate of memory T-cell responses to SARS-CoV-2 antigens.

Interpretation: These findings have implications for assessing vaccination boosting and reinfection risk among individuals recovering from COVID-19 who received corticosteroid treatment.

Since the groundbreaking Randomized Evaluation of COVID-19 Therapy (RECOVERY) clinical trial,[1] the use of corticosteroids has been recommended widely for patients with severe or critical COVID-19.[2-4] However, specific studies focusing on the effect of corticosteroids on humoral and cellular immune response in individuals 1 year after SARS-CoV-2 infection are lacking. The use of corticosteroids theoretically has the potential to impair the antigen presentation ability of antigen-presenting cells,[5] which may have detrimental effects on both humoral and cellular immune responses against viruses.[6,7] Moreover, corticosteroid therapy has been observed to delay viral clearance in severe acute respiratory syndrome coronavirus (SARS-CoV), middle east respiratory syndrome-coronavirus (MERS-CoV), and SARS-CoV-2 infections.[8,9] Overall, despite the extensive use of corticosteroids in COVID-19 treatment, our understanding of their influence on the long-term durability of humoral and cellular immune memory in survivors of COVID-19 remains limited. Therefore, we formulated the hypothesis that corticosteroid therapy during the acute phase of SARS-CoV-2 infection may influence the long-term decline of antibodies and immune memory in survivors of COVID-19. Over the past 2 years, we have established databases containing information on anti-SARS-CoV-2 antibody titers and immune memory in adults with severe COVID-19 in Wuhan, China.[10-13] In this study, we reanalyzed the database to investigate whether and how corticosteroid therapy affects the long-term durability of humoral and cellular immune responses among survivors of COVID-19.

Study Design and Methods

Study Design and Population

In this retrospective cohort study, we recruited 1,096 participants who had recovered and been discharged from the Wuhan Research Center for Communicable Disease Diagnosis and Treatment at the Chinese Academy of Medical Sciences, Wuhan, China, between January 7 and May 29, 2020.[12,14] Time 0 refers to initial infection or symptom onset at the acute phase of COVID-19. The inclusion and exclusion criteria were outlined previously, in both the 6-month and 1-year follow-up articles.[12-14] The follow-up visit for this cohort of COVID-19 survivors was conducted at the research center between December 16, 2020, and January 27, 2021 (nearly 1 year after infection), which occurred at a median duration of 347 days from the onset of symptoms [(25th percentile, 75th percentile, 336, 358)].

Venous blood was collected from all 1,096 patients at 1 year; samples from 141 patients were collected at the follow-up visits both 6 months

ABBREVIATIONS: ELISpot = enzyme-linked immunospot; E/ORF = envelope protein-open reading frame; ICS = intracellular cytokine staining; IFNγ = interferon-γ; N = nucleoprotein; NAb = neutralizing antibody; PBMC = peripheral blood mononuclear cell; RBD = receptor-binding domain; S = spike protein; TNFα = tumour necrosis factor α

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(between June 16 and September 3, 2020) and 12 months after initial infection. The microneutralization assay and T-cell functional experiments (e-Appendix 1) need to be conducted in a biosafety level 3 laboratory, and 141 samples represent the most we could test for neutralizing antibodies (NAbs) and T-cell responses based on our detection capacity. The sampling of 141 of 1,096 participants was based on the overall distribution of categories three through six of the seven-category scale, with an increased proportion of the fifth group as adjusted. The limited amount of peripheral blood mononuclear cells (PBMCs) from certain individuals prevented us from conducting T-cell functional assessment; thus, only 92 of 141 participants could be tested for intracellular cytokine staining (ICS) and with enzyme-linked immunospot (ELISpot) assays.

All patients included in the study had microbiologically confirmed SARS-CoV-2 infection, and none of participants had been reinfected by the SARS-CoV-2 virus or had received any type of COVID-19 vaccinations before the immunogenicity evaluation. To evaluate the immune response at 1 year after infection, we assessed SARS-CoV-2 antigen-specific antibodies and T-cell responses. NAbs were assessed at both 6 months and 1 year. The primary outcomes were NAbs titers and T-cell responses. T-cell responses were evaluated by the rate and magnitude of interferon-γ (IFNγ), IL-2, and tumour necrosis factor α (TNFα) generated within SARS-CoV-2-specific CD4 and CD8 T cells. The secondary outcomes encompassed IgM, IgA, and IgG antibody levels. Collectively, we analyzed the differences in long-lasting immunoreactivity of both antibodies and memory T cells after natural infection between groups receiving and not receiving corticosteroids.

Corticosteroid Therapy

The main focus of this study was to examine the effect of systemic corticosteroid use on the humoral and cellular immune response. It is important to note that no participants received inhalational corticosteroid therapy. The different preparations of corticosteroids used in the study, including methylprednisolone, prednisolone, dexamethasone, hydrocortisone, or a combination thereof were converted into methylprednisolone for the purpose of analysis. The control group comprised patients who did not receive corticosteroid treatment.

The use of therapeutic corticosteroids can affect both humoral and cellular immune responses, and the extent of this effect is influenced primarily by the dosage and duration of therapy. To investigate the specific relationship between corticosteroid therapy parameters (such as therapy duration or dosage) and the immune response, we stratified accordingly and carried out a subgroup analysis. We examined the immune response in relationship to the number of days from illness onset to the initiation of corticosteroid treatment ($\leq$ 15 days and $>$ 15 days), based on the median value. For the duration of corticosteroid therapy ($<$ 7 days, 7-10 days, and $>$ 10 days), it was stratified according to the World Health Organization management guidelines for COVID-19: patients with severe or critical COVID-19 should be given low-dose corticosteroids for 7 to 10 days.[15] And the accumulated dose of corticosteroids was separated between $\leq$ 560 mg methylprednisolone (or equivalent) and $>$ 560 mg.

Immunogenicity

In the cohort of COVID-19 survivors , we conducted a 1-year follow-up visit to assess the seropositivity and titers of SARS-CoV-2 antigen-specific antibodies. From this cohort, we randomly selected blood samples from 141 participants for neutralizing antibody tests at both the 6 month and 1-year follow-up visits. Among these participants, only 79 completed the measurement of memory T-cell responses using the interferon ELISpot assay, and 94 underwent the measurement of functional specific T cells after exposure to SARS-CoV-2 peptide pools.

The blood samples were analyzed using standardized enzyme-linked immunosorbent assays and a microneutralization assay in the laboratory. These assays, conducted at the National Health Commission Key Laboratory of Systems Biology of Pathogens and the Christophe Mérieux Laboratory, aimed to detect the levels of IgM, IgA, and IgG antibodies targeting the nucleoprotein (N), spike protein (S), and receptor-binding domain (RBD), as well as NAbs against the original SARS-CoV-2 strain, D614G, Beta (B.1.351) and Delta (B.1.617.2) strains. To determine the neutralizing antibody levels against the original Wuhan SARS-CoV-2 strains, a microneutralization assay was performed on Vero cells.

Memory T-cell responses against various components of SARS-CoV-2, including S, N, membrane protein and envelope protein-open reading frame (E/ORF) peptides, were assessed using both (ex vivo) cryopreserved and (in vitro) cultured PBMCs. The ELISpot assay was used to evaluate the responses, and cytokine-producing cells were identified to define functional specific memory T cells. The overall responses to S, N, membrane protein, and E/ORF peptide pools were defined as the sum of the background-subtracted responses to each combination of individual cytokines. The cytokines (IFNγ, IL-2, and TNFα) were analyzed using the ICS assay. The percentage of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells was determined by stimulating PBMCs from patients with the S, N, and E/ORF peptide pool, respectively. Background subtraction refers to the subtraction of the values of the negative control sample from the peptide-stimulated sample.

Statistical Analysis

Continuous variables are presented as median with (25th percentile, 75th percentile), whereas categorical variables are expressed as percentages. Clinical characteristics were compared between patients who received systemic corticosteroids (corticosteroids group) and those who did not (no corticosteroids group) using the Mann-Whitney *U* test and the χ^2 test as appropriate.

The titer of NAbs was log2 transformed and the markers of T-cell response were ln transformed for their skewed distribution. Afterward, to determine the specific effect of corticosteroids on the humoral and T-cell response, the differences of antibody titers kinetics over time, absolute antibody titers, and magnitude and rate of T-cell response were assessed by using a generalized linear model with a Gaussian link and logistic regression in patients who did and did not receive corticosteroids, where appropriate. The known or potential covariates on anti-SARS-CoV-2 antibody and T-cell responses after natural infection were included in the multivariable models, including age, sex, diabetes, malignancy, and seven-category scale at day 1. Statistical significance was set at $P < .05$, and all tests were two-tailed. The analyses were performed using SAS version 9.4 software (SAS Institute, Inc.).

Results

Patients and Corticosteroid Therapy

The schematic representation of the cohort of COVID-19 survivors is depicted in Figure 1. Blood samples were collected during the 6-month and 1-year follow-up visits to measure immunogenicity in the cohort of COVID-19 survivors. The cohort comprised 1,096 people (no corticosteroids group, n = 836; corticosteroids group, n = 260), and their levels of SARS-CoV-2 antigen-

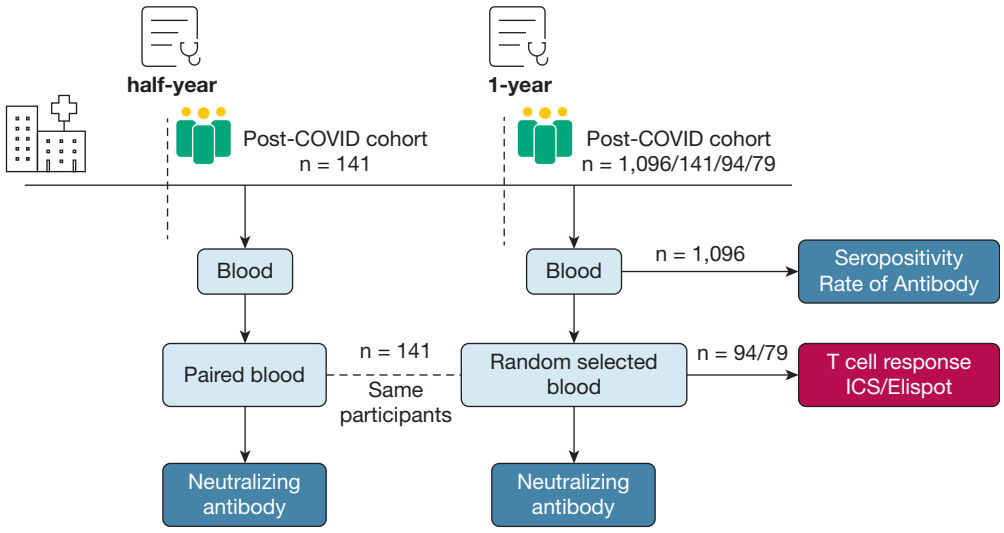

Figure 1 – *Schematic showing blood sample and flowchart of analysis of cohort of COVID-19 survivors. The blood samples for immunogenicity measurement were collected during the 6-mo and at 1-y follow-up visits. Blood collected from 1,096 participants was used to evaluate the seropositivity rate of antibodies at 1 y. Among these, samples from 141 paired participants underwent neutralizing antibody testing from 6 mo to 1 y. Of these, samples from 94 patients underwent ICS assay, whereas samples from 79 patients underwent ELISpot testing at 1 year. ELISpot = enzyme-linked immunospot; ICS = intracellular cytokine staining.*

specific antibodies in peripheral blood were determined at the 1-year follow-up visit after acute infection.

Among the participants, a subset of 141 patients (no corticosteroids group, n = 95; corticosteroids group, n = 46) were selected randomly for paired measurements of NAb titers at the 6-month and 1-year follow-up visits. As for cellular immune memory, PBMCs were collected from 94 of the 141 patients at the 1-year follow-up visit, of whom 94 patients' samples (no corticosteroids group, n = 65; corticosteroids group, n = 29) were measured by ICS assay and 79 patients' samples (no corticosteroids group, n = 56; corticosteroids group, n = 23) were measured by ELISpot assay to detect the SARS-CoV-2-specific memory T-cell responses. Detailed demographic and clinical characteristics of the cohort of COVID-19 survivors and the 141 participants are provided in Tables 1 and 2, respectively.[16]

Effect of Corticosteroids on Neutralizing Antibody Responses in the Cohort of COVID-19 Survivors

Within the cohort of COVID-19 survivors, analysis of 141 paired plasma samples revealed a relatively stable positive rate of NAbs over time. At the 6-month mark, the positive rate was 85.1% (120/141), which decreased slightly to 81.6% (115/141) at the 1-year mark (Fig 2, e-Table 1). Specifically, in the no corticosteroids group (n = 95 [67.4%]), the positive rate decreased from 81.1% (77/95) to 76.8% (73/95), whereas in the corticosteroids group (n = 46 [32.6%]), it decreased from 93.5% (43/46) to 91.3% (42/46).

Multivariate logistic regression analysis revealed that these trends and seropositivity levels were not influenced by the corticosteroid therapy strategies (e-Table 2). Furthermore, corticosteroid therapy did not affect the levels and kinetics of NAbs titers based on the 6-month and 1-year follow-up data. However, it is worth noting that in categories where the therapy was initiated more than 15 days after illness onset and the accumulated dose exceeded 560 mg, a numerical increase in NAbs titers was found, from 20.0 (12.6-39.8) to 28.2 (22.4-54.1) and from 20.0 (14.1-56.2) to 28.2 (14.1-33.9), respectively (e-Table 3). These results should be interpreted with caution because of the small sample size (n = 11). At the 1-year follow-up, NAbs against the original strain, D614 strain, Beta strain, and Delta strain were assessed in the 141 patients. The seropositive rate remained comparable with that of the no corticosteroids group across all therapy strategy categories, without statistical significance (e-Table 4).

Influence of Corticosteroids on Antigen-Specific Antibodies at the 1-Year Follow-Up After Infection

Among the 1,096 patients in the cohort of COVID-19 survivors who underwent serologic tests at the 1-year follow-up visit (e-Table 5), most patients showed negative results for short-lived IgA and IgM antibodies against SARS-CoV-2 antigens (seropositive rate range,

TABLE 1] Characteristics of Participants With or Without Corticosteroid Treatment in a Cohort of COVID-19 Survivors

Characteristic	Without Corticosteroids (n = 836)	With Corticosteroids (n = 260)	Total (N = 1,096)	*P* Value
Age, y	58.0 (48-65)	56.0 (47-64)	58.0 (48-65)	≥ .05
Sex, male	406 (49)	161 (62)	567 (52)	< .001
Education				
Primary or lower	77/749 (10)	14/230 (6.1)	91/979 (9.3)	.019
Middle school	458/749 (61)	131/230 (57)	589/979 (60)	
College or higher	214/749 (29)	85/230 (37)	299/979 (31)	
Smoking status				
No smoking	759/834 (91)	232/260 (89)	991/1094 (91)	≥ .05
Current smoking	50/834 (6.0)	23/260 (8.8)	73/1094 (6.7)	
Former smoking	25/834 (3.0)	5/260 (1.9)	30/1094 (2.7)	
Comorbidity				
Hypertension	244 (29)	77 (30)	321 (29)	≥ .05
Diabetes mellitus	120 (14)	23 (8.8)	143 (13)	.043
Heart diseases	60/835 (7.2)	16/260 (6.2)	76/1095 (6.9)	≥ .05
Cerebrovascular diseases	26 (3.1)	3 (1.2)	29 (2.6)	≥ .05
Malignant tumour	22 (2.6)	9 (3.5)	31 (2.8)	≥ .05
COPD	15 (1.8)	2 (0.80)	17 (1.6)	≥ .05
Chronic kidney disease	6 (0.70)	8 (3.1)	14 (1.3)	< .001
Highest 7-category scale during hospitalization[a]				
3 (hospitalization, not requiring supplemental oxygen)	260 (31)	29 (11)	289 (26)	< .001
4 (hospitalization, requiring supplemental oxygen)	557 (67)	177 (68)	734 (67)	
5 (hospitalization, requiring HFNC, noninvasive mechanical ventilation, or both)	18 (2.2)	51 (20)	69 (6.3)	
6 (admitted to hospital, requiring ECMO or IMV, or both)	1 (0.10)	3 (1.2)	4 (0.40)	
Duration of corticosteroids treatment, d	NA	7.0 (4.0-12)	NA	…
Accumulated corticosteroids doses	NA	200.0 (120-320)	NA	…
Duration from symptoms onset to admission, d	16.1 (12-32)	11.8 (8.7-16)	15.1 (10-25)	< .001

(Continued)

TABLE 1] (Continued)

Characteristic	Without Corticosteroids (n = 836)	With Corticosteroids (n = 260)	Total (N = 1,096)	*P* Value
Length of hospitalization, d	12.0 (8.7-17)	19.0 (14-34)	13.4 (9.5-19)	< .001
Duration from symptom onset to follow-up, d	185.0 (174-196)	185.0 (175-199)	185.0 (174-197)	≥ .05
Duration from admission to follow-up, d	162.3 (156.1-171.3)	173.0 (163.3-183.1)	164.3 (157.5-174.5)	< .0001
Duration from discharge to follow-up, d	151.5 (143-156)	152.4 (149-158)	151.6 (145-157)	.006

Data are No. (%), No./Total No. (%) when data are missing, or median (interquartile range), unless otherwise indicated. To correct for multiple comparisons between two groups of study participants with different severity scale, a Bonferroni-corrected α threshold of .0167 was used. ECMO = extracorporeal membrane oxygenation; HFNC = high-flow nasal cannula; IMV = invasive mechanical ventilation; NA = not applicable.
[a]The highest 7-category scale during the hospital stay (termed the *severity scale*) consisted of 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, but requiring supplemental oxygen; 5, admitted to hospital requiring HFNC, noninvasive mechanical ventilation, or both; 6, admitted to hospital requiring ECMO, IMV, or both; and 7, death.

0%-6.7%) (e-Fig 1). However, the seropositive rate for long-lived N, S, and RBD IgG antibodies remained relatively high across each therapy strategy (seropositive rate range, 66.4%-97.4%). Comparing the corticosteroids group and the no corticosteroids group, no significant differences were found in the seropositive rate of N, S, and RBD IgM, IgA, and IgG antibodies at the 1-year follow-up visit, even after adjusting for age, sex, diabetes, malignancy, and seven-category scale at day 1 (e-Table 6).

Further exploration of the associations between corticosteroid therapy strategies and seropositive rate revealed that only an accumulated dose of methylprednisolone exceeding 560 mg (OR, 2.99; 95% CI, 1.16-7.68) showed a protective effect on RBD IgG antibodies compared with the no corticosteroids group. However, these results should be interpreted with caution because of the small sample size in the > 560 mg subgroup (n = 27).

Regarding antibody titers (Fig 3), corticosteroids did not show a significant effect on N and RBD IgG at the 1-year follow-up visit, even after adjusting for age, sex, diabetes, malignancy, and seven-category scale at day 1. However, we observed a delayed effect of corticosteroid therapy on the decline of titers at 1 year that reached statistical significance for S IgG (β, 0.08; 95% CI, 0.04-0.12). Specifically, this effect was prominent in the categories of therapy lasting 7 to 10 days (β, 0.15; 95% CI, 0.08-0.22) and an accumulated corticosteroid dose of ≤ 560 mg (β, 0.09; 95% CI, 0.04-0.13) based on the generalized linear model with a Gaussian link (e-Table 7).

Effect of Corticosteroid Therapy on Memory T-Cell Responses and Functional Assessment at 1 Year After Infection

Memory T-cell responses to SARS-CoV-2 antigens, including N, S, membrane protein, and E/ORF, were assessed using ex vivo IFNγ ELISpot assays. A positive T-cell response was defined as a positive reaction to at least one of the SARS-CoV-2 peptide pools. At the 1-year follow-up visit, memory T-cell responses were detected in a total of 79 convalescent survivors of COVID-19. Corticosteroids did not affect the rate of T-cell responses after adjusting age, sex, diabetes, malignancy, and seven-category scale at day 1 in the logistic model (e-Fig 2). Nevertheless, subgroup analysis revealed that patients who received corticosteroid therapy showed a significantly lower rate of T-cell responses with a longer duration of therapy (> 10 days;

TABLE 2] Characteristics of 141 Participants With or Without Corticosteroid Treatment

Characteristic	Without Corticosteroids (n = 95)	Corticosteroids (n = 46)	Total (n = 141)	*P* Value
Age, y	58.0 (49.0-65.0)	53.5 (44.0-62.0)	57.0 (48.0-65.0)	≥ .05
Male	57 (60.0)	32 (69.6)	89 (63.1)	.3
Education				.012
1	6/78 (7.7)	2/43 (4.7)	8/121 (6.6)	
2	50/78 (64.1)	17/43 (39.5)	67/121 (55.4)	
3	22/78 (28.2)	24/43 (55.8)	46/121 (38.0)	
Smoking status				≥ .05
0	84 (88.4)	42 (91.3)	126 (89.4)	
1	5 (5.3)	4 (8.7)	9 (6.4)	
2	6 (6.3)	0 (0.0)	6 (4.3)	
Hypertension	26 (27.4)	15 (32.6)	41 (29.1)	.5
Diabetes mellitus	12 (12.6)	7 (15.2)	19 (13.5)	.7
Heart disease	12 (12.6)	3 (6.5)	15 (10.6)	.3
Malignant tumour	2 (2.1)	0 (0.0)	2 (1.4)	.2
COPD	2 (2.1)	0 (0.0)	2 (1.4)	.2
Chronic kidney disease	0 (0.0)	2 (4.3)	2 (1.4)	.033
BP, mm Hg				…
Systolic ≥140	23 (24.2)	10 (21.7)	33 (23.4)	.7
Diastolic ≥ 90	21 (22.1)	4 (8.7)	25 (17.7)	≥ .05
Highest seven-category scale during hospitalization[a]				< .0001
3 (hospitalization, not requiring supplemental oxygen)	44 (46.3)	4 (8.7)	48 (34.0)	
4 (hospitalization, requiring supplemental oxygen)	43 (45.3)	15 (32.6)	58 (41.1)	
5 (hospitalization, requiring HFNC, noninvasive mechanical ventilation, or both)	7 (7.4)	27 (58.7)	34 (24.1)	
6 (admitted to hospital, requiring ECMO or IMV, or both)	1 (1.1)	0 (0.0)	1 (0.7)	
Length of hospitalization, d	11.4 (7.9, 16.0)	27.3 (16.4, 51.8)	14.0 (9.8, 22.9)	< .0001
Duration from symptoms onset to admission, d	14.6 (9.7, 24.0)	11.5 (8.7, 15.6)	12.6 (9.0, 18.0)	.017

(Continued)

TABLE 2] (Continued)

Characteristic	Without Corticosteroids (n = 95)	Corticosteroids (n = 46)	Total (n = 141)	*P* Value
Duration from discharge to follow-up, d	150.5 (142.7, 153.7)	153.6 (141.6, 159.5)	151.6 (142.7, 155.6)	≥ .05
Duration from symptom onset to follow-up, d	178.5 (171.0, 192.0)	199.0 (179.0, 216.0)	182.0 (172.0, 199.0)	< .0001
Corticosteroids	0.0 (0.0, 0.0)	1.0 (1.0, 1.0)	0.0 (0.0, 1.0)	< .0001
Cumulative corticosteroid dose	NA	275.0 (180.0, 518.8)	NA	> .9
Duration of corticosteroids treatment, d	NA	9.0 (6.0, 17.0)	NA	> .9
Duration from admission to follow-up, d	159.6 (155.4, 168.5)	183.5 (169.0, 204.2)	164.1 (157.7, 178.5)	< .0001

Data are presented as No. (%), No./Total No. (%) when data are missing, or median (interquartile range), unless otherwise indicated. To correct for multiple comparisons between two groups of study participants with different severity scale, a Bonferroni-corrected α threshold of .0167 was used. ECMO = extracorporeal membrane oxygenation; HFNC = high-flow nasal cannula; IMV = invasive mechanical ventilation; NA = not applicable.

[a]The highest 7-category scale during the hospital stay (termed the *severity scale*) consisted of 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, but requiring supplemental oxygen; 5, admitted to hospital requiring HFNC, noninvasive mechanical ventilation, or both; 6, admitted to hospital requiring ECMO, IMV, or both; and 7, death.

OR, 0.06; 95% CI, 0.01-0.67 for nucleoprotein exposing) and a higher accumulated dose (> 560 mg; OR, 0.02; 95% CI, 0.00-0.35 for nucleoprotein exposing; OR, 0.06; 95% CI, 0.00-0.85 for E/ORF exposing) categories (e-Table 8). Similarly, corticosteroids made no difference to the magnitude of T-cell ELISpot responses compared with no corticosteroids generally (e-Fig 2). However, a statistically significant lower magnitude of T-cell response was observed in those with a therapy duration of > 10 days (β, –2.54; 95% CI, –4.75 to –0.32 for spike-specific T-cell response) and > 560-mg accumulated dose (β, –3.95; 95% CI, –6.69 to –1.22 for N-specific T-cell response), respectively (e-Table 9).

When it comes to the functional assessment of memory T cells, ICS for IFNγ, IL-2, and TNFα in CD4 and CD8 T cells was performed in 94 of 141 paired participants at the 1-year follow-up visit. The magnitude of ICS-specific T-cell responses was comparable between the corticosteroids and no corticosteroids groups when exposed to S and N pools (e-Fig 2). However, participants in corticosteroids group showed a higher magnitude of CD4 (β, 0.61; 95% CI, 0.10-1.12) and CD8 (β, 0.63; 95% CI, 0.11-1.15) T-cell IFNγ responses after being exposed to E/ORF peptide pools compared with the no corticosteroids group. This effect was particularly evident in the subgroup analysis of participants with initiation from illness onset of > 15 days (β, 0.78; 95% CI, 0.22-1.34; β, 0.72; 95% CI, 0.14-1.29), duration of therapy of < 7 days (β, 1.39; 95% CI, 0.68-2.09; β, 1.43; 95% CI, 0.71-2.16), and accumulated dose of ≤ 560 mg (β, 0.76; 95% CI, 0.21-1.31; β, 0.72; 95% CI, 0.16-1.29) (Fig 4) (e-Table 10).

Discussion

To our knowledge, this is the first study to explore the effect of corticosteroid therapy on long-term durability of humoral immunity and memory T-cell response in hospitalized survivors of COVID-19 infected by the Wuhan strain of SARS-CoV-2. The data from the current study showed that corticosteroid therapy had no significant effect on the waning of NAbs (seropositive rate and titers) from 6 months to 1 year, seropositivity of N, S, and RBD IgG and titer changes of N and RBD IgG at 1 year. However, corticosteroids did present a protective factor in titer decline of S IgG. For the subgroup analysis, corticosteroids also posed a statistically significant protective effect on NAbs (initiating > 15 days, > 560 mg; n = 11) and RBD IgG (> 560 mg; n = 27) in the first year. For T-cell immunity, corticosteroid therapy did not affect significantly either the rate or magnitude of T-cell responses at 1 year. However, subgroup analysis

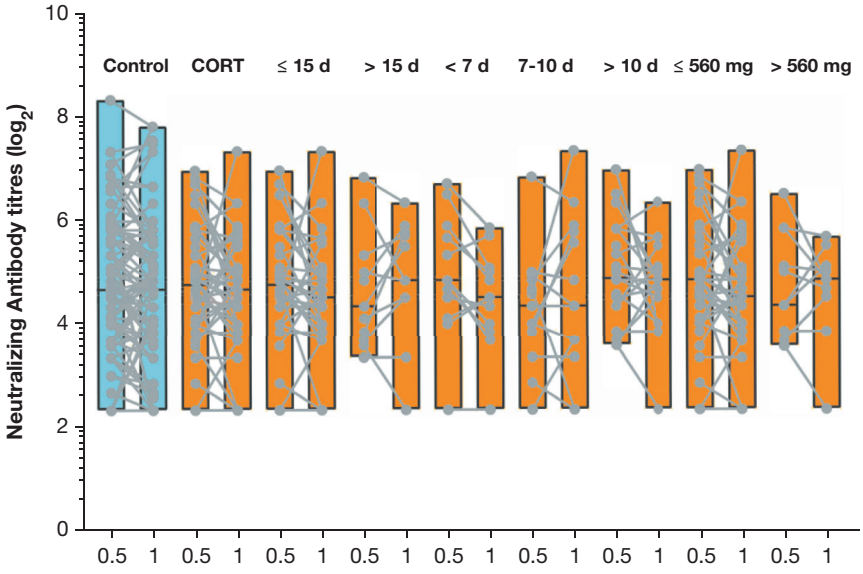

Figure 2 – *Graph showing the kinetics of neutralizing antibody titers influenced by the corticosteroids group compared with the no corticosteroids group from the 6-mo to 1-y visit for the cohort of COVID-19 survivors. Bars of each column indicate medians, each dot indicates a single participant, and the gray lines denote the individual neutralizing antibody titers and seropositivity change between two time points. All assays were performed with the use of the SARS-CoV-2 original strain from Wuhan. Logarithmic values are reported as the geometric mean concentration in the enzyme-linked immunosorbent assay analyses and as the geometric mean titer in the neutralizing antibody analyses. control = no corticosteroids group; CORT = corticosteroids; 0.5 = 6 months after SARS-CoV-2 infection; ≤ 15 d, > 15 d = initiation of corticosteroids from illness onset ≤ 15 days and > 15 days; < 7 d, 7-10 d, > 10 d = duration of therapy of corticosteroids < 7 days, 7-10 days, and > 10 days; ≤ 560 mg, > 560 mg = accumulated corticosteroid dose of ≤ 560 mg and > 560 mg.*

revealed that long therapy duration (> 10 days) and high accumulated dose (> 560 mg) lowered and impaired T-cell rate and magnitude. Furthermore, a higher IFNγ-producing CD4 and CD8 memory T-cell response to E/ORF peptide pools was observed in patients who received corticosteroid therapy.

Regarding humoral immune responses, in broad terms, corticosteroid therapy did not have a statistically significant effect on long-term seropositivity or titer changes of NAbs or SARS-CoV-2-specific antibodies within 1 year. However, subgroup analysis revealed that corticosteroids did have a protective role for NAbs and RBD IgG in the category of accumulated dose of > 560 mg, and this may be attributed to delayed viral clearance, which will be clarified later in the article. For the memory T-cell immunity, corticosteroid therapy did not have a statistically significant effect on the rate and magnitude of T-cell ELISpot responses overall, but did curtail the rate and magnitude of T-cell responses in the category of > 10-day therapy duration and > 560-mg accumulated dose, which is compatible with the latest RECOVERY trial showing that higher-dose corticosteroids significantly increased the risk of death for hospitalized patients with COVID-19 with clinical hypoxia.[17]

Functional assessment of memory T-cells showed that corticosteroid therapy resulted in higher IFNγ-producing CD4 and CD8 T-cell responses on exposure to E/ORF peptide pools. This is consistent with a previous study of virus-specific memory T-cell immunity after avian influenza A (H7N9) virus infection.[18] Zhao et al found a higher magnitude of IFNγ-producing CD4 and CD8 memory T-cell responses in patients who received corticosteroids during hospitalization. Elevated IFNγ production of T cells and durability of antibody in the corticosteroids group might be explained by prolonged viral stimulation. It was known that antibody titers and memory responses are generated in parallel in germinal centers, and a higher memory T-cell response may reflect stronger germinal center reactions.[19] SARS-CoV-2 antigen persistence resulting from delayed viral clearance may reactivate or favor the persistence of memory B and T cells.[20,21] This may be the reason why corticosteroids favor higher memory T-cell response and antibodies. These results indicate that the persistent stimulation to T cells because of delayed viral clearance may maintain the selective proliferation of the higher affinity T cells, but also may reduce the ICS sensitivity of T cells to SARS-CoV-2 antigens.[22,23] Moreover, IFNγ is the most potent macrophage-activating cytokine known. It activates classical macrophages, M1s, but inhibits alternative macrophages, M2s. Lv et al[24] reported that classically activated M1 alveolar macrophages facilitate viral spread, whereas alternatively activated M2 alveolar macrophages limit the spread. In this respect, IFNγ itself may be both the cause and effect of delayed viral clearance, leading to a perpetuating circle. The exact mechanism needs to be studied in future.

The upregulation of IFNγ in the ICS assay may indicate the polarization of subtypes of $CD4^+$ T helper cells toward the Th1 group. This is because long-lived memory Th1 cells in circulation arise from those undergoing initial

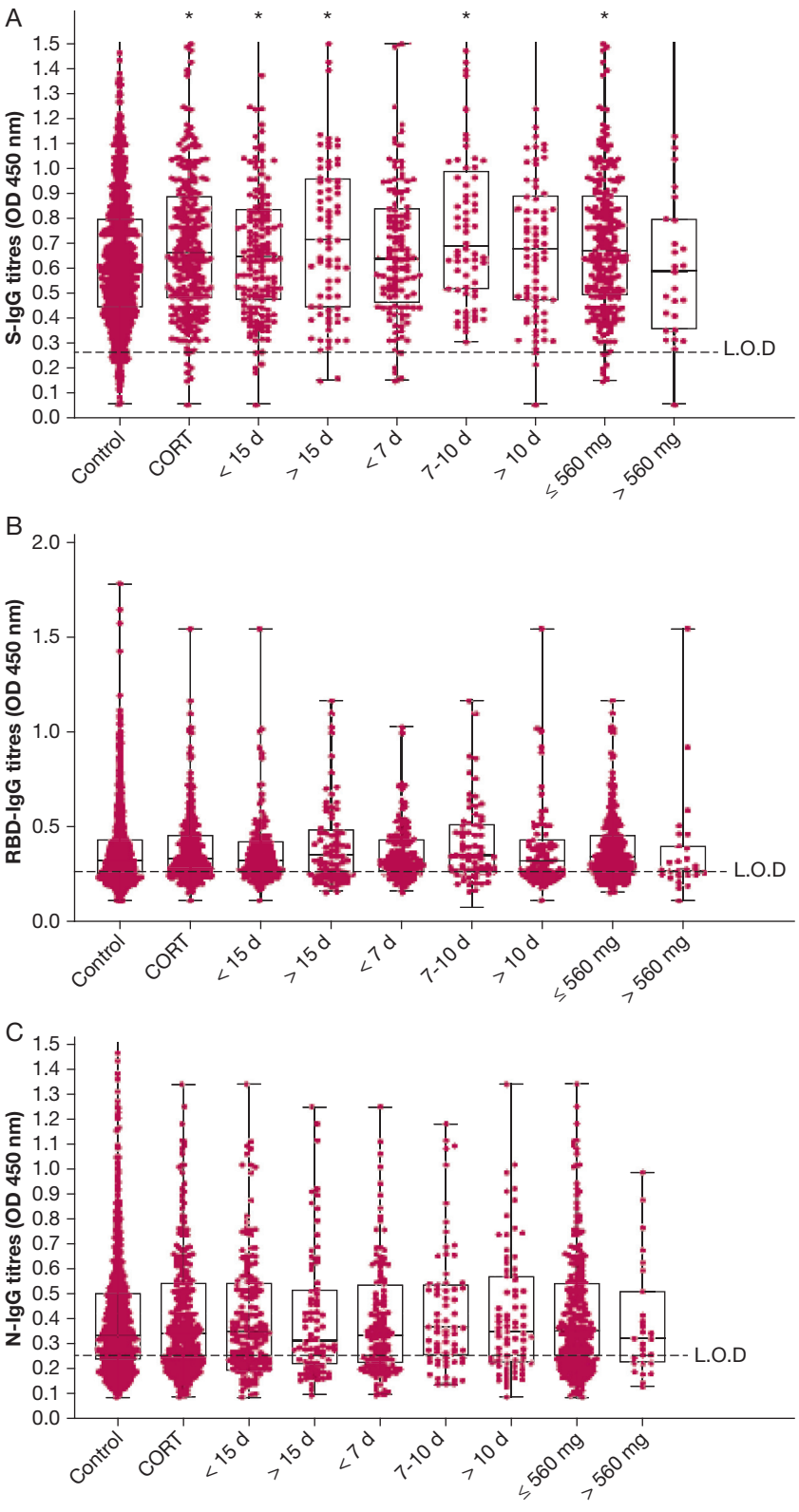

Figure 3 – *A-C, Box and whisker plots showing antibody titers for S IgG (A), RBD IgG (B), and N IgG (C) against SARS-CoV-2 antigens at the 1-y follow-up visit in the cohort of COVID-19 survivors with and without corticosteroids. Each category of patients is listed as each box. The line splitting the box in two represents the median value of all responses tested, the left edge of the box represents the lower quartile, and the right edge of the box shows the upper quartile. The values at which the horizontal lines stop are the values of the upper and lower values of the data, and each dot represents a single participant. Dashed lines present the LOD. A delayed effect of corticosteroid therapy is observed on the decline of S IgG titers at 1 y, which reached statistical significance, especially in the categories of therapy lasting 7 to 10 d and an accumulated corticosteroid dose of ≤ 560 mg. Corticosteroid therapy did not have a significant effect on N IgG and RBD IgG titers compared with the control group (no corticosteroids group). *Statistical significance, a generalized linear model with a Gaussian link is used after adjusting for age, sex, diabetes, malignancy, and seven-category scale at day 1. Control = no corticosteroids; CORT = corticosteroids; E/ORF = envelope protein-open reading frame; LOD = limit of detection; OD = optical density; M = membrane protein; RBD = receptor-binding domain; S = spike protein; ≤ 15 d, > 15d = initiation of corticosteroids from illness onset ≤ 15 days and > 15 days; < 7 d, 7-10 d, > 10 d = duration of therapy of corticosteroids < 7 days, 7-10 days, and > 10 days; ≤ 560 mg, > 560 mg = accumulated corticosteroid dose of ≤ 560 mg and > 560 mg.*

proliferation and clonal expansion in the acute phases. Consistent with the study of Gil-Etayo et al,[25] a prominent Th1 response in the acute phase of COVID-19 can be a prognostic marker for a good disease course evolution. These results also may suggest potential preferential replication of SARS-CoV-2 in T cells producing Th1-type cytokines. Further detection of Th2-type cytokines (IL-4, IL-5, and IL-10) and Th1 to Th2 ratios is needed.

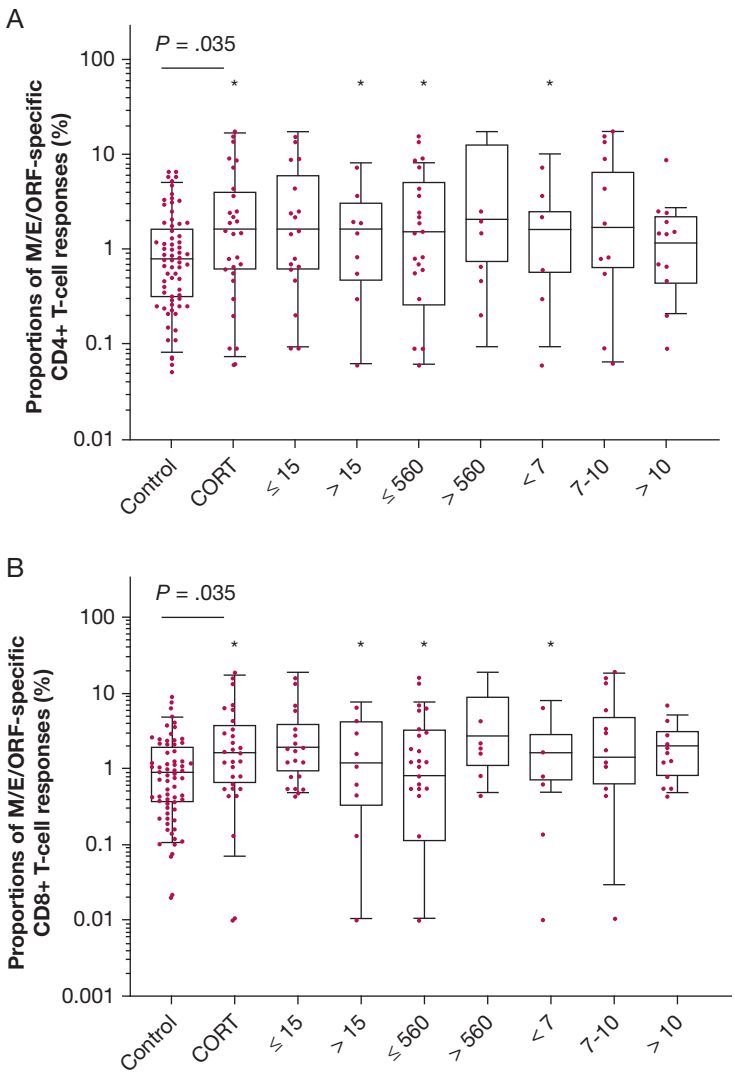

Figure 4 – *A, B, Box and whisker plots showing the proportion of antigen-specific IFNγ-producing CD4$^+$ and CD8$^+$ T cells with ICS assay in patients with and without corticosteroids at the 1-y follow-up after SARS-CoV-2 infection. Each category of patients is listed as each box. The line splitting the box in two represents the median value of all responses tested, the left edge of the box represents the lower quartile, and the right edge of the box shows the upper quartile. The values at which the horizontal lines stop are the values of the upper and lower values of the data, and each dot represents a single participant. Cytokine-producing T cells were detected by ICS after incubation with SARS-CoV-2 peptides. The responses to the SARS-CoV-2 peptide pool are depicted as the background-subtracted percentage of CD4 or CD8 T cells with IFNγ. Corticosteroid therapy showed a higher IFNγ-producing CD4 and CD8 T-cell responses after exposing E/ORF peptide pools, especially in the categories of initiation from illness onset of > 15 d, accumulated dose of ≤ 560 mg, and duration of therapy of < 7 d. Non-S means SARS-CoV-2 antigens except for spike. Spike-specific CD4$^+$ and CD8$^+$ T cells were measured as background subtracted data. *Statistically significant, a generalized linear model with a Gaussian link is used after adjusting age, sex, diabetes, malignancy, and seven-category scale at day 1. E/ORF= envelope protein-open reading frame; control = no corticosteroids; CORT = corticosteroids; ICS = intracellular cytokine staining; IFNγ = interferon-γ; M = membrane protein; ≤ 15 d, >15 d = initiation of corticosteroids from illness onset ≤ 15 days and > 15 days; < 7 d, 7-10 d, > 10 d = duration of therapy of corticosteroids < 7 days, 7-10 days, and > 10 days; ≤ 560 mg, > 560 mg = accumulated corticosteroid dose of ≤ 560 mg and > 560 mg.*

We are fully aware of the limitations of this study. First, it was an observational study and not a randomized controlled trial, although we used several statistical techniques to minimize potential bias. Second, limited availability of blood samples, especially a lack of PBMCs from certain patients, prevented us from conducting all immunologic tests; thus, only a subset of the 141 participants underwent ICS and ELISpot assay testing. Consequently, apart from enough plasma being tested for NAb titers, we opted to select the residual samples for assessing memory T-cell responses randomly. Nonetheless, ICS and ELISpot assays ultimately were conducted based on a sufficient quantity of PBMC samples, that is, 94 and 79, respectively. In addition, based on our data, a

consistent trend was observed across each type of antibody and T-cell response in the corticosteroids group, strongly supporting the credibility of the effect of corticosteroids. However, we must emphasize that the estimated effect values based on the small sample size still should be interpreted with caution. Third, for T-cell responses evaluation, some cultured PBMCs (in vitro), rather than cryopreserved PBMCs (ex vivo), were used before ELISpot analysis.[26] Herein, the culture and expansion protocol potentially could lead to bias, attributed to variations in the proliferation capacity of distinct antigen-specific T cells.

Interpretation

Our findings suggest that short-term and low-dose corticosteroid therapy administered during hospitalization does not have a statistically significant effect on the long-term dynamics of humoral immune responses, nor on the magnitude and rate of memory T-cell responses to SARS-CoV-2 antigens. However, further studies are needed to determine whether corticosteroid therapy increases the risk of reinfection. Additionally, the potential adverse effects of long-term and high-dose corticosteroid use on memory immune responses warrant further investigation.

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Additional information: The e-Appendix, e-Figures, and e-Tables are available online under "Supplementary Data."

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