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Early Use of Corticosteroid May Prolong SARS-CoV-2 Shedding in Non-Intensive Care Unit Patients with COVID-19 Pneumonia: A Multicenter, Single-Blind, Randomized Control Trial

Xiao Tang[a] Ying-Mei Feng[b] Ji-Xiang Ni[c] Jia-Ying Zhang[b] Li-Min Liu[d] Ke Hu[e] Xiu-Zhi Wu[a] Ji-Xian Zhang[f] Jun-Wen Chen[g] Jian-Chu Zhang[h] Jian Su[c] Yu-Lei Li[d] Yang Zhao[e] Jiao Xie[f] Zhou Ding[g] Xin-Liang He[h] Wen Wang[a] Rong-Hua Jin[b] Huan-Zhong Shi[a] Bing Sun[a]

[a]Department of Respiratory and Critical Care Medicine, Beijing Chao-Yang Hospital, Capital Medical University, Beijing Institute of Respiratory Medicine, Beijing, China; [b]Beijing You-An Hospital, Capital Medical University, Beijing, China; [c]Department of Respiratory and Critical Care Medicine, Yi-Chang First People's Hospital, The People's Hospital of China Three Gorges University, Hubei Province, China; [d]Department of Respiratory and Critical Care Medicine, Tianyou Hospital, Wuhan University of Science and Technology, Wuhan, China; [e]Department of Respiratory and Critical Care Medicine, Renmin Hospital of Wuhan University, Wuhan, China; [f]Department of Respiratory and Critical Care Medicine, Hubei Province Hospital of Integrated Chinese & Western Medicine, Wuhan, China; [g]Department of Respiratory and Critical Care Medicine, Xiang-Yang First people's Hospital, Hubei Province, China; [h]Department of Respiratory and Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Huazhong, China

Keywords
Coronavirus disease 2019 · Pneumonia · Corticosteroid · Outcome · Virus shedding

Abstract
Background: There is still no clinical evidence available to support or to oppose corticosteroid treatment for coronavirus disease 2019 (COVID-19) pneumonia. ***Objective:*** To investigate the efficacy and safety of corticosteroid given to the hospitalized patients with COVID-19 pneumonia. ***Methods:*** This was a prospective, multicenter, single-blind, randomized control trial. Adult patients with COVID-19 pneumonia who were admitted to the general ward were randomly assigned to either receive methylprednisolone or not for 7 days. The primary end point was the incidence of clinical deterioration 14 days after randomization. ***Results:*** We terminated this trial early because the number of patients with COVID-19 pneumonia in all the centers decreased in late March. Finally, a total of 86 COVID-19 patients underwent randomization. There was no difference of the incidence of clinical deterioration between the methylprednisolone group and control group (4.8 vs. 4.8%, $p = 1.000$). The duration of throat viral RNA detectability in the methylprednisolone group was 11 days (interquartile range, 6–16 days), which was significantly longer than that in the control group

Xiao Tang, Ying-Mei Feng, Ji-Xiang Ni, Jia-Ying Zhang, Li-Min Liu, Ke Hu and Xiu-Zhi Wu contributed equally.

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Bing Sun
Department of Respiratory and Critical Care Medicine
Beijing Chao-Yang Hospital, Capital Medical University
8 Gongti Nanlu, Beijing 100020 (China)
ricusunbing@126.com

(8 days [2–12 days], $p = 0.030$). There were no significant differences between the 2 groups in other secondary outcomes. Mass cytometry discovered $CD3^+$ T cells, $CD8^+$ T cells, and NK cells in the methylprednisolone group which were significantly lower than those in the control group after randomization ($p < 0.05$). ***Conclusions:*** From this prematurely closed trial, we found that the short-term early use of corticosteroid could suppress the immune cells, which may prolong severe acute respiratory syndrome coronavirus 2 shedding in patients with COVID-19 pneumonia. ***Trial Registration:*** ClinicalTrials.gov, NCT04273321.

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Introduction

The rapid spread of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that emerged in late 2019 [1] has been labeled a public health emergency of international concern by the World Health Organization (WHO). Globally, as of September 22, 2020, a total of 31,132,906 confirmed cases, including 962,008 deaths, have been reported to the WHO [2]. In a cohort of 179 cases with COVID-19, we have demonstrated that 73 patients underwent acute respiratory distress syndrome (ARDS), and 21 (28.8%) of them died [3, 4]. For the severe COVID-19 patients, neither lopinavir-ritonavir therapy nor remdesivir seemed to not accelerate clinical benefits from some randomized controlled trials [5, 6]. However, there was a double-blind, randomized, placebo-controlled trial of intravenous remdesivir in adults hospitalized with COVID-19, which showed it could shorten the time to recovery compared with placebo but without information about the influence on virus shedding and mortality [7].

Although the WHO does not recommend, systemic corticosteroids are routinely given to patients with COVID-19 outside of clinical trials unless they are indicated for another reason [8]. These drugs were used widely during the outbreak in China [3, 9–12]. In a retrospective cohort study, Wu et al. [13] have shown that the administration of methylprednisolone appears to have reduced the risk of death in COVID-19 patients with ARDS. However, it still lacks evidence about the details of corticosteroid application in the treatment of COVID-19 pneumonia, such as virus shedding and immunological function. There is still no clinical evidence available to support or to oppose corticosteroid treatment for COVID-19 pneumonia. We conducted a prospective, multicenter, single-blind, randomized control trial to investigate the efficacy and safety of corticosteroid given to the hospitalized patients with COVID-19 pneumonia.

Materials and Methods

Patients

Patients who were laboratory confirmed of SARS-CoV-2 infection and had pneumonia confirmed by chest computed tomography were diagnosed with COVID-19 pneumonia [8]. Patients with COVID-19 pneumonia aged 18 years or older, admitted to the general wards for less than 72 h, and able to sign informed consent were eligible for this trial. Exclusion criteria included severe immunosuppression (human immunodeficiency virus infection and long-term use of immunosuppressive agents), pregnant or breastfeeding women, corticosteroid needed for other diseases, refractory hypertension, epilepsy or delirium, glaucoma, active gastrointestinal bleeding within 3 months, refractory hypokalemia, secondary bacterial or fungal infection, unwilling or unable to participate or complete the study, and participation in other studies.

Trial Design and Oversight

This was a prospective, multicenter, single-blind, randomized, clinical control trial (ClinicalTrials.gov number, NCT04273321). Patients were recruited from the respiratory departments or infectious disease department of 7 tertiary hospitals in Beijing and Hubei province of China. After screening, eligible participants were randomly assigned (in a 1:1 ratio) to either the methylprednisolone group or control group for treatment. Randomization was stratified by the statistician of the leading site, who produced computer-generated block randomization lists with a block size of 4 patients. This was a single-blind trial; the physicians were aware of the treatment assignment, but the participants were blinded. And during the whole study period, the data collection and end point judgement were blinded, and the statisticians were also blinded during the statistical analysis.

Procedures

After informed consent was obtained, baseline data including medical history, relevant comorbidities, symptoms, vital signs, variables required for the calculation of pneumonia severity index (PSI) [14], CURB-65 [15], and sequential organ failure assessment (SOFA) [16] were collected, and baseline blood samples were also drawn.

All the participants received standard therapy of COVID-19 according to the Chinese Diagnosis and Treatment Plan for COVID-19 (trial version 6) [17]. Patients started receiving study medication 1 h after randomization. In the methylprednisolone group, 1 mg/kg per day of methylprednisolone (produced by Pfizer Manufacturing Belgium NV) dissolved in 100 mL 0.9% normal saline was administered intravenously for 7 days. In the control group, 100 mL 0.9% normal saline was administered intravenously. Clinical data were recorded on paper case record forms and then double entered in an electronic database and validated by trial staff.

End Points

The primary end point was the incidence of clinical deterioration 14 days after randomization. The secondary end points in-

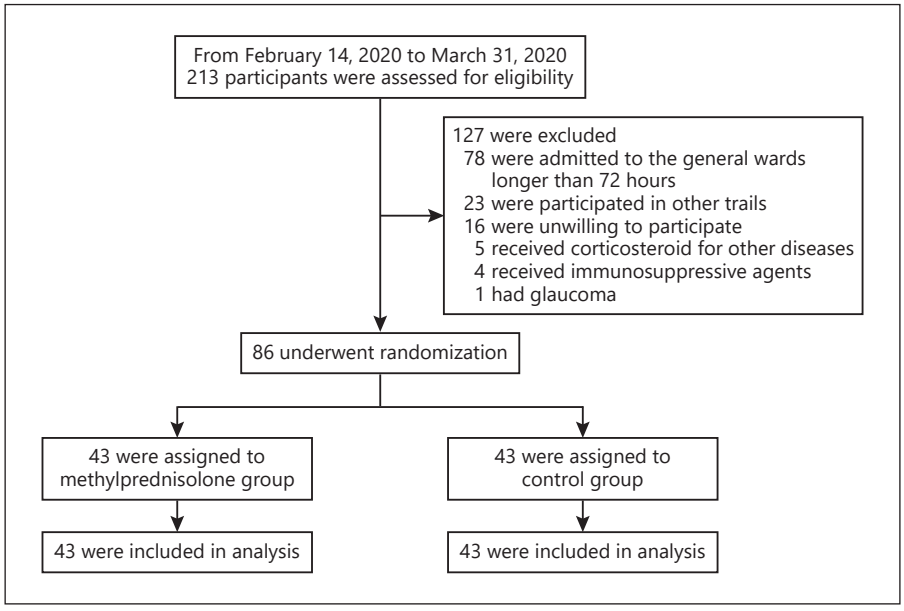

Fig. 1. Randomization and treatment assignment of patients with COVID-19. COVID-19, coronavirus disease 2019.

cluded the incidence of clinical cure 14 days after randomization, the incidence of intensive care unit (ICU) admission, in-hospital mortality, the time from randomization to clinical cure, the time from the onset to virus shedding of SARS-CoV-2 in respiratory tract samples, hospitalization duration, and complications including blood glucose abnormal, stress ulcer, and secondary infections.

The clinical deterioration fulfilled at least one of the following criteria: the clinical symptoms and signs continue to deteriorate, new pulmonary or extrapulmonary lesions appear, the chest computed tomography indicates the progress, or the patient is transferred to the ICU or is dead. The clinical cure fulfilled all of the following criteria: the clinical symptoms and signs of COVID-19 improved or alleviated (body temperature for 3 consecutive days, respiratory symptoms improved significantly, and computed tomographic images showed obvious absorption of bilateral extensive ground-glass opacification and/or consolidation), and no additional or alternative treatments were needed. The criteria of virus shedding was SARS-CoV-2-negative result of the nucleic acid tests from throat swabs for 2 consecutive times (sampling interval of at least 1 day) [17]. RT-PCR was used to test SARS-CoV-2.

Mass Cytometry

To investigate the impact of methylprednisolone on the immune cell profile, we collected peripheral blood mononuclear cells of 5 patients from the methylprednisolone group and 5 from the control group, who were hospitalized to Beijing You-an Hospital, for conducting mass cytometry analysis (see online suppl. materials; see www.karger.com/doi/10.1159/000512063 for all online suppl. material).

Statistical Analysis

According to the findings reported in the first Wuhan cohort [11], 30% of the overall population with COVID-19 pneumonia might deteriorate to a severe or critical stage. Due to a lack of corticosteroid data in COVID-19 pneumonia, we speculated corticosteroid could reduce the incidence of being severely ill from 30 to 15% based on our experience ($\alpha = 5\%$; $\beta = 20\%$). By using the superiority test, we calculated a sample size of 121 patients in each of the 2 parallel groups. Considering that 10% of the participants may be dropped, a total of 270 cases were required for this trial.

Data analysis was performed using SPSS 23.0 software. Categorical variables were summarized using frequencies and percentages, and continuous data are presented as means and standard deviations or medians and interquartile ranges. Comparisons of clinical characteristics between the 2 groups were performed by using Student's *t* test or Wilcoxon's rank sum test for continuous variables and by using χ^2 test or Fisher's exact test for categorical variables. Repeated measures analysis of the two-way ANOVA followed by Bonferroni's test or repeated measures analysis of the one-way ANOVA followed by Dunnett's test was performed for the data obtained at multiple time points. The primary and secondary outcomes were assessed using the Kaplan-Meier approach with the log-rank test. As data from all participants entered in this trial were available for the final statistical analysis, an analysis on an intention-to-treat basis was not necessary. Univariate analysis for stratification variables used logistic regression analysis, and the time variable, estimated by the Cox proportional risk model. We deemed a two-tailed p value <0.05 to be significant.

Results

Patients

The numbers of confirmed cases in China reached the peak on February 1, 2020, and gradually decreased thereafter [18]. The first patient was enrolled in this trial on February 19, 2020. Because the number of patients with COVID-19 pneumonia in all the centers decreased in late

Table 1. Clinical characteristics of patients at randomization

Characteristic	Total ($N = 86$)	Methyl-prednisolone ($N = 43$)	Control ($N = 43$)	p value
Age, median (IQR), years	56 (39–66)	57 (49–67)	55 (38–65)	0.594
Weight, median (IQR), kg	64 (58–73)	62 (60–72)	65 (56–71)	0.593
Body mass index, median (IQR), kg/m^2	23.4 (21.5–25.3)	23.9 (20.5–25.4)	23.0 (21.6–25.1)	0.792
Gender (male), *n* (%)	41 (47.7)	21 (48.8)	20 (46.5)	0.829
Smoke, *n* (%)	11 (12.9)	6 (14.3)	5 (11.6)	0.909
Time from the symptom onset to diagnosis, median (IQR), days	6 (4–10)	5 (3–10)	6 (4–10)	0.565
Time from the symptom onset to admission, median (IQR), days	7 (4–15)	6 (4–11)	10 (4–18)	0.149
Time from the symptom onset to randomization, median (IQR), days	8 (6–16)	8 (6–13)	11 (6–21)	0.131
Symptom, *n* (%)				
Fever	66 (76.7)	36 (83.7)	30 (69.8)	0.126
Cough	58 (67.4)	33 (76.7)	25 (58.1)	0.066
Sputum	23 (26.7)	17 (39.5)	6 (14.0)	0.007
Chest tightness	23 (26.7)	15 (34.9)	8 (18.6)	0.088
Chest pain	5 (5.8)	3 (7.0)	2 (4.7)	0.645
Short of breath	20 (23.3)	11 (25.6)	9 (20.9)	0.610
Dyspnea	13 (15.1)	7 (16.3)	6 (14.0)	0.763
Fatigue	22 (25.6)	12 (27.9)	10 (23.3)	0.621
Myalgia	13 (15.1)	6 (14.0)	7 (16.3)	0.763
Snot	5 (5.8)	3 (7.0)	2 (4.7)	0.645
Diarrhea	2 (2.3)	1 (2.3)	1 (2.3)	0.999
Nausea	2 (2.3)	2 (4.7)	0 (0)	0.152
Comorbidities, *n* (%)				
Chronic obstructive pulmonary disease	3 (3.5)	2 (4.7)	1 (2.3)	0.543
Asthma	2 (2.4)	1 (2.3)	1 (2.3)	0.987
Hypertension	31 (36.0)	16 (37.3)	15 (34.9)	0.758
Coronary heart disease	6 (7.0)	3 (7.0)	3 (7.0)	0.976
Diabetes	8 (9.3)	3 (7.0)	5 (11.6)	0.479
Chronic renal failure	1 (1.2)	0 (0)	1 (2.3)	0.320
Vital sign, median (IQR)				
Heart rate, beats per min	92 (84–100)	92 (90–101)	91 (80–100)	0.979
Systolic pressure, mm Hg	135 (120–146)	137 (122–143)	135 (119–147)	0.509
Diastolic pressure, mm Hg	80 (71–92)	80 (70–91)	81 (74–92)	0.194
Respiratory rate, times per min	20 (20–24)	21 (20–30)	20 (19–29)	0.481
Temperature	38.2 (37.8–38.6)	38.1 (37.8–38.5)	38.3 (37.9–38.6)	0.557
PSI score, *n* (%)				
Class I	3 (3.5)	1 (2.3)	2 (4.7)	0.780
Class II	18 (20.9)	8 (18.6)	10 (23.3)	
Class III	40 (46.5)	19 (44.2)	21 (48.8)	
Class IV	11 (12.8)	7 (16.3)	4 (9.3)	
Class V	14 (16.3)	8 (18.6)	6 (14.0)	
CURB-65, median (IQR)	0 (0–1)	1 (0–1)	0 (0–1)	0.193
SOFA score, median (IQR)	1 (0–2)	2 (1–2)	1 (0–2)	0.083
Complications, *n* (%)				
Hypoxemia respiratory failure	41 (47.7)	22 (51.2)	19 (44.2)	0.450
Abnormal liver function	13 (15.1)	6 (14.0)	7 (16.3)	0.799

CURB-65 denotes a six-point score, one point for each of confusion: urea >7 mmol/L, respiratory rate >30/min, low systolic (<90 mm Hg) or diastolic (<60 mm Hg) blood pressure, age >65 years. PSI, pneumonia severe index; SOFA, sequential organ failure assessment; IQR, interquartile range.

Table 2. Primary and secondary outcomes

Characteristic	Total (*N* = 86)	Methyl-prednisolone (*N* = 43)	Control (*N* = 43)	Regression analysis	
				OR, or HR* (95% CI)	*p* value
Primary outcome, *n* (%)					
Clinical deterioration 14 days after randomization	4 (4.8)	2 (4.8)	2 (4.8)	OR 1.000 (0.134–7.442)	1.000
Secondary outcome					
Clinical cure 14 days after randomization, *n* (%)	47 (54.7)	22 (51.2)	25 (58.1)	OR 1.326 (0.566–3.106)	0.516
Time from randomization to clinical cure, median (IQR), days	13 (10–19)	14 (10–19)	12 (9–17)	HR 1.043 (0.673–1.617)	0.850
ICU admission, *n* (%)	4 (4.8)	2 (4.8)	2 (4.8)	OR 1.000 (0.134–7.442)	1.000
Hospitalization duration, median (IQR), days	16 (11–21)	17 (13–22)	13 (10–20)	HR 1.300 (0.844–2.002)	0.235
In-hospital mortality, *n* (%)	1 (1.2)	0 (0)	1 (2.3)	OR 0.977 (0.933–1.023)	0.314
Time from virus shedding of SARS-CoV-2, median (IQR), days	9 (4–14)	11 (6–16)	8 (2–12)	HR 1.782 (1.057–3.003)	0.030

CI, confidence interval; HR, hazard ratio; ICU, intensive care unit; IQR, interquartile range; OR, odds ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. * Differences were expressed as ORs (95% CIs) analyzed by logistic regression analysis adjusted for the stratification variables, and HRs (95% CIs), estimated by Cox proportional risk model for time variable.

March, we enrolled the last patient on March 31, 2020 and terminated the trial on April 15, 2020. A total of 213 potential eligible patients were screened, and 127 of them were excluded because they did not meet the inclusion criteria (Fig. 1). Eventually, 86 patients from 7 centers (online suppl. Table 2) were randomized to either the methylprednisolone group (*n* = 43) or control group (*n* = 43) (Fig. 1). None of the patients withdrew from the trial or were excluded from the analyses because of missing or incomplete data.

As shown in Table 1, the median age of the whole cohort was 56 years (interquartile range, 39–66 years) and 41 (47.7%) patients were male. The median time from the symptom onset to randomization was 8 days (6–16 days). At randomization, 61 (70.9%) patients received oxygen therapy via a nasal cannula, while 41 (47.7%) underwent hypoxemia respiratory failure. The median arterial partial pressure of oxygen/fractional inspired oxygen was 311.5 mm Hg (236.0–354.0 mm Hg), and 41 of 86 (47.7%) patients had their arterial partial pressure of oxygen/fractional inspired oxygen below 300 mm Hg. At randomization, there was no difference in all the demographic, clinical, and laboratory characteristics between the 2 groups except that more sputum production was seen in the control group (Table 1; online suppl. Table 3).

During hospitalization, 67 (77.9%) patients in both groups were administered antivirus drugs, and 61 (70.9%) were given antibiotics. Seventy (88.1%) patients needed nasal cannula oxygen therapy, 3 (3.5%) used high-flow nasal cannula oxygen therapy, 4 (4.6%) received mechanical ventilation because of disease deterioration, and 2 of whom underwent extracorporeal membrane oxygenation. Overall, there was no difference between the 2 groups in the medicine application and respiratory support (online suppl. Table 4).

Primary Outcome

Totally, 4 (4.8%) patients showed clinical deterioration in 14 days after randomization, and there was no difference in the incidence of clinical deterioration between the methylprednisolone group and control group (4.8 vs. 4.8%; odds ratio, 1.000 [95% confidence interval, 0.134–7.442]; $p = 1.000$) (Table 2; Fig. 2a).

Secondary Outcome

As shown in Table 2, Figure 2b and c, there was no difference in the percentage of clinical cure 14 days after randomization, time from randomization to clinical cure, ICU admission, hospitalization duration, and in-hospital mortality between the methylprednisolone group and control group (all $p > 0.05$). The median time from randomization to SARS-CoV-2 shedding in the methylprednisolone group was significantly longer than that in the control group (median, 11 days vs. 8 days; hazard ratio, 1.782 [1.057–3.003]; $p = 0.030$) (Table 2; Fig. 2d).

Effect of Methylprednisolone on Immune Cell Profile

Mass cytometry analysis of blood $CD45^+$ immune cells revealed that the profile of the main cell subsets gated from the total $CD45^+$ cells have changed after treatment with methylprednisolone (Fig. 3a). Further analysis discovered that 7 days after randomization and methylpred-

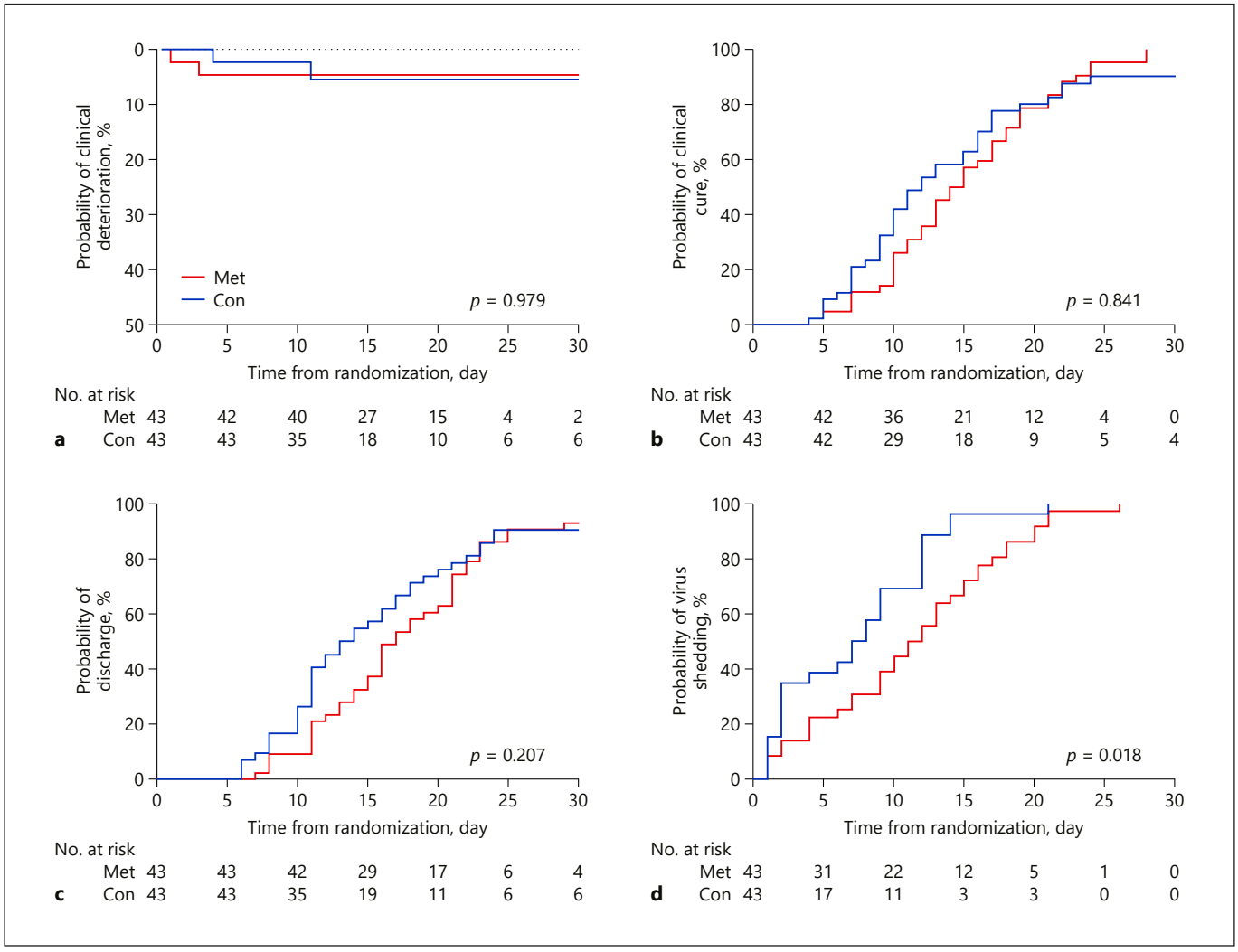

Fig. 2. Kaplan-Meier curves of the primary outcome and secondary outcomes between the Met group and Con group. Shown are the probability of clinical deterioration (**a**), clinical cure (**b**), discharge (**c**), and SARS-CoV-2 shedding (**d**) over time. The Kaplan-Meier approach with the log-rank test was used to assess the primary and secondary outcomes. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Met, methylprednisolone; Con, control.

nisolone treatment, $CD3^+$ T cells and $CD8^+$ T cells in the methylprednisolone group were significantly lower than those in the control group (both $p < 0.05$) (Fig. 3b, d); 14 days after randomization, NK cells in the methylprednisolone group were significantly lower than those in the control group ($p < 0.05$) (Fig. 3f). We also found that methylprednisolone treatment led to an increase in $CD11b^+$ myeloid cells on day 7 (Fig. 3g). In addition, $CD4^+$ and $CD8^+$ T cells were reduced on day 7 after methylprednisolone treatment as compared to day 0 (both $p < 0.05$) (Fig. 3c, d). In addition, methylprednisolone treatment did not exert effect on $CD19^+$ B cells and the other immune cells (Table 3).

Stratified by Acute Hypoxemic Respiratory Failure

We stratified patients by whether they underwent acute hypoxemic respiratory failure at randomization (online suppl. Table 5). All 4 patients showing clinical deterioration were those with respiratory failure. There was no difference in the incidence and time from randomization to clinical deterioration between the 2 groups (online suppl. Table 6). Among patients without respiratory fail-

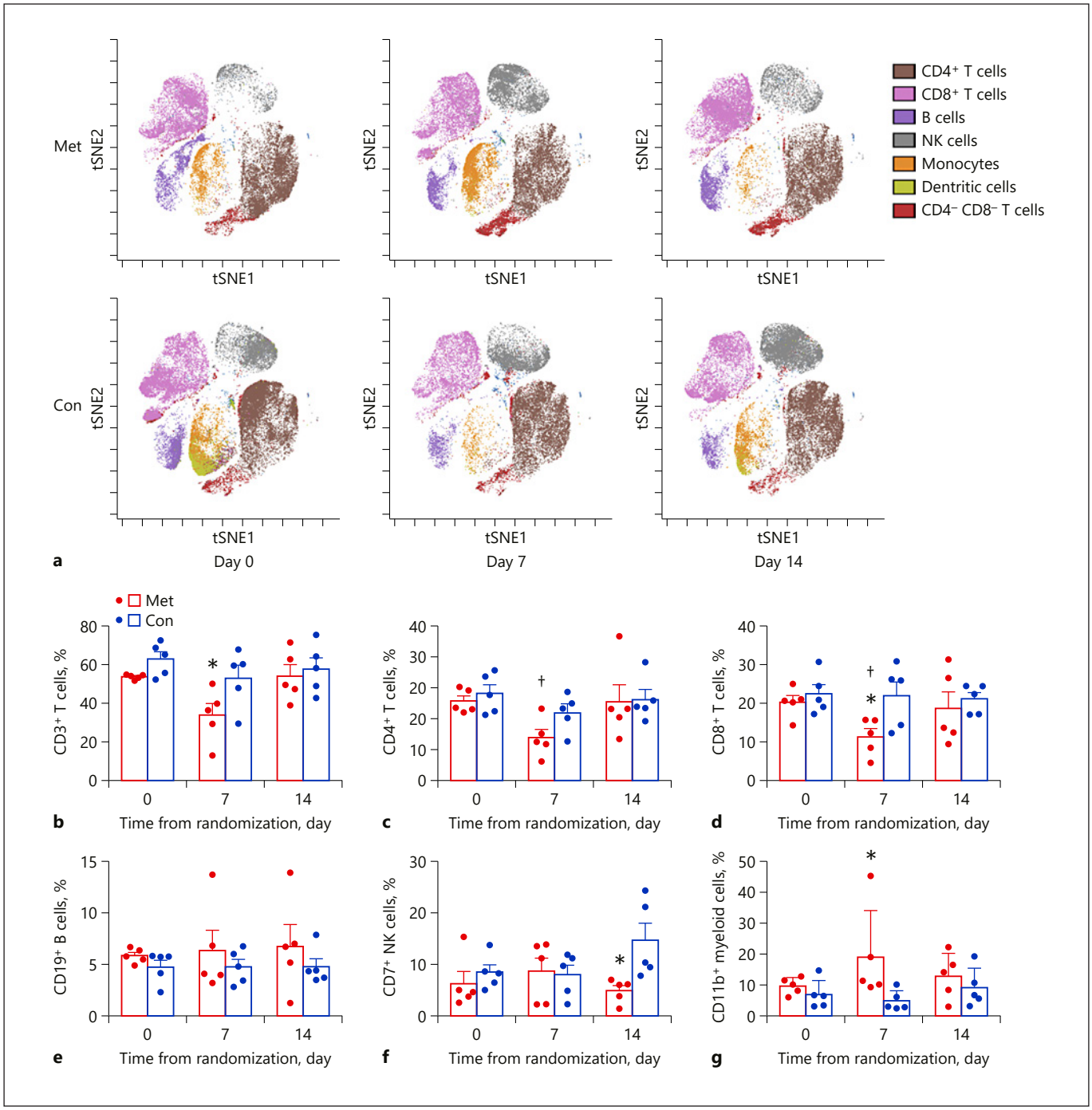

Fig. 3. Mass cytometric analysis of CD45$^+$ immune cells from peripheral blood. **a** Representative t-SNE plots of CD45$^+$ immune cells on day 0 (at randomization), 7 and 14 days after randomization derived from one COVID-19 patients from the Met group (upper panels) and one from the Con group (bottom panels) are shown. One point represents one cell, and the dark and light colors shows high expression and low expression. The comparisons of the percentages of blood CD3$^+$ T cells (**b**), CD4$^+$ T cells (**c**), CD8$^+$ T cells (**d**), CD19$^+$ T cells (**e**), CD7$^+$ NK cells (**f**), and CD11b$^+$ myeloid cells (**g**) between the Met group and Con group. Data are presented as mean ± SD. *$p < 0.05$ compared with the Con group on the same time point by repeated measures analysis of the two-way ANOVA followed by Bonferroni's test. †$p < 0.05$ compared with day 0 in the Met group by repeated measures analysis of the one-way ANOVA followed by Dunnett's test. COVID-19, coronavirus disease 2019; Met, methylprednisolone group; Con, control group.

Table 3. Effect of methylprednisolone treatment of immune cell profile determined by mass cytometry

	Methylprednisolone			Control		
	day 0	day 7	day 14	day 0	day 7	day 14
Of $CD45^+$ cells, %						
$CD3^+$ T cells	53.6±0.6	33.6±6.2*	54.0±5.8	62.8±3.8	52.9±6.7	57.8±5.7
$CD4^+$ T cells	30.7±2.0	16.4±3.3†	30.4±6.7	33.7±3.4	26.3±3.3	31.3±3.9
$CD8^+$ T cells	20.3±1.7	11.3±2.1*, †	18.7±4.3	23.4±3.2	21.9±3.6	21.1±1.7
$CD19^+$ B cells	5.8±0.3	6.3±1.9	6.8±2.0	4.7±0.7	4.8±0.7	4.8±0.8
$CD7^+$ NK cells	6.3±2.3	8.6±2.6	4.9±1.0*	8.4±1.5	8.0±1.9	14.6±3.4
$CD11b^+$ myeloid cells	9.7±1.2	18.9±6.8	12.8±3.3	6.8±2.1	4.9±1.4	9.1±2.8
$CD16^+$ neutrophils	0.06±0.02	0.85±0.71	0.35±0.21	0.23±0.013	0.08±0.05	0.32±0.14
$CD11c^+$ DCs	0.4±0.1	0.2±0.1	0.3±0.1	0.3±0.2	0.1±0.0	0.6±0.2
$CD14^+$ monocytes	9.1±0.9	8.4±0.6	9.2±3.0	4.8±1.8	3.4±1.1	6.8±2.3
Of $CD4^+$ cells, %						
$CD4^+CD45RA^+$ naïve $CD4^+$ cells	49.1±6.3	50.9±6.2	37.4±2.8	43.3±4.1	44.9±4.6	45.5±4.3
$CD4^+CD45RO^+$ memory $CD4^+$ cells	24.3±6.6	20.1±4.5	32.6±4.8	38.8±7.1	25.7±4.0	32.8±5.5
$CD4^+CD25^{++}$ regulatory T cells	3.5±0.6	3.6±0.9	3.5±0.4	2.6±0.1	3.4±0.3	2.9±0.4
$CD4^+CCR4^+$ cells	41.0±1.3	44.9±1.5	42.9±4.7	39.6±3.5	38.8±2.7	44.7±4.1
$CD4^+CCR5^+$ cells	2.1±0.4	2.8±0.4	2.3±0.3	3.7±1.2	4.3±0.6	4.7±1.1
$CD4^+CCR6^+$ cells	54.7±3.0	61.6±2.6	56.6±4.0	55.3±2.6	53.4±3.3	57.5±2.2
$CD4^+CXCR3^+$ cells	51.8±2.4	55.9±3.8	49.3±4.1	49.5±3.5	47.8±3.0	54.0±3.4
$CD4^+CXCR5^+$ cells	6.1±1.2	4.4±1.4	5.9±1.3	5.5±0.6	5.2±0.9	7.2±1.0
$CD4^+CD161^+$ $CCR6^+$ cells	2.2±0.6	2.3±0.6	3.4±0.6	4.6±1.8	3.9±0.9	4.3±1.0
Of $CD8^+$ cells, %						
$CD8^+CD45RA^+$ naïve $CD8^+$ cells	38.2±3.8	45.7±8.3	35.6±5.5	47.0±5.9	45.2±6.5	50.2±3.6
$CD8^+CD45RO^+$ memory $CD8^+$ cells	37.6±3.6	28.6±5.5	36.6±6.7	28.3±4.9	28.7±5.6	31.5±5.5
$CD8^+CCR4^+$ cells	28.1±2.8	34.0±2.5	32.3±6.8	27.2±4.0	27.1±2.1	35.5±4.1
$CD8^+CCR5^+$ cells	11.2±1.8	18.6±4.0	13.3±3.7	13.2±4.0	15.4±2.7	17.5±2.7
$CD8^+CCR6^+$ cells	37.9±2.5	49.3±2.5	44.0±6.8	41.9±4.9	45.4±2.7	50.1±1.9
$CD8^+CXCR3^+$ cells	47.5±5.3	55.5±3.1	50.1±7.2	50.8±6.3	52.1±4.4	59.9±2.8
$CD8^+CXCR5^+$ cells	2.0±0.3	2.0±0.3	1.4±0.1	2.1±0.3	1.9±0.3	2.4±0.8
Of $CD19^+$ B cells, %						
$CD5^+$ regulatory B cells	6.5±3.5	5.2±3.4	1.6±0.3	3.2±1.4	5.8±3.2	3.2±0.5
$CD27^-IgD^+$ naive B cells	50.9±9.3	42.3±7.1	51.2±4.8	57.8±3.9	50.1±7.6	61.7±1.7
$CD19^+CD27^+$ memory B cells	19.1±4.9	21.5±4.1	19.7±1.9	15.5±1.8	19.2±3.5	12.3±1.4
$CD27^+CD38^+$ plasma B cells	0.7±0.2	0.4±0.1	0.6±0.3	0.4±0.1	0.8±0.2	0.7±0.2

Data are presented as mean ± SEM. * $p < 0.05$ compared with the control group at the same time points after randomization by repeated measures analysis of the two-way ANOVA followed by Bonferroni's test. † $p < 0.05$ compared with day 0 after randomization in the methylprednisolone group by repeated measures analysis of the one-way ANOVA followed by Dunnett's test.

ure, the median time from randomization to the median time from randomization to virus shedding in the methylprednisolone group was 10 days (6–17 days), which was significantly longer than that in the control group (6 days [2–9 days]; hazard ratio, 2.730 [1.247–5.974]; $p = 0.012$). In contrast, the above effects of methylprednisolone did not exist among patients with acute hypoxemic respiratory failure (online suppl. Table 6).

Side Effects

Hyperglycemia with the need for additional therapy after randomization occurred in 3 (7.0%) patients in the methylprednisolone group and in 7 (16.3%) in the control group ($p = 0.313$). Two (4.7%) patients in the methylprednisolone group and one (2.3%) in the control group acquired ventilator-associated pneumonia after they had been transferred to the ICU and received invasive mechanical ventilation ($p = 0.557$). None of the patient in both groups had stress ulcer, gastrointestinal hemorrhage, or delirium (online suppl. Table 7). Corticosteroid-associated long-term complications were not estimated because of the short observation duration.

Discussion

This was a randomized control trial to investigate the application of corticosteroids in patients with COVID-19 pneumonia. Although with less statistical power as it was prematurely closed, our trial found that methylprednisolone added to standard therapy may not make the patients with COVID-19 pneumonia benefit from the outcomes of clinical deterioration, clinical cure, ICU admission, or mortality. However, based on the existing data and mass cytometry, we discovered that the viral shedding time in the methylprednisolone group was much longer than that in the control group. It may be associated with immune cells suppressed by the application of corticosteroid.

Because of very limited availability of evidence for harm or benefit from corticosteroids in treatment of viral pneumonia including COVID-19, the use of corticosteroids remains controversial [19, 20]. A retrospective study suggested that use of corticosteroid in critically ill SARS patients resulted in lowered mortality and a shorter hospitalization stay [21]. Another retrospective study suggested that treatment with methylprednisolone decreased the risk of death among COVID-19 patients with ARDS [13]. In contrast, several retrospective studies found no evidence of a beneficial effect of corticosteroids in severe patients with influenza A/H1N1 [22, 23] or A/H7N9 infection [24] since corticosteroids were independently associated with higher mortality. In a retrospective observational study reporting on 309 patients with critically ill MERS, corticosteroid therapy was not associated with a difference in mortality after adjustment for time varying confounders but was associated with delayed clearance of viral RNA from respiratory tract secretions [25]. These data suggest that corticosteroids may be hazardous when administered early in the context of severe viral pneumonia.

There have already been some studies about corticosteroid therapy application in critically ill patients of COVID-19, including different dosages of dexamethasone [26, 27] and hydrocortisone [28, 29]. Most results of these research studies concluded a significant clinical benefit from the application of corticosteroid in the patients of COVID-19-induced acute respiratory failure. And the meta-analysis from the WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group showed administration of systemic corticosteroids was associated with lower 28-day all-cause mortality in critically ill patients with COVID-19 [30]. However, the present study acquired an opposite result that early use of low-dose methylprednisolone may not obtain any clinical benefits from early methylprednisolone treatment in the patients of COVID-19, even prolonged the virus shedding. Considering that the patients in this trial were relatively less critical, so based on the above studies and our result, corticosteroids administered in the selected patients might help acquire better prognosis.

A pathological study of a patient who died from COVID-19 pneumonia reveals bilateral diffuse alveolar damage with cellular fibromyxoid exudates, interstitial lymphocyte infiltrates, and multinucleated syncytial cells in the intra-alveolar spaces [31]. Clinical studies suggested that dysregulation of immune response, especially T cells, might be highly involved in the pathological process of COVID-19 [32–34]. Our previous studies further demonstrated that a remarkable reduction in $CD8^+$ T cells is one of the predictors for mortality of COVID-19 patients [3]. A small prospective, randomized double-blinded, placebo-controlled trial has revealed that early steroid treatment results in a higher subsequent plasma viral load in SARS patients [35]. Similar results have also been observed in a retrospective study that corticosteroid was associated with delayed MERS coronavirus RNA clearance [25]. In the present study, our trial found $CD4^+$ cells, $CD8^+$ T, and NK cells were reduced on day 7 after methylprednisolone treatment as compared to randomization through mass cytometry. These immune cells all play an important role in the immune response of antivirus [36]. Therefore, we provided the evidence to show that methylprednisolone could retard virus shedding by suppressing immune cells in patients with COVID-19 pneumonia, such as $CD4^+$ T cells, $CD8^+$ T cells, and NK cells.

Usually, corticosteroids are prescribed for viral pneumonia patients with severe disease. Our inclusion criterion was set to recruit all hospitalized patients with confirmed diagnosis of COVID-19 pneumonia. About half of the participants demonstrated acute hypoxemia respiratory failure during randomization. In the subgroup analysis, we did not find that methylprednisolone affected clinical deterioration either in patients with or without respiratory failure. However, methylprednisolone did extend the virus shedding time and hospital days in patients without acute respiratory failure. In China, patients with COVID-19 were not allowed to be discharged until their SARS-CoV-2 tests turned out to be negative. These data suggested that COVID-19 pneumonia patients could not gain benefits in clinical improvement from the addition of methylprednisolone, while patients even without acute respiratory failure could only gain harm from such a treatment.

Corticosteroid application in treating viral pneumonia has many potential risks, such as secondary infections and other long-term complications. One retrospective study found that corticosteroid-treated patients are more likely to suffer from secondary bacterial pneumonia or invasive fungal infection than those who were not administered with corticosteroids [23]. However, another retrospective study showed that the use of corticosteroids in SARS patients was not associated with secondary lower respiratory infection and other complications [21]. In this study, we applied a short-term low-dose of methylprednisolone in the early stage among patients with COVID-19 pneumonia and noted that neither hyperglycemia nor secondary lower respiratory infection was associated with methylprednisolone treatment. It is worth noting that such incidence of methylprednisolone-related complications might be underestimated because of the small sample size.

Our trial has 2 limitations. First, the study was stopped prior to reaching the goal size due to the decreased number of cases of COVID-19 in China, and this may have blunted benefit/harm and meaningful differences in the clinical course if any. Second, we did not detect quantitative viral load measurement whereas just detected the presence or absence of SARS-CoV-2 RNA in throat swabs but not in the blood or the other specimens. Third, due to the short observation duration, it was difficult to follow up the long-term complications.

Conclusions

Due to early termination of this trial, most outcomes were difficult to be estimated because of the low statistical power. However, we found that the short-term early use of corticosteroid could suppress the immune cells, which may prolong SARS-CoV-2 shedding in patients with COVID-19 pneumonia, especially for the patients without acute respiratory failure. It was suggested that corticosteroids should not be added to standard therapy as a general treatment for the patients of COVID-19 patients; moreover, it should be evaluated according to the severity and necessity. The interpretation still needs to be further verified by a large sample size and randomized clinical trials.

Statement of Ethics

The study protocol was approved by the Ethics Committee of Beijing Chao-Yang Hospital (2020-KE-22) and all the other participating centers, and informed consent was obtained from the patients themselves or their legal guardians.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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Author Contributions

H.Z.S. and B.S. conceived the idea, designed and supervised the study, drafted the manuscript, had full access to all of the data, and took responsibility for the integrity of the data. J.X.N., Y.M.F., L.M.L., K.H., J.X.Z., J.W.C., J.C.Z., Y.L.L., Y.Z., J.S., X.L.H., J.Y.Z., Z.D., and J.X. collected data. X.T., W.W., and X.Z.W. analyzed data and performed statistical analysis. R.H.J., Y.M.F. and X.Z.W. did mass cytometry analysis. All of the authors reviewed and approved the final version of the manuscript.

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