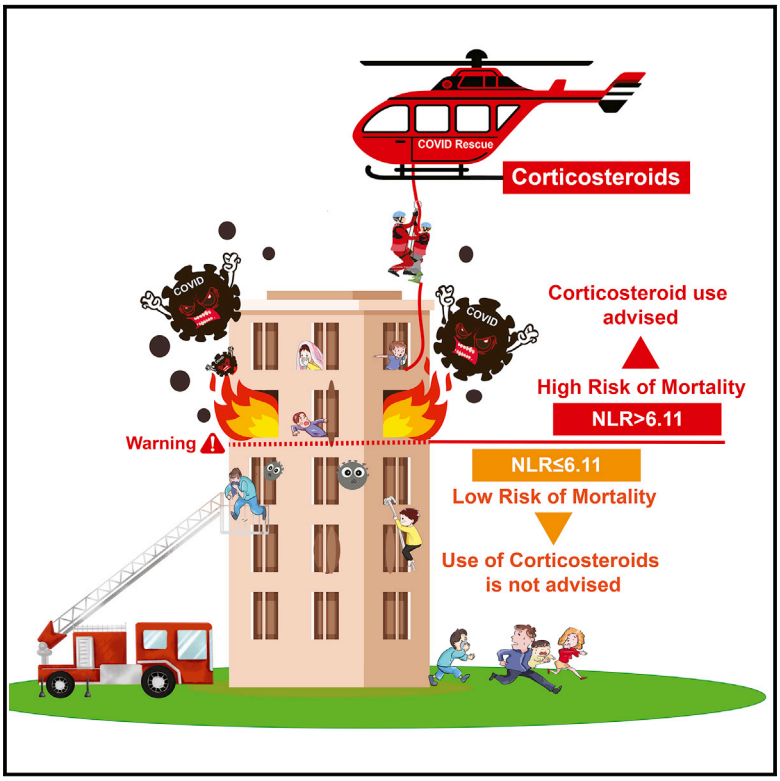
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Cell Metabolism
Clinical and Translational Report

The Neutrophil-to-Lymphocyte Ratio Determines Clinical Efficacy of Corticosteroid Therapy in Patients with COVID-19

Graphical Abstract



Highlights

- 12,862 COVID-19 cases on corticosteroid therapy or not were retrospectively studied
- NLR at admission is a key factor for patients with high or low risk of death
- An NLR > 6.11 was associated with lower mortality in patients on corticosteroids
- Corticosteroids did not reduce mortality in patients with an NLR $\leq$ 6.11 or with T2D

Authors

Jingjing Cai, Haomiao Li, Changjiang Zhang, ..., Xin Zhang, Xiao-Jing Zhang, Hongliang Li

Correspondence

yibinwang@mednet.ucla.edu (Y.W.), yangjuancat@whu.edu.cn (J.Y.), zhangxin57@whu.edu.cn (X.Z.), zhangxjing@whu.edu.cn (X.-J.Z.), lihl@whu.edu.cn (H.L.)

In Brief

While corticosteroid therapy is effective in the treatment of patients with severe COVID-19, a quantitative clinical parameter to identify such severity and which patients would respond well to corticosteroids has not been developed. Here, Cai et al. find that a simple blood test that measures the neutrophil-to-leukocyte ratio at admission discriminates high versus low mortality risk and a better response to corticosteroid therapy.

Cai et al., 2021, Cell Metabolism *33*, 258–269
February 2, 2021
https://doi.org/10.1016/j.cmet.2021.01.002

CellPress

CellPress

Cell Metabolism

Clinical and Translational Report

The Neutrophil-to-Lymphocyte Ratio Determines Clinical Efficacy of Corticosteroid Therapy in Patients with COVID-19

Jingjing Cai,[1,3,4,22] Haomiao Li,[1,3,22] Changjiang Zhang,[1,3,5,22] Ze Chen,[1,3,22] Hui Liu,[2,14,22] Fang Lei,[1,2,3] Juan-Juan Qin,[1,2,3] Ye-Mao Liu,[1,3] Feng Zhou,[3,6] Xiaohui Song,[1,3] Jianghua Zhou,[1,3] Yan-Ci Zhao,[1,3] Bin Wu,[1,3] Meiling He,[2,3] Huilin Yang,[2,3] Lihua Zhu,[1,3] Peng Zhang,[2,3,6] Yan-Xiao Ji,[3,6] Guang-Nian Zhao,[3,6] Zhigang Lu,[7] Liming Liu,[8] Weiming Mao,[9] Xiaofeng Liao,[10] Haofeng Lu,[11] Daihong Wang,[12] Xigang Xia,[13] Xiaodong Huang,[14] Xiang Wei,[15] Jiahong Xia,[16] Bing-Hong Zhang,[17] Yufeng Yuan,[18] Zhi-Gang She,[1,2,3] Qingbo Xu,[19] Xinliang Ma,[20] Yibin Wang,[21,*] Juan Yang,[1,3,*] Xin Zhang,[2,14,*] Xiao-Jing Zhang,[1,2,3,*] and Hongliang Li[1,2,3,6,23,*]

[1]Department of Cardiology, Renmin Hospital, Wuhan University, Wuhan, China
[2]School of Basic Medical Science, Wuhan University, Wuhan, China
[3]Institute of Model Animal, Wuhan University, Wuhan, China
[4]Department of Cardiology, The Third Xiangya Hospital, Central South University, Changsha 410000, China
[5]The Central Hospital of Enshi Tujia and Miao Autonomous Prefecture, Enshi 445000, China
[6]Medical Science Research Center, Zhongnan Hospital of Wuhan University, Wuhan, China
[7]Department of Neurology, The First People's Hospital of Jingmen affiliated to Hubei Minzu University, Jingmen 448000, China
[8]Department of General Surgery, Ezhou Central Hospital, Ezhou 436000, China
[9]Department of General Surgery, Huanggang Central Hospital, Huanggang 438000, China
[10]Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang, China
[11]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Changjiang University, Jingzhou, China
[12]Department of Hepatobiliary and Pancreatic Surgery, Xianning Central Hospital, Hubei Province, Xianning, China
[13]Department of Hepatobiliary Surgery, Jingzhou Central Hospital, Jingzhou, China
[14]Department of Gastroenterology, Wuhan Third Hospital and Tongren Hospital of Wuhan University, Wuhan, China
[15]Division of Cardiothoracic and Vascular Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[16]Department of Cardiovascular Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[17]Department of Neonatology, Renmin Hospital of Wuhan University, Wuhan, China
[18]Department of Hepatobiliary and Pancreatic Surgery, Zhongnan Hospital of Wuhan University, Wuhan, China
[19]Centre for Clinic Pharmacology, The William Harvey Research Institute, Queen Mary University of London, London, UK
[20]Department of Emergency Medicine, Thomas Jefferson University, Philadelphia, PA 19004, USA
[21]Departments of Anesthesiology, Physiology, and Medicine, Cardiovascular Research Laboratories, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA
[22]These authors contributed equally
[23]Lead Contact
*Correspondence: yibinwang@mednet.ucla.edu (Y.W.), yangjuancat@whu.edu.cn (J.Y.), zhangxin57@whu.edu.cn (X.Z.), zhangxjing@whu.edu.cn (X.-J.Z.), lihl@whu.edu.cn (H.L.)
https://doi.org/10.1016/j.cmet.2021.01.002

SUMMARY

Corticosteroid therapy is now recommended as a treatment in patients with severe COVID-19. But one key question is how to objectively identify severely ill patients who may benefit from such therapy. Here, we assigned 12,862 COVID-19 cases from 21 hospitals in Hubei Province equally to a training and a validation cohort. We found that a neutrophil-to-lymphocyte ratio (NLR) > 6.11 at admission discriminated a higher risk for mortality. Importantly, however, corticosteroid treatment in such individuals was associated with a lower risk of 60-day all-cause mortality. Conversely, in individuals with an NLR $\leq$ 6.11 or with type 2 diabetes, corticosteroid treatment was not associated with reduced mortality, but rather increased risks of hyperglycemia and infections. These results show that in the studied cohort corticosteroid treatment is associated with beneficial outcomes in a subset of COVID-19 patients who are non-diabetic and with severe symptoms as defined by NLR.

INTRODUCTION

Corticosteroids have been used for over half a century to treat inflammatory diseases and acute respiratory distress syndrome (ARDS). However, whether corticosteroid use is clinically efficacious for COVID-19 is still a matter of intense debate (Barnes, 2016; Cain and Cidlowski, 2017; Chotiyarnwong and McCloskey, 2020). Recent published results from a number of clinical

trials suggest a beneficial effect of dexamethasone or hydrocortisone as they reduce the risk of death and improve organ- or ventilation-support-free days among critically ill patients (Angus et al., 2020; Dequin et al., 2020; Horby et al., 2020; Prescott and Rice, 2020; Sterne et al., 2020). After a systematic review from seven randomized clinical trials of corticosteroid versus standard care in COVID-19, the WHO has published the current guidance on corticosteroids for COVID-19, recommending systemic corticosteroid therapy in patients with severe and critical COVID-19, and a conditional recommendation not to use corticosteroid therapy in patients with non-severe COVID-19 (World Health Organization, 2020a). However, the criteria for identifying severe and critical patients with COVID-19 have not been defined, which may lead to inappropriate use of corticosteroids in the treatment of the disease. Given the many side effects associated with corticosteroid use, it is critical to develop an objective, practical, and reliable clinical parameter to identify patients with severe COVID-19 who may benefit from corticosteroid therapy.

The pathophysiologic basis for corticosteroid use in a wide array of inflammatory diseases is due to its ability to suppress systemic inflammation and moderate the ensuing cytokine storm (Shang et al., 2020). A rapidly progressing pneumonia with overwhelming systemic inflammation and subsequent multi-organ damage are key features of clinical manifestations of severe COVID-19. Therefore, a therapeutic regime with corticosteroids has been attempted to mitigate the overactive inflammatory response in patients with severe pneumonia caused by SARS-CoV-2 infection. However, corticosteroid use also has the well-known risk of impairing pathogen clearance by the immune system. Finally, an absence of reliable evidence from large-scale randomized clinical trials (RCTs) further impedes its widespread application worldwide (Hui, 2018; Ye et al., 2020; Russell et al., 2020; Tang et al., 2020).

Given the key clinical manifestations of severe COVID-19 outlined above, biomarkers related to the inflammatory status of the patients should serve as good candidate indicators to define the severity and outcome among such patients. Indeed, major changes in the proportion of blood cell subsets, particularly in immune cells, have been recognized as a sensitive hallmark of systemic inflammatory states and are also closely associated with COVID-19 outcomes (Terpos et al., 2020; Xiong et al., 2020; Zhou et al., 2020). Given the immunomechanisms of corticosteroids, changes in circulating immune cell levels may be an important guide in the proper application of these drugs in patients with COVID-19. Furthermore, routine blood cell tests are one of the most commonly prescribed clinical tests available for all hospitalized patients as they are typically very low in cost and display high accuracy. Therefore, biomarkers derived from blood cell tests may serve as critical and practical indicators with broad applicability in geographic regions with different levels of medical resources. Here, we report the identification and validation of a clinical indicator from a relatively simple blood cell test, namely the neutrophil-to-leukocyte ratio (NLR), that is associated with COVID-19 severity and the outcome of corticosteroid usage at admission based on retrospective analyses in 12,862 hospitalized COVID-19 individuals. Such an indicator may assist in the clinical decision-making process concerning the initiation of corticosteroid treatment.

RESULTS AND DISCUSSION

Baseline Characteristics of the Subjects in the Training and Validation Cohorts

The study cohort consisted of subjects with COVID-19 admitted between December 30, 2019, and April 17, 2020, from 21 COVID-19 designated hospitals in Hubei, China. A total of 12,862 individuals with COVID-19 and the eligibility criteria were included in this study (Figure 1). These individuals were randomly assigned to the training and validation cohorts at a 50%/50% ratio. Their baseline characteristics are shown in Table S1. There were 25.3% of individuals on corticosteroid therapy in the entire cohort. The median age of the participants was 58 years (interquartile range [IQR], 46–68), the median heart rate was 84 (IQR, 78–96), the median respiratory rate was 20 (IQR, 19–21), and the median oxygen saturation (SpO2) was 97% (IQR, 95–98). Among these individuals, 6,226 (48.4%) were male, 1,797 (14.0%) had elevated neutrophil counts, and 2,784 (21.7%) had decreased lymphocyte counts. There were 4,285 (33.3%) individuals who had hypertension, 2,066 (16.1%) individuals had type 2 diabetes (T2D), and 1,103 (8.6%) individuals had coronary arterial disease. All baseline characteristics were comparable between the training and validation cohorts. The absolute levels of laboratory examinations in the training and validation groups are listed in Table S2.

Blood Cell Parameters Are Strongly Associated with a High Risk of Mortality

Accumulated evidence from previous clinical trials suggests that favorable immune modulation by low to moderate doses of corticosteroid usage may contribute to the beneficial effects in patients under critical conditions (Rochwerg et al., 2018; Siemieniuk et al., 2015; Villar et al., 2020). As corticosteroid application can cause a number of side effects, it is critical to identify severe patients with an objective indicator to optimize the therapeutic efficacy. Complete blood cell test is the most commonly available assay in regions with different levels of medical resources and it is closely associated with disease severity and inflammatory response. Therefore, we developed a Cox regression model to predict 60-day in-hospital mortality using ten parameters from complete blood cell counts at admission in the training dataset. The lymphocyte count reduction (HR, 4.62; 95% CI, 3.65–5.85; $p = 3.98E{-}37$) and neutrophil count increase (HR, 4.41; 95% CI, 3.30–5.89; $p = 1.22E{-}23$) were the top two factors significantly and positively associated with mortality (Table 1). In addition, LASSO Cox regression, a regularization method that creates a parsimonious model, was applied to identify critical determinants of mortality among complete blood cell tests at admission. Neutrophil and lymphocyte counts were also identified as the top two factors (Table 1). These findings were consistent with previous studies (Wilk et al., 2020; Zhang et al., 2020a), which implicated that systemic immune response was among the most critical factors related to the clinical outcomes in the subjects with COVID-19.

Considering baseline neutrophil and lymphocyte counts at admission as strong indicators of altered immune status and their significant association with COVID-19 outcomes, we further analyzed the performance of the integrated parameter, that is, a

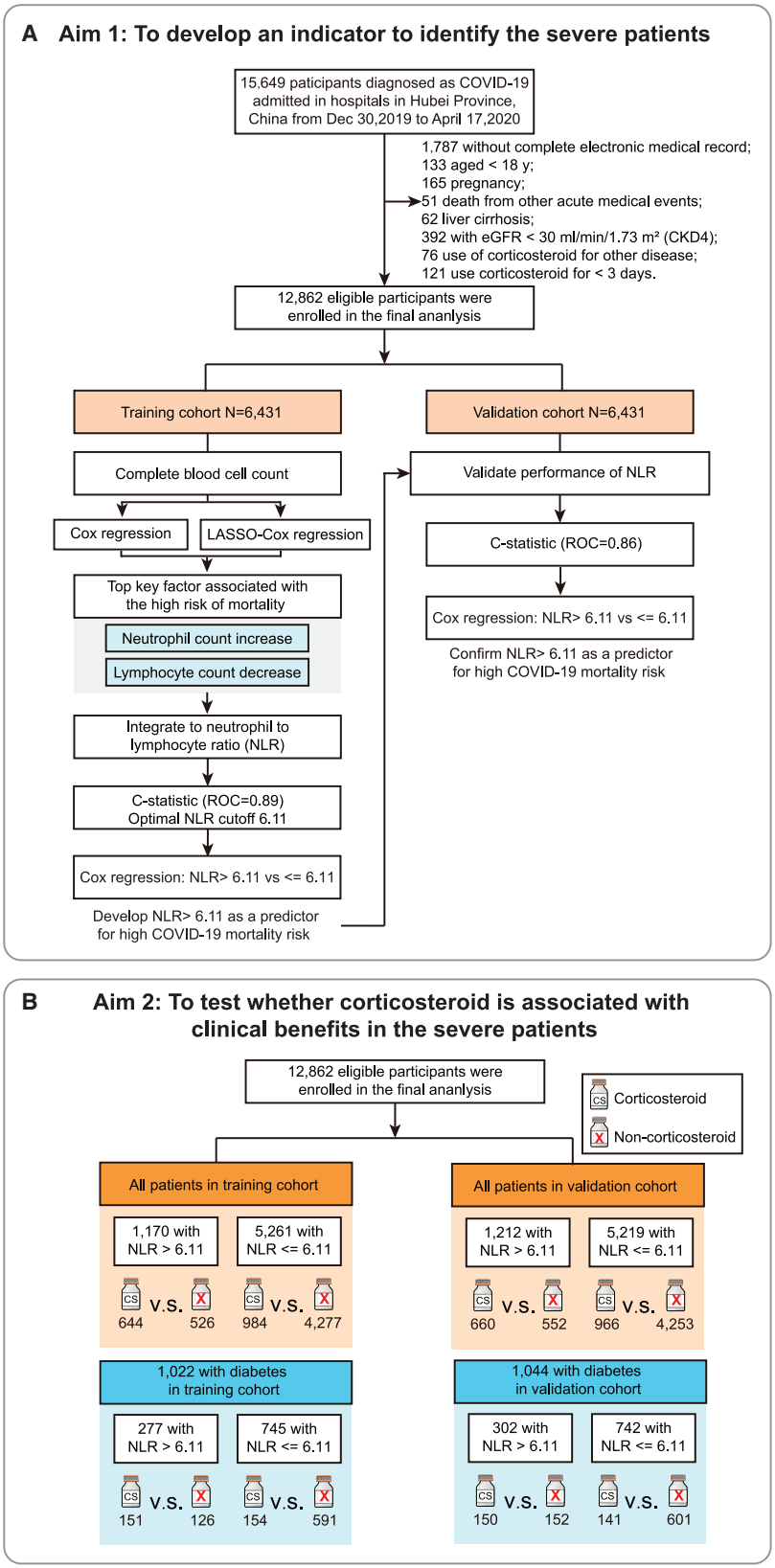


Figure 1. The Flow Chart of Patient Inclusion and Analysis Procedures in the Study

(A) A total of 15,649 individuals admitted to hospitals from December 30, 2019, to April 17, 2020, were enrolled in the study. There were 2,787 individuals not eligible for the study that were excluded. A total of 12,682 individuals were randomly and equally divided into training and validation cohorts. Screening of blood cell factors related to high risk of COVID-19 mortality was performed in the training cohort. The neutrophil-to-lymphocyte ratio (NLR) was the integrated indicator closely associated with the risk of death, and the optimal cut-off at 6.11 was developed by the highest Youden index. The capability of NLR and its cut-off at 6.11 to discriminate the high risk of death were verified in the validation cohort.

(B) The association between corticosteroid treatment and 60-day all-cause mortality was analyzed in individuals with NLR-defined high (>6.11) or low (≤6.11) risk of death in training and validation groups. In addition, the association between corticosteroid treatment and mortality was analyzed in individuals with T2D.

Table 1. The Association between Blood Cells and Mortality in Individuals with COVID-19

	Cox Regression			LASSO Cox Regression
Parameters[a]	HR	95% CI	p Value[b]	Coefficients[c]
Lymphocyte count < 0.8, 10^9/L (12,862/12,862, 100.00%)	4.62	3.65–5.85	3.98E−37	1.13
Neutrophil count > 6.3, 10^9/L (12,862/12,862, 100.00%)	4.41	3.30–5.89	1.23E−23	1.21
Platelet count < 125, 10^9/L (12,691/12,862, 98.67%)	2.96	2.38–3.70	7.48E−22	0.64
Eosinophil count > 0.52, 10^9/L (12,653/12,862, 98.38%)	2.89	0.91–9.15	7.07E−02	–
Leukocyte count > 9.5, 10^9/L (12,719/12,862, 98.89%)	1.92	1.44–2.55	7.99E−06	0.50
Hematocrit increase[d] (12,493/12,862, 97.13%)	1.90	1.10–3.25	2.02E−02	–
Erythrocyte count decrease[e] (12,663/12,862, 98.45%)	1.27	1.01–1.61	4.35E−02	–
Monocyte count > 0.6, 10^9/L (12,493/12,862, 97.13%)	1.09	0.85–1.40	5.07E−01	–
Hemoglobin decrease[f] (12,493/12,862, 97.13%)	0.93	0.73–1.19	5.75E−01	–
Basophil count > 0.06, 10^9/L (12,493/12,862, 97.13%)	0.32	0.10–1.00	5.01E−02	–

HR, hazard ratio; CI, confidence interval. The numbers in the parentheses indicate the proportion of available values for each variable.
[a]Nonparametric missing value imputation was implemented based on the missForest procedure in R.
[b]p values were calculated based on COX regression.
[c]λ = 2.05E−02 (minimum) is chosen as the optimal lambda with the minimal MSE by 10 times cross-validation. The coefficients of less important or collinearity variables shrunk to zero.
[d]Hematocrit increase was defined as hematocrit > 0.50 in male or >0.45 in female.
[e]Erythrocyte count decrease was defined as erythrocyte count < 4.3, 10^{12}/L in male or <3.8, 10^{12}/L in female.
[f]Hemoglobin decrease was defined as hemoglobin < 125 in male or <115 in female.

neutrophil-to-lymphocyte ratio (NLR), in predicting the all-cause mortality in the training cohort (Liu et al., 2020; Long et al., 2020; Qin et al., 2020). C-statistic showed the continuous value of NLR at admission had a prognostic significance for death in the training cohort, with the area under the receiver-operating characteristic curve (AUROC) value of 0.89 (95% CI, 0.87–0.91). Compared to the AUROC of lymphocyte or neutrophil counts alone, the AUROC for NLR increased by 0.05 or 0.10, respectively. These findings indicate NLR had a better performance in discriminating patients at high risk and low risk of death than either lymphocyte or neutrophil counts alone (Table S3; Figure S1A). Therefore, NLR is a superior predictor for mortality risk in individuals with COVID-19.

The maximum Youden index was used to determine the optimal cut-off value of NLR. NLR at 6.11 was identified as the optimal threshold with sufficient sensitivity (0.76) and specificity (0.87) to predict the risk of death in the training cohort (Table S3; Figure S1C). The Cox proportional hazard regression and Kaplan-Meier analysis showed the individuals with NLR above 6.11 had a significantly higher risk of mortality in the training cohort, with an HR of 16.99 (95% CI, 13.48–21.41) (Figure S2A). In the validation cohort, the NLR had an AUROC of 0.86 (95% CI, 0.84–0.88) (Table S3; Figure S1B). The survival analysis showed the individuals with NLR > 6.11 also had a higher risk of mortality than those with NLR $\leq$ 6.11 in the validation cohort, with an HR of 14.01(95% CI, 11.17–17.58) (Figure S2B).

Primary Outcomes Based on NLR-Defined Patient Severity

In the entire population of the study cohort, the individuals with corticosteroid treatment had 4.36-fold higher incidence of death than those without corticosteroid treatment (95% CI, 3.79–5.02; p < 2.00E−16). After adjusting for time-varying exposure, the use of corticosteroid was associated with decreased mortality, with adjusted HR 0.64 (95% CI, 0.51–0.79; p = 5.79E−05). However, after adjusting time-dependent confounders, this association did not reach the significant threshold, with adjusted HR 0.17 (95% CI, 0.02–1.44; p = 1.03E−01). This result indicates corticosteroid therapy may not benefit all individuals with COVID-19 and further subgroup analyses are needed.

The NLR is a well-established indicator of systemic inflammatory status (Laforge et al., 2020; Liao et al., 2020) and the therapeutic target of corticosteroids is associated with their capability to suppress systemic inflammation and the ensuing cytokine storm (Shang et al., 2020). Therefore, we further evaluated the association between corticosteroid therapy and mortality in the COVID-19 individuals stratified by NLR > 6.11 versus NLR $\leq$ 6.11 at admission. The clinical characteristics in both sub-cohorts were listed in Tables S4 and S5. In the strata with NLR > 6.11 in the training cohort, after adjusting for time-varying exposure and confounders, both Cox time-varying exposure model (adjusted HR, 0.56; 95% CI, 0.41–0.77; p = 2.90E−04) and MSM analysis (adjusted HR, 0.47; 95% CI, 0.26–0.86; p = 1.36E−02) indicated that corticosteroid treatment was associated with a lower risk of all-cause mortality (Table 2). Once the NLR $\leq$ 6.11, there was no consistent association between corticosteroid use and the risk of death using either Cox with time-varying exposure model or MSM analysis (Table 2). The use of corticosteroids was also associated with reduced mortality in individuals with an NLR > 6.11 in the validation cohort. These results indicated that a value of NLR above 6.11 at admission appropriately stratified patients for disease severity, as well as clinical outcome from corticosteroid treatment.

We also tested the effect of corticosteroid treatment on COVID-19 patients who developed an NLR > 6.11 after admission; that is, at a later time during hospitalization. However, as only 552 out of 12,862 (4%) patients had demonstrated an ascending trend in NLR value and reached NLR > 6.11 after admission, our data did not show a significant association

Table 2. The Association of Corticosteroid Therapy with 60-Day All-Cause Mortality in Individuals with NLR > 6.11 or ≤6.11

	Incidence			Cox with Time-Varying Exposure		Marginal Structural Model	
Corticosteroid versus Non-Corticosteroid	IR (100 person-day)	IRR (95% CI)	p Value[a]	aHR (95% CI)[b]	p Value	aHR (95% CI)[c]	p Value
Training Cohort							
CS versus non-CS in NLR ≤ 6.11 (984 versus 4,277)	0.11 versus 0.02	7.02 (4.64, 10.62)	<2.00E−16	1.95 (1.04, 3.63)	3.61E−02	1.22 (0.31, 4.71)	7.76E−01
CS versus non-CS in NLR > 6.11 (644 versus 526)	0.65 versus 0.51	1.27 (1.01, 1.60)	3.85E−02	0.56 (0.41, 0.77)	2.90E−04	0.47 (0.26, 0.86)	1.36E−02
Validation Cohort							
CS versus non-CS in NLR ≤ 6.11 (966 versus 4,253)	0.10 versus 0.02	5.10 (3.45, 7.54)	<2.00E−16	0.56 (0.27, 1.18)	1.29E−01	2.29 (0.95, 5.49)	6.40E−02
CS versus non-CS in NLR > 6.11 (660 versus 552)	0.52 versus 0.52	1.01 (0.80, 1.28)	9.01E−01	0.49 (0.34, 0.71)	1.81E−04	0.52 (0.32, 0.84)	7.72E−03

IR (100 person-day), incidence rate; IRR, incidence rate ratio; NLR, neutrophil-to-lymphocyte ratio; aHR, adjusted hazard ratio; CI, confidence interval; CS, corticosteroid.

[a]p values were calculated by R package "fmsb." The significant probability of the result of null-hypothesis testing.

[b]In all the time-varying Cox models, corticosteroid use was considered as time-varying exposure; aHR and p value were calculated. The adjustment factors included age, respiratory rate, SBP, SpO2, diabetes mellitus, coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, neutrophil count, lymphocyte count, erythrocytes, C-reactive protein, procalcitonin, BUN, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

[c]In all the marginal structural models, CURB-65 pneumonia severity score, neutrophil, lymphocyte, and SpO2 were considered as time-varying confounders; aHR and p value were calculated. The adjustment factors included diabetes mellitus, coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, erythrocytes, C-reactive protein, procalcitonin, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

between corticosteroid therapy and mortality in this particular subset of patients (Table S6).

Sensitivity Analyses of NLR Cut-Off at 6.11 for Risk Stratification

To further validate the performance of risk stratification using NLR 6.11 as the cut-off value, we performed the following additional subgroup studies and sensitivity analyses: (1) individuals with different NLR values; (2) individuals with mechanical ventilation; (3) individuals with methylprednisolone; (4) randomly removing patients from two hospitals; and (5) modifications to adjusting confounders, such as gender and hypertension. Both Cox with time-varying exposure and MSM analyses were conducted on those subgroups for sensitivity analyses.

The NLR cut-off points selected for the sensitivity tests either prioritized sensitivity (0.90) over specificity (0.68) or chose specificity (0.90) over sensitivity (0.70) (Figure S1). In the sub-cohort with NLR > 3.43, corticosteroid treatment was associated with lower mortality in the Cox with time-varying exposure model (adjusted HR, 0.50; 95% CI, 0.35–0.71; p = 9.44E−05). However, this association was not supported by the MSM analysis. There was no consistent association between corticosteroid therapy and all-cause mortality in the sub-cohort with an NLR ≤ 3.43 by either Cox with time-varying exposure model or MSM analysis. Therefore, a cut-off value of NLR > 3.43 did not stratify the benefit from corticosteroid therapy for hospitalized COVID-19 patients. In addition, corticosteroid treatment was also not significantly associated with a reduction in mortality in the subpopulation with 3.43 < NLR ≤ 6.11 (Table 3).

As the NLR 7.33 is above the optimal threshold value for predicting death risk and effectiveness of corticosteroid treatment (i.e., 6.11), not surprisingly, the individuals with NLR > 7.33 had significantly lower incidences of all-cause mortality after corticosteroid treatment versus the non-treated ones as demonstrated by either Cox with time-varying exposure model (adjusted HR, 0.47; 95% CI, 0.32–0.70; p = 1.85E−04) or MSM analysis (adjusted HR, 0.53; 95% CI, 0.31–0.88; p = 1.39E−02). However, when all the individuals with an NLR ≤ 7.33 were included, neither the Cox with time-varying exposure model nor MSM analysis showed a significant association between corticosteroid treatment and all-cause mortality. Of note, there was a tendency of protective effect for corticosteroid treatment in individuals with 6.11 < NLR ≤ 7.33. However, due to the limited sample size in this subgroup, the statistical tests did not reach a significant threshold (Table 3).

Sensitivity analyses showed that significant clinical benefits for corticosteroid treatment in the individuals with NLR > 6.11 remained in the subgroup with mechanical ventilation, methylprednisolone, and after modified participating hospitals or following different confounder adjustments (Table 3).

E-value analysis was conducted to assess the robustness of the association between corticosteroid use and all-cause mortality in the Cox with time-varying exposure model and MSM model. When analyzing the association between corticosteroid

Table 3. Subgroup and Sensitivity Test for Primary Outcomes among Individuals with COVID-19

	Cox with Time-Varying Exposure		Marginal Structural Model	
	aHR (95% CI)[a]	p Value	aHR (95% CI)[b]	p Value
Stratified by Different Cutoffs				
CS versus non-CS in NLR ≤ 3.43 (574 versus 3,455)	0.91 (0.29, 2.85)	8.73E−01	5.28 (1.28, 21.76)	2.14E−02
CS versus non-CS in NLR > 3.43 (1,052 versus 1,350)	0.50 (0.35, 0.71)	9.44E−05	0.48 (0.19, 1.16)	1.02E−01
CS versus non-CS in 3.43 < NLR ≤ 6.11 (392 versus 798)	0.34 (0.11, 1.02)	5.52E−02	0.72 (0.21, 2.44)	5.95E−01
CS versus non-CS in NLR ≤ 7.33 (1,093 versus 4,389)	0.72 (0.39, 1.35)	3.10E−01	1.74 (0.80, 3.80)	1.64E−01
CS versus non-CS in NLR > 7.33 (533 versus 416)	0.47 (0.32, 0.70)	1.85E−04	0.53 (0.31, 0.88)	1.39E−02
CS versus non-CS in 6.11 < NLR ≤ 7.33 (127 versus 136)	0.49 (0.13, 1.76)	2.72E−01	0.62 (0.11, 3.50)	5.89E−01
Subgroup with Mechanical Ventilation				
CS versus non-CS in NLR ≤ 6.11 with ventilation machine (101 versus 137)	0.15 (0.03, 0.68)	1.40E−02	1.30 (0.18, 9.27)	7.96E−01
CS versus non-CS in NLR > 6.11 with ventilation machine (42 versus 148)	0.26 (0.13, 0.53)	1.82E−04	0.41 (0.17, 0.99)	4.63E−02
MP Subgroup versus Non-CS				
MP versus non-CS in NLR ≤ 6.11 (763 versus 4,253)	0.60 (0.25, 1.40)	2.37E−01	2.65 (0.98, 7.15)	5.41E−02
MP versus non-CS in NLR > 6.11 (503 versus 552)	0.45 (0.29, 0.71)	5.22E−04	0.47 (0.26, 0.86)	1.52E−02
After Random Removal of Two Hospitals				
CS versus non-CS in NLR ≤ 6.11 (909 versus 4,214)	0.53 (0.24, 1.18)	1.22E−01	1.99 (0.76, 5.19)	1.61E−01
CS versus non-CS in NLR > 6.11 (638 versus 545)	0.48 (0.33, 0.71)	1.81E−04	0.50 (0.30, 0.83)	7.38E−03
Adjustment Factors Included Gender and Hypertension				
CS versus non-CS in NLR ≤ 6.11 (966 versus 4,253)	0.56 (0.26, 1.17)	1.24E−01	2.26 (0.94, 5.43)	6.90E−02
CS versus non-CS in NLR > 6.11 (660 versus 552)	0.49 (0.34, 0.71)	1.77E−04	0.52 (0.32, 0.85)	8.57E−03

NLR, neutrophil-to-lymphocyte ratio; aHR, adjusted hazard ratio; CI, confidence interval; CS, corticosteroid; MP, methylprednisolone.

[a]In all the time-varying Cox models, corticosteroid use was considered as time-varying exposure; aHR and p value were calculated. The adjustment factors included age, respiratory rate, SBP, SpO2, diabetes mellitus, coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, neutrophil count, lymphocyte count, erythrocytes, C-reactive protein, procalcitonin, BUN, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

[b]In all the marginal structural models, CURB-65 pneumonia severity score, neutrophil, lymphocyte, and SpO2 were considered as time-varying confounders; aHR and p value were calculated. The adjustment factors included diabetes mellitus, coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, erythrocytes, C-reactive protein, procalcitonin, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

use and all-cause mortality in the individuals with an NLR ≤ 6.11, the E-value was 2.97 in Cox with time-varying exposure model and 3.87 in the MSM analysis using the validation cohort. When analyzing the association between corticosteroid use and all-cause mortality in the individuals with NLR > 6.11, the E-value was 3.50 in Cox with time-varying exposure model and 3.26 in MSM analysis (Table S7). These E-values were greater than the estimated confounders for COVID-19 mortality; therefore, it is unlikely that a potential unmeasured confounder could have a considerably greater effect on COVID-19 mortality than these known risk factors.

Corticosteroid Regimen in Individuals Stratified by NLR Cut-Off at 6.11

Among the 3,254 individuals who received corticosteroids, the vast majority received methylprednisolone, which accounted for 97.1% of the corticosteroid-treated individuals, followed by prednisolone (10.7%) and hydrocortisone (1.0%). Methylprednisolone was also the most frequently administered corticosteroid across the NLR subgroups in both training and validation cohorts. The median duration of corticosteroid treatment was 6.0 (IQR, 4.0–11.0) days. The median time when corticosteroid therapy was initiated was 1.0 (IQR, 0.0–4.0) day after admission. The median daily dosage was relatively low, at 40.0 (IQR, 31.1–40.0) mg methylprednisolone-equivalent dosage, and the median accumulated dose was 240.0 (IQR, 128.0–440.0) mg. The corticosteroid category, therapeutic duration, dosage, and days of hospitalizations were not significantly different between the training and the validation cohorts. With regard to corticosteroid treatment, individuals with NLR > 6.11 had earlier drug initiation time, higher accumulated dosage, and longer treatment duration than those below the NLR cut-off (Tables 4 and S8). According to recent published evidence, the WHO guidance recommends 6 mg dexamethasone (equivalent of 32 mg methylprednisolone) orally or intravenously daily or 50 mg hydrocortisone intravenously every 8 h for 7 to 10 days in patients with severe and critical COVID-19 (World Health Organization, 2020a). Coincidentally, in our study, the median daily dosage was 40 mg methylprednisolone-equivalent dose and the median duration was 7 days among individuals who received corticosteroid treatment. Therefore, our study cohort of individuals with severe

Table 4. Corticosteroid Regimen in the Individuals with NLR > 6.11 or ≤6.11

		Training Cohort			Validation Cohort		
Corticosteroid Use[a]	Total (n = 3,254)	Total (n = 1,628)	NLR > 6.11 (n = 644)	NLR ≤ 6.11 (n = 984)	Total (n = 1,626)	NLR > 6.11 (n = 660)	NLR ≤ 6.11 (n = 966)
Methylprednisolone, n (n%)	3,158 (97.1%)	1,581 (97.1%)	632 (98.1%)	949 (96.4%)	1,577 (97.0%)	653 (98.9%)	924 (95.7%)
Prednisolone, n (n%)	347 (10.7%)	181 (11.1%)	68 (10.6%)	113 (11.5%)	166 (10.2%)	60 (9.1%)	106 (11.0%)
Hydrocortisone, n (n%)	33 (1.0%)	14 (0.9%)	11 (1.7%)	3 (0.3%)	19 (1.2%)	10 (1.5%)	9 (0.9%)
Other CCs, n (n%)	420 (12.9%)	216 (13.3%)	108 (16.8%)	108 (11.0%)	204 (12.6%)	94 (14.2%)	110 (11.4%)
Duration of CCs, median (IQR), days	6.0 (4.0–11.0)	6.0 (4.0–11.0)	7.0 (4.0–12)	6.0 (3.0–10.0)	7.0 (4.0–11.0)	7.0 (4.0–12.0)	6.0 (4.0–11.0)
Duration of CCs for survivor, median (IQR), days	6.0 (4.0–11.0)	6.0 (4.0–11.0)	7.0 (4.0–13.0)	6.0 (3.0–10.0)	7.0 (4.0–11.3)	7.0 (4.0–13.0)	6.0 (4.0–11.0)
Duration of CCs for nonsurvivor, median (IQR), days	6.0 (4.0–10.0)	6.0 (4.0–10.0)	7.0 (4.0–10.0)	5.0 (4.0–11.0)	6.0 (4.0–10.0)	6.0 (4.0–10.0)	7.0 (3.0–12.0)
In-hospital days for survivor, median (IQR), days	25.0 (18.0–35.0)	25.0 (17.0–34.0)	29.0 (20.0–40.0)	23.0 (17.0–32.0)	25.0 (18.0–35.0)	28.0 (19.0–39.5)	24.0 (17.0–32.0)
In-hospital days for non-survivor, median (IQR), days	13.0 (8.0–21.0)	12 (7.8–19.0)	12.0 (7.0–19.0)	14.0 (8.0–21.0)	14.5 (9.0–23.0)	14.0 (8.0–22.0)	19.0 (12.0–25.0)
Follow-up days, median (IQR), days	24.0 (16.0–33.0)	23.0 (16.0–33.0)	24.0 (14.0–35.0)	23.0 (16.0–31.0)	24.0 (16.0–34.0)	24.0 (15.0–36.0)	23.5 (17.0–32.0)
Accumulated dose, median (IQR), mg	240.0 (128.0–440.0)	240.0 (123.5–417.0)	280.0 (160.0–520.0)	200.0 (120.0–360.0)	240.0 (140.0–447.0)	284.0 (160.0–530.2)	220.0 (120.0–400.0)
Daily dose, median (IQR), mg	40.0 (31.1–40.0)	40.0 (31.1–40.0)	40.0 (34.5–48.5)	40.0 (30.0–40.0)	40.0 (31.1–40.0)	40.0 (34.0–54.1)	40.0 (30.0–40.0)
Days from illness onset to CCs, median (IQR), days	11.0 (8.0–17.0)	11.0 (8.0–17.0)	12.0 (8.0–17.3)	11.0 (8.0–17.0)	12.0 (8.0–16.0)	12.0 (8.0–16.0)	11.0 (8.0–17.0)
Days from admission to CCs, median (IQR), days	1.0 (0.0–4.0)	1.0 (0.0–4.0)	1.0 (0.0–3.0)	2.0 (0.0–5.0)	2.0 (0.0–4.0)	1.0 (0.0–3.0)	2.0 (1.0–5.0)
Survival/death/censor, n	2,459/450/345	1,228/240/160	390/183/71	838/57/89	1,231/210/185	419/157/84	812/53/101

CCs, corticosteroids; NLR, neutrophil-to-lymphocyte ratio; IQR, interquartile range.
[a]An individual can be taking more than one corticosteroid. Methylprednisolone equivalent data are presented.

COVID-19 received appropriate corticosteroid treatment in good compliance with the WHO guideline.

Adverse Events of Corticosteroid Treatment

In the previous clinical trials, the adverse events observed from corticosteroid therapy included hyperglycemia, infection, gastroduodenal bleeding, and hypernatremia (Annane et al., 2009; Siemieniuk et al., 2015). Here, we analyzed the incidences and risks of corticosteroid-related adverse events. In the training cohort, the incidences of corticosteroid-correlated adverse events were significantly higher in the corticosteroid group than the non-corticosteroid group in strata with an NLR ≤ 6.11, including hyperglycemia requiring treatment (IRR, 2.83 [2.31–3.47]; IR, 0.31 versus 0.11; p < 2.00E−16), infection requiring acceleration of antibiotics (IRR, 6.85 [6.03–7.78]; IR, 1.47 versus 0.21; p < 2.00E−16), gastrointestinal hemorrhage (IRR, 3.87 [1.74–8.65]; IR, 0.02 versus 0.01; p = 3.66E−04), fungal infection needing antifungal treatment (IRR, 3.58 [2.24–5.72]; IR, 0.06 versus 0.02; p = 1.25E−08), and hypernatremia (IRR, 3.05 [1.98–4.71]; IR, 0.06 versus 0.02; p = 1.11E−07) (Table 5). Similarly, in the strata with NLR > 6.11, the incidences of the above adverse events were significantly elevated in the individuals with corticosteroid treatment compared to those without corticosteroid. After adjusting for time-varying exposure and confounders, the treatment of corticosteroid was significantly associated with higher risks of hyperglycemia and infection (and thus accelerated antibiotic use) in both NLR subgroups by the Cox with time-varying exposure and the MSM analysis (Table 5). Thus, in individuals with NLR > 6.11, despite a significantly lowered risk of 60-day all-cause death from corticosteroid treatment, the adverse events of hyperglycemia and infection were higher and should be closely monitored. Among individuals

Table 5. The Adverse Events of Corticosteroid Therapy in Patients with NLR ≤ 6.11 and >6.11

	Incidence			Cox with Time-Varying Exposure		Marginal Structural Model	
Corticosteroid versus Non-Corticosteroid	IR (100 Person-Day)	IRR (95% CI)	p Value[a]	aHR (95% CI)[b]	p Value	aHR (95% CI)[c]	p Value
Training Cohort							
Hyperglycemia Requiring Treatment							
CS versus non-CS in NLR ≤ 6.11	0.31 versus 0.11	2.83 (2.31, 3.47)	<2.00E−16	2.99 (2.24, 3.99)	8.12E−14	5.12 (1.52, 17.26)	8.44E−03
CS versus non-CS in NLR > 6.11	1.14 versus 0.41	2.80 (2.18, 3.60)	<2.00E−16	2.89 (2.28, 3.67)	<2.00E−16	2.77 (1.93, 3.98)	3.79E−08
Acceleration of Antibiotics							
CS versus non-CS in NLR ≤ 6.11	1.47 versus 0.21	6.85 (6.03, 7.78)	<2.00E−16	2.85 (2.35, 3.45)	<2.00E−16	8.70 (1.37, 55.22)	2.18E−02
CS versus non-CS in NLR > 6.11	2.37 versus 0.74	3.20 (2.61, 3.92)	<2.00E−16	1.75 (1.41, 2.17)	3.69E−07	1.64 (1.26, 2.13)	2.57E−04
Gastrointestinal Hemorrhage							
CS versus non-CS in NLR ≤ 6.11	0.02 versus 0.01	3.87 (1.74, 8.65)	3.66E−04	2.02 (0.64, 6.32)	2.29E−01	5.94 (0.65, 54.72)	1.16E−01
CS versus non-CS in NLR > 6.11	0.08 versus 0.07	1.14 (0.61, 2.13)	6.91E−01	1.74 (0.81, 3.75)	1.57E−01	1.44 (0.53, 3.90)	4.72E−01
Need for Antifungal Treatment							
CS versus non-CS in NLR ≤ 6.11	0.06 versus 0.02	3.58 (2.24, 5.72)	1.25E−08	1.02 (0.15, 6.71)	9.85E−01	0.96 (0.01, 72.68)	9.85E−01
CS versus non-CS in NLR > 6.11	0.21 versus 0.14	1.51 (0.98, 2.33)	6.09E−02	1.72 (0.68, 4.33)	2.51E−01	0.90 (0.26, 3.08)	8.70E−01
Hypernatremia							
CS versus non-CS in NLR ≤ 6.11	0.06 versus 0.02	3.05 (1.98, 4.71)	1.11E−07	2.32 (0.83, 6.53)	1.09E−01	0.34 (0.02, 4.69)	4.19E−01
CS versus non-CS in NLR > 6.11	0.37 versus 0.31	1.19 (0.88, 1.62)	2.67E−01	0.50 (0.30, 0.83)	7.36E−03	0.31 (0.13, 0.78)	1.21E−02
Validation Cohort							
Hyperglycemia Requiring Treatment							
CS versus non-CS in NLR ≤ 6.11	0.36 versus 0.11	3.28 (2.70, 3.99)	<2.00E−16	3.65 (2.74, 4.85)	<2.00E−16	3.83 (2.63, 5.59)	3.08E−12
CS versus non-CS in NLR > 6.11	0.99 versus 0.38	2.60 (2.02, 3.34)	1.18E−14	3.18 (2.45, 4.13)	<2.00E−16	3.14 (1.92, 5.14)	5.55E−06
Acceleration of Antibiotics							
CS versus non-CS in NLR ≤ 6.11	1.53 versus 0.21	7.40 (6.51, 8.42)	<2.00E−16	2.49 (2.05, 3.03)	<2.00E−16	3.77 (3.02, 4.72)	<2.00E−16
CS versus non-CS in NLR > 6.11	2.44 versus 0.68	3.59 (2.93, 4.39)	<2.00E−16	2.08 (1.70, 2.55)	1.06E−12	2.11 (1.62, 2.75)	2.99E−08
Gastrointestinal Hemorrhage							
CS versus non-CS in NLR ≤ 6.11	0.02 versus 0.01	1.89 (0.87, 4.11)	1.01E−01	2.19 (0.54, 8.83)	2.70E−01	0.63 (0.08, 5.33)	6.75E−01
CS versus non-CS in NLR > 6.11	0.03 versus 0.06	0.54 (0.23, 1.24)	1.38E−01	0.16 (0.02, 1.29)	8.60E−02	–	–
Need for Antifungal Treatment							
CS versus non-CS in NLR ≤ 6.11	0.08 versus 0.02	4.15 (2.70, 6.39)	1.96E−12	–	–	–	–
CS versus non-CS in NLR > 6.11	0.19 versus 0.09	1.97 (1.21, 3.21)	5.80E−03	1.69 (0.66, 4.30)	2.74E−01	2.87 (0.95, 8.74)	6.28E−02

(*Continued on next page*)

Table 5. *Continued*

	Incidence			Cox with Time-Varying Exposure		Marginal Structural Model	
Corticosteroid versus Non-Corticosteroid	IR (100 Person-Day)	IRR (95% CI)	p Value[a]	aHR (95% CI)[b]	p Value	aHR (95% CI)[c]	p Value
Hypernatremia							
CS versus non-CS in NLR $\leq$ 6.11	0.07 versus 0.02	3.16 (2.04, 4.88)	4.73E−08	0.69 (0.19, 2.52)	5.72E−01	1.15 (0.15, 8.91)	8.94E−01
CS versus non-CS in NLR > 6.11	0.29 versus 0.30	0.94 (0.69, 1.30)	7.20E−01	0.52 (0.28, 0.96)	3.73E−02	0.51 (0.22, 1.18)	1.15E−01

IR (100 person-day), incidence rate; IRR, incidence rate ratio; NLR, neutrophil-to-lymphocyte ratio; aHR, adjusted hazard ratio; CI, confidence interval; CS, corticosteroid.

[a]p values were calculated by R package "fmsb." The significant probability of the result of null-hypothesis testing.

[b]In all the time-varying Cox models, corticosteroid use was considered as time-varying exposure; aHR and p value were calculated. The adjustment factors included age, respiratory rate, SBP, SpO2, diabetes mellitus, coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, neutrophil count, lymphocyte count, erythrocytes, C-reactive protein, procalcitonin, BUN, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

[c]In all the marginal structural models, CURB-65 pneumonia severity score, neutrophil, lymphocyte, and SpO2 were considered as time-varying confounders; aHR and p value were calculated. The adjustment factors included diabetes mellitus, coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, erythrocytes, C-reactive protein, procalcitonin, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

with an NLR $\leq$ 6.11, corticosteroid therapy was not associated with a detectable survival benefit, but rather a higher incidence of adverse events in the corticosteroid treatment group than those without corticosteroid therapy.

Associations of Corticosteroid Therapy with Outcomes in Individuals with T2D

Given the significantly elevated risk of hyperglycemia by corticosteroid treatment and the high prevalence of pre-existing diabetes in hospitalized COVID-19 patients, it is clinically important to examine the influence of corticosteroid in subjects with T2D. This is a highly relevant and urgent issue as patients with T2D are at a markedly higher risk of death after SARS-CoV-2 infection (Zhou et al., 2020), and this risk can be further exacerbated when the blood glucose is poorly controlled (Zhu et al., 2020). In our validation cohort, 1,044 individuals had pre-existing T2D. The baseline characteristics of the subjects with T2D are shown in Table S10. Among them, 291 cases (aged 66.0 [57.0–72.0] years; 57.7% males) received corticosteroid therapy and the remaining 753 cases (aged 64.5 [57.0–71.0] years; 53.0% males) did not receive corticosteroids (Table S10). The occurrence of other comorbidities in these diabetic individuals was comparable between the corticosteroid and the non-corticosteroid groups. Within this diabetic sub-cohort, individuals who received corticosteroids exhibited more severe pathological features and higher frequencies of lymphocyte decrease and elevations of leukocyte count, neutrophil count, CRP, procalcitonin, D-dimer, and other organ injury markers than those who did not receive corticosteroids (Table S10).

Also in the same diabetic sub-cohort, the death rate was significantly higher in the individuals with corticosteroid therapy (IRR, 3.46 [2.41–4.98]; IR, 0.46 versus 0.13; p = 9.77E−13) compared to those without corticosteroids in the validation cohort (Table 6). However, when we balanced time-varying exposure and confounders, the use of corticosteroids did not display any significant association with the risk of death (Table 6). We further tested whether corticosteroid therapy is associated with reducing the risk of mortality in individuals with NLR > 6.11 (Table 6). In addition, corticosteroid treatment was also not significantly associated with changes in mortality when NLR 6.11 was used as the cut-off point in either the training or the validation cohort (Tables 6 and S11). This result indicates that the individuals with T2D have more complex pathophysiologic processes and NLR is no longer sufficient for patient risk stratification and determination of corticosteroid therapy initiation.

Regarding the adverse events in the COVID-19 individuals with T2D, the crude incidences of hyperglycemia requiring treatment (IRR, 3.18 [2.63–3.84]; p < 2.00E−16), infection requiring acceleration of antibiotics (IRR, 7.12 [5.52–9.19]; p < 2.00E−16), fungal infection (IRR, 3.88 [2.22–6.77]; p = 2.94E−07), and hypernatremia (IRR, 2.24 [1.38–3.62]; p = 7.39E−04) were all significantly higher in the corticosteroid-treated individuals than the non-corticosteroid counterparts (Table 6). After balancing time-varying exposure and confounders, Cox with time-varying exposure model and MSM analysis consistently demonstrated a significant association between corticosteroid use and elevated risks of hyperglycemia and infection requiring acceleration of antibiotics in the validation cohort (Table 6). The need for antifungal treatment was also tightly associated with corticosteroid use in the MSM analysis (Table 6).

Conclusions

Our study establishes the clinical evidence that the NLR at admission is a highly practical and cost-effective parameter for patient risk stratification. In addition, low-dose corticosteroid treatment is associated with an improved 60-day all-cause mortality in individuals with severe COVID-19, as defined by the NLR. In individuals with a low inflammatory status (i.e., NLR $\leq$ 6.11) or individuals with pre-existing T2D, corticosteroid therapy should be used with caution given significant adverse events with no

Table 6. Association of Corticosteroid Therapy with 60-Day All-Cause Mortality and Adverse Events in Patients with Pre-existing Type 2 Diabetes in the Validation Cohort

Corticosteroid versus Non-Corticosteroid in T2DM patients	Incidence			Cox with Time-Varying Exposure		Marginal Structural Model	
	IR (100 Person-Day)	IRR (95% CI)	p Value[a]	aHR (95% CI)[b]	p Value	aHR (95% CI)[c]	p Value
CS versus non-CS in validation patients (291 versus 753)	0.46 versus 0.13	3.46 (2.41, 4.98)	9.77E−13	0.96 (0.53, 1.73)	8.87E−01	1.90 (0.93, 3.89)	7.82E−02
CS versus non-CS in NLR ≤ 6.11 (141 versus 601)	0.18 versus 0.03	5.37 (2.41, 11.98)	4.17E−06	0.77 (0.13, 4.73)	7.81E−01	2.20 (0.30, 16.12)	4.36E−01
CS versus non-CS in NLR > 6.11 (150 versus 152)	0.81 versus 0.65	1.25 (0.83, 1.88)	2.84E−01	0.81 (0.42, 1.59)	5.45E−01	0.88 (0.38, 2.04)	7.60E−01
Side Effects (CS versus Non-CS, 291 versus 753)							
Hyperglycemia requiring treatment	2.93 versus 0.92	3.18 (2.63, 3.84)	<2.00E−16	3.26 (2.54, 4.19)	<2.00E−16	2.88 (1.76, 4.74)	2.92E−05
Acceleration of antibiotics	1.94 versus 0.27	7.12 (5.52, 9.19)	<2.00E−16	3.23 (2.38, 4.38)	4.69E−14	4.39 (3.13, 6.15)	<2.00E−16
Gastrointestinal hemorrhage	0.02 versus 0.02	0.99 (0.27, 3.66)	9.88E−01	–	–	–	–
Need for antifungal treatment	0.22 versus 0.06	3.88 (2.22, 6.77)	2.94E−07	2.52 (0.52, 12.08)	2.49E−01	6.63 (1.84, 23.91)	3.82E−03
Hypernatremia	0.22 versus 0.10	2.24 (1.38, 3.62)	7.39E−04	0.37 (0.12, 1.09)	7.17E−02	0.33 (0.04, 2.40)	2.71E−01

IR (100 person-day), incidence rate; IRR, incidence rate ratio; aHR, adjusted hazard ratio; CI, confidence interval.

[a]p values were calculated by R package "fmsb." The significant probability of the result of null-hypothesis testing.

[b]In all the time-varying Cox models, corticosteroid use was considered as time-varying exposure; aHR and p value were calculated. The adjustment factors included age, respiratory rate, SBP, SpO2, coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, neutrophil count, lymphocyte count, erythrocytes, C-reactive protein, procalcitonin, BUN, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

[c]In all the marginal structural models, CURB-65 pneumonia severity score, neutrophil, lymphocyte, and SpO2 were considered as time-varying confounders; aHR and p value were calculated. The adjustment factors included coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, erythrocytes, C-reactive protein, procalcitonin, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

discernable improvement in clinical outcomes. Our study paves the way for designing successful RCTs for investigating the therapeutic effects of corticosteroid therapy in individuals with COVID-19 at different risks of mortality.

Limitations of Study

The retrospective design is the main limitation of our study. First, while multiple statistical methods were applied to adjust time-varying exposure and confounders, these adjustments may not be adequately balanced and bias could still occur in the presence of unmeasured confounding; however, as E-values were greater than estimated confounders for COVID-19 mortality, we reason that it is unlikely that an unmeasured confounder would have a substantially greater effect on COVID-19 mortality than these known risk factors. Second, the cut-off of NLR for risk stratification was established mainly from in-hospital individuals in the Chinese population. The optimal cut-off value may need to be recalculated in individuals outside of China to account for potential differences in virus strains, population-specific background, and the different normal range reference of blood tests. Third, due to the limited number of patients, we did not demonstrate whether corticosteroid treatment protects patients who developed NLR > 6.11 at a point after initial hospital admission. More extensive prospective studies are needed to address this question. Fourth, due to the relative shortage of medical resources under such an urgent condition, we were not able to assess viral concentration or clearances rates regarding different treatment groups. Thus, our current study cannot answer whether virus clearance is affected by corticosteroid therapy. Fifth, the inherent limitation of observational research makes it hard to define the causal effects of corticosteroid use on the reduced mortality of individuals with COVID-19 or individuals with an NLR > 6.11 at admission. Sixth, we failed to estimate the risk of individuals taking different types of corticosteroids or with different therapeutic durations through head-to-head comparisons owing to the limited sample size. Finally, we did not adjust the variability in the threshold of initiating corticosteroid therapy in different hospitals; e.g., individuals may begin corticosteroid earlier than when ARDS became apparent in some hospitals, while in other hospitals initiation of corticosteroid treatment may have occurred at 24–48 h after the onset of ARDS. These limitations highlight the urgent need for prospective studies and RCTs to validate NLR as an important indicator of COVID-19 severity and as a guide for initiating more proper corticosteroid treatment.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead Contact
  - Materials Availability
  - Data and Code Availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Study design and participants
- METHOD DETAILS
  - Data collection and complication evaluation
  - Outcomes and definition
  - Select determinants among CBC associated with primary outcome
  - Cox proportional hazards model accounting for time-varying exposure
  - Marginal Structural Cox Proportional Hazards Model
  - Sensitivity analyses
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cmet.2021.01.002.

ACKNOWLEDGMENTS

We thank the individuals who participated in this study and thank clinicians who helped to collect and review clinical information. This work was supported by grants from the National Key Research & Development Program of China, China (2016YFF0101504, 2016YFF0101505, 2020YFC2004700, 2020YFC2004702, and 2020YFC0845500), the Special Foundation for Emergency Research on Prevention and Control of COVID-19 of Guangdong Province, China (2020B1111330003), the National Science Foundation of China, China (81630011, 81970364, 81970070, 81970011, 81770053, 81970364, 81870171, 81700356, and 81570412), the Hubei Science and Technology Support Project, China (2019BFC582 and 2018BEC473), and Medical Flight Plan of Wuhan University, China (TFJH2018006).

AUTHOR CONTRIBUTIONS

J.C., Haomiao Li, C.Z., Z.C., and H. Liu designed the study, collected and analyzed data, and drafted the manuscript. B.W., F.L., J.-J.Q., Y.-M.L., and F.Z. performed the statistical analysis and interpreted data. X.S., J.Z., Y.-C.Z., M.H., H.Y., L.Z., P.Z., Y.-X.J., G.-N.Z., Z.L., L.L., W.M., X.L., H. Lu, D.W., X.X., X.H., and X.W. collected, reviewed, interpreted, and checked clinical information, laboratory, and radiological data. X.Z., J.Y., Y.-X.J., G.-N.Z., J.X., B.-H.Z., Y.Y., and Z.-G.S. edited the manuscript and provided valuable suggestions for study design and data analysis. Y.W., J.Y., X.Z., X.-J.Z., and Hongliang Li contributed equally, designed the project, edited the manuscript, and supervised the study. All authors have approved the final version of this paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: August 12, 2020
Revised: October 15, 2020
Accepted: December 30, 2020
Published: January 5, 2021

REFERENCES

Angus, D.C., Derde, L., Al-Beidh, F., Annane, D., Arabi, Y., Beane, A., van Bentum-Puijk, W., Berry, L., Bhimani, Z., Bonten, M., et al.; Writing Committee for the REMAP-CAP Investigators (2020). Effect of hydrocortisone on mortality and organ support in patients with severe COVID-19: the REMAP-CAP COVID-19 Corticosteroid Domain Randomized Clinical Trial. JAMA *324*, 1317–1329.

Annane, D., Bellissant, E., Bollaert, P.E., Briegel, J., Confalonieri, M., De Gaudio, R., Keh, D., Kupfer, Y., Oppert, M., and Meduri, G.U. (2009). Corticosteroids in the treatment of severe sepsis and septic shock in adults: a systematic review. JAMA *301*, 2362–2375.

Barnes, P.J. (2016). Kinases as novel therapeutic targets in asthma and chronic obstructive pulmonary disease. Pharmacol. Rev. *68*, 788–815.

Cain, D.W., and Cidlowski, J.A. (2017). Immune regulation by glucocorticoids. Nat. Rev. Immunol. *17*, 233–247.

Capelastegui, A., España, P.P., Quintana, J.M., Areitio, I., Gorordo, I., Egurrola, M., and Bilbao, A. (2006). Validation of a predictive rule for the management of community-acquired pneumonia. Eur. Respir. J. *27*, 151–157.

Chotiyarnwong, P., and McCloskey, E.V. (2020). Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. Nat. Rev. Endocrinol. *16*, 437–447.

Dequin, P.F., Heming, N., Meziani, F., Plantefève, G., Voiriot, G., Badié, J., François, B., Aubron, C., Ricard, J.D., Ehrmann, S., et al.; CAPE COVID Trial Group and the CRICS-TriGGERSep Network (2020). Effect of hydrocortisone on 21-day mortality or respiratory support among critically ill patients with COVID-19: a randomized clinical trial. JAMA *324*, 1298–1306.

Gönen, C.S.S.M. (2013). Optimal cutpoint estimation with censored data. J. Stat. Theory Pract. *7*, 14.

Haneuse, S., VanderWeele, T.J., and Arterburn, D. (2019). Using the E-value to assess the potential effect of unmeasured confounding in observational studies. JAMA *321*, 602–603.

Horby, P., Lim, W.S., Emberson, J.R., Mafham, M., Bell, J.L., Linsell, L., Staplin, N., Brightling, C., Ustianowski, A., Elmahi, E., et al.; RECOVERY Collaborative Group (2020). Dexamethasone in hospitalized patients with Covid-19 - preliminary report. N. Engl. J. Med. Published online July 17, 2020. https://doi.org/10.1056/NEJMoa2021436.

Hui, D.S. (2018). Systemic corticosteroid therapy may delay viral clearance in patients with Middle East respiratory syndrome coronavirus infection. Am. J. Respir. Crit. Care Med. *197*, 700–701.

Laforge, M., Elbim, C., Frère, C., Hémadi, M., Massaad, C., Nuss, P., Benoliel, J.J., and Becker, C. (2020). Tissue damage from neutrophil-induced oxidative stress in COVID-19. Nat. Rev. Immunol. *20*, 515–516.

Liao, D., Zhou, F., Luo, L., Xu, M., Wang, H., Xia, J., Gao, Y., Cai, L., Wang, Z., Yin, P., et al. (2020). Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. Lancet Haematol. *7*, e671–e678.

Liu, Y., Du, X., Chen, J., Jin, Y., Peng, L., Wang, H.H.X., Luo, M., Chen, L., and Zhao, Y. (2020). Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J. Infect. *81*, e6–e12.

Long, L., Zeng, X., Zhang, X., Xiao, W., Guo, E., Zhan, W., Yang, X., Li, C., Wu, C., Xu, T., et al. (2020). Short-term outcomes of COVID-19 and risk factors for progression. Eur. Respir. J. *55*, 2000990.

Mathur, M.B., Ding, P., Riddell, C.A., and VanderWeele, T.J. (2018). Web site and R package for computing E-values. Epidemiology *29*, e45–e47.

National Health Commission of China (2020). New Coronavirus Pneumonia Prevention and Control Program. http://www.nhc.gov.cn.

Prescott, H.C., and Rice, T.W. (2020). Corticosteroids in COVID-19 ARDS: evidence and hope during the pandemic. JAMA *324*, 1292–1295.

Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., Xie, C., Ma, K., Shang, K., Wang, W., and Tian, D.S. (2020). Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin. Infect. Dis. *71*, 762–768.

Rochwerg, B., Oczkowski, S.J., Siemieniuk, R.A.C., Agoritsas, T., Belley-Cote, E., D'Aragon, F., Duan, E., English, S., Gossack-Keenan, K., Alghuroba, M.,

et al. (2018). Corticosteroids in sepsis: an updated systematic review and meta-analysis. Crit. Care Med. *46*, 1411–1420.

Russell, C.D., Millar, J.E., and Baillie, J.K. (2020). Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet *395*, 473–475.

Shang, L., Zhao, J., Hu, Y., Du, R., and Cao, B. (2020). On the use of corticosteroids for 2019-nCoV pneumonia. Lancet *395*, 683–684.

Siemieniuk, R.A., Meade, M.O., Alonso-Coello, P., Briel, M., Evaniew, N., Prasad, M., Alexander, P.E., Fei, Y., Vandvik, P.O., Loeb, M., and Guyatt, G.H. (2015). Corticosteroid therapy for patients hospitalized with community-acquired pneumonia: a systematic review and meta-analysis. Ann. Intern. Med. *163*, 519–528.

Sin, D.D., Man, S.F., and Tu, J.V. (2003). Inhaled glucocorticoids in COPD: immortal time bias. Am. J. Respir. Crit. Care Med. *168*, 126–127.

Sterne, J.A.C., Murthy, S., Diaz, J.V., Slutsky, A.S., Villar, J., Angus, D.C., Annane, D., Azevedo, L.C.P., Berwanger, O., Cavalcanti, A.B., et al.; WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group (2020). Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: a meta-analysis. JAMA *324*, 1330–1341.

Tang, C., Wang, Y., Lv, H., Guan, Z., and Gu, J. (2020). Caution against corticosteroid-based COVID-19 treatment. Lancet *395*, 1759–1760.

Terpos, E., Ntanasis-Stathopoulos, I., Elalamy, I., Kastritis, E., Sergentanis, T.N., Politou, M., Psaltopoulou, T., Gerotziafas, G., and Dimopoulos, M.A. (2020). Hematological findings and complications of COVID-19. Am. J. Hematol. *95*, 834–847.

VanderWeele, T.J., and Ding, P. (2017). Sensitivity analysis in observational research: introducing the E-value. Ann. Intern. Med. *167*, 268–274.

Villar, J., Ferrando, C., Martínez, D., Ambrós, A., Muñoz, T., Soler, J.A., Aguilar, G., Alba, F., González-Higueras, E., Conesa, L.A., et al.; dexamethasone in ARDS network (2020). Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. Lancet Respir. Med. *8*, 267–276.

Waljee, A.K., Mukherjee, A., Singal, A.G., Zhang, Y., Warren, J., Balis, U., Marrero, J., Zhu, J., and Higgins, P.D. (2013). Comparison of imputation methods for missing laboratory data in medicine. BMJ Open *3*, e002847.

Wilk, A.J., Rustagi, A., Zhao, N.Q., Roque, J., Martínez-Colón, G.J., McKechnie, J.L., Ivison, G.T., Ranganath, T., Vergara, R., Hollis, T., et al. (2020). A single-cell atlas of the peripheral immune response in patients with severe COVID-19. Nat. Med. *26*, 1070–1076.

World Health Organization (2020a). Corticosteroids for COVID-19: living guidance. https://www.who.int/publications/i/item/WHO-2019-nCoV-Corticosteroids-2020.1.

World Health Organization (2020b). Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: interim guidance. https://www.who.int/publications/i/item/10665-331501.

Xiong, C., Jiang, L., Chen, Y., and Jiang, Q. (2020). Evolution and variation of 2019-novel coronavirus. bioRxiv. https://doi.org/10.1101/2020.01.30.926477.

Ye, Z., Wang, Y., Colunga-Lozano, L.E., Prasad, M., Tangamornsuksan, W., Rochwerg, B., Yao, L., Motaghi, S., Couban, R.J., Ghadimi, M., et al. (2020). Efficacy and safety of corticosteroids in COVID-19 based on evidence for COVID-19, other coronavirus infections, influenza, community-acquired pneumonia and acute respiratory distress syndrome: a systematic review and meta-analysis. CMAJ *192*, E756–E767.

Zhang, X., Tan, Y., Ling, Y., Lu, G., Liu, F., Yi, Z., Jia, X., Wu, M., Shi, B., Xu, S., et al. (2020a). Viral and host factors related to the clinical outcome of COVID-19. Nature *583*, 437–440.

Zhang, X.J., Qin, J.J., Cheng, X., Shen, L., Zhao, Y.C., Yuan, Y., Lei, F., Chen, M.M., Yang, H., Bai, L., et al. (2020b). In-hospital use of statins is associated with a reduced risk of mortality among individuals with COVID-19. Cell Metab. *32*, 176–187.e4.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet *395*, 1054–1062.

Zhu, L., She, Z.G., Cheng, X., Qin, J.J., Zhang, X.J., Cai, J., Lei, F., Wang, H., Xie, J., Wang, W., et al. (2020). Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. Cell Metab. *31*, 1068–1077.e3.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
R-3.6.3	R Foundation for Statistical Computing	https://www.r-project.org/
SPSS statistics 23.0	IBM Corporation	http://www.spss.com.hk/software/statistics/
Adobe illustrator CC 2019	Adobe company	https://www.adobe.com/cn
glmnet-4.0-2	Jerome Friedman et al.	https://www.jstatsoft.org/article/view/v033i01
timeROC-0.4	Paul Blanche	https://cran.r-project.org/web/packages/timeROC/index.html
tableone-0.11.1	Kazuki Yoshida	https://github.com/kaz-yos/tableone
doBy-4.6.6	Søren Højsgaard	https://cran.r-project.org/web/packages/doBy/index.html
survival-3.1-12	Terry M Therneau et al.	https://cran.r-project.org/web/packages/survival/index.html
rms-6.0-0	Frank E Harrell Jr	https://cran.r-project.org/web/packages/rms/index.html
nnet-7.3-14	Brian Ripley et al.	https://cran.r-project.org/web/packages/nnet/index.html
car-3.0-8	John Fox et al.	https://cran.r-project.org/web/packages/car/index.html
mgcv-1.8-31	Simon Wood	https://cran.r-project.org/web/packages/mgcv/index.html
Hmisc-4.4-0	Frank E Harrell Jr et al.	https://cran.r-project.org/web/packages/Hmisc/index.html
survey-4.0	Thomas Lumley	https://cran.r-project.org/web/packages/survey/index.html
MASS-7.3-51.6	W. N. Venables et al.	https://cran.r-project.org/web/packages/MASS/index.html
landest-1.0	Layla Parast	https://cran.r-project.org/web/packages/landest/index.html
Matrix-1.2-18	Douglas Bates et al.	https://cran.r-project.org/web/packages/Matrix/index.html

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to the Lead Contact, Hongliang Li (lihl@whu.edu.cn).

Materials Availability

No new reagents or materials were generated in this study.

Data and Code Availability

The data related to the findings of this study will be available from the corresponding author after publication upon reasonable request. The research team will provide an email address for communication once the data are approved to be shared with others. The proposal with specific aims, statistical plans, and other information/materials may be required to guarantee the rationale of requirements and the security of the data. The patient-level data, but without names and other identifiers, will be shared after review and approval of the submitted proposal and any related requested materials.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study design and participants

In this multi-centered, retrospective cohort study, participants diagnosed with COVID-19 and consecutively admitted to the 21 hospitals in Hubei, China, that were designated to treat COVID-19 individuals were enrolled. A total of 15,649 individuals admitted to hospitals from December 30th, 2019 to April 17th, 2020, were enrolled in the study. Among these individuals, 2,787 individuals aged less than 18, with eGFR < 30ml/min/1.73 m^2, with liver cirrhosis, with pregnant, with severe medical conditions leading to death, including acute myocardial infarction, acute pulmonary embolism, and stroke, taking corticosteroid for other diseases or less than 3 days, use of corticosteroid of other illnesses, or transferred to other hospitals were excluded (Figure 1). A total of 12,862 individuals were randomly and equally assigned to the training and validation cohorts. The final date of follow-up was April 26th, 2020, for both groups. A total of 469 individuals remaining in the hospital at the end of follow-up were treated as censored individuals and 399 individuals died during hospitalization in the training cohort. 489 individuals remaining in the hospital at the end of follow-up were treated as censored individuals, and 388 individuals died during hospitalization in the validation cohort. The criteria of the individuals

with T2D in the validation cohort were (1) the individuals with a medical history of diagnosed T2D; or (2) the individuals without a medical history of T2D, but with uncontrolled blood glycemic levels after admission and a new diagnosis of T2D was made in the medical record. The 89 individuals with T2D who remained in hospitals at the end of the follow-up date were treated as censored.

The throat-swab specimens and/or computerized chest tomography (CT) were examined for all individuals upon admission. COVID-19 was diagnosed by clinical manifestations, chest CT, and real-time RT-PCR according to the WHO interim guidance and the New Coronavirus Pneumonia Prevention and Control Program (5th edition) published by the National Health Commission of China (National Health Commission of China, 2020; World Health Organization, 2020b). The central ethics committee approved the study protocols and procedures were accepted or approved by each collaborating hospital. Ethics committees granted a waiver of the requirement for documentation of informed consent for analyzing existing data without interfering with patient treatment.

METHOD DETAILS

Data collection and complication evaluation

We collected individuals' demographic information, clinical characteristics, medical history, laboratory tests, radiological reports, therapeutic intervention, and outcome data at admission and during hospitalization at each hospital site. The age, gender, and clinical symptoms (fever, cough, fatigue, and dyspnea) were extracted from individuals' electronic medical records. Medical histories comprising the coexistence of chronic obstructive pulmonary disease (COPD), T2D, hypertension, coronary heart disease, cerebrovascular disease, chronic liver disease, chronic kidney disease, cancer, heart failure, and autoimmune diseases were reviewed and extracted. The laboratory examination data included a complete blood count, C-reactive protein (CRP), procalcitonin, D-dimer, and serum biochemical test for liver, kidney, heart, and coagulation dysfunction were obtained from the laboratory informatics system. The unilateral and bilateral lesions in chest CT scan images were analyzed based on the radiological report data. We extracted and analyzed individuals' medications and interventions during hospitalization according to the doctor's advice. Personal identification information (e.g., name and ID) of the study subjects were anonymized and replaced with a coding system before data extraction. We collected data on the daily dose, starting time, duration of each corticosteroid, and converted it to methylprednisolone-equivalent dose (Table S9). Data were reviewed and confirmed by experienced physicians and were double-checked to guarantee accuracy.

Outcomes and definition

The primary endpoint was evaluated in this longitudinal cohort, which was 60-day all-cause death. The hyperglycemia requiring treatment, infection needing acceleration of antibiotics, fungal infection needing antifungal medication, gastrointestinal hemorrhage, and hypernatremia were also recorded and analyzed as adverse events. The increases of variables were defined as above their upper limits of normal (ULN). The decreases of variables were defined below their lower limits of normal (LLN) according to their normal ranges in each hospital site. The primary endpoint and adverse events were reviewed and confirmed by a team of certified physicians to ensure accuracy. All individuals who remained in hospitals at the end of the follow-up date were treated as censored data.

Select determinants among CBC associated with primary outcome

To gain estimates of the primary outcome (60-day all-cause mortality) in ten blood cell-related variables, we performed Cox regression and LASSO Cox regression models in the training cohort. The covariates included blood cell variables comprised leukocyte counts, neutrophil counts, lymphocyte counts, monocyte counts, basophil counts, eosinophil counts, platelet counts, erythrocyte counts, hemoglobin concentrations, and hematocrit. The hazard ratio (HR), 95% confidence intervals (CI), p values, and coefficients calculated by various models were listed in Table 1. As neutrophil and lymphocyte counts are the top two factors related to mortality; we further established a Cox model on the index of neutrophil to lymphocyte ratio (NLR) as a potential predictive parameter for all-cause mortality in the training cohort. The area under the ROC curve for the NLR-Cox model was measured with consideration of censored data, and the maximum Youden index was used for selecting an optimal cutpoint in the training cohort (Gönen, 2013).

Cox proportional hazards model accounting for time-varying exposure

Immortal time bias may arise when individuals were waiting to receive corticosteroid therapy (Sin et al., 2003). When determining clinical outcomes as a time to event, we performed a Cox proportional hazards model accounting for time-varying exposure that adjusted immortal time bias with corticosteroids as a time-varying exposure. When analyzing the association of corticosteroids uses with all-cause mortality and adverse events among individuals divided by the optimal cut-off value in the training and validation cohorts, we treated corticosteroid initiation as time-varying exposure and adjusted for time-varying exposure and confounders in subgroup analysis. The adjusted confounders were selected by LASSO regression, which is strongly covariates affect the mortality in individuals with SARS-CoV-2 infection, namely, age, respiratory rate, SBP, SpO2, diabetes mellitus (not considered as a confounder in the DM subgroup analysis), coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, neutrophil count, lymphocyte count, erythrocyte, C-reactive protein, procalcitonin, BUN, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose. All individuals who remained in hospitals at the end of the follow-up date were treated as censored data.

Marginal Structural Cox Proportional Hazards Model

Since the time-related changes in patient condition impact the initiation or stop of corticosteroid therapy and thus confound the associations between corticosteroid use and outcomes, we performed marginal structural model (MSM) analysis via the inverse probability of treatment weighting (IPTW) to mitigate time-varying confounders (Zhang et al., 2020b). CURB-65 pneumonia severity score (confusion, blood urea nitrogen, respiratory rate, SBP, and age) (Table S12) (Capelastegui et al., 2006), neutrophil counts, lymphocyte counts, and SpO2 levels were time-varying confounders when analyzing the relationship between corticosteroid use with the primary outcome and adverse events. Other variables that strongly affected the mortality (selected by LASSO regression model) in individuals with COVID-19 were further adjusted in all subgroups, namely, diabetes mellitus (not considered as a confounder in the DM subgroup analysis), coronary arterial disease, cerebrovascular disease, chronic kidney disease, heart failure, neoplastic disease, leukocyte count, erythrocytes, C-reactive protein, procalcitonin, creatinine, D-dimer, ALT, AST, LDL-c, creatine phosphokinase, creatine phosphokinase-MB, platelet count, and fasting blood glucose.

The stabilized weights for MSM were calculated based on IPTW by multiplying the treatment weights and the censoring weights, where the treatment weights were first derived for each subject to estimate their probability to take corticosteroid therapy at a time, and the censoring weights were calculated to assess the early time dropout of subjects. The treatment weights were varying until the first day of corticosteroid treatment. The time-varying intercept was assumed as a smooth function and estimated using spline smoothing. The generalized additive model was conducted to evaluate the effect of corticosteroids on the results with confounders adjusted. Stabilized weights were pooled into the MSM analysis to calculate the associations between corticosteroid therapy and clinical outcomes. All individuals who remained in hospitals at the end of the follow-up date were treated as censored data.

Sensitivity analyses

To validate the corticosteroid treatment is associated with a reduced risk of mortality in the individuals with NLR > 6.11, we performed validation using Cox with time-varying exposure and MSM models on the following subgroups in the validation cohort: (1) individuals with NLR $\leq$ 3.43 or > 3.43, 3.43 < NLR $\leq$ 6.11, NLR $\leq$ 7.33 or > 7.33, and 6.11 < NLR $\leq$ 7.33; (2) individuals with mechanical ventilation; (3) individuals with methylprednisolone; (4) randomly removing two hospitals; and (5) modified adjusting confounders, e.g., gender and hypertension.

To evaluate the potential effect of unmeasured confounding in the association between corticosteroid use and all-cause mortality, E-value analysis was performed using the methodology developed by VanderWeele and Ding (Haneuse et al., 2019; Mathur et al., 2018; VanderWeele and Ding, 2017). The E-value is an alternative approach to sensitivity analyses for unmeasured confounding in our studies that avoids making assumptions that, in turn, require subjective assignment of inputs for some formulas.

QUANTIFICATION AND STATISTICAL ANALYSIS

Categorical variables were presented as frequency, and continuous variables were described as median (interquartile range, IQR). Means or medians for continuous variables were compared using independent group t tests when the data were normally distributed; otherwise, the Mann-Whitney test was used. Proportions for categorical variables were compared using the Chi-square test. The Fisher exact test was used when the data were limited. The baseline characteristics were analyzed using the LASSO regression model to identify critical determinants of all-cause mortality in the training cohort. The LASSO model was performed using R/glmnet software, and the optimal value of λ was determined via 10-fold cross-validations. A C-statistic for the Cox model was applied to show the performance of NLR (area under of receiver-operating characteristic curve [AUROC]) in predicting the risk of mortality in the training dataset. The selection of the optimal NLR cut-off point was based on the highest Youden index. The NLR cut-off points selected for the sensitivity tests either prioritized sensitivity over specificity or chose specificity over sensitivity. To account for the missing data on the laboratory variables, we used non-parametric missing value imputation, based on the missForest procedure in the R. A random forest model based on the rest of the variables in the dataset was constructed to predict the missing values with an estimation of the internally cross-validated errors (Waljee et al., 2013; Zhu et al., 2020). The p values were 2-sided, and an alpha level of 0.05 was used to define statistical significance. Results from all multivariable analyses are reported as hazard ratios (HR) with 95% confidence intervals (CIs) or coefficients. All statistical parameters are indicated in the figure legends or table footnote. All analyses were conducted using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) or SPSS version 23.0 (IBM, Armonk, NY, USA.