Peer Review File

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First round peer review

Reviewer A

I read with interests a manuscript in title "Efficacy of low-dose corticosteroids in patients with acute respiratory distress syndrome: a prospective observational study". The authors performed a prospective observational study using the data from the CHARDS study by comparing several clinical outcomes between ARDS patients who recieved low-dose corticosteroids and patients without steroids. They showed that low-dose corticosteroids may improve the outcome by improving survival. I have several comments and suggestions as follows:

Abstract

Comment 1: Total number of subjects who were enrolled in this study should be 289 patients because the authors only focused on patients who received low dose corticosteroids compared to the control group (without corticosteroids treatment)

Reply 1: Thank the reviewer for the advice. According to Reviewer C & E, we analyzed clinical characteristics and outcomes of other groups in supplement material (Table S3-4, S6-7).

Comment 2: The results were so weird that patients with low-dose corticosteroids had a trend towards increases ICU and hospital mortality and longer length of stay but they concluded that giving low dose corticosteroids may reduce mortality in ARDS patients (according to cox regression analysis). Please clarify.

Reply 2: Thank the reviewer for requesting clarification. We consider that low-dose corticosteroids may associate with some factors such as immunosuppression, which increased risk of mortality. Therefore, there is no significant difference in mortality between these two groups. The univariate Cox regression did not show that low-dose corticosteroid was a protective factor for the risk of death (HR 0.82, 95%CI 0.54-1.25, p=.348, Table S8). Then, we performed multivariate Cox regression to adjust for

confounders to identify whether low-dose corticosteroids are a protective factor. And significant difference was found (HR 0.63,95%CI 0.39-0.98, p=.041, Figure S9).

Methods

Comment 3: Many factors are associated with the outcomes of ARDS patients for example lung protective ventilation strategy, prone positioning, and neuromuscular blocking agents. Did the authors concern for these factors?

Reply 3: Thank the reviewer for the question. As general management, lung protective ventilation strategy was applied in mechanically ventilated patients. However, none of these adjunctive measures were explicitly demanded in our observational study. The respective proportions between low-dose group and control group for neuromuscular blocking agents at least 1 hour were 12.9% vs 35.4%, p=.000, for lung recruitment manoeuvre were 5.4% vs 23.1%, p=.000, for prone position ventilation were 13.4% vs 21.5%, p=.082, for ECMO were 11.2% vs 6.2%, p=.172. Though, there was some significant difference in NMBAs and RM between two groups, the impact of NMBSs and RM was limited due to the great heterogeneity of them.

Comment 4: What is the KS normality test? Please use the full name.

Reply 4: Thank the reviewer for the question. Kolmogorov–Smirnov normality tests were performed for checking data normality.

Results

Comment 5: The authors separate patients who received corticosteroids into 3 groups including 1. low-dose group, 2. high-dose group, and 3 impulse group but they didn't show any results and/or discussion about the last 2 groups so I would suggest them to remove these 2 groups from the study.

Reply 5: Thank the reviewer for the suggestions. We added some data in supplemental materials (Table S3-4, S6-7).

Comment 6: The number of patients who received high-dose corticosteroid is quite large (55 patients) so I think it's worth to take a look on the clinical outcomes in this group whether high-dose corticosteroids led to poor outcomes such as increased mortality, longer LOS, or adverse events such as myopathy.

Reply 6: Thank the reviewer for the suggestions. There are 41 patients received high-dose corticosteroids. The outcome of this group was added in Table S6. Regrettably, we did not have detailed data on myopathy.

Comment 7: What is the oxygenation index? Please clarify or describe in methods.
Reply 7: Thanks for pointing out the problem. We are sorry for this mistake. we have modified our text as advised (see Page 9, line173 and Page 12, line 246)

Comment 8: Half of patients with pneumonia were undetermined etiology. Could it be possible to be ARDS-mimics such as acute interstitial lung disease, acute eosinophilic pneumonia or some diseases that good response to corticosteroids.
Reply 8: Thank the reviewer for the question. Whether it is interstitial lung disease or eosinophilic pneumonia is comprehensively judged by clinicians based on the symptoms, imaging data, and laboratory findings. However, we don't know whether these diagnoses exist indeed. Because we don't know whether these patients have performed laboratory examinations to exclude these diseases, such as BALF cell classification or pathology.

Comment 9: Baseline characteristics between the two groups were unequal that may lead to the difference in the outcomes. Please discuss. In addition, other important factors for example lung protective ventilation strategy, prone positioning, neuromuscular blocking agents, plateau pressure and/or driving pressure did not mentioned in this study.
Reply 9: Thank the reviewer for the question. We performed 1:2 propensity score matching to minimize the influence of the different baseline characteristics between two groups. The initial driving pressure were not significantly difference between two groups (13.0 vs 12.0, p=0.183).

Comment 10: Although there was no statistical significant difference in mortality rate and length of stay between the two groups but patients in low-dose corticosteroids group had a trend toward increases in mortality rate. I was wondering why cox regression model demonstrated that low-dose corticosteroids may improve outcomes. Please clarify.

Reply 10: Thank the reviewer for the question. We answered this question in comment #2.

Discussion

Comment 11: Again, the ICU and hospital mortality rate between patients with low-dose corticosteroids and control groups were 43.1% vs 38.8% and 44.6% vs 40.6%, respectively. In addition, patients in low-dose corticosteroids group had also longer length of stay in ICU and hospital so why they conclude that low-dose corticosteroid has a protective role in ARDS.

Reply 11: Thank the reviewer for the question. Although there is no significant difference in mortality between two groups, multivariate Cox regression showed decreased HR for low-dose corticosteroids. In addition, hospital mortality showed downward trend in low-dose group in propensity score matching samples (27.5% vs 42.5%, p=.110). The difference in length of stay in ICU and hospital may be due to survivor bias, the survivors were enrolled in low-dose group easily leading the longer length of stay.

Comment 12: Other limitations should be also mentioned for example no data on ventilator management, definite etiologies of ARDS in many patients were not proven, etc.

Reply 12: Thank the reviewer for the suggestion. We added related discussion in the manuscript and Table1 (Page14, line 295-296).

Figure 2

Comment 13: Please check the Kaplan-Meier curve for survival rate because the mortality rate in the group of low-dose corticosteroids was higher than the control group (according to Table 3).

Reply 13: Thanks for pointing out the problem. We checked the Figure 2. The difference mortality between Figure 2 and Table 2(Table S5 now) was because they showed different endpoints. 30-days mortality in low-dose corticosteroids and control groups are 32.3% (21/65) vs 36.6% (82/224), respectively.

Figure 3

Comment 14: Again, please check the Kaplan-Meier curve for probability of nosocomial infection because the rate of nosocomial infection in patients with low-dose corticosteroids was lower than control group (21.5% vs 26.3%, respectively) but the curve seem that low-dose corticosteroids had higher rate of nosocomial infection.
Reply 14: Thanks for pointing out the problem. We checked and modified the Figure 3.

Table 1
Comment 15: Some ventilator settings and physiologic variables should be provided for example tidal volume (per PBW), plateau pressure, PEEP level.
Reply 15: Thank the reviewer for the question. We provided such data in Table1.

Table 2
Comment 16: Most of clinical outcomes in this table demonstrated that patients with low-dose corticosteroids had poorer outcome (higher intubation rate, higher mortality rate, longer length of stay) but why the end result showed that low dose corticosteroids may improve outcomes. Please clarify.
Reply 16: We thank the reviewer for requesting clarification. We answered this question in comment #2 and #11.

Table 3
Comment 17: Please check total number of patients in this table (N = 292) why it's different from table 1 and 2 (N = 289).
Reply 17: Thanks for pointing out the problem. We check and modified Table (Table S10 now).

Reviewer B

The authors searched in a prospectively collected database of ARDS patients those who received low dose corticosteroids and those who received no corticosteroids (control group). The two groups were compared for mortality in univariate and multivariate analyses. In univariate analysis mortality was higher in the corticosteroid than in the control group (44.% vs. 40.6%, p=0.656). However, in multivariate Cox regression analysis mortality was lower in the corticosteroid group (HR, 0.62; 95% CI, 0.39-0.98;

p=.041).

Major comments

Comment 1: The major limitation of the study is that corticosteroids were administered mostly to patients with baseline immunosuppression. Approximately half of patients with low dose corticosteroids were immunosuppressed, whereas only 17% of patients in the control group were immunosuppressed. Thus, the conclusion is not that corticosteroids given to ARDS patients improve (or do not change, after the caveats suggested above) mortality, but that corticosteroids administered mostly to patients who already need corticosteroid is associated with no change (or according to the authors, improved) survival.

Reply 1: Thank the reviewer for the sharp comment. We performed 1:2 propensity score matching to investigate the question. In matched sample, hospital mortality showed downward trend in low-dose group in propensity score matching samples (27.5% vs 42.5%, p=.110). And the multivariate Cox regression showed significant decreased HR for low-dose group (Table 4. HR 0.48,95%CI 0.24-0.97, p=.040). Therefore, we believe our conclusion still hold.

Comment 2: pg. 8: “To eliminate potential confounding factors, Cox regression analysis was performed. Administration of low-dose corticosteroids showed a lower risk of death in some of subgroups including patients with intrapulmonary ARDS, mechanical ventilation and patients without immunosuppression (shown in Figure 4 and Table 3)”. However, Figure 4, at least as I tend to understand it, does not indicate the HR of treatment with corticosteroids in different subgroups, but rather the effect of different risk factors (i.e., high SOFA score, age, intrapulmonary versus extrapulmonary) on mortality. Otherwise, if the different HRs shown indicate the effect of corticosteroids in different subgroups (ie., old and young, high and low SOFA, etc.), the HR of treatment with corticosteroids in old and also in young patients (or in high and also in low SOFA score, etc.) should be shown.

Reply 2: Thanks for spotting our mistakes. It should be “Variable” not “Subgroup” in figure 4(Table 4 now), and we modified it.

Comment 3: The present reviewer does not understand how it is possible that the point

estimate indicated a higher mortality in the corticosteroid group than in the control group yet in the multivariate analysis the opposite, even at a statistically significant level, occurs (lower mortality in the corticosteroid group).

Reply 3: Thank the reviewer for requesting clarification. We consider that low-dose corticosteroids may associate with some factors such as immunosuppression, which increased risk of mortality. Therefore, there is no significant difference in mortality between these two groups. The univariate Cox regression did not show that low-dose corticosteroid was a protective factor for the risk of death (Table S8, HR 0.82, 95%CI 0.54-1.25, p=.348). Then, we performed multivariate Cox regression to adjust for confounders to identify whether low-dose corticosteroids are a protective factor. And significant difference was found (Table S9. HR 0.63,95%CI 0.39-0.98, p=.041).

Comment 4: In table 3 it says that there were 209 patients with mechanical ventilation, however there were 289 patients with ARDS in the present study. It seems that 80 (28%) of 289 were not receiving mechanical ventilation. Were there patients with ARDS that were not on mechanical ventilation?

Reply 14: Thank the reviewer for the question. Not all patients with ARDS received mechanical ventilation. In total, 228/527 (43.3%) ARDS patients initially received NPPV or HFNC. And some of them were not intubated until they were transferred to general wards. We analyzed the outcomes of these patients in another manuscript we are working on.

Comment 5: Values in Fig. 4 and table 3 do not coincide. In fig. 4, what does “ARDS severity” mean?

Reply 5: Thank the reviewer for the question. Patients with ARDS were categorized on the day of ARDS diagnosis based on their PaO2/FIO2 ratio into mild (200< PaO2/FIO2≤ 300mmHg), moderate (100<PaO2/FIO2≤200mmHg), and severe (PaO2/FIO2 ≤100mmHg) based on the Berlin Definition

Comment 6: Define what variables included in the multivariate analysis.

Reply 6: Thank the reviewer for the question. All variables we considered were included in Table 3&4 and they were analyzed in Table 5 & S10. Variables were included based on the clinical observation and previous literature.

Minor comments

Comment 7: pg. 5 Replace impulse by pulse.

Reply 7: Thanks for pointing out the problem. We replaced this word.

Comment 8: pg. 6. It says that the “other corticosteroids group was not statistically discussed". In reality groups that were not statistically discussed included as well the "high dose corticosteroids" and the "pulse corticosteroid".

Reply 8: According to Reviewer C & E, we analyzed outcomes of other groups in supplement material. we added some data (see Table S3-4, S6-7)

Comment 9: pg. 7. The sentence “The outcomes of the two groups are shown in Table 2. The ICU and hospital patient mortality rates were 39.8% and 41.5%, respectively, in both groups” is not well understood, as refers to the entire population rather than to the “two groups” (low dose corticosteroids and control group), in which case to figures would be lacking.

Reply 9: Thanks for pointing out the problem. We revised the wrong statement (see Page10, line194-195).

Comment 10: Whenever LOS is mentioned, survivors and non survivors should be separated (LOS may be longer because survivors live longer, or because non survivors stay longer in the ICU, which has different interpretation).

Reply 10: Thank you for your advice. We added data in manuscript (see Page 109, line200-204).

Reviewer C

Comment 1: In methods section, the authors stated that they included patients diagnosed with ARDS in this study. However, in the results section, the authors showed the results only from patients in low-dose corticosteroids group and control group, those without corticosteroids. Although they are not the main results in this study, the analyses from high-dose corticosteroids group and other-dose of corticosteroids group need to be presented in the supplemental materials at least.

Reply 1: Thank you for your constructive advice. And we added some tables in supplemental material (Table S3-4, S6-7).

Comment 2: The number of patients treated with other-dose of corticosteroids, 192 patients (36.4%), is too high to be simply excluded. As mentioned in comment #1, the baseline characteristics and clinical outcomes need to be described as well, at least in the supplemental materials.
Reply 2: Thank you for your constructive advice. And we added some tables in supplemental material (Table S4).

Comment 3: Since the type of corticosteroids may affect the clinical outcome, (there are several articles showing that the hydrocortisone may be associated with worse outcome in ARDS because of the more potent mineralocorticosteroids effect which enhances water reabsorption), the type of corticosteroids such as hydrocortisone, methylprednisolone, and dexamethasone, needs to be described in table 1 and in the results section.
Reply 3: Thank the reviewer for the question. This is a limitation of our study, as certain. We initially designed the dose of methylprednisolone equivalent in the Case Report Form, we inappropriately overlooked the type of corticosteroids. Therefore, such data cannot be provided.

Comment 4: It is well known that the timing of administration of corticosteroids is important in ARDS, because the late administration is associated with worse clinical outcome. It would be interesting to see if there is a difference in the clinical outcome between the groups which started steroids within 3 days from the diagnosis of ARDS and those started steroids after 3 days.
Reply 4: Thank the reviewer for the suggestion. We are interested with this issue, too. However, the number of patients who had continuously applied low-dose corticosteroid after 3 days was too few to conduct further analysis($n<10$).

Comment 5: In page #6 and line #113, it would be better to show the percentage limited to the low-dose corticosteroids group. I mean, it would be better to show the percentage as 55/65 than 55/527. Likewise, in page #6 and line #117, it would be better to show

the percentage limited to the high-dose steroid group.

Reply 5: Thank you for your kind advice. And we have modified our text as advised (see Page 8, line 156 & 160)

Comment 6: In the study, 17 hospitals were participated. Were the patients treated with corticosteroids recruited from all the 17 hospitals? It is necessary to show the distribution of hospitals of patients treated and not treated with corticosteroids. This is important because, if the patients treated with corticosteroids are confined to several hospitals, it is hard to know whether the positive effect of corticosteroids come from the steroid itself or from the characteristics of hospitals such as expertise in the treatment of ARDS.

Reply 6: We added new figures as supplemental material to show the distribution of patients according to their hospitals. And we considered that patients treated with low-dose corticosteroids were not confined to few hospitals, showing a balanced distribution approximately (see Figure S1a-c).

Comment 7: In table 1, and in page #7 and line #128, the authors used the “oxygen index”. Does it mean PaO2? Please, describe the definition of “oxygen index” in the manuscript.

Reply 7: We are sorry for this mistake. we have modified our text as advised (see Page 9, line 173)

Comment 8: The proportion of intrapulmonary ARDS seems to be much higher than other ARDS studies. Is there any reason for this? The characteristics of this cohort, including high proportion of intrapulmonary ARDS, seems to be described in the discussion section.

Reply 8: Thank the reviewer for the question. Actually, this is a limitation of our study. Most of the included ICUs are respiratory ICUs, and the risk factors may be constrained to intrapulmonary elements. Therefore, the extrapulmonary element induced ARDS patients may have been underrepresented.

Comment 9: The proportion of Immune compromized hosts is too high particularly in the low-dose corticosteroid group. It would be better to show how many patients were

treated with corticosteroids before the diagnosis of ARDS in each group. And this needs to be discussed more in the discussion section as the main limitation of this study.
Reply 9: Thanks for pointing out the problem. We performed 1:2 propensity score matching to address the limitation. In matched sample, hospital mortality showed downward trend in low-dose group in propensity score matching samples (27.5% vs 42.5%, p=.110. Table 2). And the multivariate Cox regression showed significant decreased HR for low-dose group (HR 0.48,95%CI 0.24-0.97, p=.040. Table 4). Therefore, we believe this question may no longer be the limitation of the study.

Comment 10: In table 1, 3 patients in low-dose corticosteroids group and 8 patients in the non-corticosteroids group had chronic cardiac insufficiency. However, according to the Berlin definition of ARDS, cardiac causes of pulmonary edema should be excluded. How did you exclude the cardiac cause of pulmonary edema in these patients?
Reply 10: Thank the reviewer for the question. Differential diagnosis ARDS must be distinguished from acute heart failure. It mainly relys on the judgment based on the heart failure biomarkers BNP, echocardiography, chest CT and even PICCO if necessary.

Comment 11: In table 2, about 75% of patients were not intubated until the end of study. However, for me, this raised another question. To diagnose ARDS according to the Berlin definition, PEEP or CPAP of at least 5 cmH2O is required. All patients who were not intubated in this study were supported with NIV at the time of ARDS diagnosis? Or did you make a diagnosis of ARDS in patients treated with high flow nasal cannula and included them in the present study? Please, describe this in the manuscript.
Reply 11: Thank the reviewer for requesting clarification. Patients who were not intubated were initially supported with NPPV for 15 minutes to confirm PaO2/FiO2 ratio (CPAP≥5cmH2O), and then switch to HFNC or not based on the opinion of physician. Patients who were managed with NPPV for at least 6 hours were termed as NPPV group, while patients with 6 hours HFNC treatment were in HFNC group. We added this in the text (see Page 6, line 104-108). For more details, (DOI: 10.1186/s13054-020-03112-0) is a published article of our study.

Comment 12: Table 2, it seems to be better to show the “duration of mechanical

ventilation", instead of "duration of intubation", because the duration of intubation may be prolonged with delayed tracheostomy. And I recommend that to show 28-day ventilator free days and 28-day ICU free days in table 2 as well.
Reply 12: Thanks for pointing out the problem. We would like to express "mechanical ventilation" rather than "intubation" in fact. And we updated the table2.

Comment 13: In methods section, the authors stated that the evaluation was performed at days 0-14, 21, and 28. Therefore, the clinical outcomes such as 30-day survival and 30-day nosocomial infection rates should be revised to 28-day outcomes.
Reply 13: 30-days survival probability and 30-days nosocomial infection rates were accurate. Because the endpoints, such as, Length of stay, outcome and nosocomial infection, were recorded whenever it happened. The Evaluation at days 0-14,21,28 included laboratory examination, management of ventilation, use of adjunctive measures, fluid balance and so on.

Comment 14: Interestingly, in figure 3, the low-dose corticosteroids group showed tendency of lower nosocomial infection with p-value of 0.087. As it conflicts with common belief, it needs to be explained in the discussion section.
Reply 14: Thank the reviewer for the question. We considered that the difference was not statistically significant. In addition, this tendency disappeared in matched sample (Table 2). Therefore, the results should not be over-discussed in our opinion.

Comment 15: In figure 4, the authors performed Cox regression analysis. However, they did not state the analysis was for which outcome variable. It was for hospital mortality? Or for other outcome variable? It should be stated in the figure legends.
Reply 15 Thanks for pointing out the problem. The outcome variable was hospital mortality. We modified figure legends (Table 4 now).

Comment 16: Is the hazard ratio in figure 4 was adjusted for other co-variants? If is adjusted, the authors should describe which variables were used for the adjustment. And if not, they should perform multivariate analysis using factors that might affect clinical outcomes.
Reply 16: Thanks for pointing out the problem. We misled the reviewer by our wrong

term. “Subgroup” was replaced by “variables”, co-variants in Cox regression were presented in this Figure.

Comment 17: Likewise, in table 3, was it adjusted for co-variants? I think it needs to be adjusted for co-variants and the authors should describe which variables were used for the adjustment.

Reply 17: Thank the reviewer for the question. All variables we considered were included in Table 3&4 and they were analyzed in Table 5 & S10. Variables were included based on the clinical observation and previous literature.

Comment 18: In page #8 and line #168, the authors stated that “our results show that low-dose corticosteroid therapy may play a protective role in ARDS patients except in influenza-related ARDS”. However, in page #7 and line #132, the most common identified pathogen in this cohort was influenza virus. This contradiction should be revised.

Reply 18: Thanks for pointing out the problem. We noted that contradiction about influenza-related ARDS. In our opinion, influenza-related ARDS was just a part of all ARDS (48/289, 16.6%), The representativeness and influence of this part might be limited. Importantly, there was insufficient evidence for evaluating the effect of corticosteroids on patients with influenza-related ARDS, so we deleted “except in influenza-related ARDS” in manuscript.

Comment 19: In line with comment #1 and #2, as a reader of this article, I wonder the nosocomial infection rate was increased in Nosocomial infection patients with high-dose corticosteroids or pulse therapy group.

Reply 19: Thank the reviewer for the question. We added Table S6 to investigate the issue. There was no significant difference in Nosocomial infection rate between high-dose corticosteroids and control group (22.0% vs 26.3%, p=.554) in our limit sample. The number of patients in pulse groups is too few to conduct further analysis (n=8).

Comment 20: Because of the non-interventional (observational) design, the steroid treatment protocols such as type, dose, timing of starting corticosteroids, and tapering methods are heterogenous. I think this is the main drawback of the present study. Please,

discuss this limitation more in the discussion section.

Reply 20: Thanks for pointing out the problem. We added some more discussions in the revised manuscript (see Page 14, line 282-289).

Reviewer D

The authors of this study present the results of a prospective multicentre observational study drawing data from 16 hospitals in China on the use of corticosteroids in ARDS and the association of various dose ranges with outcome. While the data is well presented and the manuscript is clearly written and discussed, I have a number of concerns about the handling of data and potential sources of bias. I also have concerns about the conclusions of the study.

Major comments

Comment 1: This study is touted as a prospective observational study, though the methods and missing data would suggest a retrospective design. Was there a prospective study protocol and statistical analysis plan? This should be linked and included with the submission.

Reply 1: Thanks for pointing out the problem. To tell the truth, we just have a unpublished protocol and CRF in Chinese without statistical analysis plan. What's more, previous study have been published (DOI: 10.1186/s13054-020-03112-0) may provide more details about design and analysis of our study.

Comment 2: Patients were excluded from analysis if they died on the day of inclusion. I suspect this introduced a significant selection bias and downwardly skewed the mortality of the cohort. Can the authors explain the decision to use this exclusion criterion?

Reply 2: Thank the reviewer for the question. We have opposite view on this point. If patients died on the day of inclusion, there must be such a strong fatality factor that most treatment fail. If these patients were enrolled, it may lead to inappropriate negative evaluation of treatment measures.

Comment 3: Page 5 line 82 – "Missing or poor-quality data were removed before data

analysis began." How exactly was missing data handled, what approach was used to determine missingness and the degree of missingness threshold for removal? How can we be assured this did not introduce bias?
Reply 3: Thank the reviewer for the question. Due to oversight, a few data could not be analyzed due to lack of endpoints such as hospital length of stay. We just removed these cases (n=7). We don't think this was enough to introduce new bias.

Comment 4: Page 6, line 121 – "Other corticosteroid administrations were not further statistically discussed (n=192, 121 36.4%)" This is a large number of patients to exclude. In what way did these steroid regimens fall outside the pre-defined thresholds for low, high, and pulse dose regimens? What is the rationale for ignoring this large proportion of the study population? Can the authors provide assurance that this did not introduce selection bias? Furthermore, these patients were effectively excluded from the study though they are depicted as being included in the STROBE diagram. These patients were excluded and the STROBE diagram should reflect this. Was the decision to exclude these patients made based on a prospective analysis plan or was the decision made post-hoc? Please provide supporting evidence in the prospective analysis plan.
Reply 4: Thanks for pointing out the problem. We do, however, excluded a large number of patients, it was a post-hoc decision. To probe the efficacy of low-dose corticosteroid, a standard of dosage should be ensured first in order to minimize the heterogeneity within a group. And there was a large variant of dosage of corticosteroid in other corticosteroid group, further analysis basis on this group cannot provide any clinically meaningful results. Therefore, in contrast, we excluded these patients in order to reduce bias.

Comment 5: Figure 4, and the Results section – "Multiple Cox regression analysis identifies low-dose corticosteroid as a protective factor in patients with ARDS (HR, 0.62; 95% CI, 0.39-346 0.98; p=.041)." This confidence interval is quite close to 1 and in the context of no significant difference in hospital mortality, I would suspect this is a type I error. This concern is further exacerbated by the observational nature of this study, the multiple possible sources of selection bias, the small number of patients in the low-dose corticosteroid group, and the baseline imbalance between the corticosteroid group and patients who did not receive steroids. Can the authors provide

stronger rationale to explain why this questionable finding is included as the main result of the study?

Reply 5: Thank the reviewer for the sharp comment. We performed 1:2 propensity score matching to investigate the question. In matched sample, hospital mortality showed downward trend in low-dose group in propensity score matching samples (Table 2. 27.5% vs 42.5%, p=.110). And the multivariate Cox regression showed significant decreased HR for low-dose group (Table 4. HR 0.48,95%CI 0.24-0.97, p=.040). Therefore, we believe our conclusion still hold.

Comment 6: Page 8 line 171 – "Second, low-dose corticosteroids do not increase the nosocomial infection rate". This is an observational study and as such, causality cannot be established. This statement should be revised.

Reply 6: Thanks for pointing out the problem. We revised the text (Page12, line 237).

Minor comments

Comment 7: Which hospitals participated in this study? Include specific names in the supplement.

Reply 7: Thank the reviewer for the suggestion. We added the data in supplemental materials (see Figure S1).

Comment 8: Page 9 line 194-195 – "This potential benefit can be explained by the anti-inflammation function of corticosteroids, by which the "cytokine storm" experienced in ARDS was relieved." It seems this sentence is a carry-over from the COVID literature. I am not aware of any evidence supporting "cytokine storm" in all-cause ARDS. Further, the cytokine storm theory of COVID-19 is now widely questioned. See: (DOI: 10.1016/S2213-2600(20)30366-0, 10.1001/jamainternmed.2020.3313, 10.1172/jci.insight.140289)

Reply 8: Thanks for pointing out the problem. Our poor choice of words may mislead the reviewer on this point. However, systemic inflammation in patients with ARDS have been widely reported (DOI: 10.1056/NEJMra1608077 and DOI: 10.1056/NEJMe068033). This is a theoretical basis for the corticosteroids therapy for ARDS. And we modified the word in the text (see Page13, line261).

Comment 9: Were any data on ICU-acquired weakness collected?
Reply 9: Thank the reviewer for the suggestion. Regretfully, we did not investigate the morbidity of ICU-acquired weakness and unable to provide exact data on that.

Reviewer E

This is a prospective observational study of ARDS patients among 17 hospitals in China, assessing the effect of low-dose corticosteroids (LDC) in mortality (in-hospital and ICU), length-of stay ICU and in-hospital and intubation rate. The study shows no significant difference in mortality and intubation rates; but a longer ICU and hospital stay as well as duration of mechanical ventilation in LDC.
Studies about immunregulatory and anti-inflammatory treatments, especially corticosteroids which have shown so conflicting results, are immensely important in ARDS, positive and negative studies equally. Therefore, this study will add to this field and provide more evidence for future recommendations.

Major concerns:

Comment 1: 63% of patients (n=192) with corticosteroids are excluded from this analysis: there need to be valid arguments for this approach as it introduces a major bias in the analysis: Were these patients excluded because the exact dose was not known or any other reason? It also needs to be shown that the excluded patients do not significantly differ in their baseline demographics as compared the included ones (f.e. in the Online Supplement).
Reply 1: Thanks for pointing out the problem. We do, however, excluded a large number of patients, it was a post-hoc decision. To probe the efficacy of low-dose corticosteroid, a standard of dosage should be ensured first in order to minimize the heterogeneity within a group. And there was a large variant of dosage of corticosteroid in other corticosteroid group, further analysis basis on this group cannot provide any clinically meaningful results. Therefore, in contrast, we excluded these patients in order to reduce bias. Their baseline demographics were added in Table S7. The reason for different apply of corticosteroids may be part of the reason for the inconsistent baseline data. For example, patients may be divided in other corticosteroid group because they

had oral corticosteroid due to history of autoimmune diseases. Therefore, there was significant difference in immunosuppression between two groups.

Comment 2: The manuscript compares 65 LDC patients with 224 patients with no corticosteroids. If the primary focus is to analyse the LDC alone, then I`d suggest to match these with propensity scores 1:2 to controls (no corticosteroids) according to age, gender, PF ratio and ICU scores (to adjust for the severity of the disease) and run all analysis in this cohort. It will eliminate some of the introduced bias and enhance the validity of your results. As a secondary analysis, the 65 LDC patients can still be compared to the 224 patients without corticosteroids. If your primary goal is to compare treatment with corticosteroids (CS) in ARDS versus no CS, then you could analyse all with CS, independently of the dose, and 2° only the subgroup with LDC to a propensity-score matched control group.

Reply 2: Thank the reviewer for the constructive suggestion. We performed 1:2 propensity score matching to eliminate bias. In matched sample, hospital mortality showed downward trend in low-dose group in propensity score matching samples (Table 2. 27.5% vs 42.5%, p=.110). And the multivariate Cox regression showed significant decreased HR for low-dose group (Table 4. HR 0.48,95%CI 0.24-0.97, p=.040). Therefore, we believe our conclusion still hold.

Comment 3: The main result is: there is no significant difference in mortality and intubation rates between LDC and no-corticosteroids, but ICU and in-hospital stay as well as duration of mechanical ventilation longer in LDC. Also, although not revealing a significant p, there is a trend towards higher ICU and in-hospital mortality in LDC (comparison of percentages). Therefore, it is not really correct to report these results as a positive-effect study. Nevertheless, this should not encourage authors because to finally understand if CS are beneficial or not we need positive and negative studies.

Reply 3: Thanks for pointing out the problem. In matched sample, hospital mortality showed downward trend in low-dose group in propensity score matching samples (27.5% vs 42.5%, p=.110). So, this should still be a positive effect.

Minor concerns

Comment 4:

Introduction:

Line 48: “recommended routine treatment for ARDS”, this is true but it is a weak one based on research recommendation only.

Reply 4: Thanks for pointing out the problem. We revised the text (Page5, line86).

Comment 5:

Methods:

Line 62: the informed consent was required by patient OR the next of kin or both?

Reply: Yes, the informed consent was required by both patient and at least one of the next of kin.

Line 82: “missing or poor-quality data removed”: this needs to be specified how this criteria was defined and how many patients were affected. Are these the n=7 in Fig 1?

Reply: Yes, we removed 7 patients because of missing key data.

Comment 6:

Results:

As above in major concerns

Line 125: both groups incorporates LDC and no-corticosteroids?

Reply: Yes, we clarified the text.

Line 128: I would strongly recommend to start with describing the oxygenation as PF ratio and then the oxygenation index.

Reply: Thanks for pointing out the problem. We are sorry for this mistake. we have modified our text as advised

Line 140: The length of the median follow-up needs to be reported? How many patients were lost for follow-up? Additionally, for a consistent structure the reporting of the outcomes in line 140 should be moved under paragraph 4. Mortality analysis (which could be “outcomes and mortality”)

Reply: Thanks for pointing out the problem. We modified the structure. The study was just focus on hospitalization, follow-up was not involved.

Line 142: The information in these 2 sentences is doubled and could be summarized in 1 sentence.

Reply Thanks for pointing out the problem. We deleted the first sentence.

Line 153: The number available for Cox regression analysis in subgroups of LDC is

way too small. Therefore these results should be treated with utmost caution. The risk of death is for ICU and in-hospital combined?Fig 4: I believe this means multivariate not multiple Cox regression. Additionally, the analysed number of patients (n=xx) per subgroup needs to be included as well as the compared groups in the title. There should be no results reported in the title of the figure. Is it in-hospital or ICU mortality or combined (this needs to be clarified in title)? I understand it is a univariate analysis: LDC as the factor in the respective subgroups to determine the HR? If this is correct, the title needs to be adapted. Small comment: I doubt that ARDS severity is significant as the 95%CI includes 1.

Reply: Thanks for spotting our mistakes. It should be "Variable" not "Subgroup" in Fig 4(Table 4 now), and we modified figure and legend.

Table 1: The PF ratio needs to be included as primary marker of oxygenation in ARDS.

Reply: Thanks for pointing out the problem. We modified the table.

Table 3: multivariate analysis: Are all these factors analysed together? If so, I`d strongly recommend to first report the univariate analysis with their HR, and then the multivariate analysis (same table). Again, there is a major bias the analysis by comparing 65 versus 224 patients (correct? All is 292 patients).

Reply: Thank the reviewer for the question. All variables we considered were included in Table 3&4 and they were analyzed in Table5 & S10.

Comment 7:

Discussion:

There is a quite the difference in the percentage of immunosuppressed patients of 49 vs 17% (LDC vs no-CS). This fact will introduce a bias and should be discussed.

Reply: Thank the reviewer for the question. As mentioned in response#2, we performed 1:2 propensity score matching to eliminate bias.

Line 169: "LDC with protective role in ARDS": not sure how this conclusion can be drawn given the results? (as well as line 194: "potential benefit")

Reply: Thank the reviewer for the question. We considered that the analysis base on the matched sample further supported the conclusion.

Line 205: univariate Cox regression?

Reply: I mean 2 test in mortality here.

Line 211: data covered 14 days? Earlier it said that FU up to 28 days and in Kaplan

Meier curves it is past 30 days. This information needs to be homogenized.
Reply: Thanks for pointing out the problem. The detailed data covered 1-14,21 and 28 days. And the endpoints, such as, Length of stay, outcome and nosocomial infection, were recorded whenever it happened. So different time nodes were showed in different results.
Limitations: needs to include that 64% were not analysed if this stays that was.
While the excess hospital acquired infections are reported as a potential side effect of CS, ICU acquired weakness and delirium are not but equally common. These might influence the longer ICU and hospital stay and should be discussed.
Reply: Thank the reviewer for the question. We have mentioned this view in discussion (Page13, line263-266)
Conclusion: See point 3 in major concerns

Reviewer F

Comment 1: Background: Suggest including the ARDSNet RCT ("Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome" in NEJM in 2006) This is the largest RCT examining what is generally considered low dose steroids (< 2 mg/kg/d).
Reply 1: Thank the reviewer for the question. As far as we know, there are different definitions of low or high dose steroids. In LUNG SAFE study (10.1001/jama.2016.0291), high-dose corticosteroids was defined as doses that were equal to or greater than the equivalent of 1 mg/kg of methylprednisolone. We adopted this definition.

Comment 2: Methods: Many studies use a slightly higher dose of methylprednisolone – i.e. start with 2 mg/kg/d in divided doses – and many clinicians continue to think of this as low dose. In contrast, the dosage used by Bernard and others was truly high dose at 30 mg/kg doses 4 times per day. Accordingly, I do not think the groups accurately reflect international thinking – specifically group 2 being defined as "high-dose" for any dose > 1 mg/kg/d. I suggest performing an additional analysis that defines low dose as < 2 mg/kg/d.
Reply 2: Thank the reviewer for the question. We set cut-off dosage also based on the

series of study conducted by Meduri GU except LUNG SAFE study. Thanks for the suggestion again.

Comment 3: Results: The relationship between LOS and treatment group should be further explored as to the impact of other factors. For example, patients with immunosuppression were significantly more common (p = .000) in the low dose group. Suggest you perform multivariable analysis to explore interactions.
Reply 3: Thank the reviewer for the comment. We performed 1:2 propensity score matching to investigate the question. In matched sample, hospital mortality showed downward trend in low-dose group in propensity score matching samples (27.5% vs 42.5%, p=.110). And the multivariate Cox regression showed significant decreased HR for low-dose group (Table 4. HR 0.48,95%CI 0.24-0.97, p=.040). Therefore, we believe our conclusion still hold.

Comment 4: Discussion: Several points deserve emphasis. Since the is not randomized, interpretation must be cautious. You have made several statement to that effect. The relationship of low dose steroids and longer ICU and hospital LOS is unexpected and might be related to a higher proportion of immunosuppressed patients – suggest commenting on this and if possible performing multivariable analysis to explore the interaction.
Reply 4: As mentioned in Response#3, we performed propensity score matching to eliminate bias.

Second-round peer review

Reviewer A

I would like to thanks the authors for sending the revised manuscript. Many comments were appropriately responded; however, I still have some comments as follows:

Comment 1: Line 186-191: The authors performed 1:2 propensity-matched analysis to minimize the effect of some factors such as immunosuppresion and they mentioned that mechanical ventilation variables were not included in the matching protocol. Did they

do the sample size calculation?

Reply 1: Thank the reviewer for the question. Truth be told, the sample size calculation was not conducted. We just try to make the most of our observational data by propensity score matching. Then, the mechanical ventilation was not included in the matching because there was no significant difference between the two groups before and after the propensity score matching.

Comment 2: It seems that immunosuppression was strongly associated with poor outcomes in this cohort so it's worth to mention it in the discussion.

Reply 2: We appreciate the reviewers request for additional discussion. And we increased the discussion of this topic (Page 13, Line 269 -270).

Comment 3: According to table 3 and 4, It's not cleat at all that which factors have been put in the uni- and multi-variate analyses? Which factors were used for adjustment? In general, significant factors (which one that P-value < 0.1) from univariate analysis will be brought into multivariate model.

Reply 3: We thank the reviewer for requesting clarification. We must admit that we did not perform multivariate analysis according to general standard. The inclusion variables were mainly based on clinical observations and previous literature rather than univariate analysis.

Reviewer B

I appreciate for your hard work to revise the manuscript. I have 2 major and 1 minor comments.

Major

Comment 1: According to the present study, the mortality benefit was observed only in the low-dose corticosteroid group, while the incidence of nosocomial infection was comparable between the variable doses of corticosteroid groups. How would you explain these results? It would be better to describe why the mortality benefit was observed only in the low-dose corticosteroid group in the discussion section.

Reply 1: We appreciate the request for explanation. Results should be interpreted with caution given the limited number of patients especially in high-dose group. Our attitude,

as a result, does not emphasize the role of high-dose corticosteroid rather than negating it. We hope to perform further study with larger sample size in the future. We added to the discussion (Page 14, Line 295-296).

Comment 2: Although the authors matched baseline characteristics between the low-dose corticosteroid group and the control group using propensity score matching, it is an undeniable limitation of this study that there is a huge difference in the proportion of immune compromised patients between the two groups in the original population. It would be better to describe this limitation in the discussion section.
Reply 2: We appreciate the reviewers request for additional discussion. And we increased the discussion of this topic (Page 14, Line 289-291).

Minor
Comment 3: In line #140, please describe the KS normality test with full term.
Reply 3: Thank you for pointing out this error. We have modified our inappropriate abbreviation as advised (See Page 8, Line 144)

Reviewer C

Comment 1: I think the authors have addressed most of the criticisms posed. However, I still have some doubts about the results and how they are presented.
Consider mentioning the control group and its sample size in the Abstract.
Reply 1: Thank you for your advice and we modified our abstract.

Comment 2: Adding the size of the different groups (65, 41, 8, 192) as it appears in the text does no add the overall sample size of 303.
The corticosteroid group is 192 patients in the text (Results) and 189 in Table S4.
Reply 2: Thank you for reading the manuscript so carefully. We are sorry for our careless mistake. This mistake was due to the initial mislabeling, which resulted in a grouping error. In fact, the three patients were excluded. And we modified it (See Page 9, Line 169).

Comment 3: How many patients were receiving mechanical ventilation in the original

groups and how many in the matched groups. I do not see this information in Table 1.
Reply 3: Thank you for your advice. We added data in Table 1.

Comment 4: If there are many non intubated patients in the study population, explain in detail how this (i.e., the diagnosis of ARDS in patients without mechanical ventilation) may impact the interpretation of the results.
Reply 4: Thank the reviewer for requesting clarification. Patients who were not intubated were initially supported with NPPV for 15 minutes(CPAP≥5cmH2O) to confirm PaO2/FiO2 ratio, and then switch to HFNC or not based on the opinion of physician. Therefore, all patients were evaluated for positive pressure ventilation and met the Berlin criteria. Though some patients were not intubated, there is no impact on the results. (DOI: 10.1186/s13054-020-03112-0), was published by our team, provides more details about the diagnosis of ARDS.

Comment 5: Explain for what variables was the matched group matched.
Reply 5: We thank the reviewer for requesting explanation. According to the original sample, variables with difference between the groups, such as, Intrapulmonary ARDS, D0 PCT and Immunosuppression, were included for propensity matching (P-value < 0.10 = . At the same time, we verify that there was no new difference emerging.

Comment 6: Why was mechanical ventilation excluded as a matching variable?
Reply 6: Thank the reviewer for the question. We didn't exclude this variable deliberately. Only because there was no significant difference between the two groups before and after the propensity score matching. Therefore, the variable was not considered.

Comment 7: The authors state that mechanical ventilation did not have a relationship with mortality. This is unusual.
Reply 7: We are sorry for we didn't fully understand the question. We did not come to this conclusion. There was a significant difference in hospital mortality between patients with or without mechanical ventilation (51.2% vs 16.3%, p=0.000).

Comment 8: Under the heading "Mortality analysis" it says that "The hospital patient

mortality in two groups were 27.5% and 42.5%. There were no significant differences in mortality and intubation rates…". I think this is a bit confusing. Maybe it would be more clear: "The hospital mortality in the two groups (27.5% and 42.5%) was not significantly different", or maybe "The hospital patient mortality in the two groups was 27.5% and 42.5%. This difference in mortality was not significantly different", or any other sentence the author may choose.
Reply 8: Thanks for pointing out the problem and we revised the manuscript (Page 10, Line 199-201).

Comment 9: When "intubation rate" in mentioned, is this a patient baseline characteristic or is it an outcome?
Reply 9: Thank the reviewer for the question. In present study, many patients received mechanical ventilation (159/289, 55.0%) on first day. Therefore, intubated rate largely depends on the patient's condition rather than administration of corticosteroid. After consideration, we moved the intubated rate from Table 2 to Table1 and revised Table S5-S7.

Comment 10: Tables S1 and S2. Please consider giving the information provided for each of the groups, not just the two groups combined.
Reply 10: We are thankful for this suggestion and revised the Table S1 and S2.

Comment 11: Table 5. Is this the mortality in the matched group? Please, clarify.
Reply 11: We are sorry for the unclear expression. Table 5 showed the mortality in the matched group and we modified the title.

Comment 12: Table 1. Consider providing the mortality in the original groups (n=65 and n=224).
Reply 12: Thank the reviewer for the suggestion. We provided the mortality in Table S5.

Comment 13: Table 1. Correct "driving pressurea".
Reply 13: Thanks for pointing out the problem and we fixed the error.