Author’s response to reviews

Title: High hemoglobin is associated with increased in-hospital death in patients with chronic obstructive pulmonary disease and chronic kidney disease: A retrospective multicenter population-based study

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Author’s response to reviews:

Dear editor,

Thank you for your message.

We have carefully read the reviewers’ comments on our manuscript and think that these comments are very constructive and have been of tremendous benefit.
In the revised manuscript, we have made some modifications/corrections in accordance with the reviewers’ feedback. Furthermore, we have asked an expert, native English speaker to edit the language. We hope that these changes will help us meet the requirements for publication in your journal.

In the following section, the text shown in red corresponds to new text that has been incorporated into the revised manuscript, and all of our responses are marked in blue.

Thank you for your time and careful work.

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Reviewer reports:

Brian Hobbs (Reviewer 1): Summary:

The authors report results from a large, retrospective, multi-hospital association study of hemoglobin (Hb) levels and in-hospital mortality in COPD patients with and without CKD. The primary conclusion is that high Hb levels (compared to the hemoglobin group with the lowest mortality) are associated with a higher risk of in-hospital death in COPD patients with comorbid CKD. The study has major limitations related to the definition of COPD (unclear if spirometric or based on diagnostic coding) and the lack of available data to adjust for severity of COPD in multivariable regression. That said, the authors present intriguing preliminary data that should be replicated and validated, preferably in data sets with cause-specific mortality, spirometry, supplemental oxygen utilization, and medications.

Major:

1) The title and the running title are misleading with regards to the conclusions one can make from the reported investigation. High hemoglobin was not shown to "increase" in-hospital death as no causal-modeling or functional studies were done to prove that hemoglobin itself is the reason for the observed increases in mortality. High hemoglobin is merely "associated" with in-hospital deaths, and may be a marker for another process contributing to the observed increase in mortality, as the authors state as a limitation in the discussion. Throughout the manuscript, the
authors need to adjust language that says Hb "increases" the risk of death to make it clear they are reporting an association, not a causation.

Response: Thank you for your feedback. Following your suggestion, we have modified the original title to “High hemoglobin is associated with increased in-hospital death in patients with chronic obstructive pulmonary disease and chronic kidney disease: A retrospective multicenter population-based study”.

Correspondingly, we changed the original running title to “High Hb associates with increased in-hospital deaths in COPD with CKD”.

In addition, we reviewed the whole passage to avoid such inaccurate descriptions.

2) How was COPD defined in this study? There are details on classification and stratification of CKD, but no details are given for COPD determination.

Response: Thank you for your question. In this study, COPD was determined based on clinical diagnostic coding. The related information has been incorporated into the current version of manuscript.

As with the classification and stratification, due to the retrospective, multicenter features of this study, pulmonary function- and blood gas-related data were lacking, which constituted another limitation of this study. To avoid misleading readers, we have described this limitation in the revised manuscript as follows:

“Second, because the data were derived from retrospective electronic medical records, supplemental oxygen use, pulmonary function and blood gas analysis indices were not available, and the severity of hypoxia and patient conditions were not evaluated.”

3) The covariates in the regression model are quite limited. Were any data available regarding COPD severity (such as supplemental oxygen use or FEV1 level)? It seems that if COPD is defined by ICD diagnostic coding, then supplemental oxygen use should also be available.

Response: Thank you for your comment. Due to a large amount of data derived from retrospective electronic medical records, neither supplemental oxygen use nor FEV1 was available from the electronic medical records. This limitation in the Discussion section was revised as follows:

“Second, because the data were derived from retrospective electronic medical records, supplemental oxygen use, pulmonary function and blood gas analysis indices were not available, and the severity of hypoxia and patient conditions were not evaluated.”
4) The authors missed an opportunity to compare hemoglobin cutoffs for persons with and without COPD in the CCS-AKI study, thus it is unclear if any of the observations are specific to COPD or are representative of patient with CKD overall. This is a major limitation of the investigation, particularly since elevated hemoglobin levels are already known to be associated with increased mortality in CKD. The authors note that the KDIGO group recommends that Hb levels should not exceed 13 g/dL. Thus, one question from the reported investigation would be whether this upper bound for Hb levels should be more liberal in COPD, where the current manuscript suggests threshold of >14 in advanced CKD and >17 in all CKD. It do not think that is an appropriate interpretation of the current study, but it is hard to definitively draw another conclusion without a comparison of the association of in-hospital mortality with Hb levels in persons with CKD, stratified by COPD status (and severity).

Response: Thank you for your concern. The original design aimed to compare the results from previous studies on COPD (Respir Care. 2013.58:1204-1212; N Engl J Med. 1983.308:1045-1049; Chest. 2005.128:1201-1208), and thus, only data of patients with COPD was extracted in this branch study of CCA-AKI. We could not compare hemoglobin cutoffs for patients with or without COPD. In addition, the recommendation that the Hb levels should not exceed 13 g/dL in CKD patients by the KDIGO guideline was based on results from several randomized controlled trials, the characteristics of which were markedly different from ours. Our data are closer to a real-world situation, and our manuscript suggests an Hb threshold of >14 in advanced CKD and >17 in all CKD. Therefore, although the Hb upper limit is higher in our study than the KDIGO guideline, it cannot be concluded that the upper bound for Hb levels should be more liberal in COPD than in CKD, as the reviewer denoted. Our results indicated only the importance of the upper limit of Hb in the COPD population.

Minor:

5) Introduction, pg 4, line 87, grammatical error: "…comorbid polycythemia is low, which contributes to the…" should read, "…comorbid polycythemia is low, which is attributed to the…"

Response: Thank you for your careful review. In the revised manuscript, we have replaced the original citation from the related reference.

6) The p value threshold of P <0.05 does not properly account for the multiple testing performed for analysis of each bin of mean hemoglobin level to a reference hemoglobin. The authors should consider more strict adjustment for multiple testing.
Response: Thank you for your valuable comment. If multiple testing is performed, the \( \alpha \) level should be adjusted according to the number of comparisons (i.e., based on the number of the subgroups). If the enrolled patients were subgrouped according to every 1 g/dL interval, there should be 11 subgroups from \( \leq 9 \) g/dL to \( >17 \) g/dL in total. Correspondingly, an adjusted \( \alpha' \) level of 0.0045 could be considered the threshold for statistical significance.

Although we did not perform sampling in the COPD population from CCS-AKI, the enrolled sample size reached 47,209 cases. Notwithstanding the large number of enrolled patients, if these subgroups, based on every 1 g/dL interval, were further divided according to non-CKD and CKD or according to non-CKD, early CKD and advanced CKD, some of the subgroups would suffer from a small sample size and a small number of death cases, which would entail difficulties in multiple testing. Based on this consideration, we have plotted Figure 3 to show the overall mortality trends based on Hb stratification, rather than performing multiple testing. According to the tendencies indicated by Figure 3, we performed statistical analysis of the relationship between different Hb intervals and mortality in the subsequent multivariable logistic regression model.

7) Results, all: The mortality rate throughout the results should have accompanying statistics when two groups are compared. For example, in lines 197-198, "the death rate showed an increasing tendency, which reached 3.8% and 4.0% within the 16-17 g/dL and higher than 17 g/dL groups, respectively" should be clarified to state if 3.8% and 4.0% are significantly different. I suspect they are not different and "increasing tendency" may be misleading.

Response: Thank you for your concern.

As in our response to the previous comment, the current Figure 3 showed only the general tendencies of the relationships between mortality and Hb stratification, and we did not perform statistical analysis. Therefore, in the revised manuscript, we have removed all the descriptions related to statistical significance, and the revised result section is as follows:

“Compared with the subgroups with Hb < 10 g/dL, the death rate decreased among those with Hb 10-16 g/dL in both the non-CKD and CKD groups (Fig 3A). The death rate tended to further decrease in the non-CKD patients with increased Hb. However, in the CKD group, the death rate showed a U-shaped distribution pattern with changes in Hb. Hb levels and mortality rate were inversely proportional when the Hb level was above 16 g/dL (Fig 3A). Unsurprisingly, this U-shaped pattern was more noticeable in the advanced CKD group (Fig 3B and Supplementary Fig 2).”
8) Results, pg 8, line 192: "With an increase in Hb levels, the death rate decreased gradually." These are cross-sectional data and "gradually" may imply a longitudinal aspect to the investigation. It would be better to say, "Hb levels and mortality rate were inversely proportional."

Response: Thank you for your feedback. In the revised manuscript, we have made the suggested change.

9) Results, pg 8, lines 195-200: A lot of this text is extraneous and could more concisely describe the U-shaped relationship of Hb level and mortality, which is best visualized in Figure 3.

Response: We agree with your suggestion. In the revised manuscript, we have removed some of the detailed description and described the general tendencies shown in Figure 3 (please also refer to our response to comment 7).

10) Results, pg 9, line 201: The authors talk about "correlation" between Hb and death rate, though no correlation statistics are given. I suggest either a correlation analysis be performed and plotted, or a choice of different words.

Response: Thank you for your careful review. As in the response to comment 7, we did not perform correlation statistical analysis. According to your comment, we have modified the original description and instead, focused on the description of the tendencies shown in Figure 3.

11) Results, pg 9, line 208: "independent correlation" is not the correct wording and I suggest the authors refer to this analysis as "independent association."

Response: Thank you for your feedback. In the revised manuscript, we have changed the original description to the following:

“Taken together, the above results suggested that the independent association between high Hb and in-hospital mortality was primarily due to complications of advanced CKD…”

12) Results, pg 9, line 210: "correction factors" should be "covariates"

Response: Thank you for your feedback. We have made the suggested change in the revised manuscript as follows:
“Furthermore, the independent correlation between a high Hb level and in-hospital mortality was tested using a multivariate logistic regression model that included age (≤55 years, 56-75 years and >75 years) and CCI scores as the covariates.”

13) Results, pg 9, line 215: p value missing for the OR
Response: Thank you for your feedback. We have added the p value for the OR as follows:

“For the CKD group, however, Hb levels > 17 g/dL were associated with a higher risk of death, with an OR of 2.085 (95% CI 1.019 - 4.264, P=0.044) (Fig 4B).”

14) Results, pg 9, line 219: “>7” should be “>17”
Response: We apologize for our carelessness. In the revised manuscript, we have corrected the mistake.

15) Results, pg 9, line 215: p value missing for the second OR
Response: Thank you for your careful review. In the revised manuscript, we have added the p value (please also refer to our response to your 13rd comment).

16) Figure 3: These lines are an inaccurate representation of the data, which is discussed according to hemoglobin bins in the main text. Additionally, the data as currently plotted do not allow the visualization of the error about the mortality percent at each level of hemoglobin. Since the data were binned according to mean hemoglobin, these data would be more accurately represented by three set of box plots in each hemoglobin bin.
Response: Thank you for your comment. We agree that the addition of Hb standard errors or the use of Hb ranges might increase the amount of the data compared to the use of mere mean values. However, the purpose of Figure 3 in this study was to focus on the general tendencies of the relationship between mortality and Hb, rather than statistical analysis among different Hb bins. Plotting Figure 3 required a certain criterion to divide the Hb subgroups. Even in the same patient, however, the Hb outcomes might fluctuate during the hospital stay. For instance, a patient within the 12-13 g/dL subgroup might present with an Hb level of 10 g/dL or 15 g/dL. Therefore, if the error bars had been added, there would have been no method for subgrouping based on Hb. We admit that the use of Hb means for figure plotting was somewhat inaccurate. However, this method could be the only feasible way to show the general tendency of the
relationship between Hb and mortality after our careful thinking. Therefore, we did not revise Figure 3, except for the removal of the original labels indicating significant differences.

17) Figure 4: The Y axis scale should for OR should be log, not linear, such that 0.5 and 2 are equally spaced around 1. This explains the upward skew of the 95% CI around the OR.

Response: Thank you for your constructive comment. In the revised manuscript, the Y axis scales have been transformed to log scales, as follows.

18) Given the large 95% CI around the OR at the tails of the hemoglobin distributions, a table showing the number of deaths per hemoglobin bin in each CKD stratum would be helpful.

Response: Thank you for your constructive comment. Following your suggestion, we have added the number of deaths per hemoglobin bin to the revised Figure 4 (please also refer to our response to comment 17).

Spyridon Fortis (Reviewer 2): This study/manuscript examined the association of Hg levels with mortality in COPD patients with CKD and no-CKD. My main concern is why hospital mortality was chosen as the main outcome. Authors need to explain that. The manuscript will benefit from English language editing.

Major comments:

Introduction

- There are studies have shown that polycythemia is a poor prognostic factor. Those need to be discussed in the introduction (e.g. PMID:16162707). Regardless that, the introduction does not give a clear message. I would write that the studies examined association of Hg and mortality in COPD showed conflicting results. We wanted to examine the association of Hg and mortality in COPD. However, Hg can be affected by CKD, which is common, and Hg can also affect mortality in CKD. therefore we examined separately the association of Hg with mortality in non-CKD and CKD patients.

Response: Thank you for your constructive comment. Following your suggestion, we have revised the introduction section. In the revised manuscript, the focus of this section has been directed to the fact that previous studies reported only that polycythemia is not associated with poor prognosis of COPD and that reports on the possible relationship between them are lacking.
The main changes are as follows:

“The association of polycythemia and adverse outcomes is not well understood in patients with COPD. Several previous studies indicated a neutral or protective role of polycythemia [2, 7, 8]. In COPD patients with chronic respiratory failure, polycythemic subjects seemed to have a higher survival rate than normocytic subjects [9]. Similar results were observed in studies using hematocrit as a polycythemic index. In a small sample cohort study, the hematocrit level was comparable in survivors and nonsurvivors [10]. In another COPD cohort including 2,524 patients, the 3-year survival was 24% when the hematocrit was < 35% and 70% when the hematocrit was ≥ 55% [11].

However, the potential detrimental effects of polycythemia have been implicated in several other chronic conditions, one of which is chronic kidney disease (CKD).”

In addition, we have added a description regarding the relationship between CKD and Hb, as follows:

“CKD is a common comorbidity in the COPD population [4, 6, 19]. Given the effect of CKD on Hb, does CKD affect the Hb distribution among COPD patients? Furthermore, due to the detrimental role of high Hb in the CKD population, does high Hb pose a more serious threat to COPD patients with CKD than to those without CKD? In this hospital population-based study, we aimed to compare the difference in Hb distribution in non-CKD and CKD patients with COPD and then separately study the relationship between high Hb and in-hospital mortality.”

There are also contradictory statements: page 4 line 83 "Hemoglobin (Hb) abnormalities including anemia and polycythemia are common in the COPD population" and then at line 87 "However, the prevalence of comorbid polycythemia is low, which contributes to the widespread prescription of long-term oxygen therapy". I think that the authors meant to write that recent studies have shown that polycythemia prevalence rates are lower compared to those in earlier studies likely due to widespread oxygen use.

Response: Thank you for your feedback. We have modified the original description to the following:

“Hemoglobin (Hb) abnormalities, including anemia and polycythemia, are common in the COPD population [2-4]. …However, polycythemia prevalence rates reported in recent studies are lower than those in earlier studies and range from 6-10% in the COPD population [2, 7, 8], likely due to widespread prescription of long-term oxygen therapy [6].”
In contrast to COPD, a high Hb concentration is an adverse factor in several chronic conditions, one of which is chronic kidney disease (CKD). needs to be rephrased.

Response: Thank you for your feedback. We have rephrased the problematic sentence as follows:

“However, the potential detrimental effects of polycythemia have been implicated in several other chronic conditions, one of which is chronic kidney disease (CKD).”

Last paragraph in the introduction needs to include clear objectives and should be rewritten

Response: Thank you for your feedback. In the revised manuscript, we have rewritten the objectives of this study as follows:

“In this hospital population-based study, we aimed to compare the difference in Hb distribution in non-CKD and CKD patients with COPD and then separately study the relationship between high Hb and in-hospital mortality. The reference Hb intervals in the COPD population were also explored.”

Methods

- Did the study include only COPD patients hospitalized for AKI (this was an AKI registry)? that needs to be clarified.

Response: Thank you for your feedback. The dataset includes all COPD patients with or without AKI. We have added this information to the “Method-- Study subjects” as follows:

“This dataset includes all hospitalized COPD patients with or without AKI.”

- What missing in excluded subjects --> Hg, creatinine

Response: Thank you for your feedback. In total, 25,653 admissions had no Hb records, and 18,278 had no creatinine records. These excluded subjects accounted for approximately 40% of the original 110,305 subjects (Fig 1). This limitation was denoted in the “Discussion” in this revised edition as follows:

“In addition, approximately 40% of subjects were excluded due to incorrect or incomplete medical records (Fig 1), which might cause selection bias.”
“CKD was defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m2 based on serum creatinine, according to the KDOQI guidelines [20]. In-hospital death was the main outcome, and the Hb interval at the minimum death rate was defined as the reference interval. The Charlson Comorbidity Index (CCI) was used as the primary risk index for in-hospital death [2, 21]. Considering the inconsistency in the International Classification of Disease (ICD) standards among the enrolled study centers, ICD codes and diagnostic nomenclature were both used to calculate CCI. The mean Hb level during hospitalization for each patient was used as the Hb level for subsequent analysis.”

Results

- "The lowest death rate was among patients with Hb levels within the 15-16 g/dL interval at 0.5%, and the highest death rate was among those with Hb levels above 15-16 g/dL at 0.7%" --> ?
Response: Thank you for your feedback. This sentence describes the tendency of Hb distribution in patients without CKD. To be concise, this content has been replaced by the following content in this revised manuscript:

“Compared with the subgroups with Hb < 10 g/dL, the death rate decreased among those with Hb 10-16 g/dL in both the non-CKD and CKD groups (Fig 3A). The death rate tended to further decrease in the non-CKD patients with increased Hb. However, in the CKD group, the death rate showed a U-shaped distribution pattern with changes in Hb. Hb levels and mortality rate were inversely proportional when the Hb level was above 16 g/dL (Fig 3A). Unsurprisingly, this U-shaped pattern was more noticeable in the advanced CKD group (Fig 3B and Supplementary Fig 2).”

- "However, in the CKD group, the death rate showed a typical U-shaped distribution pattern with increases in Hb"-- should be "However, in the CKD group, the death rate showed a typical U-shaped distribution pattern with changes in Hb"

Response: Thank you for your feedback. We have made the suggested change in the revised manuscript.

- Authors should report the number of subjects in each group/statum. It is possible that the higher mortality rate in Hg>17 is due to small sample size. They can add a table with n for each strata in the supplement and report that in the results as well.

Response: Thank you for your constructive comment. The number of subjects in each group has been added to the revised Figure 4. As shown in Fig 4B, 275 patients had a > 17 g/dL Hb level, and 11 of these patients died in the hospital.

Discussion

- Another limitation of the study is that more than 50% of participants were excluded

Response: Thank you for your thorough consideration. As shown in Figure 1, there were 44,174 patients with incorrect or incomplete medical records who were excluded from the original 110,305 subjects. In addition to the 40% of patients excluded due to data quality, other exclusion criteria were related to study design. Thus, we think other exclusions play a smaller role in selection bias.

Following your constructive comment, we have added the limitation in the Discussion section as follows:
“In addition, approximately 40% of subjects were excluded due to incorrect or incomplete medical records (Fig 1), which might cause selection bias.”