Author's response to reviews

Title: The Prevalence of Anemia and Its Association with 90-day Mortality in Hospitalized Community-acquired Pneumonia

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Version: 2 Date: 21 January 2010

Author's response to reviews: see over
Dear Ms. Titmus,

Thank you for the opportunity to respond to the reviewer concerns re: our manuscript examining anemia and its association with 90d mortality in patients hospitalized with community-acquired pneumonia. Our responses our described below in blue font. We believe we have adequately addressed all concerns and the manuscript is much improved as a result. We hope that you find our changes acceptable and stand ready to make any additional changes as needed.

Best Regards,

Eric B. Milbrandt, MD, MPH

**Reviewer: Marya Zilberberg**

**Reviewer’s report:**
Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. I have a major problem with the way the authors define anemia: at least borderline anemia threshold Hb is \(<= 13\) g/dL. By most definitions 13 g/dL is a normal value for men, and for women it is 12 g/dL and above. This is an important misclassification and the authors either need to explain why they used such definition and how the resulting misclassification has affected their estimates or re-do the analyses with more appropriate thresholds. While the authors attempt to explain this more fully in the text on page 5, their explanation does not address my concerns. I personally would be much more comfortable with Hb < 13 g/dL for men and <12 g/dL for women, with normals being \(>/=\) to the corresponding value. However, if you show me that it did not affect your results that much, I am willing to live with my discomfort.

Along the same lines, when the authors state on page 1 that “nearly 4 in 5 were anemic…” and “3 in 4 survivors…”, are they referring to the 13 g/dL threshold? If so, this may be overstating the magnitude of the problem.

We are sorry for the confusion this created. We intended for borderline anemia to describe subjects that were on the cusp of developing anemia, but realize in doing so that we overstated the magnitude of the problem. We have changed the focus of our results to those who have at least mild anemia, as defined by a hemoglobin <=12 g/dL.

We considered having different threshold for different genders, but felt this would be unnecessary since one could easily determine the proportion of each gender that met various hemoglobin thresholds according to the data presented in the right most columns of table 2. Again, our intent was not to say any particular hemoglobin threshold was “the definition of anemia”, but to instead explore the full spectrum of changes in hemoglobin values across important subgroups.

2. In the Statistical Analysis section of the Methods the authors talk about models for anemia development and the 90-day mortality. The 90-day mortality model includes the additions of
anemia and transfusions. How were these factors analyzed? After all anemia and transfusions are likely not independent of each other. In other words did the analysis assume independence of these events, which is not a correct approach, and if not, how was their interdependence handled statistically? If you considered them independent and put them in the same regression models, you need to check for their correlation, and, if positive, re-run a separate model including each one.

We agree. Our multivariable logistic regression models for 90d mortality used backward stepwise variable selection with those variables listed in the statistical analysis section being eligible for inclusion. Because we also suspected that anemia and transfusion would not be independent, we initially included not only these variables but an interaction term as well. The p-value for the interaction term was highly non-significant (p=1.0), so there appeared to be no interaction, and the variable was dropped from the final model (as was the transfusion variable with its p-value of 1.0). To be certain of our results, we also ran models including each variable separately (anemia w/o transfusion and transfusion w/o anemia). Our results were unchanged by this approach.

3. On page 8 when talking about enrollment numbers: it is unclear why you are jumping between 1,838 who had a Hb on day 1 and 1,893 with a Hb ever during hospitalization. By inference, it looks like you are including anyone with a Hb drawn ever during hospitalization. If so, how is baseline Hb defined? To avoid this confusion I recommend that you define your inclusion and baseline Hb criteria more explicitly in the Methods.

We apologize for the confusion this created and have clarified this in the methods.

The hemoglobin value obtained on day 1 was defined as the baseline “hemoglobin on presentation” value. The final hemoglobin measured in the hospital was the discharge value.

For analyses in which a baseline value was necessary (determining the prevalence of anemia on presentation and stratifying the subsequent course of hemoglobin values by presenting hemoglobin category (figure 3)), we limited our analysis to those subjects with day 1 hemoglobin values obtained (n=1838). For analyses not requiring a baseline value (determining the prevalence of anemia during hospitalization (figure 2), risk factors for its development, and the association with 90d mortality), we included all subjects who had hemoglobin measured at least once during their hospital stay (the “inpatient CAP analysis cohort”, n=1893).

4. In figure 3, referred to on page 9, the group with the lowest baseline Hb seems to peter out around day 10 – is this because they are all dead or discharged or is this an error in the graph?

Figure 3 is limited to those with a day 1 baseline hemoglobin measurement. Only 20 subjects were in the lowest baseline category. By day 10, few of those subjects remained in the hospital, being either dead or discharged. As noted in the figure legend, we censored figure 3 when there were <5 observations/category/day to reduce the effect of outliers.

5. Similar to my suggestion in 3 above, please, define what you accepted as discharge Hb in the Methods.

We apologize for the confusion this created and have clarified this in the methods. The final hemoglobin measured in the hospital was the discharge value.
6. On page 9 second line from bottom: “As expected” should be removed, as this is editorializing.

Done.

7. On page 10: again, anemia and transfusions are likely collinear and are by no means independent. You need to address this issue in your modeling, as it is likely not valid to include both anemia and TF as independent covariates into the same model.

We agree. Our multivariable logistic regression models for 90d mortality used backward stepwise variable selection with those variables listed in the statistical analysis section being eligible for inclusion. Because we also suspected that anemia and transfusion would not be independent, we initially included not only these variables but an interaction term as well. The p-value for the interaction term was highly non-significant (p=1.0), so there appeared to be no interaction, and the variable was dropped from the final model (as was the transfusion variable with its p-value of 1.0). To be certain of our results, we also ran models including each variable separately (anemia w/o transfusion and transfusion w/o anemia). Our results were unchanged by this approach.

8. Again on page 10 I am concerned that you are overstating the prevalence of anemia given your threshold definitions for Hb.

We agree that including those with baseline anemia unintentionally overstated the prevalence. We have changed the focus of our results to those who have at least mild anemia, as defined as hemoglobin <=12 g/dL.

9. Your Discussion section is good. I would like some additional discussion of why you think chronic respiratory disease may be protective from development of anemia, since several studies have found a fairly high prevalence of anemia in COPD patients, though these were not confined to hospitalized patients. Some have in fact proposed that anemia in COPD develops during hospitalization and that may artificially inflate its prevalence estimate in this population. At any rate, this point is of interest and bears some discussion.

We were surprised by this as well. Unfortunately, we have no explanation for the finding. We have added the following to the limitations paragraph:

We are unable to explain why chronic respiratory disease appeared protective for the development of anemia in our models when it is known to be a risk factor for anemia in the outpatient setting [37]. Importantly, this observation did not seem to be due differential transfusion rates in our cohort.

10. Finally, now that I have read the entire paper, I would suggest that you go back to the abstract and amend your conclusions. In my opinion, you have found that not all levels of anemia are clinically important, at least the way you have defined this importance relative to the 90-day mortality rate. Therefore, for a clinician it may be more helpful to know what levels of anemia he/she should worry about. Also, once you have addressed my TF issue, this should be brought out in the abstract as well.

We have modified the abstract (and paper) to focus on subjects with at least mild anemia (<=12 g/dL), since the literature shows this to be associated with increased mortality. We have also changed our work (abstract, discussion, conclusions) to highlight the exact threshold (<=10
g/dL) that our particular study found to be associated with 90d mortality in CAP in order to give clinicians a level that he/she should worry about. We have not added anything about transfusions to the abstract since there did not seem to be much of a story to tell.

Reviewer: Paula Peyrani
Reviewer’s report: This is a very interesting article trying to describe the natural history of anemia and CAP. However, I have major concerns with some sections of this manuscript. Please see my comments below.

1. MAJOR COMPULSORY REVISIONS
TITLE
Comment #1. The natural history of anemia in hospitalized CAP is difficult to be assessed.

Even though most of the patients had several hemoglobin assessments performed during hospitalization, the fact that prior medical history regarding hemoglobin values and values after discharge are not available makes very difficult to analyze the so called natural history. Instead it is more a descriptive evaluation of anemia status of hospitalized patients with CAP, on admission, during hospitalization, and at discharge. Given this scenario, I think that the association between anemia and mortality at 90 days becomes the relevant point and the title should be modified reflecting so. Examples would be “Association of Anemia with 90-day mortality in hospitalized patients with CAP” or already giving a hint about the results “Anemia is Associated with Increased 90-day mortality in hospitalized patients with CAP”.

We agree with your assertions. While a true natural history cannot be described without premorbid and post-discharge values, the evolution of changes in hemoglobin values during hospitalization remains an important and little known piece. Because our work addresses important knowledge gaps in this area while also highlighting the association between anemia and mortality, we have modified the title to read:

The Prevalence of Anemia and Its Association with 90-day Mortality in Hospitalized Community-acquired Pneumonia

ABSTRACT
Comment #2. Objective missing in the abstract. Even though it can be inferred from the background paragraph, the objective of the study should be described and written in the abstract.

The objective is now clearly stated in the abstract.

BACKGROUND
Comment #3. Natural history of anemia as primary objective is difficult to assess from available data. In relationship with what it was already mentioned in comment #1, I would strongly suggest to modify the primary objective to the evaluation of the association of anemia with 90-day mortality and keep the descriptive section of hemoglobin as a secondary objective.

We agree with your assertions. While a true natural history cannot be described without premorbid and post-discharge values, the evolution of changes in hemoglobin values during hospitalization remains an important and little known piece. Because our work addresses important knowledge gaps in this area while also highlighting the association between anemia...
and mortality, we have kept both objectives primary, but no longer use the words “natural history”. Instead we use the words “prevalence”, “development”, and “progression” in the paper. We hope you find these changes acceptable.

METHODS
Comment #4. Study outcomes not defined in M&M

The authors defined how anemia was classified and how mortality data was captured. It is not clear from this section if the analysis of variables independently associated with moderate to severe anemia was performed with hemoglobin values at any time during hospitalization (as stated for 90-day mortality) or at other time. Since different denominators are being used for the study outcomes, it may help if the paragraph of clinical definitions and outcome variables is divided in study definitions and study outcomes specifying for each study outcomes when it was assessed.

We have modified the statistical analysis section to better describe which hemoglobin values were used for each outcome and analysis and well as the number of subjects for each analysis. We have also clarified which values were baseline and which were discharge values in the clinical definitions section.

RESULTS
Comment #5. Anemia over time. Interpretation/discussion given in the results section. Page 9. Second sentence to the last: “Over time, hemoglobin value converged….due in part to the 176 subjects that received at least one blood transfusion”. Author’s interpretation of results should not be given in this section.

We have modified these sentences to avoid interpretation and simply state how many subjects received a transfusion and what the pretransfusion hemoglobin value was.

Comment #6. Variables independently associated with the development of moderate to severe anemia. Odd ratios should not be interpreted as relative risk/risk ratio. The authors concluded that “white race and chronic respiratory disease were associated with lower odds of developing anemia”. Odds ratios should not be interpreted as relative risk or risk ratio when the outcome has a high prevalence such in this case where at least moderate anemia was found in 27% of patient during hospitalization and 16% at discharge. One solution could be to use a different statistical methodology to obtain risk ratio. Otherwise, I think this should be stated as a limitation of the study

We apologize for this confusing statement. We only intended to describe the association of race and respiratory disease with the development of moderate to severe anemia. We have modified the sentence to read:

White race and the presence of chronic respiratory disease were inversely associated with moderate to severe anemia.

DISCUSSION
Comment #7. Discussion about possible pathogenesis of results is not included. The authors found that moderate to severe anemia is associated with increased 90-day mortality but they are not discussing why they think this association occurred. This may be the section to have some more detailed analysis/speculation regarding cytokines production in CAP and the link with anemia.
Thank you for this opportunity. We have added a paragraph to the discussion which examines the link between cytokines, anemia, and mortality.

2. MINOR ESSENTIAL REVISIONS

RESULTS

Comment #8. Page 8. Study population and outcomes. Sentence starting: “After day 1, hemoglobin values… subjects that remained hospitalized each day” should read “… subjects who remained…”

Done.

Comment #9. Page 8. Study population and outcomes. Sentence starting: “Of the 1893 in…, 114 (6.0%) has positive…” should read “…114 (6.0%) had positive…”

Done.

Comment #10. Page 9. Anemia over time. Sentence stating: “Over time, hemoglobin values…, 176 (9.3%) subjects that received…” should read “…176 (9.3%) subjects who received…”

Done.

Comment #11. Page 9. Anemia over time. Sentence starting: “Of those that were transfused…” should read “…Of those who were transfused…”

Done.

Comment #12. Page 10. Anemia and its association with 90d mortality. Sentence starting: “This increased risk persisted… to subjects that survived…” should read “…to subjects who survived…”

Done.

DISCUSSION

Comment #13. Page 10. Anemia over time. Sentence starting: “Consequently, … depending on hemoglobin values for those that were not…” should read “…depending on hemoglobin values for those who were not…”

Done.

TABLES

Comment #14. Table 2. Page 23. Suggest to modify for each row title: “Anemia on …” to “Hemoglobin on…” No anemia is included as part of the results so technically this would not be Anemia.

Done.

3. DISCRETIONARY REVISIONS

DISCUSSION
Comment #15. Page 12. Last paragraph. Sentence starting: “In our study, the presence of moderate to severe anemia was independently associated with 90d mortality…” Suggest to add the word increased before 90d.

Done.

Comment #16. Page 13. Limitations. I would not consider as a limitation that outpatients were not included since it is stated in the title and M&M section that this were hospitalized patient with CAP.

Removed.

Comment #17. Page 13. I think it is not appropriate to suspect that acute GI bleed was uncommon just due to the reason of patients consenting to participate in the study. I would remove this explanation.

Done.