Author's response to reviews

**Title:** Macrocytosis may be associated with mortality in chronic hemodialysis patients: a prospective study

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**Author's response to reviews:** see over
Macrocytosis is associated with mortality in chronic hemodialysis patients

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Dear editorial board at BMC,

Below is our response to each of the reviewer concerns. Their suggestions and comments have also been used to submit a revised manuscript, with changes noted in red text. Any sections that were removed are also discussed in the comments below. We appreciate the extremely helpful reviewer suggestions and we hope that you accept the changes as noted below.

A. Reviewer 1: Robert Means

1. Over emphasis on erythropoietin resistance aspect of findings

   a. Pg. 11: We modified the conclusion to only state the association of macrocytosis with mortality, and eliminated the conclusion statement on clinical associations. Prev. Pg. 10: We also removed the paragraph on inflammation, darbepoetin alfa and macrocytosis as we felt it detracted from the primary purpose of this paper as stated by the reviewer.

   b. Pg. 8: We did keep a reference to the ratio in paragraph 2 of the discussion, but primarily as a means of relating macrocytosis to mortality.

   c. Pg. 9: Any paragraph in the discussion referring to associations with macrocytosis was adjusted to again focus on mortality. This includes the paragraph on IV iron.
2. Darbepoetin/hemoglobin ratio as a mortality predictor
   a. Pg. 8: We added a sentence to the last paragraph of the results section specifically stating that the darbepoetin alfa to Hb ratio was not associated with mortality in this study.

3. Lack of B12/folate levels in all patients as a limitation
   a. Pg. 10: We have specifically stated the lack of B12 and Folate levels in the limitations section of the discussion
   b. Prev. Pg. 8: We have moved the previous B12/Folate paragraph (as a potential cause of macrocytosis) to the limitations section as an accompaniment to this limitation

4. Background of figures
   a. All figures are now consistent: with a white (clear) background.

B. Reviewer 2:

1. Multivariate Cox regression model over-fitting
   a. We do agree with the reviewer on this point, and we have adjusted our models in the following ways:
      i. Rather than use each individual clinical condition, we calculated a baseline Charlson-age comorbidity index (CACI) which includes diabetes, CAD, stroke, cancer and PVD amongst other conditions grouped together to come up with a “score” of morbidity. This has been examined as an effective tool in dialysis patients and allows us to incorporate several clinical conditions into one variable.
      ii. Pg. 5: Regarding this index, we included a paragraph on it in the methods section along with two references. We added its clinical significance to the results section, the baseline characteristics section, and the associations table. We also included a brief theory as to its significance in the discussion.
      iii. We reduced the number of variables in our multivariate model. We only examined macrocytosis with the age-adjusted comorbidity index (2 total variables). As a result, we removed table 4. from the study altogether. We did modify the
limitation section statement about multivariate model use in light of the above changes.

2. Choice of variables, more clear statement of goal of study
   
   a. We have reworded sections of the abstract, introduction and discussion of the paper to emphasize the purpose of this study which is to see if macrocytosis is associated with mortality. We have also removed the causation sentence from the discussion as appropriately recommended.

   b. We appreciate the input regarding choice of variable. We elected in our multivariate Cox analysis to only use variables associated with macrocytosis and mortality. The only variable that fit this restriction was the age-adjusted CCI. We removed previous reference in the methods section to use of other variables that predict death in other dialysis cohorts. However, to identify that our population is similar to others that have been studied with respect to predictors of death, we added a statement in the discussion emphasizing this point and maintained the references already stated in the previous paper.

3. Reporting one of multivariate effect estimates in abstract
   
   a. Pg. 2: We reported the multivariate effect estimate using the age-adjusted CCI.

4. Make the abstract conclusion less strong
   
   a. Pg. 2: We rephrased the abstract conclusion to be less strong

   b. Pg. 1: We also made the title less strong to reflect the change in abstract conclusion and more closely resemble the conclusion of the paper.

5. Table 1. Footnote explanation RE: continuous variables
   
   a. We have moved the description of continuous variables (mean ± standard deviation) to the footnotes section of the table

6. Table 2: clarify in footnote which variables in multivariate logistic regression model
   
   a. We added a footnote clarifying this point in the table

7. Explanation why only hemodialysis patients were included in the study
a. Pg. 4: We elected to replace the sentence “In our peritoneal and hemodialysis population” with “In our hemodialysis population”. This negates the need to explain why PD patients were excluded from the study within the paper itself. While it is true that macrocytosis is also highly prevalent in peritoneal dialysis patients in Halifax, we did not incorporate them into this study because of the route of administration of darbepoetin alfa (subcutaneous in PD patients, and IV in hemodialysis patients). Subcutaneous and IV doses are not interchangeable, thus, different routes of administration of darbepoetin would potentially confound the results of the study. We elected not to put this in the paper, as we feel it will interfere with the main message which is this significant finding in hemodialysis patients. Macrocytosis in peritoneal dialysis patients may be the subject of future study.

8. Spelling out abbreviation CBC

a. Pg. 5: We have added “complete blood count” to the methods section of the article

9. Results of table 3 inclusion in Table 1

a. We appreciate that this is a rather simple table, but elected to keep it separate, as it is not a baseline characteristic rather an outcome measures table.

10. Figure 1: use of cross-sectional cohort

a. We agree with the reviewer and have removed cross-section from the study entirely. Specifically, we referred to the study as prospective cohort in the abstract, methods and Figure 1.