Author's response to reviews

Title: The importance of iron in long-term survival of maintenance hemodialysis patients treated with epoetin-alpha and intravenous iron: analysis of 9.5 years of prospectively collected data

Authors:

Victor E Pollak (vpollak@migs.com)
Jonathan A Lorch (lorchj@mail.rockefeller.edu)
Rakesh Shukla (Rakesh.Shukla@uc.edu)
Supriya Satwah (supriya.satwah@gmail.com)

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Author's response to reviews: see over
Dear Dr. Alexandersson

Please find a revised version of our manuscript.

We have attempted to respond, on a point by point basis, to the issues and concerns raised by the three reviewers. Our responses follow.

We trust that you will find the revised manuscript satisfactory for acceptance.

With kind regards, and wishes for the New Year

Victor E. Pollak, M.D.
Reviewer 1: Francesco Locatelli  
Responses to Reviewer’s report:  
However the Authors should better explain the conclusions on the light of the recommendations of the K/DOQI Guidelines:  
- Hb concentration: it is known that lower Hb concentrations are associated with higher morbidity and mortality: each 1g/dl greater Hb concentration is associated with reduction of relative risk of mortality of 4%, but there is not significant difference for patients with Hb concentration of 12 g/dl or greater compared with an Hb concentration of 11 to 11.9 g/dl (reference group). The Authors should better discuss the data respect the benefits for Hb > 11g/dl and for Hb > 12 g/dl. The data derived from multivariate analysis in Table 4 indicate that, as compared with a Hb level >120 g/L, the Hazard Ratio (HR) for a Hb level 100.1-110 g/L was 1.69 (95% CL 1.31-2.14). This provides convincing evidence that survival was better with a Hb >120 g/L. We agree that the HR in Table 4 does provide convincing evidence that survival was better with Hb >120 g/L. We also agree that the KDOQI guidelines may need revision; this view is supported by reference #23.

- Iron therapy: it is known that a greater TSAT and Ferritin levels are positively associated with better anemia control minimizing the dose of ESA needed to achieve the range Hb levels. We agree. The data in Table 4 show that “greater TSAT and Ferritin levels are positively associated with” lower mortality. The Authors should illustrate the benefits and the risks for TSAT and Ferritin beyond the targets recommended in the K/DOQI. The HRs in Table 4 confirm that serum ferritin levels up to >1000 µg/L are not associated with a higher mortality, and that the mortality was greater with ferritin levels <600 µg/L than with levels >1000 µg/L. Two recent reviews (cited as references #35 & #36) concluded that an evidence based guideline for an upper limit for ferritin is not available. Reference #37 provided evidence that serum ferritin levels up to 1200 µg/L were NOT associated with an increase in all cause mortality. Again, we suggest that the KDOQI (and NICE) guidelines may need revision.

- About ESA dose: the Authors didn’t discuss at all the recent paper by Streja et Al. describing that higher platelet count is associated with lower iron stores and greater prescribed rHuEPO (AJKD 2008 Oct; 52 (4): 727-36). We thank the reviewer for drawing attention to this important paper. A short paragraph has been added to the discussion, on page 15, to address the issue raised in this paper (now reference # ) and in a recent paper on the subject in women with iron deficiency anemia (now reference # )
Reviewer 2: Michal Mysliwiec
Responses to Reviewer’s report:

The Authors should better describe the clinical state of their patients, particularly some comorbidities like heart failure and infections which have been known to be associated with higher death rate in HD patients with high hemoglobin level. The same deals with LVH, did the Authors observe any changes in LVMI over the years of observations in dependence of hemoglobin level? Was quality of life of the patients studied

it is interesting to note that of patients with Hb ≤120 g/L 591/1314 (45%) died, whereas of those with Hb >120 g/L only 107/432 (24.7%) died.

Systematic observations in LVH and LVMI were not made.

Quality of life was not studied.

There are to much discussion on posible changes in cytokines and only superficial explanation of the results on real clinical grounds.

We believe that the discussion on cytokines is important; it provides one possible explanation for some of the findings reported in the literature and discussed in this paper. Because of the possible importance of cytokines in the genesis of favorable and unfavorable outcomes, studies are currently underway with colleagues at Rockefeller University to address the possible relationship of cytokine levels to iron, TSAT, and clinical outcomes in HD patients. When completed they may throw light on this issue “on real clinical grounds”.

Recently published guidelines state that no patient should not be routinely maintained at greater than 13 g/dL of hemoglobin level, which is considered to be the upper threshold in ESA-treated patients. The FDA has recently issued a black box warning that recommended that HB in pats with kidney disease not rise beyond 12 g/dL. [www.FDA.gov.cder/drug/advisory/RHE2007.htm](http://www.FDA.gov.cder/drug/advisory/RHE2007.htm). Both these warnings should be mentioned in the discussion section.

These have been mentioned in the body of the manuscript. We looked at the data on 47 patients with Hb >130 g/L of whom 9 (19.1%) died. In this very small sample there does not therefore seem to be any evidence for concern about adverse effects of this relatively high Hb level. And the recent study by Gilbertson (reference #23) showed clearly that there was not an increased risk of death in patients with a Hb >125 g/L. We respectfully suggest that published guidelines may need revision.
Reviewer 3: Anatole Besarab  
Responses to Major & Minor Issues Raised in Reviewer’s report:

A. Major  
1. I take issue with the comment that the NHCT, CHOIR, and CREATE, and Canadian LV remodeling studies were “relatively short-term” or did not examine mortality explicitly. Mortality was part of the composite end point and the studies were designed to see a difference in end-points based on the “available best estimates” present at the time of the design. The reviewer is correct to take issue with this point. The sentence (and the 3 accompanying references) has been deleted.

2. The studies with CERA were designed to maximize iron sufficiency. The characterization that “few reports on the effects of EPO and newer ESAs have addressed the need for adequate iron” is improper. The CERA study (reference #10) was “designed to maximize iron sufficiency”. At the time of selection, patient adequate iron status was defined if serum ferritin was $\geq 100 \text{ OR TSAT was } \geq 20$ (i.e., not if serum ferritin was $\geq 100 \text{ AND TSAT was } \geq 20$). For TSAT at the onset of the study the 25% IQR was 20% in the CERA group, 20.3% in the darboepoitin group; i.e., approximately 25% had a TSAT $\leq 20$ before the test ESA was started. The 25% IQRs for serum ferritin were, respectively, 108 and 129; and therefore a significant number of patients had a ferritin $\leq 100$ at onset. Taken together these data suggest that perhaps 30% of patients were iron deficient at study start. Moreover, iron had been administered intravenously at baseline in only 14% of patients.

Definition of the iron status of the patients at the start of the studies in references #3 and #2 is less precise. In #3, TSAT levels at outset of 25.2±11.8 and 24.6±10.1 and ferritin levels of 167.8±157.2 and 179.2±171.5 (large Standard deviations) point to a significant number with TSAT $\leq 20$ and/or ferritin $\leq 100$. Iron had been administered intravenously at baseline in only 1.6 and 2.6% of patients. In reference #2, the median TSAT at outset was 23.9 and 25.1, the mean ferritin 174.4±148.3 and 189.4±157.7 (medians 131 and 128), figures compatible with a significant number of patients who must have had TSAT $\leq 20$ and/or ferritin $\leq 100$. Whether iron had been administered intravenously at baseline is not mentioned, other than to record that 525 of patients in one group and 42% in the second had received “at least one dose of intravenous iron”, and iron administration during the study is not mentioned. The reports cited as references #6, 7, 8, 9 strongly reinforce the view that iron deficiency was NOT excluded at the onset of the studies in references #2, 3, and 10. We therefore respectfully disagree with the reviewer that it is improper to state that few reports on the effects of EPO and newer ESAs have addressed the need for adequate iron”.

The authors are however correct that the guidelines have been too cautious about “proper” iron usage. Ref 15 actually has to be taken in the context of other papers by
this group of authors and it should be noted that maximal survival in the DaVita data set occurs at a ferritin of 900, above the current recommended upper limit. This paper specifically does not caution about iron use but makes the point that ferritin is a bad marker of actual iron stores.
We agree. Reference #15 appears to have been the important source for guidelines on an upper limit for ferritin. Later work by these authors (references # 33, 35, 37) clearly shows that guideline limits <1000 are not consistent with currently available published data; this is confirmed in the present manuscript.

3. In the discussion, the authors again refer to the absence of iron in the CKD RCTs but in all fairness, delivering iron in these patients (as in CAPF/CCPD) is quite different from HD patients. I think all comments regarding whether iron was not appropriately given in these trials is not germane in the presentation at hand and should be removed.
We agree that the method of iron delivery in CKD and in HD patients differs. The important point, however, relates to the iron status of these patients. If indeed iron insufficiency occurs in patients without renal disease, and is prevalent in a significant proportion with CKD, this suggests that iron insufficiency is likely to be prevalent in a moderate to high proportion of patients at the start of a course of HD treatment. We respectfully suggest that comments about iron in these trials are indeed germane.

4. There is no specifics given about the iron or EPO algorithms used at the three dialysis units other than they conformed with guidelines and with external pressures from CMS. For instance was iron sufficiency maintained by a load-hold approach or by a maintenance iron regimen?
Virtually all patients were given IV iron by a “load-hold” approach. We assume that the phrase “load-hold” approach means that patients were given a course of IV iron, that TSAT was followed, and that another course was given when the TSAT fell below the acceptable range.

5. In the analysis we are told that EPO doses and iron doses were administered and we are told of a dose conversion when patients participated in equivalence studies using other ESAs. However we are not told whether the protocol was thrice weekly i.v. epoetin alfa administration ~99% OF THE TIME. If the latter, since iron was administered ~ 1/3 as often as epoetin alfa, it appears that “maintenance” was practiced most of the time?
For the majority of patients EPO was administered three times a week. When iron was given it was usually ordered three times a week for ten doses.

6. It is expected that certain parameters will not be normally distributed but presentation of mean values in table 2 allows the reader to determine which way the skewness points.
We agree that the presentation of mean values and interquartile ranges in Table 2 “allows the reader to determine which way the skewness points”.

7. It also would be useful to see an overall Kaplan Meier survival curve. Based on
the # of EPO doses give, the average weeks of participation is 80.1 weeks which is much lower than the actual median weeks of survival (or time to censure). An overall Kaplan Meier survival curve has been included as a new Figure 1. The remaining figures have been re-numbered.

8. Comorbid conditions could have had better granularity if there were 3-4 subgroups rather than 2, i.e. no comorbid conditions, 1-2, 3-4, > 4. Since HIV had such a bad prognosis during the time of the study (i.e. pre HAART) wonder whether it should not have been looked at separately. Would the multivariate model results changed if there was more or less granularity?

As the number of patients was relatively small (n=1774), we chose for analysis 2 co-morbidity subgroups. The primary focus of the paper is unlikely to be affected significantly by dividing co-morbidity into 3 groups because: (1) co-morbidity is NOT the primary focus of the paper and (2) the impact of adjusting for the co-morbidity variable on other factors' relationship with survival is NOT large.

We thank the reviewer for drawing attention to the HIV patients, who were in fact in the study post HAART. We looked at the issue and found that the mean (and median) survival for HIV patients was 872 (839) days; for non-HIV patients it was 924 (751) days.

9. The effect of iron dose per month is to me, in part, a comorbidity effect of underlying illness. Requirements for more than 455 mg/mo of Fe indicate ongoing losses (usually GI, on occasion vascular access) and of course would be confounding by indication.

The reviewer raises a good point. We did examine the nature of the primary renal disease causing ESRD in the group receiving ≥455 mg iron/month, and found no difference between these and the remaining patients. We also examined the prevalence of 0, 1, and ≥2 co-morbid conditions in the group receiving >455 mg iron/month, and found no difference between these and the remaining patients. We also examined the prevalence of the following specific disease conditions

<table>
<thead>
<tr>
<th>ICD</th>
<th>Disease</th>
</tr>
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<tbody>
<tr>
<td>530</td>
<td>Esophagitis</td>
</tr>
<tr>
<td>531-533</td>
<td>Peptic ulcer w/ or s/ hemorrhage</td>
</tr>
<tr>
<td>535</td>
<td>Gastritis/duodenitis w/ or s/ hemorrhage</td>
</tr>
<tr>
<td>552.12</td>
<td>Diverticulosis w/ hemorrhage</td>
</tr>
<tr>
<td>569.3</td>
<td>Hemorrhage of rectum and anus</td>
</tr>
<tr>
<td>578</td>
<td>Hematemesis</td>
</tr>
<tr>
<td>578.1</td>
<td>Blood in stool</td>
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<tr>
<td>578.9</td>
<td>Hemorrhage of GI tract</td>
</tr>
</tbody>
</table>

and of procedures and complications associated with vascular access in the group receiving ≥455 mg iron/month and in those receiving ≤455 mg/month, and found no difference between the two groups.
The now iron group may have had specific characteristics different from the other 3 groups. Was this specifically looked for?
Yes. The no iron group was more frequent among those entering the study in 1998-1999 (14.2%) than in those entering in the later years, 2000-2006 (9.8%)

10. Again, when looking at the survival curves for iron, TSAT, and ferritin, what this reviewer would want to see on each graph is the amount of iron given to each subgroup over the time at risk. For instance did the lowest serum iron and TSAT groups receive the most or least iron compared to the high groups. Similarly for serum ferritin.
To address this we counted the number of patients who received each of 4 IV iron dose levels (including the iron level) in each of 4 TSAT levels, and added the results to the legend to Figure 6. In the text the following sentences were added to the text: “No explicit relationship was observed between TSAT level and IV iron administered. For example, 17% of patients with TSAT levels ≤20% had received no iron IV, but 51% had received >455 mg/month.”
And similarly for serum ferritin where 4 IV iron dose levels (including the iron level) in each of 5 ferritin levels were counted, and the following sentences were added to the text: No explicit relationship was observed between serum ferritin level and IV iron administered. For example, 40% of patients with serum ferritin levels >600 µg/L had received IV iron >455 mg/month but 18% had received no IV iron.

11. I am not certain I understand what the authors mean by EPO alone had no effect on survival [page 12. last paragraph]. Was the effect a positive or negative one?
Could not find this particular statement. We believe the effect of EPO on survival is explicitly stated at several points in the paper.

12. I could find no presentation of table 7 perhaps it was inadvertently cut in the operation that reduplicated the problem under Minor comments, item 11.
Table 7 is included in the revised manuscript

13. I would delete the entire 3rd paragraph under “hemoglobin: except the first sentence and combine it with the first sentence of the next. That paragraph can be condensed to the last 2 sentences.
We respectfully disagree, and believe that the material in question should not be deleted.

14. On page 15, the whole paragraph on cytokines can be reduced.
We would, respectfully, suggest that this paragraph be not shortened. Please refer also to comments made on this point in reply to Reviewr #2,

B. Minor
1. INTRO: 2nd paragraph of the Background is peripheral to the population studies and can be eliminated.
We consider that this paragraph, which presents evidence on the prevalence of iron deficiency in CKD patients before any HD treatment is needed, is important background to the study and for readers of the manuscript. Respectfully, we believe it should be retained.

2. I do not have reference 19 in front of me so the authors should summarize the characteristics in this paper.

Reference 19, which addresses the electronic medical record used in this study, describes the patient characteristics in detail and, published in an on-line journal, is easily accessible to the reader.

3. Most readers will not know what the omnibus test for interaction (page 9, line 6) is.
   This has been clarified by deleting the word “omnibus”

4. Could the ethnic distribution have been broken out as: white/black/Hispanic/other?
   Have added the phrase “18% were Hispanic” to the description of patients on page 9.

5. In table 4, unable to determine the absolute # of subject with the reference value compared to the other value. Please provide!
   The number of subjects for each reference value has been added as a footnote to Table 4.

6. The difference in survival at 1000 days was 75% vs. 60% in the low vs. high EPO tertiles. This is a 20% difference. I would not characterize this as “rather small” [page 10 last line].
   The reviewer is correct about the difference between the high and low EPO tertiles. The term “rather small”, which was used in a statistical sense is inappropriate from a clinical point of view. The following clause “but survival differences between low, medium, and high EPO groups were rather small” has therefore been deleted.

7. Figure 3 looks impressive but this phenomenon is well known at this point and the figure can be deleted.
   We too believe that Figure 3 is impressive. It represents one of the essential core elements of the study, and must be retained. Incidentally, it also presents evidence from a 9.5 year study, that having a Hb>120 g/L is a relatively good prognostic indicator - at variance with current guidelines and the FDA ‘black box’ warning.

Table 6 has no data in 2 of the cells.
   The cells in Line O in Table 6 are blank because this Line O is the reference line for lines A through N. It is customary to leave the HR blank for the reference. this can be done or, if the editor prefers, “reference” can be inserted in the HR column.
There is a similar situation with Line L in Table 5. It should be treated in similar manner.