Reviewer’s report

Title: Plasma isofurans concentrations are associated with erythropoiesis-stimulating agent resistance in maintenance hemodialysis patients

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Reviewer: Vincenzo Bellizzi

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In this manuscript Dr Rivara and colleagues present an observational study on 253 patients from an RCT, the PATH study evaluating the effect of antioxidant therapy on oxidative stress in MHD, with the aim to evaluate the association of plasma markers of oxidative stress and inflammation with ESA resistance in MHD. The topic is novel and of interest, data seem well analysed and possibly these information may have relevant clinical implication. Nonetheless, the relationship between markers of oxidative stress and ERI is complex and more details may improve its comprehension.

Major Comments

1. The study enrolled 253 out of 353 patients from the PATH study, either treated or untreated control subjects. Authors performed an analysis based on the time-averaged exposure, that is measuring the levels of markers of Oxidative Stress at the baseline and at the end of the study; thus, in some subjects enrolled in the present study, the OS is influenced by the intervention treatment of PATH. It seems more adequate to include in such time-averaged analysis only control subjects of PATH (no effect of treatment). On the contrary, changes of the markers of OS with treatment should be better correlated with ERI changes (see below).

2. To better understand the differences among subgroups at baseline (Table 1), either data on Kt/V, PTH, transferrin saturation, serum albumin, mean corpuscular volume of RBC and % of patients on ESA for each ERI quartile, or normal values for markers of inflammation and oxidative stress, or statistical differences (p for trend; p vs. reference) should be added.

3. For each analysis it is reported a model adjusted for possible factors of ESA resistance. Some major factor of ESA resistance in MHD, however, have not been considered; at least Kt/V, PTH and Ferritin should be included in the adjusted analysis. Maybe a multivariate analysis including all the factors associated with ESA resistance could be useful.

4. The cubic splines correlation was used instead of linear correlation because the relationships between the markers of oxidative stress and ERI is not linear. Authors should give some more details on the modalities they evaluated the non-linearity. What does it means “Unadjusted restricted cubic splines … were superimposed on scatter plots”? Also, statistical differences should be added for Figures 2 and 3.
5. It would be very interesting to know the relationship between baseline exposition factors and ERI changes along the study; this might suggest a possible impact on clinical outcome.

6. Besides the higher quartile, also the middle-low quartile of isofuran is significantly different from reference (Table 3); this means that the relationship between ERI and isofuran is not-linear. Authors should comment on this point for the possible interpretation of mechanisms leading from OS to ESA resistance.

7. Authors associate the relatively high isofuran levels with a greater tissue oxygen tension due to higher ESA resistance. This mechanism needs more discussion; indeed, haemoglobin levels were not different among ERI quartiles and it’s not clear why tissue oxygen tension should be greater with higher ESA resistance. Authors should deeply address this central issue, taking into account also the non-linear relationship between isoprostan and ERI.

8. Authors state that they cannot conclude on the causality of the association and further studies should test if intervention to decrease oxidative stress leads to reduction of ERI. I would suggest two analyses. First, the relationship between markers of OS at baseline and changes of ERI along the study might add information on causality. Second, this study comes from a RCT where antioxidant therapy was assigned to MHD patients vs. controls; the comparison of ERI changes between intervention group (treatment for OS) and controls might assess such effect of intervention to decrease oxidative stress on the reduction of ERI.

Minor Comments

1. The Title of the paper should not include the conclusion of the study.

2. In Table 1 it seems there is a mistake in the limits of ERI quartiles (first or second) and, as well, the median ESA dose for the first quartile should be checked.

3. Isofuran correlation with PCR is very weak or even absent.

4. The long introduction on the mechanism how inflammation influences the ESA resistance can be reduced improving the readability of the discussion.

5. The last conclusion of the discussion, “Additionally, lipid ……” seems too speculative and not fully supported by data and can be deleted.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests