Author's response to reviews

Title: Predicting erythropoietin resistance in hemodialysis patients with type 2 diabetes

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Author's response to reviews: see over
We thank the editors and reviewers for their valuable comments, which helped us to improve our manuscript.

According to the suggestions, we have prepared a revised version of the manuscript. In the following paragraphs, we repeat each question by the reviewers, and provide our answers accordingly.

We have highlighted the changes in the revised version of the manuscript. We feel that we could answer and comment appropriately to all of the issues raised by the reviewers.

~~~~~~~~~~~~~~~~~REFEREE COMMENTS~~~~~~~~~~~~~~~~~

REFEREE #1:

R1: The authors define ESA Resistance Index as a ratio of weekly (weight-adjusted) ESA dose to Hb level. This (widely used) definition is only valid when both ESA dose and Hb level are in a steady state. However, we do not know if that condition was satisfied in the study cohort. Based on the information available in the literature, this condition is very rarely reached in HD patients. In that case, a more appropriate definition of ERI would be one to account for extended, time-lagged effect of ESA on Hb, for example, a ratio of average ESA dose received over months 1-3 to the average Hb achieved over months 2-4 (at least three months should be taken into account).

A1: The analyses in the current study were conducted within the 4D baseline parameters. We did a cross sectional approach because all data were available at baseline. Unfortunately, although we would have liked to, we were not able to obtain all measurements that would be required for such a time-lagged statistical approach due to the large number of patients included in the study and the associated costs.

R2: ESA Resistance is a continuous (not discrete) diagnostic concept. ROC analysis, as performed by authors, is predominantly applicable to binary classification problems. I am not sure it is appropriate here. However, if the authors decide to report the results of the ROC analysis, they should provide the best achieved sensitivity and specificity and the associated diagnostic threshold.
A2: We agree with the reviewer and deleted the ROC analyses accordingly.

R3: The authors include hemoglobin among the clinical markers of ESA resistance. Hemoglobin is already functionally used in the dependent variable in the model. Using it as an independent covariate is questionable. It is therefore not a surprise that low hemoglobin is predictive of ESA resistance, this is known per definition.

A3: We agree and therefore deleted hemoglobin from our analyses. The results of our multivariate analyses without inclusion of hemoglobin are presented in the revised table 3 at page 18 and in the text of the results section at pages 7 and 8.

R4: With the number of covariates included, it would be valuable to know if any of them were correlated or, generally speaking, functionally related to each other.

A4: We have evaluated potential correlations and selected variables in the model based on previous knowledge in the literature. For example, we tested the lipid profile and found strong correlations between total cholesterol, LDL and HDL; however, for our ESA model we restricted the lipid variables to LDL to avoid too much collinearity.

R5: Either do not report the ROC analysis or report it as described in comment 2.

A5: We agree and deleted the ROC analyses from our manuscript accordingly.

REFEREE #2:

R1: The definition of ESA resistance is inflated and based more on statistical criteria than on clinical relevance. Defining the patients in the upper quartile as ESA resistant, the frequency of ESA resistance was extended, by definition, to 25% of enrolled patients.

Moreover, the correspondent cut-off values were not given in the manuscript. I advice to give this information in table 1.

A1: We have done so and added the ESA resistance cut-off values to table 1. Additionally, to comply with the reviewers request, we provided the cut-off values in the manuscript on page 5, lines 6-8. The reason why we chose the upper quartile as ESA resistant, was population based, because in our cohort we only had a limited number of patients that would achieve the KDOQI Guidelines for ESA resistance (ESA dose of 300 IU/kg/week and not achieving Hb 11-12 g/dl; Locatelli et. al., NDT 2007). We used a population based approach in line with similar procedures in previous important studies: the investigators from the TREAT study (“Erythropoietic Response and Outcomes in Kidney Disease and Type 2 Diabetes” Solomon et al. NEJM 2010) and the authors from the RISCAVID study (“Anaemia and resistance to erythropoiesis-stimulating agents as prognostic factors in haemodialysis patients: results from the RISCAVID study. Panichi et al. NDT 2011) used the definition of ESA resistance with patients belonging to the upper quartile.

R2: It is unclear whether the analysis is limited to the baseline data of the 4D study or whether the analysis is extended to the follow-up data.
A2: The analyses in the current study were conducted within the 4D baseline parameters. We did a cross sectional approach because all data were available at baseline. See also answer to reviewer 1 with regard to this issue.

R3: It isn’t mentioned how the authors managed the missing data that are likely distributed in a different manner among the explored predictors. For this reason I suggest to insert this information, variable by variable, in tables 1 and 2.

A3: This study was originally a randomized controlled trial. Data collection was performed in a uniform manner and supervised by a central coordinating center. In case of missing data, queries were sent out and the information collected subsequently. Luckily, the amount of missing data was extremely small and mainly restricted to some biomarkers. These had few missing data < 10%. We included the numbers of patients with the available parameters in Table 1. In addition, we extended Table 1 to all parameters used in our study in order to provide a comprehensive overview.

R4: To give weight to this outcome variable, it should be advisable to explore its link with the primary outcome of the 4D study.

A4: We agree that this is of interest. We found a significant association of ESA resistance with the primary outcome of combined cardiovascular events. In patients with ESA resistance, the incidence of CVE was increased by 33% as compared to non-resistant patients during the median follow-up of 4 years (HR 1.33, 95% CI 1.06-1.67, p=0.013). Mortality of epo-resistant patients was significantly increased by 44% as compared to non-epo-resistant patients. The mortality was highest within the first year of follow-up, in which epo-resistant patients showed a 77% increased death rate (HR 1.77, 95% CI 1.23-2.54, p=0.002). We included this information at page 6, lines 18 -22 of the revised manuscript.

R5: I advice to explore the percent iron saturation in place of the simple iron concentration in the blood.

A5: We agree and revised our analyses accordingly. We now have included the iron saturation in place of the simple iron concentration. The mean iron saturation was 22% in all patients and 23% in the non-resistant as compared to 18% in the resistant patients. We also explored iron saturation within our multivariate models. The novel results are included in the revised version of the manuscript in Table 1.

R6: In Table 1, there aren’t the values of the outcome variable of this study and of the some relevant predictors used in the models 1 (ace-inhibitors, heart rate), 2 (urea, potassium, iron and ferritin) and 3 (ADMA, PTH, 25OH vitamin D and osteocalcin).

A6: We thank the reviewer for the valuable advice. We revised our manuscript accordingly and present all relevant predictors in table 1. Furthermore, we added the ESA resistance cut-off values to table 1 and we provided the cut-off values in the manuscript on page 5, lines 6-8.

R7: In Table 2, please check the unit of measure of ferritin.

A7: We thank the reviewer for the advice and and corrected the unit for ferritin accordingly.
**R8:** In Table 3, it is strange that the increase of urea concentration reduces the risk of ESA resistance. Moreover, the authors did not discuss why they hold the predictors of model 1 in model 3, although they lost statistical significance. It is also strange that ferritin and CRP are not selected in models 2 and 3, respectively.

**A8:** We thank the reviewer for the valuable comments. In line with all further requests, we have revised our analyses and applied stepwise selection procedures throughout. After obtaining the final model of patient history, the laboratory parameters were added and selection procedures applied to obtain model 2. To the final model 2, the biomarkers were added and again, selection procedures applied to obtain the final model 3. In our novel analyses, CRP and albumin remained in the final model, with albumin also partly representing inflammation. The reason why ferritin dropped may be due to collinearity with CRP and albumin. In the revised multivariate analyses low urea concentrations were not associated with ESA resistance. However, a low urea concentration may partly represent protein energy wasting (ref Fouque et al; Kidney int. 2008). In our study, markers of protein energy wasting e.g. low albumin, low BMI and low cholesterol were predictive of ESA resistance.

**R9:** Figures 1-3 on ROC analyses are unnecessary because they can be replaced by only one row in table 3. This is relevant to obtain a more synthetic paper.

**A9:** We agree and have deleted the figures from the manuscript.

**REFEREE #3:**

The authors aimed to identify predictors for ESA resistance and to develop a prediction model for the risk stratification in a large number of hemodialysis patients with type 2 diabetes. The prediction model consisting of easily obtainable parameters seems useful in clinical practice of hemodialysis.

**R1:** Please add the data of ESA-sensitive patients and ESA-resistant patients in Table 1.

**A1:** We have done so and added the data of ESA-sensitive patients and ESA-resistant patients in Table 1. In the revised version of the manuscript, we now present the data of the complete study population as well as of the ESA-sensitive patients and ESA-resistant patients in separate rows of Table 1.

**R2:** ADMA, 25(OH) vitamin D, or osteocalcin was shown only in Table 2. These should be included also in Table 1.

**A2:** We have done so and added the parameters to Table 1.

**R3:** It seems inappropriate to include hemoglobin in logistic regression analyses for ESA resistance, because ERI was calculated by patient’s hemoglobin value.

**A3:** We agree with the reviewer and deleted hemoglobin from the analyses. The novel analyses are presented in the Tables 2 and 3 at pages 17 and 18.
R4: Please add the discussion on the relationship between serum potassium and ESA resistance.

A4: We have done so. In our study, higher potassium levels were associated with resistance to ESAs. Patients in whom it is difficult to maintain potassium levels within the physiological range are often inadequately dialysed. This group of patients are frequently found to suffer from malnutrition (e.g. low BMI) and an increased inflammatory state, which are also, associated with ESA resistance. Furthermore, in 4D we found that the use of ACEI is associated with higher potassium levels. Therefore, the link between higher potassium levels and ESA resistance might be confounded by a higher use of ACEI's. Please find the revision on page 10, lines 4-10.

R5: The style of reference list should be fixed.

A5: We did the correction. Please find the revised reference style on page 12-15.

Editorial Comments:

E1: Trial Registration Number

Can you please include the trial registration number from the 4D study.

A1: The study was designed in 1997 and started in 1998. At that time, a general registration was not yet required. However, the study was registered at the German medical authority (BfArM; registration number 401 3206). The sponsor protocol ID and clinical trial unique identified number was CT-981-423-239. The results of the study are published and available at http://www.ncbi.nlm.nih.gov/pubmed/16034009. We have inserted this information including the trial identifier number on page 2, lines 22-25.

E2: Ethical Approval

Can you please include the full name of the ethical committee that granted approval for your study, listing a reference number if obtained.

A2: The protocol was approved by the ethics committee of the University of Würzburg (Ethik-Kommission bei der Medizinischen Fakultät der Universität Würzburg, Institut für Pharmakologie und Toxikologie Versbacher Str. 9 97078 Würzburg). Please find the added information on page 4, lines 16-17.
Thank you again for your comments and suggestions, by which we believe the manuscript has much improved.

Yours sincerely,

Dr. A. Schneider, on behalf of all co-authors