

Supplemental data to Heinze et al.

‘The use of erythropoietin stimulating agents in renal transplant recipients with hemoglobin above 12.5g/dl is associated with elevated mortality’

Sensitivity analyses of multivariable Cox regression

Part 1: including deaths before day 90 into analyses.....	1
Part 2: sensitivity analysis for multiple imputation.....	4
Part 3: tests of interactions of covariates in final model with log of time	9
Part 4: tests of nonlinearity of covariates in final model	12
Part 5: Assessment of interactions	15
Part 6: Follow-up extended to 31 Dec 2008.....	16
Part 7: Adjusted hazard ratio plots excluding the cardiovascular disease variables.	19
Part 8: Adjusted hazard ratio plots with ESA prescription ‘never turned off’: ESA users remain in the user group even if treatment was stopped.	21
Part 9: Adjusted hazard ratio plots with deaths and graft failure counted as events (transplant survival).	23
Part 10: Including primary indication for transplantation as additional baseline covariates into the model.....	25

Part 1: including deaths before day 90 into analyses

In the original analysis, deaths before day 90 were excluded from analysis. We compared results obtained by including these deaths to our original conclusions.

Variables selected by purposeful selection algorithm:

Dialysis status, number of antihypertensive drugs, cerebrovascular disease, peripheral vascular disease, coronary heart disease, cholesterol level, type of immunosuppressive regimen, use of calcineurin inhibitors, diabetes status, age at transplantation, cold ischemia time, donor age, sum of HLA mismatches.

Results of analysis on hemoglobin and ESA use:

Fig. 1: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (excluding deaths up to day 90), black: analysis including deaths up to day 90

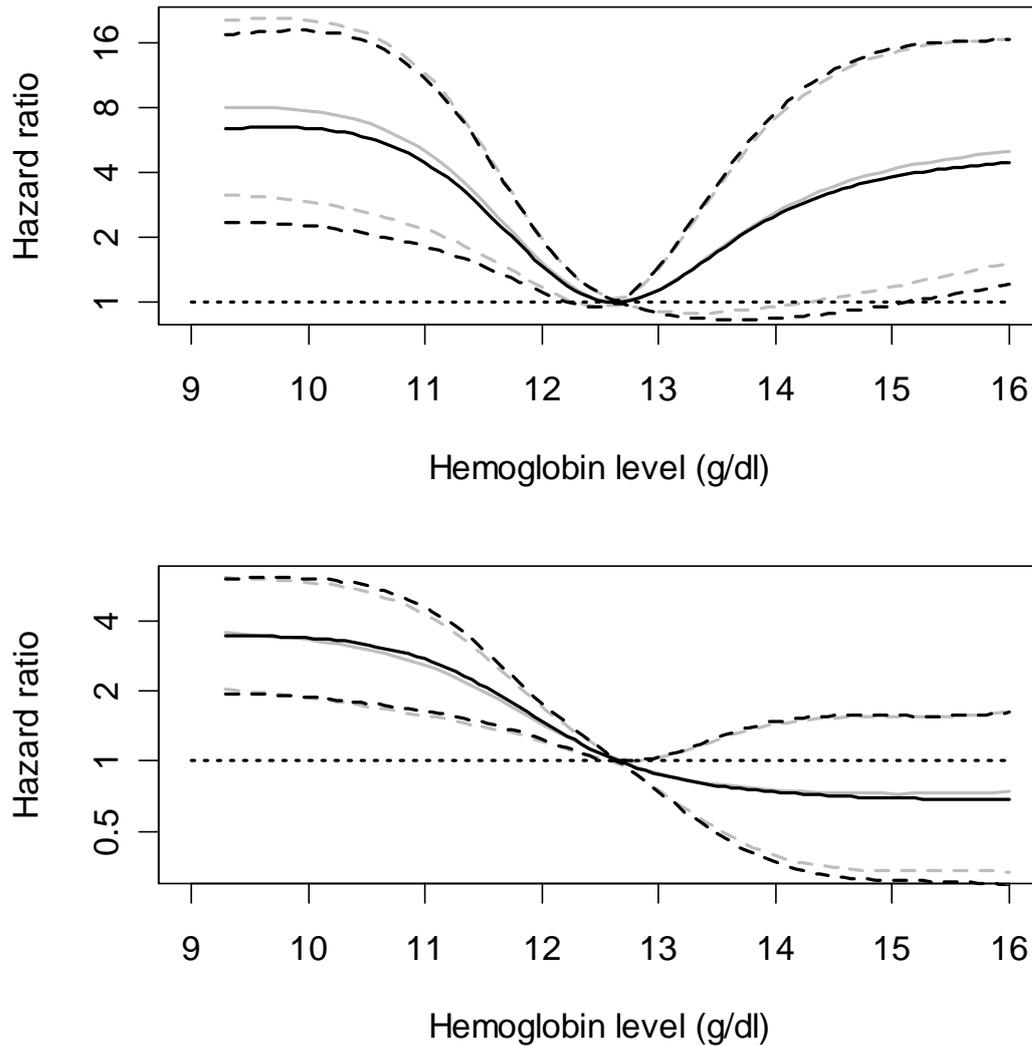
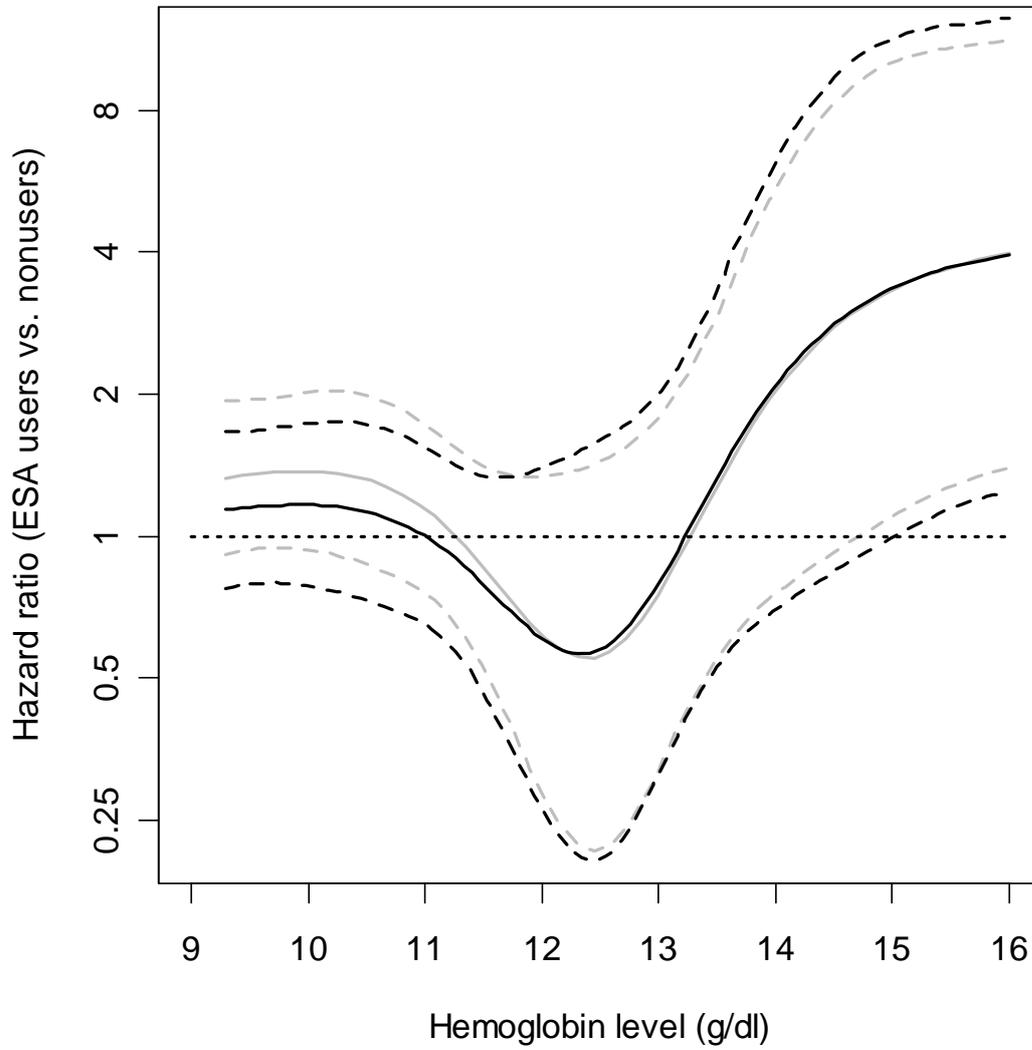


Fig.2: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (excluding deaths up to day 90), black: analysis including deaths up to day 90



Part 2: sensitivity analysis for multiple imputation

For each variable in the final model, we generated non-randomly missing values by randomly deleting values above the median. The proportion of values additionally deleted was equal to the proportion of missing values in the original data set. The data set was multiply imputed and reanalysed. The impact of non-randomly missing data on our conclusions on hemoglobin and ESA use was assessed by comparing the results of this additional analysis to the original results, as well as to results obtained by complete-cases-only analysis.

Purposeful selection algorithm on complete-case-only data selected the following variables:

Dialysis status, type of immunosuppressive regimen, number of antihypertensive drugs, sum of HLA mismatches

Complete records on these variables and on hemoglobin levels were available for N=1386 patients, with 208 events.

Fig. 3: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis using complete cases only (N=1386, 208 events)

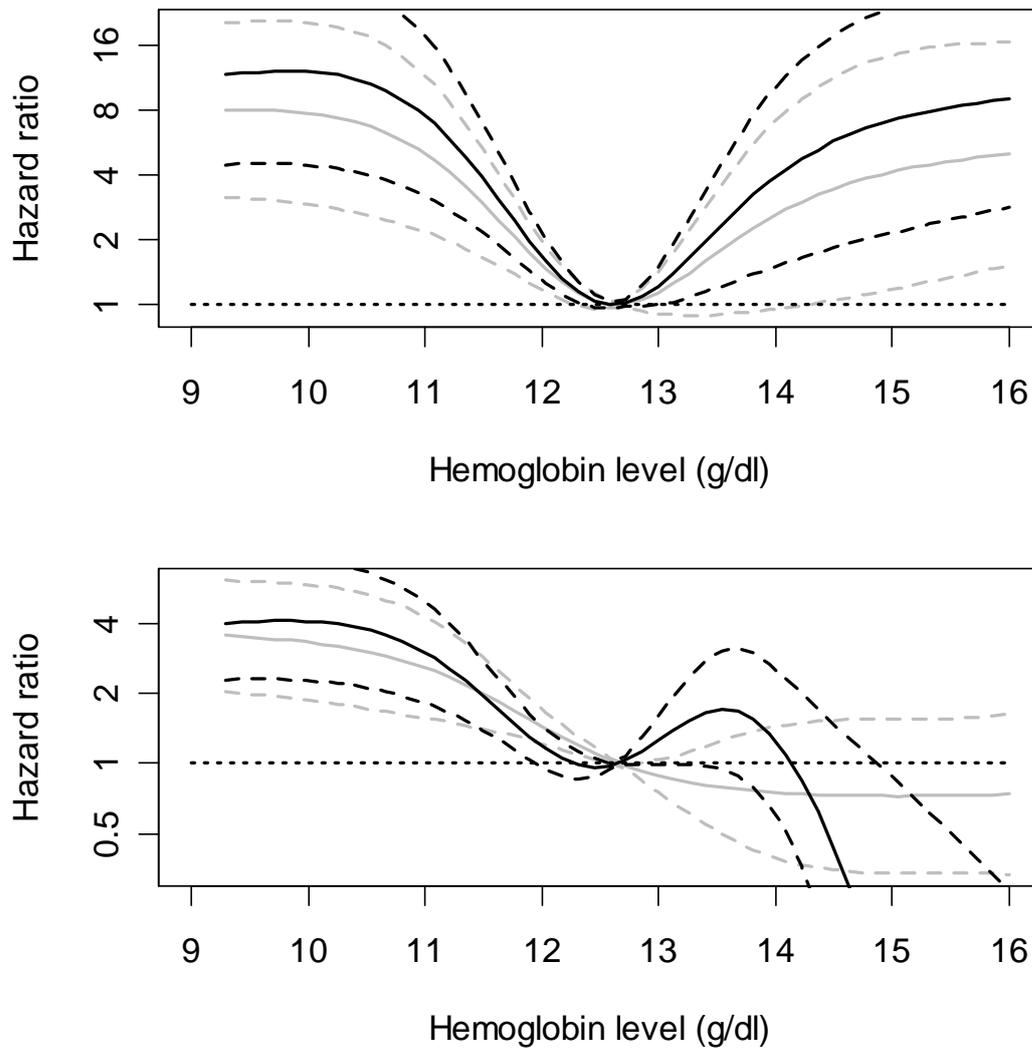


Fig.4: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis using complete cases only (N=1386, 208 events)

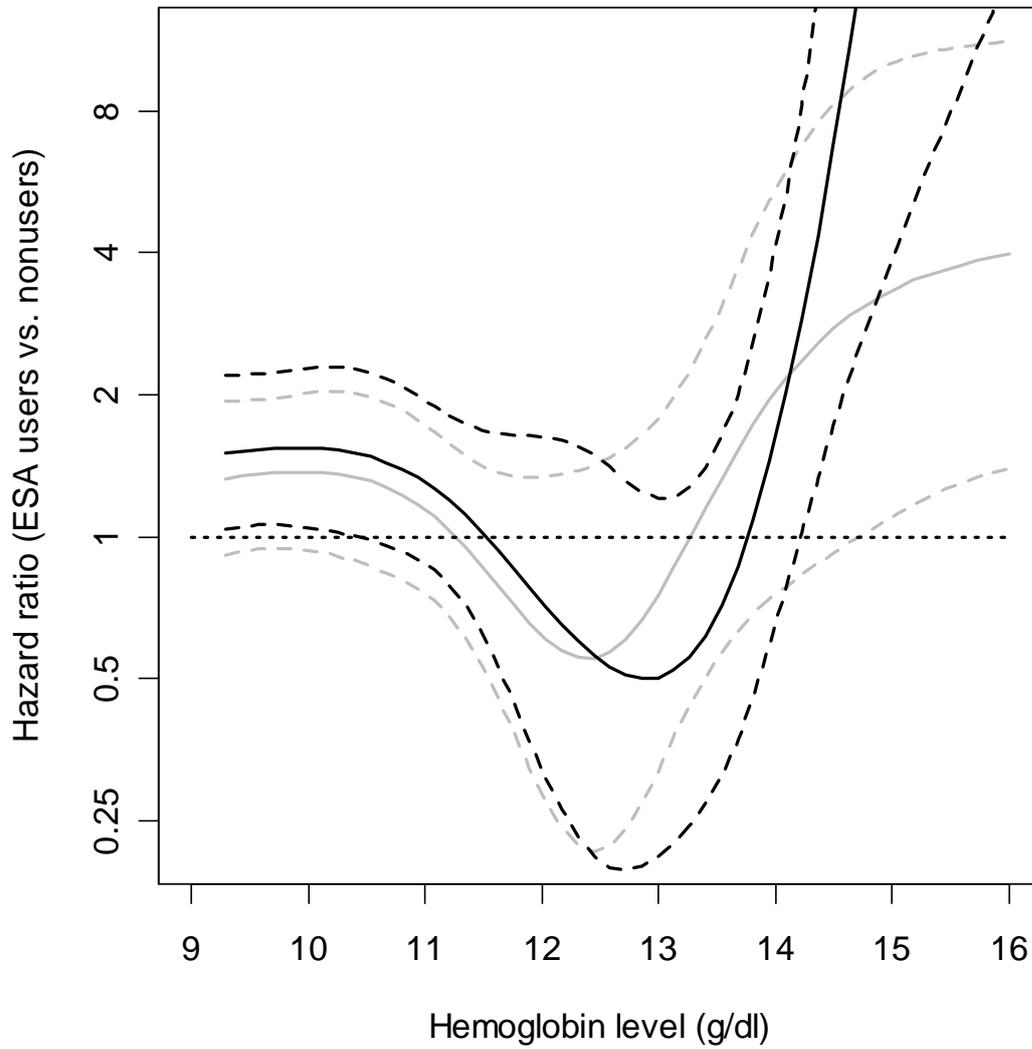


Fig. 5: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis after multiple imputation of artificially generated nonrandomly missing data

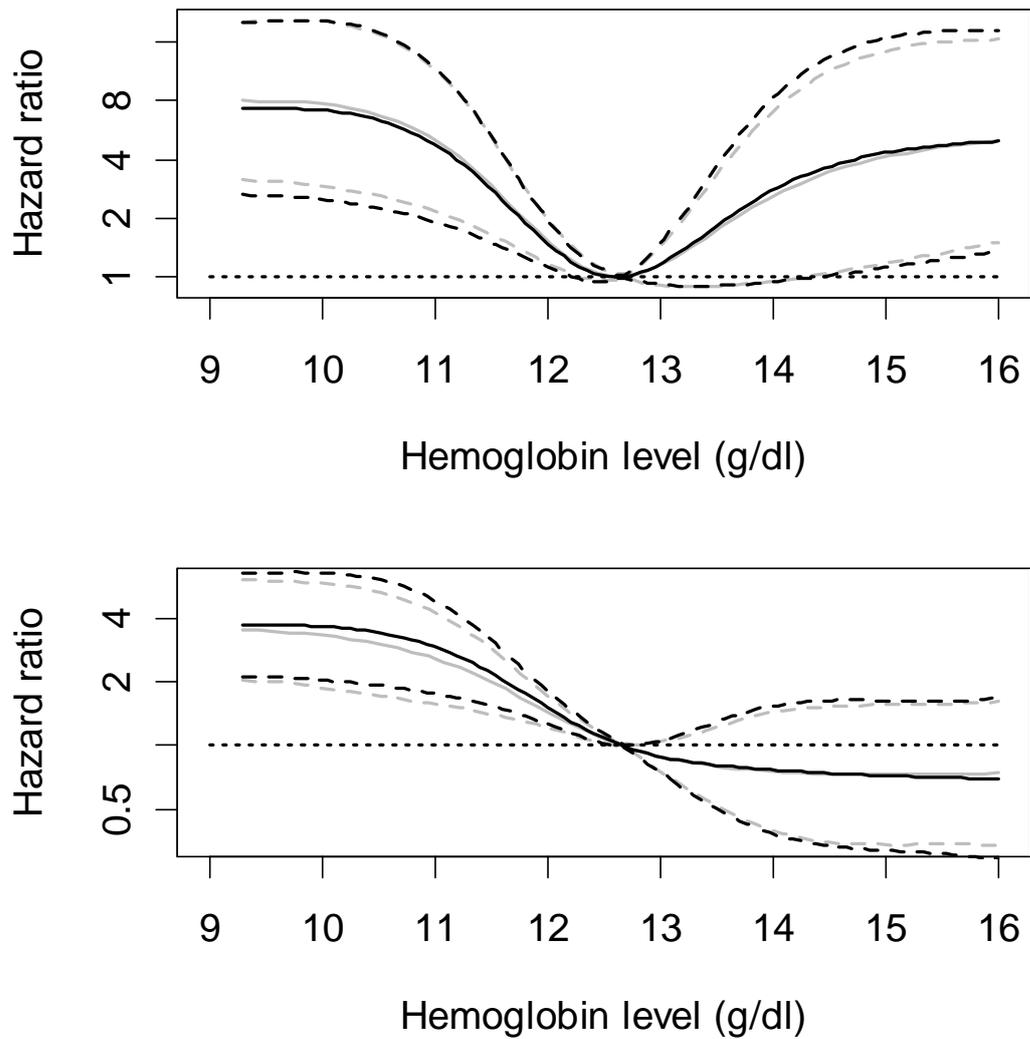
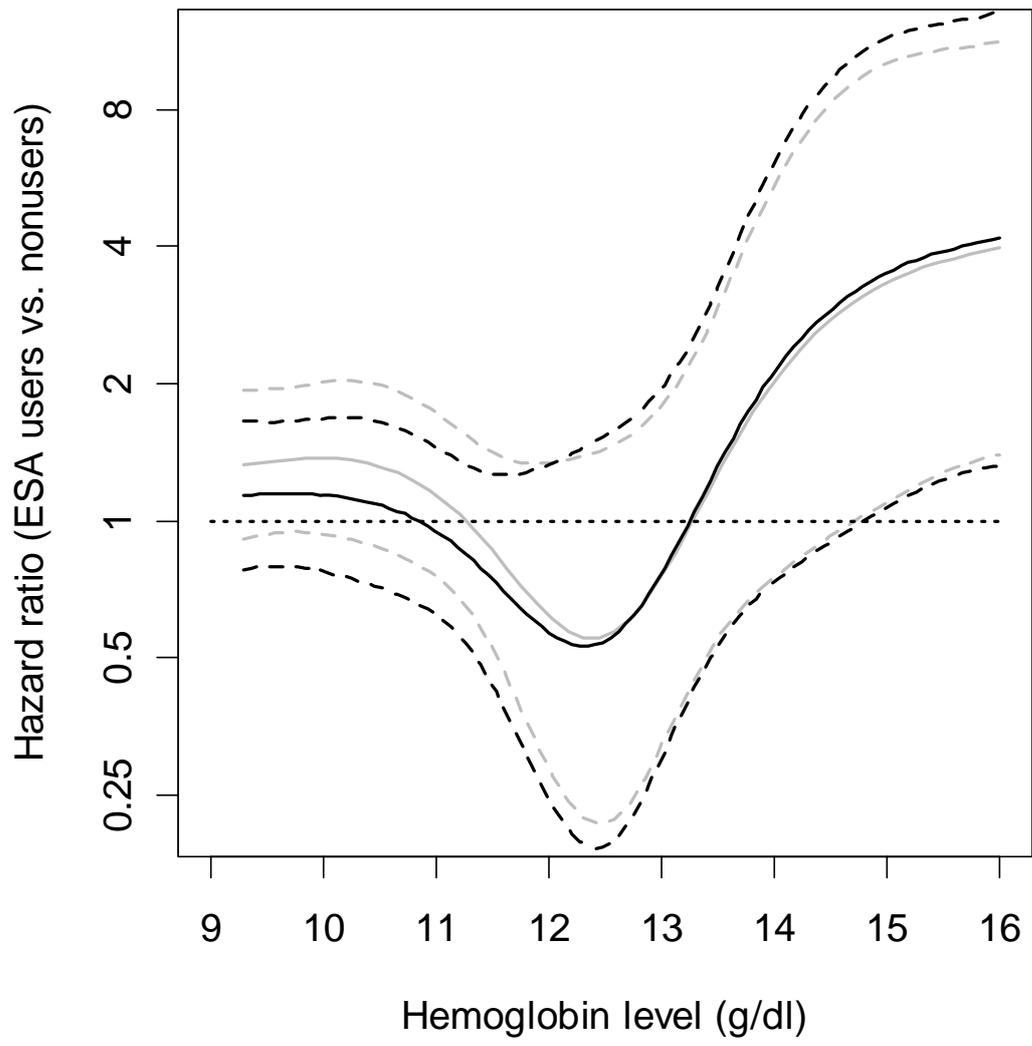


Fig.6: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis after multiple imputation of artificially generated nonrandomly missing data



Part 3: tests of interactions of covariates in final model with log of time

To assess the proportional hazards assumption, we included interactions of each variable in the model with log of time. The significance of these interactions was assessed by controlling a false discovery rate of 5%. Significant interactions were included into the model and the main results reassessed and compared to the original results.

Two variables showed *fdr*-corrected *p*-values (“*q*-values”) of their interaction with log of time lower than 5%: Cholesterol level (*q*-value 0.0157), and number of antihypertensive drugs (*q*-value 0.0154). We included interactions of these variables with log of time into the model and recomputed the adjusted hazard ratios, which are depicted and compared to the original analysis in Fig. 7 and 8.

Fig. 7: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis including time-dependent effects for cholesterol level and number of antihypertensive drugs

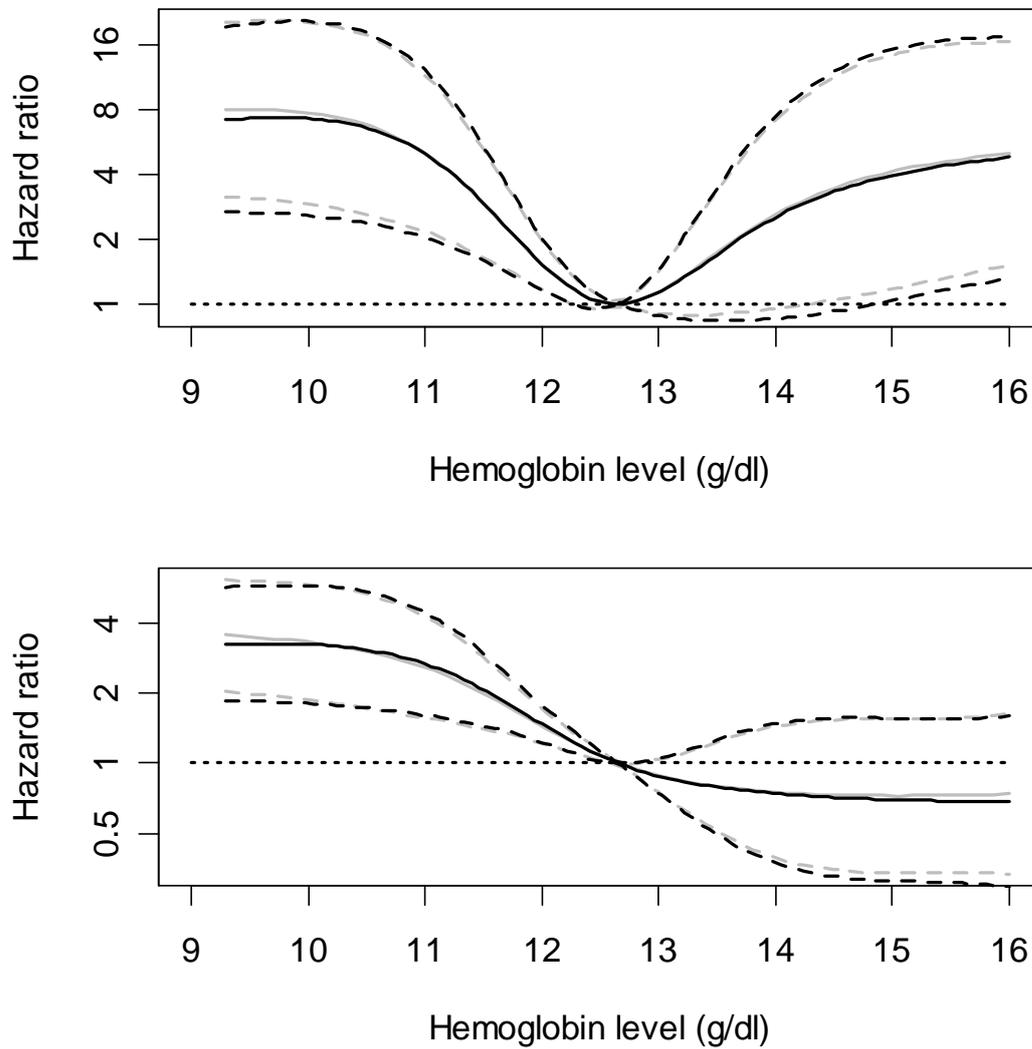
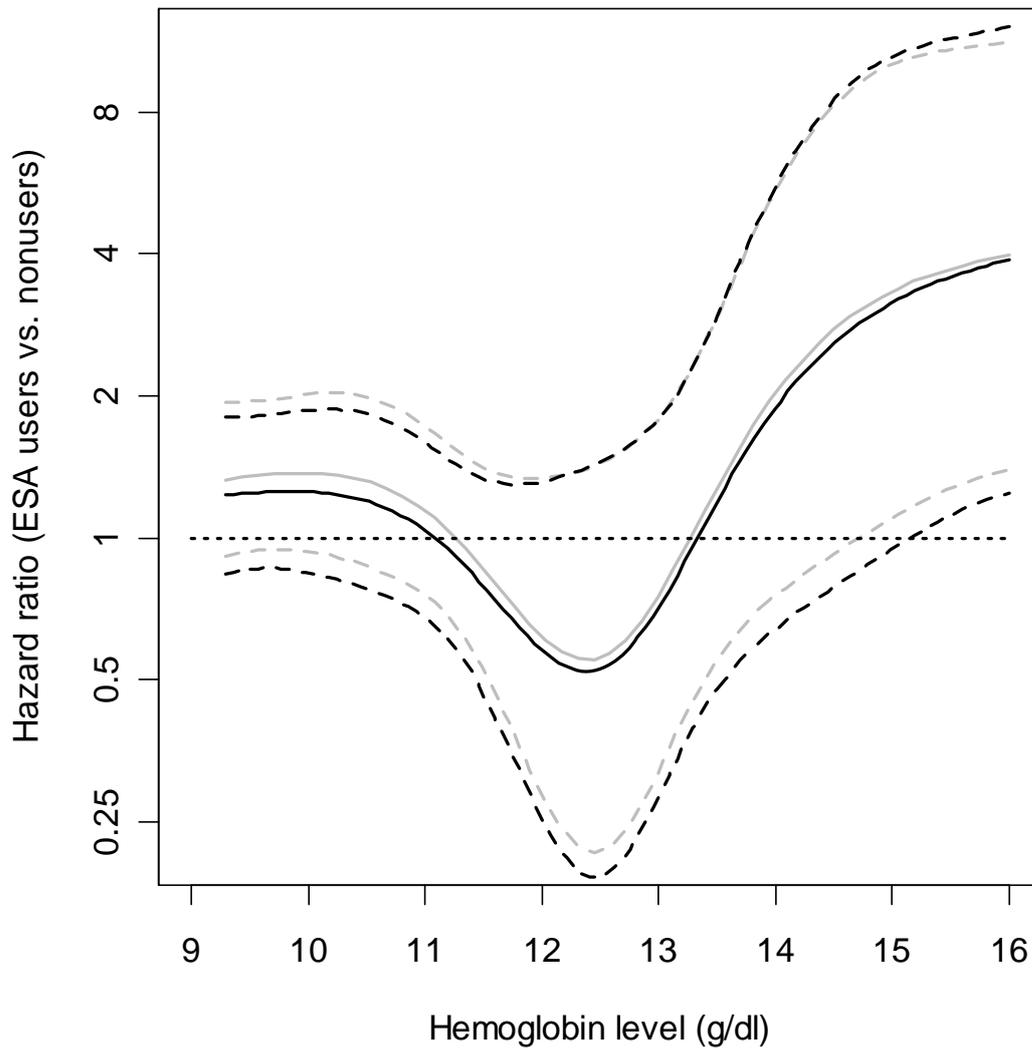


Fig. 8: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis after including time-dependent effects for cholesterol level and number of antihypertensive drugs



Part 4: tests of nonlinearity of covariates in final model

Nonlinearity of covariates in the final model was tested by likelihood ratio tests of the model treating a covariate as nonlinear (by restricted cubic splines) and the original final model. Significance was assessed at a false discovery rate of 5%. Significant nonlinear effects were included into the model, and results were compared with the original results.

Cholesterol level showed a nonlinear effect ($p=0.0221$). Accounting for this nonlinear effect by restricted cubic splines led to a slight change in the hazard ratio estimates of HB and ESA use, as can be seen in Fig. 9 and 10:

Fig. 9: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis including nonlinear effects for cholesterol level

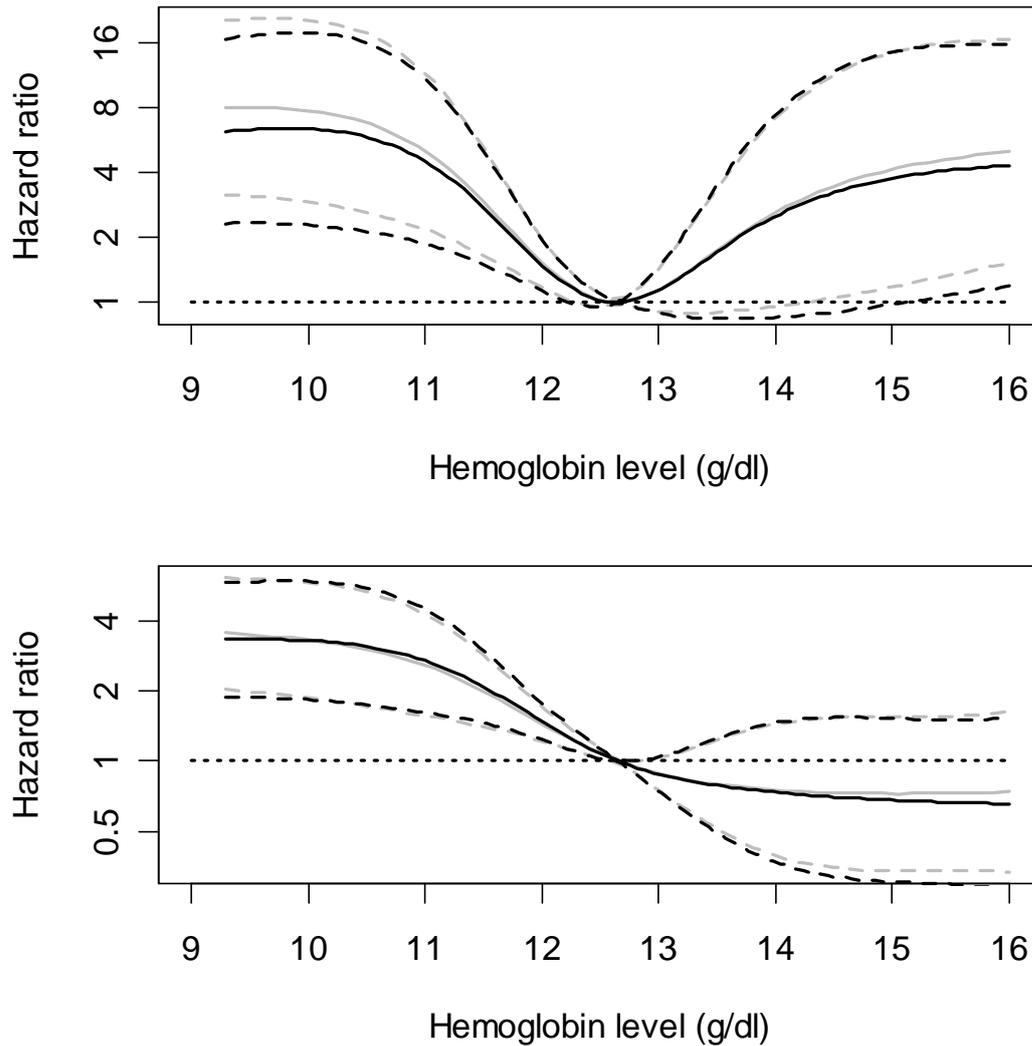
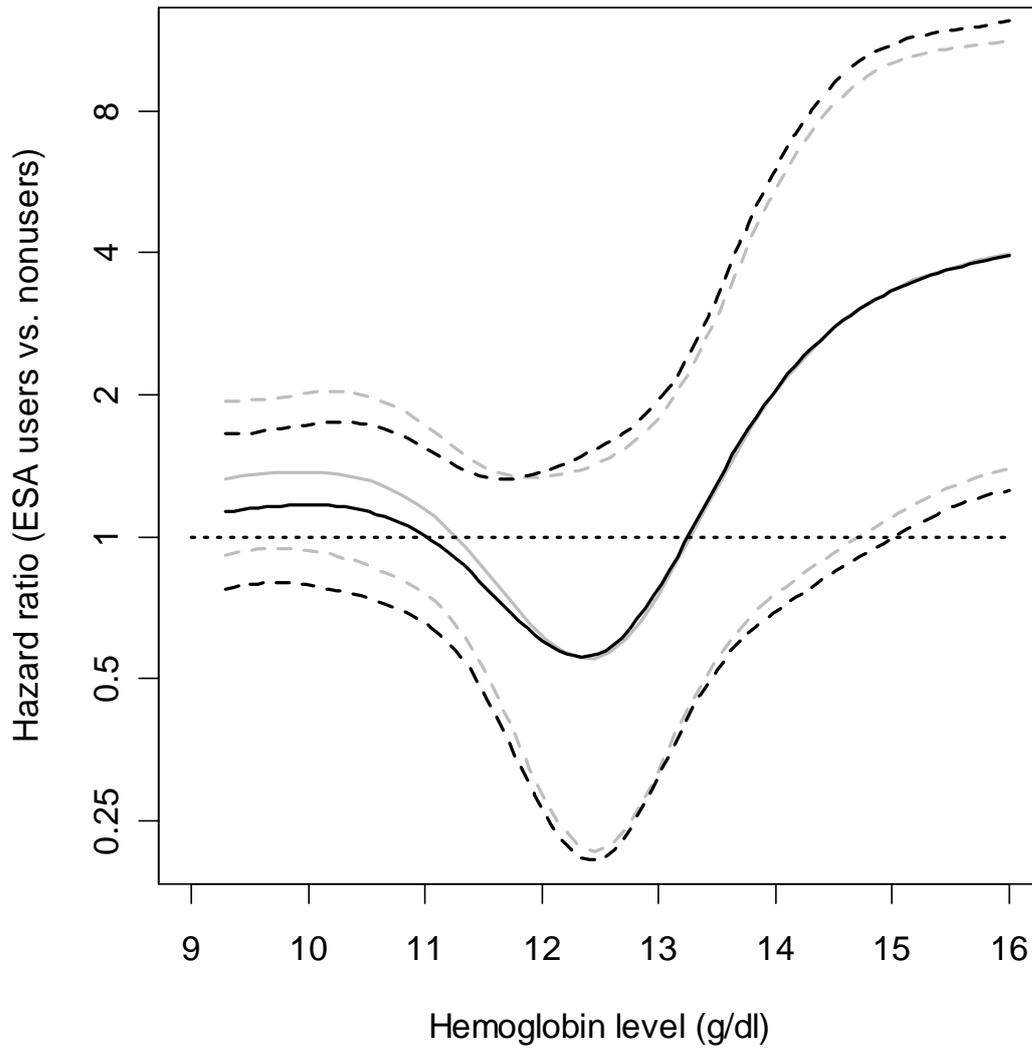


Fig. 10: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (multiple imputation; N=1794, 286 events), black: analysis after including nonlinear effects for cholesterol level



Part 5: Assessment of interactions

Pairwise interactions of covariates in the final model were tested by including respective product terms into the model. Significance was assessed at a false discovery rate of 5%. No interactions were significant at the specified level.

Part 6: Follow-up extended to 31 Dec 2008.

We used follow-up data until 31 Dec 2008 and rerun the Cox regression analyses. In total, 313 events were identified. By means of comparison of the adjusted hazard ratio plots (Fig. 4 and 5 in original paper), we see high agreement of the new results with those of the original analysis.

The purposeful selection algorithm selected the variables: dialysis status, vascular diseases, heart failure, coronary heart disease, cholesterol level, type of immunosuppressive regimen, diabetes, age at transplantation, cold ischemia time.

Fig. 11: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis including follow-up data until 31 Dec 2008 (313 events).

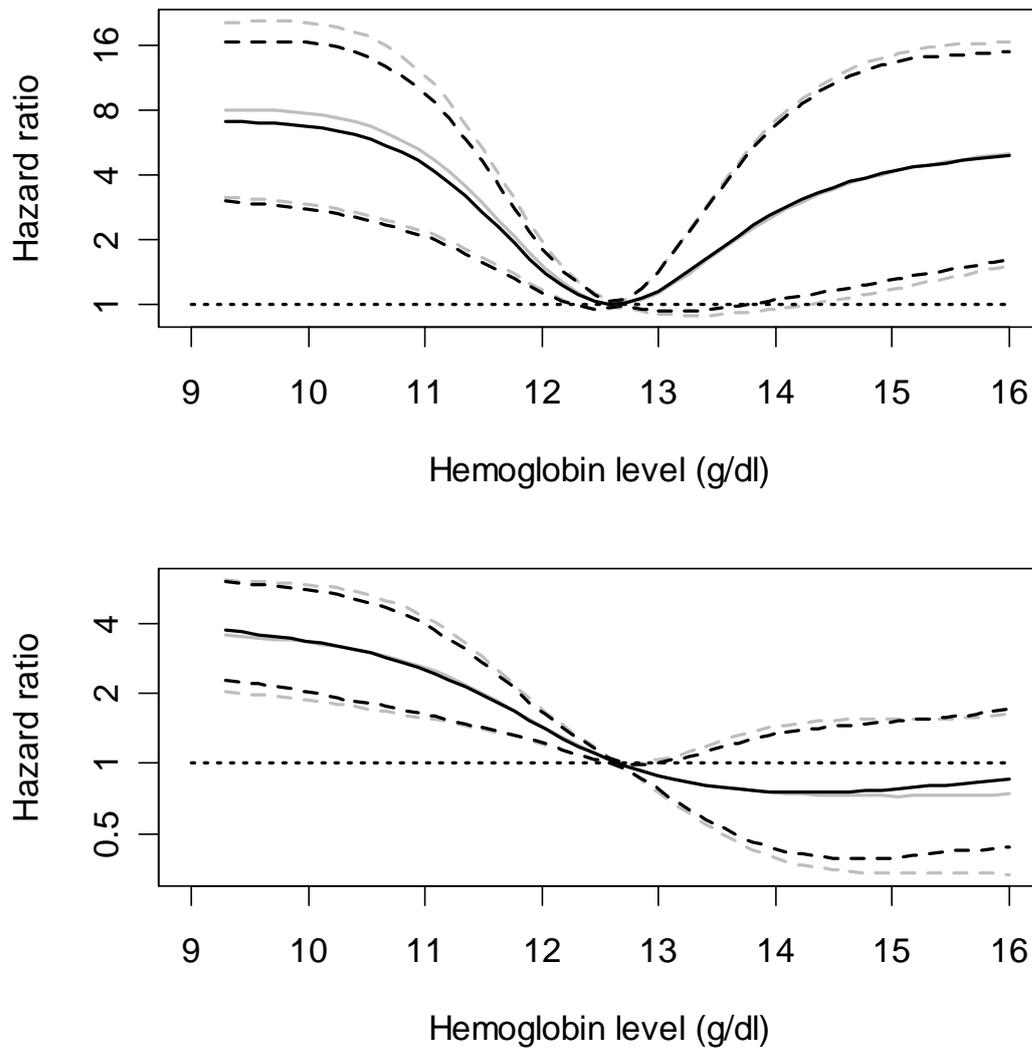
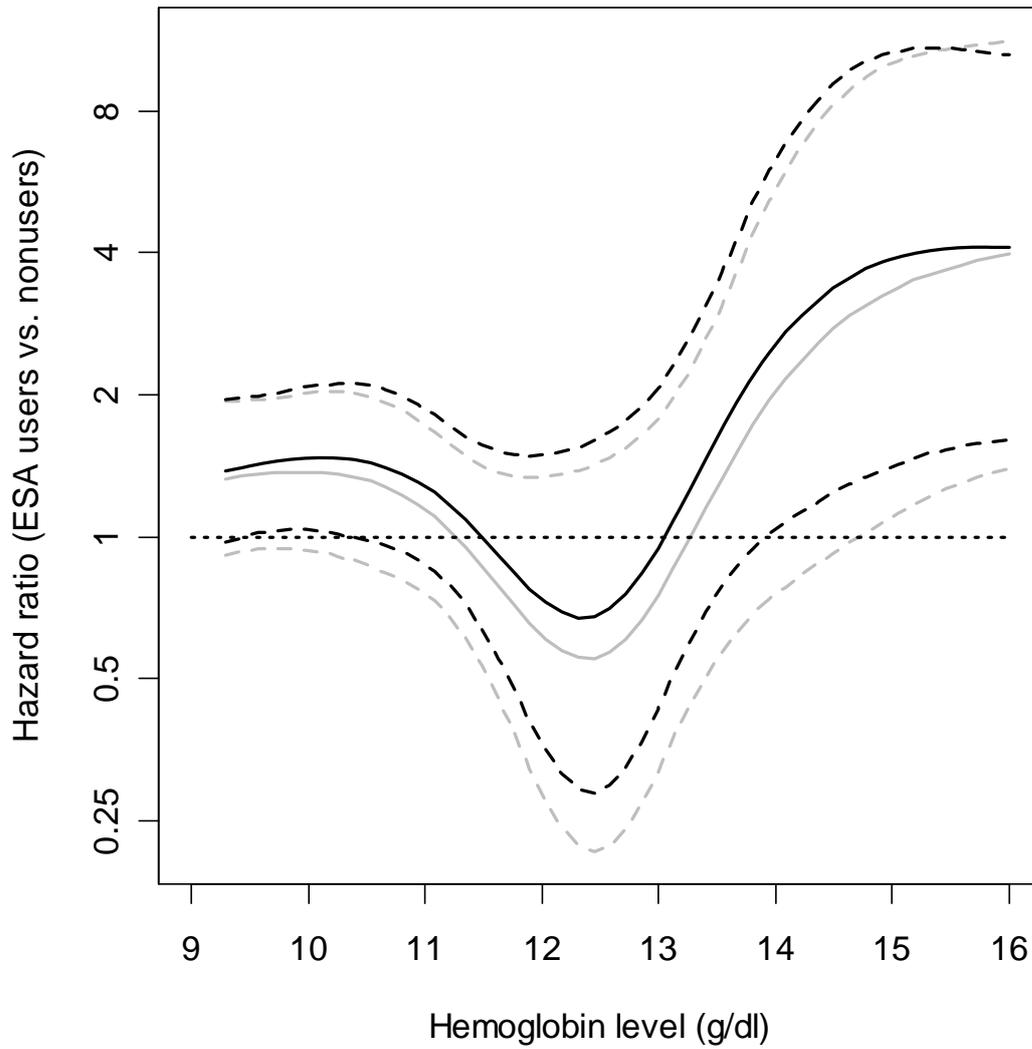


Fig. 12: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: similar, but follow-up until 31 Dec 2008 (313 events)



Part 7: Adjusted hazard ratio plots excluding the cardiovascular disease variables.

Fig. 13: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis excluding cardiovascular disease variables from final model.

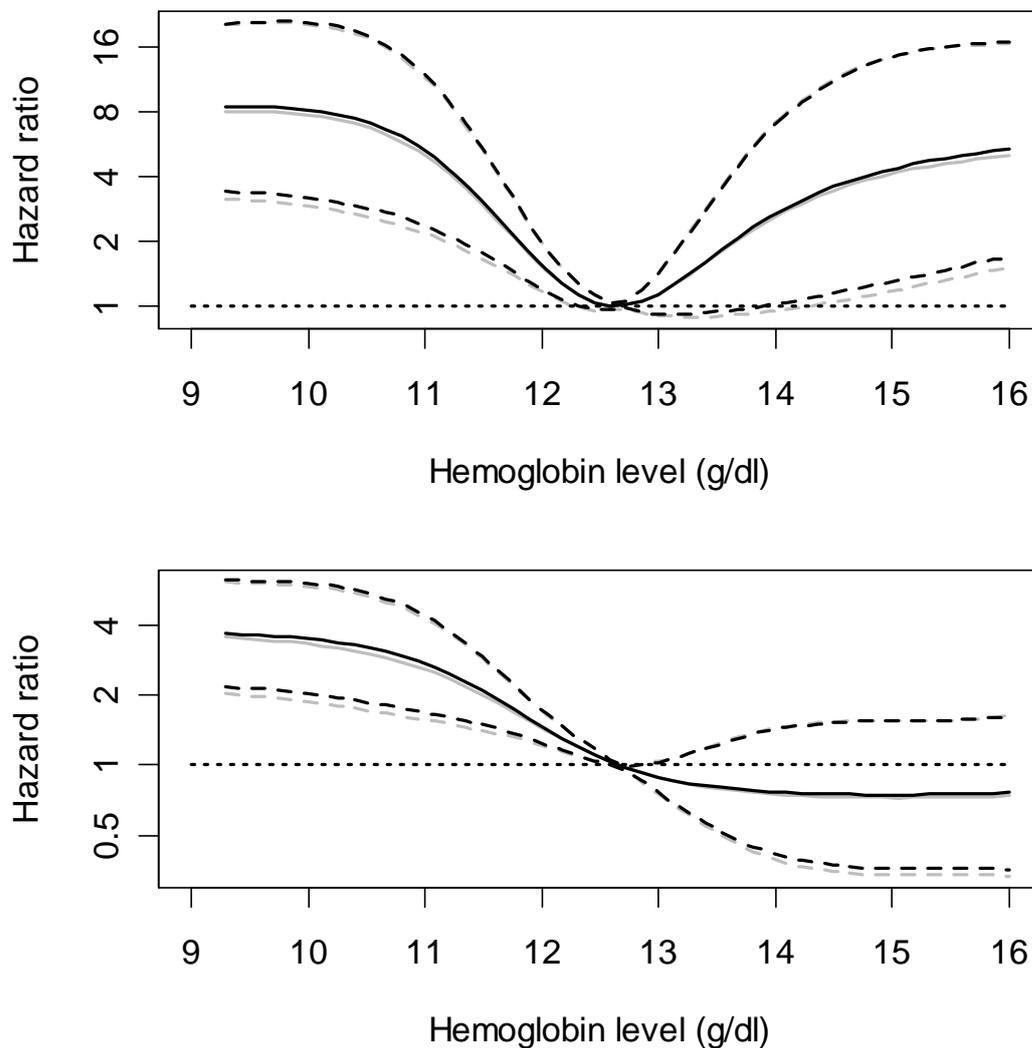
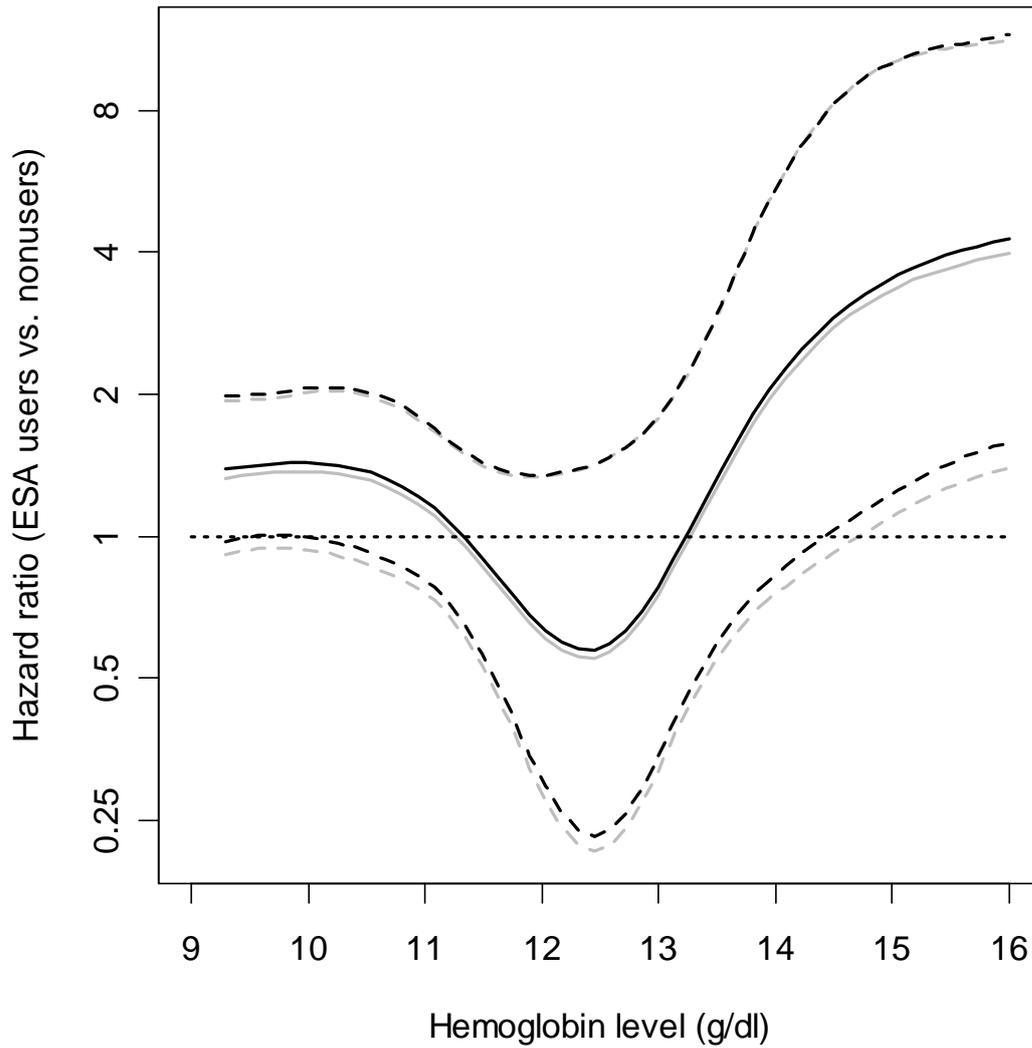


Fig. 14: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis excluding cardiovascular disease variables



Part 8: Adjusted hazard ratio plots with ESA prescription 'never turned off': ESA users remain in the user group even if treatment was stopped.

Fig. 15: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis with ESA users left in ESA user group even after the time point where treatment is stopped.

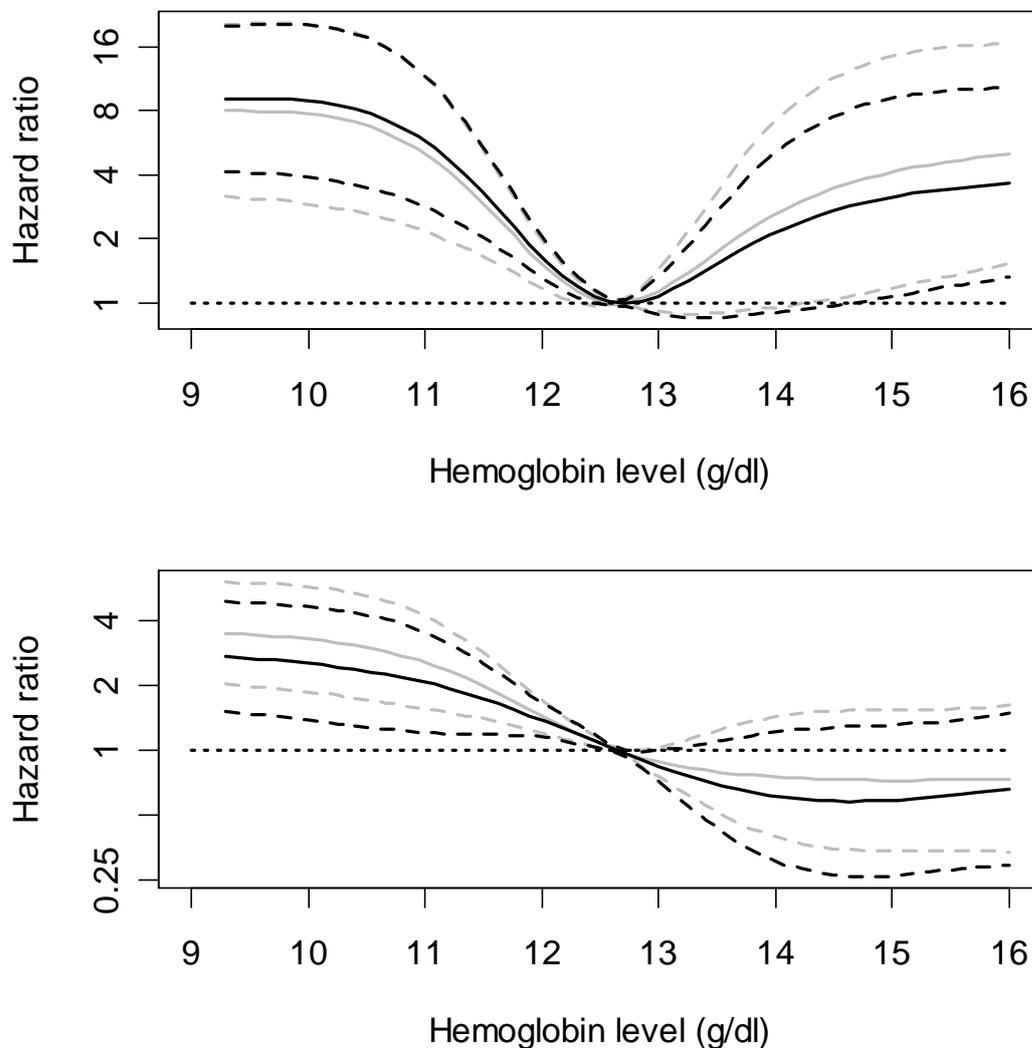
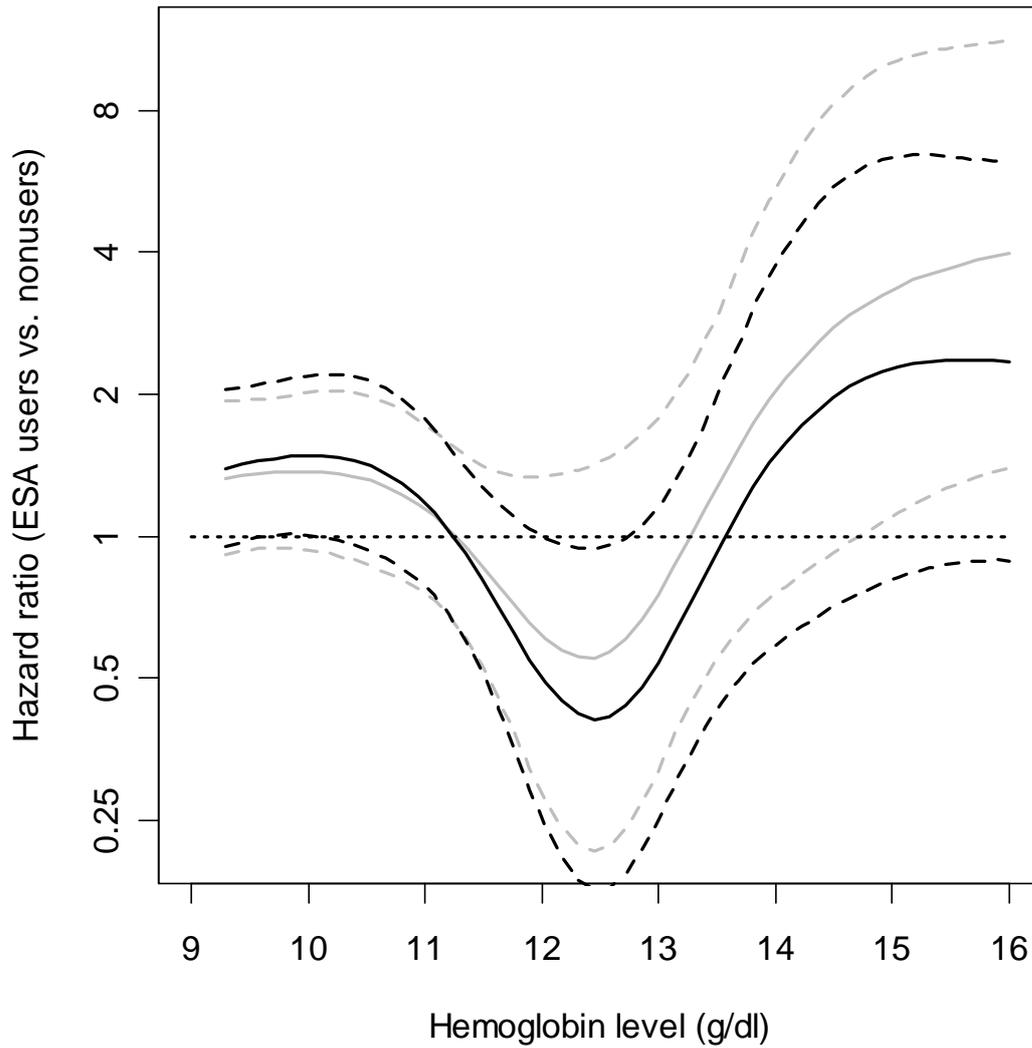


Fig. 16: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis with ESA users left in ESA user group even after the time point where treatment is stopped.



Part 9: Adjusted hazard ratio plots with deaths and graft failure counted as events (transplant survival).

Figure 17: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis of patient survival (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis of transplant survival (graft loss and death counted as event; N=1794, 367 events).

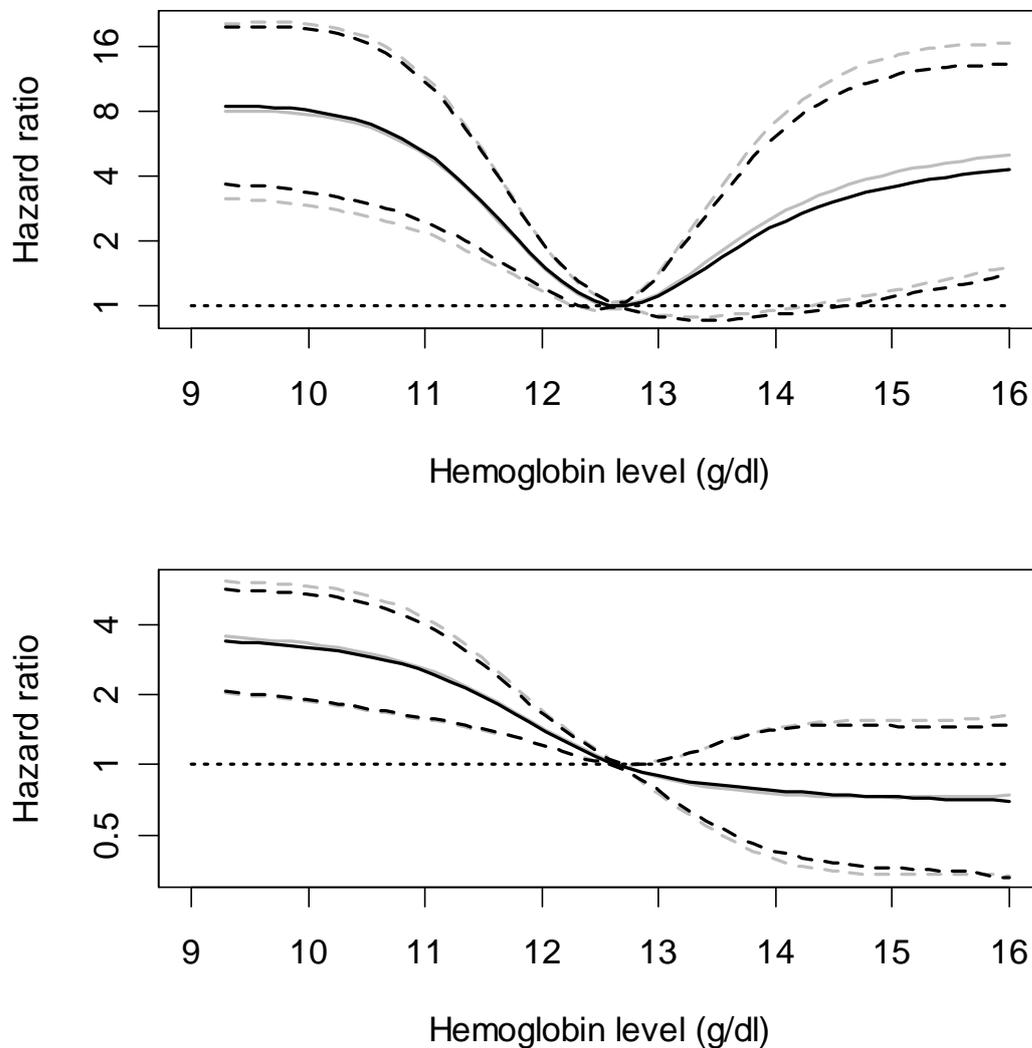
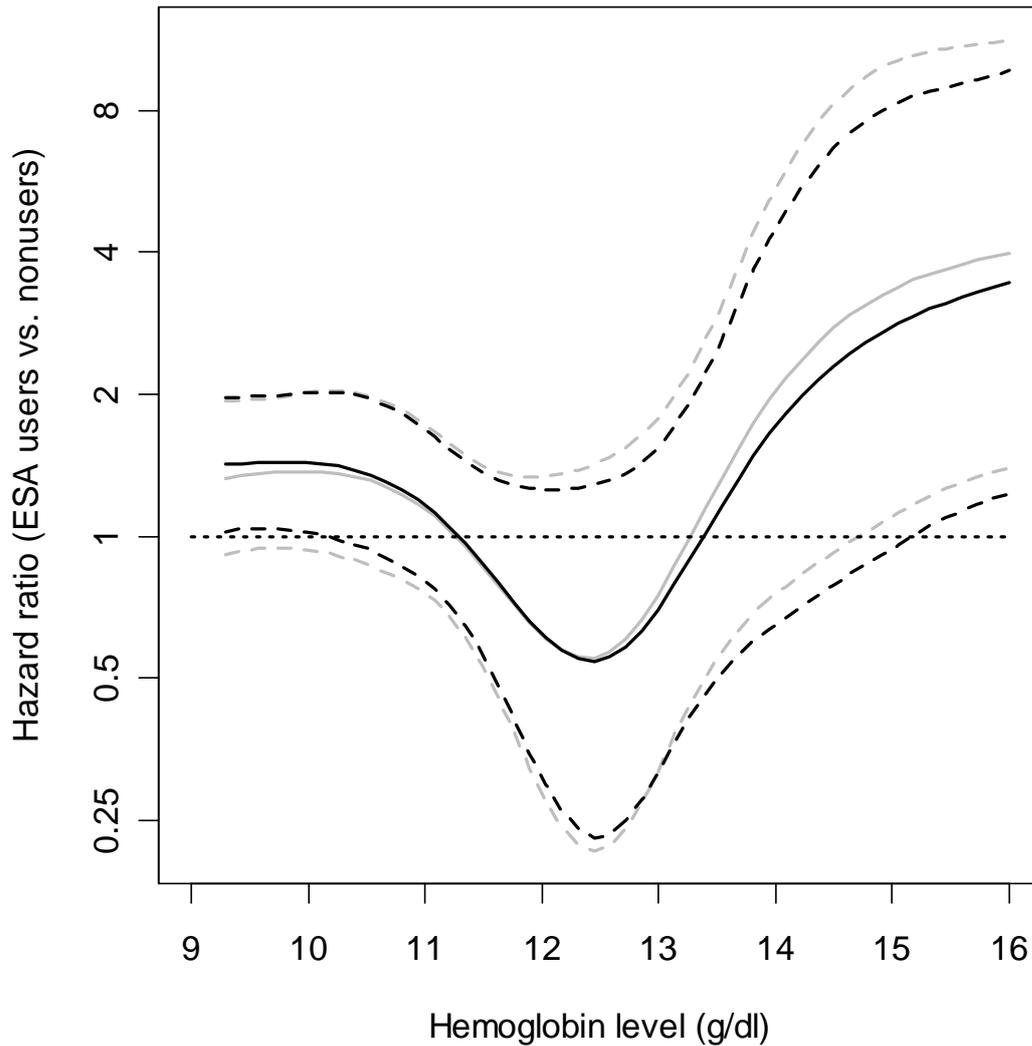


Figure 18: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis of patient survival (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis of transplant survival (graft loss and death counted as event; N=1794, 367 events).



Part 10: Including primary indication for transplantation as additional baseline covariates into the model

Figure 19: Adjusted hazard ratio for ESA users (top) and non-users (bottom) at various hemoglobin levels vs. a reference level of 12.5 g/dl. Gray: original analysis of patient survival (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis additionally adjusting for primary indication for transplantation (diabetes, immune mediated, PCKD, or other).

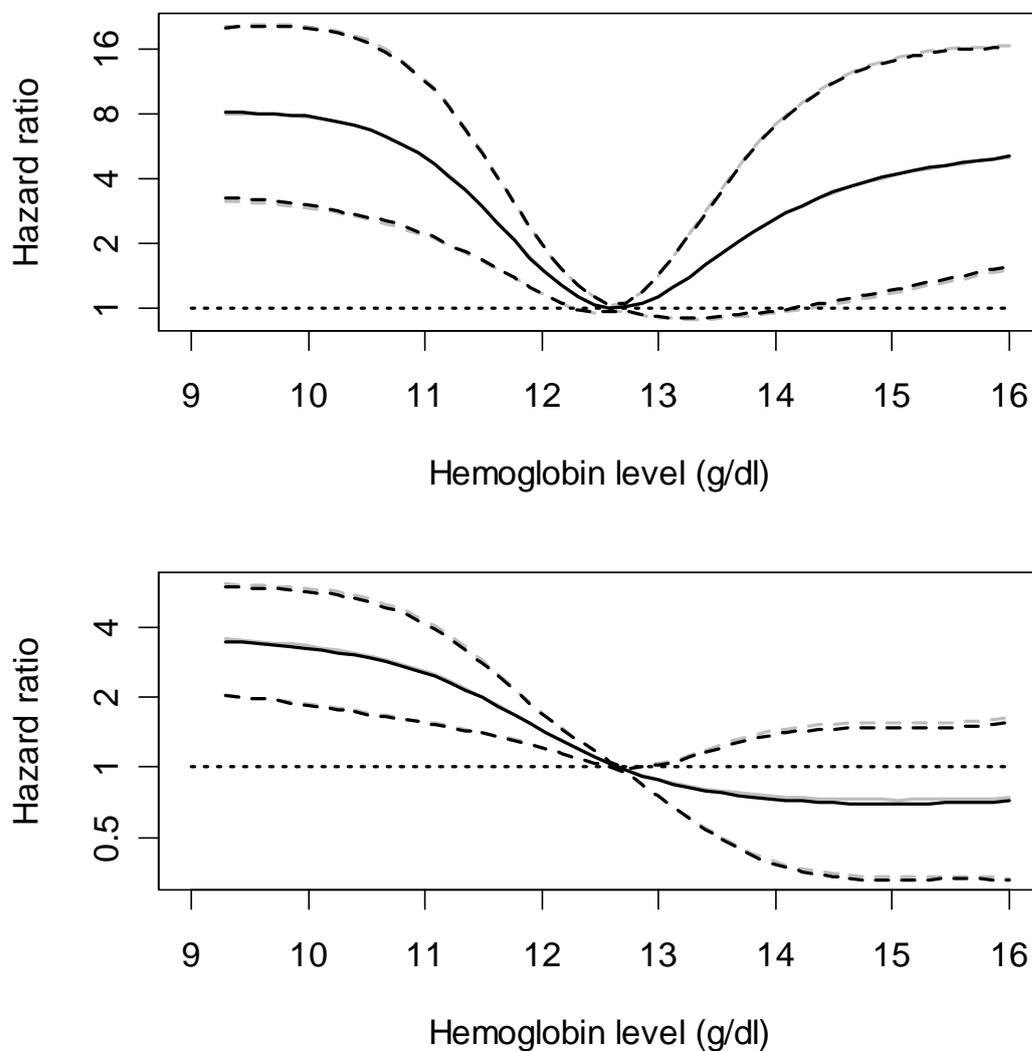


Figure 20: Adjusted hazard ratios of ESA users vs. non-users at various hemoglobin levels. Gray: original analysis of patient survival (multiple imputation; N=1794, 286 events, follow-up until 31 Dec 2004), black: analysis additionally adjusting for primary indication for transplantation (diabetes, immune mediated, PCKD, or other).

