Association of ESA hypo-responsiveness and haemoglobin variability with mortality in haemodialysis patients

Alexander Kainz\textsuperscript{1,2}, Bernd Mayer\textsuperscript{3}, Reinhard Kramar\textsuperscript{4} and Rainer Oberbauer\textsuperscript{1,2,4}

\textsuperscript{1}Department of Nephrology, KH Elisabethinen, 4010 Linz, Austria, \textsuperscript{2}Department of Nephrology, Medical University of Vienna, 1090 Vienna, Austria, \textsuperscript{3}Emergentec Biodevelopment GmbH, 1010 Vienna, Austria and \textsuperscript{4}Austrian Dialysis and Transplant Registry, Linz, Austria

Correspondence and offprint requests to: Alexander Kainz; E-mail: alexander.kainz@meduniwien.ac.at

Abstract

Background. Anaemia is a common complication in dialysis patients. In most cases, it is treated with erythropoietin-stimulating agents (ESA). It is not entirely clear whether the variability of haemoglobin caused by changing ESA response is associated with increased mortality. Therefore, we conducted a retrospective cohort study to evaluate ESA responsiveness and haemoglobin variability in association with mortality.

Methods. We used the Austrian dialysis and transplant registry, and identified 932 patients who were on maintenance haemodialysis in the years 2005–08 with recorded weekly ESA doses and haemoglobin concentrations. ESA response was defined as a positive regression slope over the observation period. Cox regression analysis with spline functions and purposeful variable selection algorithms were used.

Results. Adjusted Cox regression analysis showed an increased mortality risk in subjects with wide ranges of haemoglobin variability (from <10 to >12 g/dL) (HR = 2.38, 95% CI 1.20–4.71, \(P = 0.013\)). Furthermore, patients that never reached haemoglobin levels >10 g/dL despite ESA therapy exhibited the highest risk of mortality (HR = 6.37, 95% CI 2.15–18.82, \(P < 0.001\)). ESA hypo-responsiveness was associated with increased risk of mortality in the low as well as high haemoglobin ranges \(\text{HR} = 2.06, 95\% \text{ CI} 1.49–2.86 \text{ at haemoglobin of 9.5 g/dL and HR} = 1.64, 95\% \text{ CI} 0.68–3.92 \text{ at 13.5 g/dL both vs. 11 g/dL (reference). ESA dose equivalents >16 000 units per week were associated with increased mortality in ESA responders (HR = 1.30, 95\% CI 1.02–1.64). However, in hypo-responders, mortality is not associated with ESA dose (HR = 1.02, 95\% CI 0.87–1.20) [both at weekly ESA dose of 20 000 units vs. 16 000 (reference)].

Conclusions. These findings suggest that the risk of mortality of haemodialysis patients requiring ESA therapy is lowest if the haemoglobin concentration is stably maintained in the range between 10 and 12 g/dL with weekly ESA dose equivalents <16 000 units.

Keywords: Cox regression; erythropoietin; maintenance haemodialysis; mortality risk; spline function

Introduction

Anaemia is a common phenomenon in patients on haemodialysis. Among the major causes are iron deficiency \[1\] due to blood loss through dialysis filters or line blood retention, infections, and hypo-responsiveness to erythropoietin-stimulating agents (ESA) \[2\]. ESA hypo-responsiveness is defined as the requirement of higher than average doses of ESA to achieve an increase of haemoglobin concentrations.

ESAs became available in the early 1990s of the last century. Initially, only recombinant human erythropoietin (rhEPO) and, subsequently, drugs with longer half-life were approved for clinical use \[3,4\]. Although ESA use in patients with chronic kidney disease and subjects on dialysis were studied extensively, the optimal target haemoglobin concentration as well as the required ESA dose and dosing interval to achieve this concentrations remain elusive (NHS, CREATE, CHOIR and TREAT) \[5–8\].

Furthermore, the impact of haemoglobin variability on patient survival still remains controversial. Haemoglobin variability occurs in almost all subjects on haemodialysis and is caused by several factors including variable ESA doses, blood loss, intermittent infections and others \[9–15\]. This variability is also associated with an increased mortality if it is >1 g/dL \[16\]. Another study performed by Gilbertson and colleagues suggested that the absolute haemoglobin levels, namely those <11 g/dL, are the main factor for increased risk of death \[17\]. However, ESA responsiveness was not taken into account by these studies. In a review written by Kalantar-Zadeh and Aronoff, it is suggested that haemoglobin variability is influenced by ESA response \[15\]. Other studies could find an association with ESA responsiveness and mortality if adjusted for haemoglobin variability, especially ESA hypo-responsiveness that led to higher risk of death \[18\].
We therefore investigated the association of ESA response and haemoglobin variability with mortality in a cohort of haemodialysis patients from the Austrian dialysis and transplant registry. This registry holds complete longitudinal entries of all Austrian haemodialysis patients since 1970 [19].

Materials and methods

Patient population

We investigated patients with reported ESA doses and haemoglobin levels in the Austrian dialysis and transplant registry. In this database, 932 dialysis patients are recorded with corrected weekly ESA doses and haemoglobin levels from the years 2005–08. For 246 patients, BMI values were missing, which were imputed by multiple imputation through a linear regression.

ESA response

In order to calculate the ESA response of each patient, a mixed linear model for longitudinal data was designed with haemoglobin as dependent and ESA dose as independent variable [20]. In case of darbepoetin, corrected weekly ESA doses were computed by multiplication by 200 [21].

A random intercept was applied. The parameter estimate for ESA dose representing the slope of the regression line was used as ESA response. A positive parameter estimate (positive slope of haemoglobin over the observation period of 4 years) was used as proxy for adequate ESA response, whereas a negative value (declining slope of haemoglobin over time) defined ESA hypo-responsiveness.

Demographic data

Variables for demographic data were compared between the two ESA response groups, and displayed as mean and standard deviation or frequency for normal distributed data or categorical data, respectively. Continuous variables were tested with Student’s t-test and discrete variables with chi-square test when appropriate.

Classification of patients

Classification was done as described previously but with changed thresholds for the groups [17]. Briefly, the haemoglobin level range was divided in three groups, namely Group L for levels <10 g/dL, Group M for levels from 10 to 12 g/dL and Group H for levels >12 g/dL. Furthermore, the patients were classified in six different variability groups by their minimum and maximum haemoglobin level throughout the analysis period: LL, LM, LH, MM, MH and HH.

Outcome

Patient survival time was defined as time of ESA use during the years 2005–08 until transplantation or death of the patient. Only death was counted as an event.

Cox proportional hazard models

We calculated the hazard ratio (HR) for haemoglobin levels with three different time-dependent models. First was the unadjusted model, where only the variability groups (LL–HH) were used as independent variable. Group MM was used as reference in all models. The second model was adjusted for age, vintage of dialysis and sex. The third model was additionally adjusted for ESA responsiveness. Additionally, we chose confounder variables by using the purposeful selection algorithm for the multiple imputation analyses [22]. In this algorithm, variables are chosen by their significance (P < 0.05) in a multivariable model or by changing the log hazard ratio of haemoglobin variability group or ESA responsiveness by >25%. The proportional hazard assumption was tested by inspection of the correlation of Schoenfeld residuals to rank of failure time.

Furthermore, we analysed the change of hazard ratio in dependence of the haemoglobin level. Because it was evident from earlier studies that dependency is different for different ESA responsiveness, we used two groups as described above [23]. The Cox model was modelled by using restricted cubic spline curves with four knots, which were at 5th, 25th, 75th and 95th percentile of the haemoglobin range [24,25].

For all statistical tests, a P-value <0.05 was considered significant. The statistical analysis was conducted using SAS for Windows 9.2 TS1M0 (SAS Institute, Inc., Cary, NC, USA).
Results

Patient population
The demographic data of the patients are displayed in Table 1. None of the variables was significantly different between the two ESA response groups.

Unadjusted Cox models
The analysis of the unadjusted model (not taking ESA responsiveness into account) showed that the MM variability group exhibited the lowest risk of death. Any variability of haemoglobin as well as HH and LL groups was associated with increasing risk of dying (Figure 1A, Webtable 1).

Adjusted Cox models
When the analysis was adjusted for covariables derived by the purposeful selection algorithm, age, sex and vintage of dialyses results remained virtually identical except that the hazard ratio of the LL nearly doubled (5.97, 95% CI 2.70-13.21) (Figure 2B, Webtable 2). When ESA response was added as potential confounder, again, hazard ratios remained almost unchanged, but the 95% CI increased slightly (Figure 1C, Webtable 3). Data remained virtually identical when missing BMIs were imputed (Figure 1D, Webtable 4).

The risk of death depending on the achieved haemoglobin concentrations
The calculation of hazard ratio dependence in the range of 9.3–13.6 g/dL resulted in an optimum haemoglobin level of 11.02 g/dL for patients who were classified as ESA hypo-responders. Below and above this value, the hazard ratio increased up to significant 2.41 (95% CI 1.64–3.53) and non-significant 1.64 (95% CI 0.69–3.92), respectively (Figure 2A).

Patients with ESA response, i.e. a slope greater than zero, exhibited a numerically higher mortality with lower haemoglobin levels (1.66; 95% CI 0.83–3.31), but the hazard ratio decreases steadily with the haemoglobin level (Figure 2B). However, due to a few outcomes in patients with high haemoglobin level and positive ESA response, the confidence interval widened (0.15; 95% CI 0.003–7.56) (Table 2).

Risk of mortality and weekly ESA dose
The Cox regression adjusted for haemoglobin level, ESA dose, age and vintage of dialysis showed an association of ESA dose and hazard of dying, which was not linear in...
For both ESA response groups, the hazard ratio increased with increasing ESA doses (Figure 3, Webfigure 2). However, for the hypo-responding group, the hazard ratio did not reach statistical significance.

Similar results were obtained, when stratifying patients by their baseline haemoglobin level (Webfigure 3–6).

An analysis considering incident and prevalent patients separately resulted in virtually identical results with the exception of the spline function for incident hypo-responders where hazard ratio was highest for an ESA dose of 10 000 U per week (Webfigure 7–10).

### Discussion

This study showed that ESA responders requiring low weekly doses exhibit the lowest risk of mortality. Above weekly doses of 16 000 corrected units of ESA, this benefit is lost. As it is more likely for patients with more severe anaemia to get higher doses, their hazard ratio for mortality is more influenced by the achieved haemoglobin value. This was also shown by Regidor and colleagues, who divided the ESA dose in intervals of 6000 U and the patients’ cohort for this model by the haemoglobin level.
value of 12 g/dL [18]. The hazard ratio varied from ~0.4 to 0.8 in the analysed ESA dose range. These findings fit well to our results. We calculated hazard ratios of mortality from 0.34 to 1.12 and 0.57 to 1.35 in the hypo-responder and responder group, respectively. It has to be mentioned, however, that we did not compare the results of ESA users to patients who did not receive ESA.

As evident from Webfigure 1, patients with lower haemoglobin values received larger doses of ESA. This was also shown by other studies [26–29]. López-Gómez reported also greater co-morbidity in patients with severe anaemia. It is also known from other studies that some co-morbidities like antecedents of malignant neoplasm are associated with EPO responsiveness [29]. Cytokines like IL6 are induced by malignant tumours and may impair erythropoiesis. Also, TNF-α is known to inhibit this pathway [30].

The National Kidney Foundation (NKF) and the Food and Drug Administration (FDA) recommend different target levels for haemoglobin in patients with terminal kidney disease treated by haemodialysis [31,32]. The NKF recognizes also the importance of individualizing the treatment of anaemia. In respect to different ESA responsiveness in different patients, our data would support the NKF statement. The optimal range of target haemoglobin levels in our analysis of haemodialysis patients was 11 g/day. Furthermore, ESA hypo-responders showed an increased risk of mortality with higher haemoglobin levels, and ESA responders actually exhibited a decreased risk.

In contrast to the study performed by Yang and colleagues, we did not calculate haemoglobin variability by the residual standard deviation of a linear regression for the periodical measured haemoglobin levels [16]. Instead, we used a similar approach as Gilbertson et al., i.e. dividing the patients into low, medium and high haemoglobin level categories for each calendar year [17]. Yang’s analysis revealed an increasing hazard ratio for patients with increasing haemoglobin variability. We also found a relationship of haemoglobin variability with mortality, but the highest risk could be attributed to subjects that remained always <10 g/dL.

Killpatrick and colleagues used the haematocrit value to study the association of ESA response and mortality [33]. They showed also a decrease of the hazard ratio for increasing response to ESA, which were divided in quartiles. A significant hazard ratio of 0.41 (95% CI 0.20–0.87) was achieved for the highest quartile in comparison to the lowest, and the second and third quartiles revealed a non-significant hazard ratio. This is in concordance to our result, where the ESA hypo-responder showed a greater risk of death than patients from the ESA responding group when low ESA doses were administered. However, Killpatrick did not show a significant hazard of death for ESA responders to whom higher doses were applied. A reason for this might be the usage of restricted splines in the Cox model which allows calculating a hazard ratio for a continuous exposure variable (ESA dose), whereas Killpatrick included ESA dose as categorical variable (quartiles) in their Cox model.

One limitation of our dataset is that variables describing nutritional status or inflammation are missing. It is well accepted that dietary status is strongly associated with outcomes [34]. Regidor et al. adjusted their analysis for several covariables from the malnutrition–inflammation complex syndrome and found these to be associated with mortality [18]. We have only BMI at baseline and type of diabetes in our dataset and could not find any association with baseline BMI and mortality. This might be because of different distribution of BMI in the study population in contrast to studies from the USA. CDC has reported an obesity rate, defined by a BMI >30 kg/m², for more than one-third of the people [35]. In our case, the prevalence of obesity is ~20%.

Another limitation may depend on the definition of haemoglobin variability, which in our study does not discriminate between frequent and annual variation, i.e. moving from one category into another. However, an analysis with residual standard deviation used as haemoglobin variability as was introduced by Yang et al. resulted only in a marginal change of the hazard ratio [16].

It has been shown by Brunelli et al. that a history-adjusted marginal structural model may be used to analyse the association of haemoglobin variables and outcome [36]. Also discussed in another study of Brunelli and colleagues, it may be essential to analyse incident and prevalent patients from studies performed in different decades separately to account for time-dependent confounding [37]. In accordance to these papers, we also found that incident subjects exhibited a lower HR for mortality with higher ESA doses in hypo-responders, suggesting that it takes several months of dialysis treatment to increase the mortality risk.

The primary strength of our study is the longer follow-up time compared with several other studies [18,29,33]. For evaluating potential confounders, we used the purposeful selection algorithm, which has the advantage to include variables not only significant in a bivariate model, but also variables which change the predictor’s hazard ratio by a certain amount, namely 25%. Therefore, no confounders are missed in the model as could have happened by evaluating only a bivariate model. For the variables haemoglobin and ESA response, we used restricted spline modelling in the Cox proportional hazard regression. This was done, as it had to be assumed that the hazard rate of mortality over the range of haemoglobin and ESA dose may likely not be following a linear function. Restricted splines allowed for flexible modelling of the hazard ratios over the different exposure [25].

In summary, our data suggest that a corrected weekly ESA dose up to 16 000 units with achieved haemoglobin levels ~11 g/dL exhibited the lowest mortality risk. Haemoglobin variability as well as ESA hypo-response causing low haemoglobin levels was associated with a numerically increased risk of mortality compared with patients with stable haemoglobin levels between 10 and 12 g/dL. Furthermore, ESA response requiring >16 000 units per week was also associated with an increased risk of death in ESA responders.

**Supplementary data**

Supplementary data is available online at http://ndt. oxfordjournals.org.
Acknowledgements. This study was supported by the Austrian Science Fund (FWF P-21436) and Austrian Academy of Science (OELZELT EST370/04). We are indebted to the administrators and all contributors of the Austrian Dialysis and Transplant Registry.

Conflict of interest statement. None declared.

References


Received for publication: 5.1.10; Accepted in revised form: 30.4.10