Sex-specific association of time-varying haemoglobin values with mortality in incident dialysis patients

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Abstract

Background. Previous studies in dialysis patients showed an association between haemoglobin levels and all-cause mortality, however, without addressing sex differences.

Methods. We followed up 235 incident dialysis patients of the region of Vorarlberg in a prospective cohort study applying a time-dependent Cox regression analysis using all the measured laboratory values for up to more than 7 years. In total, 12 242 haemoglobin measurements with a median of 47 (range 3–270) per patient were available to evaluate the impact of haemoglobin levels and their variability on all-cause mortality in a sex-stratified analysis. Non-linear P-splines were used to allow a flexible modelling of the association with mortality.

Results. We observed an inverse relationship between the increasing haemoglobin values and the decreasing risk of mortality. The linear component of the non-linear spline was highly significant for both men (P = 0.00005) and women (P = 0.0000000052). The non-linear component was also significant but less pronounced than the linear component. The inverse relationship was clear to see for haemoglobin levels of up to 12 g/dL in women, which reached a plateau for the higher values of haemoglobin. For men, an inverse trend was observed but clearly attenuated when compared to women. After adjustment for additional parameters of inflammation and malnutrition as well as diabetes, the linear component was more significant in women (P = 0.0018) than in men (P = 0.023).

Conclusions. This study applied for the first time a time-dependent Cox regression analysis over a long-term observation period of several years using all available measurements. Besides the methodological advantages, our data indicate a sex-specific linear as well as non-linear effect of haemoglobin levels on all-cause mortality, which was markedly more pronounced in women.

Keywords: end-stage renal disease; haemoglobin; mortality; prospective observational study; time-dependent Cox regression

Introduction

An optimal management of anaemia in chronic kidney disease (CKD) has been associated with lower morbidity and mortality [1–4]. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines recommend treating anaemia to a target haemoglobin level between 11 and 12 g/dL, but also state that haemoglobin >13 g/dL should be avoided [5]. Several studies observed low haemoglobin values to be associated with increased risk of death [1,6–10]. However, studies are controversial whether haemoglobin levels above the treatment target also increase risk of mortality [11–14]. Clinical trials indicated an increased risk of adverse outcomes for higher compared with lower haemoglobin targets [11,15–22], whereas some observational studies found an incremental improvement in survival with higher haemoglobin levels in dialysis patients [2,6,10,23]. Recent studies have shown a large intrapatient haemoglobin variability (Hb-Var) during a given observation period, which complicates maintaining haemoglobin levels within the target range over time [24–27]. Furthermore, two recent studies showed that Hb-Var itself seems to be associated with an increased mortality risk [28,29].

Despite a large number of investigations, only a few studies considered sex-specific differences in anaemia treatment [30]. To our knowledge, studies dealing with Hb-Var over time in men compared to women and the accompanied risk of mortality do not exist.

Our single-centre study investigated the sex-specific association of haemoglobin values with mortality in a prospective observational inception cohort of 235 incident dialysis patients followed up for a period of more than 7 years. Haemoglobin levels as well as all other covariates recorded during the entire observation period were considered in the time-dependent Cox regression modelling that takes into account that risk factors may change over time. This resulted in 12 242 different haemoglobin measure-
ments during the entire observation period which were used in the analysis and risk estimation that can be considered as a weighted average of short-term effects on mortality. Furthermore, non-linear splines were used to allow a flexible modelling of the association with mortality.

**Materials and methods**

**INVOR Study**

The Study of Incident Dialysis Patients in Vorarlberg (INVOR Study) is a single-centre, prospective, observational cohort study of incident haemodialysis and peritoneal dialysis patients in Vorarlberg, the westernmost province of Austria counting ∼400 000 inhabitants. The study was approved by the local ethics committees, and all patients enrolled in the study provided written informed consent. All incident dialysis patients starting chronic dialysis treatment between 1 May 2000 and 30 April 2006 were enrolled. Patients having a malignant tumour at initiation of dialysis were excluded. A total of 235 patients were included in the study.

Patients were treated according to the European Best Practice Guidelines in place at the time of treatment (http://www.ndt-educational.org/guidelines.asp).

**Data description**

Clinical, laboratory and medication data were collected prospectively starting at the time of initiation of dialysis. These data included age, sex, height, weight, body mass index, diabetes status, primary cause of end-stage renal disease, smoking status and time between the first nephrological visit and initiation of dialysis. Type of and change in renal replacement therapy (haemodialysis, peritoneal dialysis and kidney transplantation) were recorded and considered as time-dependent treatment status for data analysis. Also, vascular access procedures and the type of vascular access (native fistula, graft or central venous catheter) for haemodialysis were evaluated.

Information on the following comorbidities were collected before initiation of dialysis and during the entire observation period thereafter: coronary artery disease (including myocardial infarction, percutaneous transluminal coronary angioplasty, aortocoronary bypass, angiographically proven coronary stenosis ≥50%, sudden cardiac death), cerebrovascular disease (including ischaemic cerebral infarction, transient ischaemic attack/prolonged reversible ischaemic neurologic deficit (PRIND), ultrasound-proven carotid stenosis, carotid artery surgery), peripheral arterial disease (significant ultrasound- or angiographically proven vascular stenosis, percutaneous transluminal angioplasty, peripheral bypass, amputation) and mesenterial infarction. Duration of hospitalization and mortality data including death causes (autopsy proven in 33%) were evaluated.

Laboratory parameters were recorded continuously during the study period and measured in a central laboratory. Among others, haemoglobin, creatinine, high-sensitivity C-reactive protein, albumin, calcium, phosphorus, ferritin and erythrocytes were measured.

The entire medication history was documented including all drugs, duration of intake and doses patients received during the study period and even the time before dialysis treatment. Laboratory parameters were measured in different time intervals—most of them once to twice monthly (haemoglobin, erythrocytes, creatinine, calcium, phosphorus) and a few of them every 3 months (albumin, C-reactive protein and ferritin). The patients had a median number of 47 haemoglobin measurements in the follow-up period (with a minimum of 3 and a maximum of 270 measurements) resulting in 12 242 different haemoglobin measurements during the entire observation period which were used in the time-dependent Cox regression modelling described below.

**Study outcome**

The outcome of interest was all-cause mortality. No patient was lost to follow-up.

**Statistical methods**

At baseline, categorical data were compared using χ² test, and continuous variables were analysed using an unpaired t-test or the non-parametric Wilcoxon rank-sum test. Haemoglobin values between men and women over the entire observation period were compared using a linear mixed effect model taking into account the repeated measurements for each patient.

To investigate the influence of haemoglobin levels on all-cause mortality, a time-dependent Cox proportional hazards model [31] was used allowing all variables to vary over different measurements during the whole observation time for each patient. That is, each time span between two successive measurements enters the model independently. Each covariate that entered the model was updated at the time they were measured and modelled in a time-dependent fashion. If not all variables were measured at a particular date, the respective missing values were replaced by the values measured at the last observation of this variable (‘last observation carried forward’). To account for a possible correlation of values within one patient, robust variances were estimated, which were grouped for each patient. The proportional hazards assumption was tested for each model by testing for zero slopes of scaled Schoenfeld residuals.

In order to evaluate the functional form of the haemoglobin effects, non-linear P-splines of degree 3 were estimated [32]. A spline of degree 3 is a linear combination of cubic functions, which can fit virtually any smooth curve to the data. Therefore, the analysis was not restricted to a potential linear relationship of haemoglobin with risk of mortality. To keep the number of parameters estimated at a minimum, the minimum number of knots for a nonlinear P-spline was chosen [degrees of freedom (df) = 2]. The spline term can be split into its linear and non-linear components, which can each be tested separately. For the linear term, a hazard ratio (HR) can be estimated, whereas the non-linear component can be depicted in a plot of the logHR. Furthermore, the predicted HRs and their 95% confidence intervals (CI) were calculated for several levels of haemoglobin with 12 g/dL as reference category. Graphical plots of P-splines were also standardized to a log hazard ratio of 0 for haemoglobin values equalling 12 g/dL.

To evaluate the effect of Hb-Var, the standard deviation of haemoglobin measurements was calculated for each time span using the current and all preceding values for each patient. Thus, the standard deviation of haemoglobin as a surrogate for Hb-Var is also modelled in a time-dependent fashion and is added to the Cox model linearly as a further covariate. As an additional sensitivity analysis, Hb-Var was also calculated in a time-varying manner using all values measured during the 6 months (and not the entire period) before the current observation period to yield a consistent time span for each patient. All the applied Cox models, including the time-varying haemoglobin measurements as well as Hb-Var, were additionally adjusted for age and sex; however, if the analyses were separated by men and women, the adjustments were made only for age. An extended model was also conducted, additionally adjusting for the time-varying variables of the type of renal replacement therapy including type of vascular access, diabetes mellitus, C-reactive protein, albumin, calcium and phosphorus. Due to the correlation of these factors with inflammation and malnutrition, adding them as proxies in the model adjusts for the presence of inflammation and malnutrition at least partially. Additional sensitivity analysis was conducted to adjust for cardiovascular events before the start of renal replacement therapy as well as hospitalization days per patient-year in a time-dependent manner, and finally, one analysis performed a censoring at the time of transplantation.

All analyses were conducted in R [33] using the ‘survival’ package [34].

**Results**

**Patient characteristics**

Table 1 presents the baseline demographic and laboratory characteristics as well as comorbidities before the start of dialysis treatment of the 235 incident dialysis patients (146 men and 89 women). The median follow-up time was 35.1 months, ranging from 24 days to ∼7.5 years. During this period, 82 patients died (34.9%), and this frequency was similar in men and women (34.9% and 34.8%). Thirty-eight patients died of cardiovascular disease (51.0% and 38.7% of the death causes in men and women, respectively), 22 died of a fatal sepsis (25.5% and 29.0%, respectively).
Table 1. Clinical characteristics of patients at baseline and during follow-up

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 235)</th>
<th>Men (n = 146)</th>
<th>Women (n = 89)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>61.7 ± 14.0</td>
<td>61.6 ± 14.4</td>
<td>61.9 ± 13.4</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>26.1 ± 4.5</td>
<td>26.2 ± 4.1</td>
<td>25.9 ± 5.0</td>
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<tr>
<td><strong>Start of dialysis with</strong></td>
<td></td>
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<tr>
<td>Haemodialysis, n (%)</td>
<td>197 (83.8%)</td>
<td>127 (87.0%)</td>
<td>70 (78.7%)</td>
</tr>
<tr>
<td>Central venous catheter, n (%)</td>
<td>32 (16.2%)</td>
<td>18 (14.2%)</td>
<td>14 (20.0%)</td>
</tr>
<tr>
<td>Native fistula, n (%)</td>
<td>133 (67.5%)</td>
<td>96 (75.6%)</td>
<td>37 (52.9%)***</td>
</tr>
<tr>
<td>Graft, n (%)</td>
<td>32 (16.2%)</td>
<td>13 (10.2%)</td>
<td>19 (27.1%)***</td>
</tr>
<tr>
<td>Peritoneal dialysis, n (%)</td>
<td>38 (16.2%)</td>
<td>19 (13.0%)</td>
<td>19 (21.3%)</td>
</tr>
<tr>
<td><strong>Year of start of dialysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000–03</td>
<td>122 (51.9%)</td>
<td>78 (53.4%)</td>
<td>44 (49.4%)</td>
</tr>
<tr>
<td>2004–06</td>
<td>113 (48.1%)</td>
<td>68 (46.6%)</td>
<td>45 (50.6%)</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, n (%)</strong></td>
<td>82 (34.9%)</td>
<td>47 (32.2%)</td>
<td>35 (39.3%)</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>154.0 ± 22.7</td>
<td>154.7 ± 22.5</td>
<td>152.8 ± 23.1</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>83.0 ± 12.3</td>
<td>83.1 ± 12.6</td>
<td>82.9 ± 11.9</td>
</tr>
<tr>
<td><strong>Laboratory parameters at baseline</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Ferritin (ng/mL)</td>
<td>174.3 ± 206.5 (44.0; 111.0; 234.0)</td>
<td>191.5 ± 197.6 (57.8; 129.5; 249.3)</td>
<td>147.1 ± 218.2** (36.0; 75.0; 185.5)</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>3.24 ± 5.34 (0.30; 0.98; 3.00)</td>
<td>3.43 ± 5.64 (0.40; 0.97; 3.04)</td>
<td>2.93 ± 4.81 (0.30; 1.06; 3.05)</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>1.98 ± 0.61 (1.57; 1.94; 2.30)</td>
<td>2.06 ± 0.63 (1.60; 1.95; 2.40)</td>
<td>1.87 ± 0.56 (1.47; 1.90; 2.12)</td>
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<tr>
<td>Calcium (mmol/L)</td>
<td>2.12 ± 0.27</td>
<td>2.09 ± 0.27</td>
<td>2.18 ± 0.26*</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>3.71 ± 0.65</td>
<td>3.69 ± 0.70</td>
<td>3.73 ± 0.58</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>7.28 ± 6.24 (5.50; 6.80; 8.60)</td>
<td>7.67 ± 6.28 (5.70; 7.30; 8.73)</td>
<td>6.65 ± 2.09*** (5.10; 6.10; 8.10)</td>
</tr>
<tr>
<td>Erythrocytes (T/L)</td>
<td>3.73 ± 0.62</td>
<td>3.68 ± 0.62</td>
<td>3.82 ± 0.61</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.17 ± 1.72</td>
<td>11.13 ± 1.74</td>
<td>11.22 ± 1.69</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>154.0 ± 22.7</td>
<td>154.7 ± 22.5</td>
<td>152.8 ± 23.1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>83.0 ± 12.3</td>
<td>83.1 ± 12.6</td>
<td>82.9 ± 11.9</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD (25, 50 and 75 percentiles in the case of non-normal distribution) or number (%).

*p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001—comparison between men and women.

aCoronary artery disease: myocardial infarction, percutaneous transluminal coronary angioplasty, aortocoronary bypass.

bCardiovascular disease: myocardial infarction, percutaneous transluminal coronary angioplasty, aortocoronary bypass, coronary artery stenosis ≥50%, ischaemic cerebral infarction, transient ischaemic attack/PRIND.

cPeripheral arterial disease: arterial stenosis, percutaneous transluminal angioplasty, peripheral bypass, amputation.

dFollow-up time was calculated as the time from the start of dialysis until the patient died or the end of the observation period was reached.

Most of the baseline laboratory parameters were similar in men and women. Serum creatinine (P < 0.0005) and ferritin (P < 0.01) were significantly higher in men than in women, whereas serum calcium (P < 0.05) was lower. Haemoglobin values did not differ significantly between men and women with a mean (SD) of 11.13 (1.74) g/dL versus 11.22 (1.69) g/dL, if only the measurements at the start of dialysis were considered. Taking all measurements during the whole observation period into account, haemoglobin values were slightly different between men and women: mean (SD): 12.38 (1.55) g/dL in men, 12.07 (1.47) g/dL in women.

Supplementary Table 1 shows the patient characteristics during the whole observation period into account, haemoglobin values were slightly different between men and women: mean (SD): 12.38 (1.55) g/dL in men, 12.07 (1.47) g/dL in women.

Cox regression analysis

A non-linear P-spline on the age- and sex-adjusted model (blue line in Figure 1) showed a significant linear inverse relationship between the increasing haemoglobin values and the decreasing risk of all-cause mortality for the entire study group. A sex-stratified analysis pointed to clear differences in this relationship between men and women.

The linear component of the non-linear spline was highly significant for both men (P = 0.00005) and women (P = 0.00000000052) (Table 2), which underscored the overall inverse trend. The non-linear component was also significant in both men and women but less pronounced than the linear component. This implies that a simple linear model and thus a single HR as a result would not be sufficient to describe the relationship between haemoglobin values and mortality.
time to event. Figure 1C (blue line) and Table 2 clearly showed an inverse relationship up to values of 12–13 g/dL in women, which reached a plateau for the higher values of haemoglobin. For men (blue line in Figure 1B), an inverse trend was observed but clearly attenuated when compared to women. HRs were significantly increased for haemoglobin values of 10 g/dL and lower in women compared to the reference of 12 g/dL. For men, the risk for low haemoglobin values was also increased and decreased for higher values, although partially lacking statistical significance (Table 2).

Additional adjustment for parameters accounting for inflammation and malnutrition as well as diabetes attenuated all regression estimates. The overall form of the association, as shown in the P-splines (red lines), however, remained very similar in women, including the significant non-linear components (Table 2). In this extended adjustment model, the linear component of the haemoglobin association with all-cause mortality was still significant (P = 0.0018) for women, as well as the corresponding increased HRs for haemoglobin values of 10 mg/dL and lower. In men, the linear relationship was less pronounced (P = 0.023).

The increasing Hb-Var, estimated by the time-varying standard deviation of haemoglobin measurements for each patient, was significantly associated with higher mortality risk in men when the analysis was adjusted for age (P = 0.00068). After including extended adjustment, this association vanished (P = 0.15).

**Sensitivity analysis**

We performed several sensitivity analyses: one analysis adjusted for cardiovascular events before the start of renal replacement therapy, one analysis adjusted for hospitalization days per patient-year in a time-dependent manner which might reflect the severity of comorbidities, one analysis performed a censoring at the time of transplantation and another analysis with time-varying Hb-Var calculated from the last 6 months (and not the entire period) before the current observation period. All additional analyses did not reveal any substantial differences in HRs compared to our primary analysis (see Supplementary Table 2).

**Discussion**

The study at hand used a time-dependent Cox regression analysis of a single-centre inception cohort of dialysis patients followed up for more than 7 years. It used all information of haemoglobin levels available from the entire observation period to model the association of haemoglobin—
bin levels and all-cause mortality with flexible modelling techniques. We observed a pronounced inverse association of haemoglobin levels with all-cause mortality which was not entirely linear. Patients with haemoglobin levels of 10 g/dL or below had an increased risk of mortality, and this risk did not increase in the case of higher haemoglobin levels of 13 g/dL. The most important observation was a pronounced difference in risk by haemoglobin levels between men and women.

**Time-dependent modelling and Hb-Var**

First of all, it is important to point out that we used in our analysis the entire data collected through the whole observation period. That means we did not only consider haemoglobin levels from the baseline of the study or the first 6 months of observation but the levels of haemoglobin as well as all other covariates recorded during the entire observation period. This results in a depth of data never used before in dialysis patients over such a long observation period.

The use of a time-dependent modelling has several advantages since it does not consider only one or a few data points but the full spectrum of data in each patient that occurred during follow-up. Studies which include only a baseline value or values of the first few months consider usually the most instable phase of treatment when a patient starts dialysis treatment. This vulnerable phase is associated with a very high mortality and does not necessarily reflect the more stable latter phases of treatment with often much better laboratory values. It furthermore avoids censoring of data in the case of kidney transplantation since this new phase of treatment can be considered as a time-dependent covariate. As we discussed recently [35], censoring of the data has major disadvantages such as loss of power due to loss of follow-up time, informative bias due to the health status influencing the probability of transplantation and hardly any comparable studies due to tremendous differences in transplantation rates in various countries.

Some recent studies indicate that the phenomenon of Hb-Var may have an adverse impact on patient outcomes [28,29]. In a retrospective study, Gilbertson et al. found persistently and transiently low haemoglobin levels and highly variable haemoglobin levels to be a risk factor for death. Patients who consistently maintained haemoglobin values within the recommended target range had the lowest risk to die [28]. Another retrospective study observed that each 1 g/dL increase in the residual standard deviation of

<table>
<thead>
<tr>
<th>Age- and sex-adjusted measurements</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin, linear part</td>
<td>0.739 (0.656, 0.833) 0.711 (0.602, 0.838) 0.651 (0.564, 0.752)</td>
<td>0.739 (0.656, 0.833) 0.711 (0.602, 0.838) 0.651 (0.564, 0.752)</td>
<td>0.739 (0.656, 0.833) 0.711 (0.602, 0.838) 0.651 (0.564, 0.752)</td>
</tr>
<tr>
<td>Haemoglobin, non-linear part</td>
<td>0.017</td>
<td>0.017</td>
<td>0.017</td>
</tr>
<tr>
<td>Hb-Var</td>
<td>2.205 (1.449, 3.354) 0.00022</td>
<td>2.466 (1.465, 4.151) 0.000068</td>
<td>1.877 (0.962, 3.661) 0.065</td>
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<table>
<thead>
<tr>
<th>Exact haemoglobin levels (g/dL)</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>8</td>
<td>3.619 (1.384, 9.459)</td>
<td>1.905 (0.585, 6.201)</td>
<td>19.308 (3.867, 96.403)</td>
</tr>
<tr>
<td>10</td>
<td>1.708 (1.175, 2.482)</td>
<td>1.418 (0.872, 2.306)</td>
<td>2.506 (1.395, 4.504)</td>
</tr>
<tr>
<td>12</td>
<td>1.000 (0.879, 1.137)</td>
<td>1.000 (0.842, 1.188)</td>
<td>1.000 (0.846, 1.182)</td>
</tr>
<tr>
<td>14</td>
<td>0.640 (0.483, 0.848)</td>
<td>0.565 (0.406, 0.788)</td>
<td>0.761 (0.481, 1.205)</td>
</tr>
<tr>
<td>16</td>
<td>0.425 (0.145, 1.245)</td>
<td>0.325 (0.082, 1.294)</td>
<td>0.575 (0.180, 1.839)</td>
</tr>
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<tr>
<th>Extended adjustments</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin, linear part</td>
<td>0.830 (0.711, 0.968) 0.806 (0.670, 0.971) 0.758 (0.637, 0.902)</td>
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<td>0.830 (0.711, 0.968) 0.806 (0.670, 0.971) 0.758 (0.637, 0.902)</td>
</tr>
<tr>
<td>Haemoglobin, non-linear part</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Hb-Varb</td>
<td>1.624 (0.889, 2.965) 0.098</td>
<td>1.612 (0.837, 3.103) 0.15</td>
<td>1.332 (0.345, 5.133) 0.68</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Exact haemoglobin levels (g/dL)</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1.707 (0.639, 4.562)</td>
<td>0.796 (0.163, 3.871)</td>
<td>9.281 (2.010, 42.864)</td>
</tr>
<tr>
<td>10</td>
<td>1.266 (0.845, 1.897)</td>
<td>0.925 (0.505, 1.695)</td>
<td>2.248 (1.204, 4.197)</td>
</tr>
<tr>
<td>12</td>
<td>1.000 (0.891, 1.122)</td>
<td>1.000 (0.859, 1.164)</td>
<td>1.000 (0.842, 1.187)</td>
</tr>
<tr>
<td>14</td>
<td>0.749 (0.557, 1.007)</td>
<td>0.732 (0.516, 1.037)</td>
<td>0.816 (0.466, 1.429)</td>
</tr>
<tr>
<td>16</td>
<td>0.534 (0.194, 1.469)</td>
<td>0.443 (0.130, 1.512)</td>
<td>0.888 (0.234, 3.373)</td>
</tr>
</tbody>
</table>

For each model, estimated HRs are shown for the linear component and the time-varying Hb-Var of the non-linear P-spline as well as HRs for several exact haemoglobin levels compared to the reference (12 g/dL).

*No additional adjustment for sex.

bHaemoglobin variability was calculated as standard deviation of haemoglobin measurements based on each time span using the current and all preceding values for each patient.
Haemoglobin was associated with a 33% increased death rate even after adjusting for multiple covariates [29]. Fishbane et al. described the phenomenon of haemoglobin cycling in haemodialysis patients by reporting the frequency of haemoglobin fluctuation across the target range and the magnitude of change over time [26]. Similar findings were described by Ebben et al. in a large population study, which demonstrates that haemoglobin levels of ~90% of the patients fluctuate across the NKF KDOQI target range of 11–12.5 g/dL. In order to be able to describe the fluctuation patterns, they defined six groups of patients according to their haemoglobin level fluctuation during a 6-month period: patients who had stable values during this time span in the given haemoglobin intervals (<11, 11–12.5 and ≥12.5 mg/dL), patients whose measurements covered two adjacent intervals and patients whose values covered all three defined intervals [25]. The increasing number of measurements over a longer period of time, however, increases the probability of changes in the haemoglobin categories. Applying the categories by Ebben et al. to our data and restricting the cycling definition to the first 6 months of observation resulted in half of the patients being categorized into the highest cycling category (values changing from <11 g/dL to values ≥12.5 g/dL). Extending the time interval for the cycling definition to 2 years or even the entire observation period did not leave any patients in the reference category but almost all patients in the highest cycling group. Therefore, this categorization is less applicable in the case of long-term observation data. It results in a collapsing of information into categories instead of using the entire information which could otherwise also be applied in a time-dependent Cox regression model as in our study. Since this model considers at least partially the fluctuations over time, it was not surprising that the standard deviation of haemoglobin values as a measure of Hb-Var had only a rather small influence on mortality, if any.

**Sex-specific differences for haemoglobin and mortality**

There are similarities among sexes in the basic underlying physiology of anaemia in CKD, but evidence is accumulating with respect to the differential responses to anaemia between the sexes. However, a few studies to date have addressed the implications of sex differences in anaemia in CKD in sufficient detail, and the underlying physiology remains speculative [36]. Interestingly, the usual difference in haemoglobin levels between men and women of ~1 g/dL was much smaller in our patients, in whom we observed only a sex difference of 0.31 g/dL when all (n = 12,242; men: 7690, women: 4552) the measured haemoglobin levels were considered during the entire observation period. This can be explained by the fact that the haemoglobin target to treat is the same in men and women.

The presented data reveal an inverse association between haemoglobin values and risk of all-cause mortality, which is primarily present in women. For women, the risk was shown to be linearly decreasing for increasing haemoglobin values up to ~12–13 g/dL with no further change in risk for higher values. This was much less pronounced in men, which suggests that women on renal replacement therapy are more vulnerable to anaemic effects. This might point to differences in CKD compared to physiological conditions, where healthy women are known to shift the haemoglobin oxygen dissociation curve to reduce oxygen affinity for haemoglobin secondary to higher levels of 2,3-diphosphoglycerate [37]. Whether CKD also influences 2,3-diphosphoglycerate levels or properties is less well understood.

**Haemoglobin above target levels**

The NKF KDOQI guidelines do not only recommend treating anaemia to a target haemoglobin level between 11 and 12 g/dL, but also state that haemoglobin >13 g/dL should be avoided [5]. However, this threshold is presently heavily debated caused by inconsistencies of studies [11–14]. Observational studies observed an incremental improvement in survival with higher haemoglobin levels in dialysis patients [2,6,10]. Several single trials as well as a meta-analysis of nine randomized controlled clinical trials [38], however, showed an increased risk of adverse outcomes for higher compared with lower haemoglobin targets, especially in patients with pre-existing cardiovascular disease. Of note, the vast majority of our observation time was prior to the publication of the latter studies. Interestingly, in our data, an increasing risk for haemoglobin values >13 g/dL could not be observed. This is probably an effect of individualized target levels, which means that higher levels were mainly aimed for in younger active patients without severe cardiovascular problems. Although our study is small, it considers all measured haemoglobin levels as well as the time exposed to these levels over a long-term observation period. This depth of data could guide future studies in large cohorts to consider the long-term time of exposure to high or low haemoglobin levels.

**Strengths and limitations of the study**

A limitation of this study is the relatively small sample size. Thus, our observations should stimulate further large-scale prospective studies analysing the impact of sex on specific risk factors on mortality as well as the use of long-term analysis of time-dependent variables.

Due to its design as an observational study, a complete adjustment for all individual factors influencing the achievement of haemoglobin levels over time and mortality is not possible. Individualized therapy related to the patient’s comorbidities contributes to anaemia therapy and attained haemoglobin values. Nevertheless, we statistically adjusted for the main risk factors which have been extensively shown to be associated with low haemoglobin levels and all-cause mortality, respectively.

Despite its limited sample size, our study has several notable strengths. It is a single-centre study with uniform laboratory measurements of high frequency and continuity collected over a period of up to 7 years. By analysing haemoglobin on mortality risk in a time-varying modelling framework, we were able to include all measurements over the whole observation period covering the haemoglobin fluctuation over time. Furthermore, little data exist on
sex differences of haemoglobin values in patients on dialysis, and to our knowledge, there have been no studies to date investigating the effect of HB-Var over time in men compared to women. Of course, our findings can only be the starting point for further and larger studies evaluating the sex-specific effect using longitudinal data.

Conclusions

Our prospective observational cohort study of 235 incident dialysis patients considered the entire information collected on haemoglobin and HB-Var over a period of more than 7 years and observed a sex-specific linear as well as non-linear effect of haemoglobin levels on all-cause mortality when time-varying modelling was applied.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

Acknowledgements. The study was supported by an unrestricted grant from Amgen.

Conflict of interest statement. None declared.

References

The safety and efficacy of intravenous ferric carboxymaltose in anaemic patients undergoing haemodialysis: a multi-centre, open-label, clinical study

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Abstract

Background. Patients with chronic kidney disease (CKD) often present with iron depletion and iron deficiency anaemia (IDA) because of frequent blood (and iron) loss. Therapy consists of repletion of iron stores and intravenous (i.v.) iron has become the standard care in this setting. However, older i.v. iron preparations have their limitations. This study primarily investigated the safety, and also the efficacy, of ferric carboxymaltose (FCM), a next-generation i.v. iron formulation, given as a bolus-push injection in patients with CKD undergoing maintenance haemodialysis (HD).

Methods. Patients (aged 18–65 years) with IDA undergoing HD received 100–200 mg of iron as FCM via an i.v. bolus-push injection into the HD venous line, two to three times weekly for ≤6 weeks. Safety assessments included incidence of adverse events (AEs). Treatment responders were patients attaining ≥1.0 g/dl increase in haemoglobin (Hb) from baseline at any time during the study. Enrolled patients (safety population) receiving ≥1 dose of study medication were included in the efficacy analyses [intent-to-treat (ITT) population].

Results. Of 163 patients enrolled, 150 (92%) completed the study. The mean ± SD total cumulative dose of iron as FCM administered was 2133.3 ± 57.7 mg. In total, 193 AEs were reported in 89 out of 163 (54.6%) patients. Almost three-quarters of patients (73.6%) received erythropoietin-stimulating agents (ESAs), but the dose remained stable during the study. Serious AEs occurred in 12 out of 163 (7.4%) patients and two patients died; none of these was considered by the investigator to be related to the study medication. Only five out of 163 (3.1%) patients discontinued study medication due to an AE. Overall, 100 out of 162 (61.7%; ITT population) patients were treatment responders, and mean Hb levels increased from 9.1 ± 1.30 g/dl at baseline to 10.3 ± 1.63 g/dl at follow-up.

Conclusions. FCM is well-tolerated and effective in the correction of Hb levels and iron stores in patients with IDA undergoing HD. As changes in anaemia treatment other than i.v. FCM (e.g. increased ESA doses) were not permitted during the study, the clinically relevant increase in Hb in the majority of patients can be solely attributed to efficient iron utilization. The incidence of AEs was as expected for this population.

Keywords: clinical trial; efficacy; ferric carboxymaltose; haemodialysis; iron deficiency anaemia; safety

Introduction

Annual blood losses of around 2.5 l place patients with chronic kidney disease (CKD) undergoing haemodialysis (HD) at particularly high risk of iron store depletion with subsequent iron deficiency anaemia (IDA) [1,2]. Iron deficiency can be defined as absolute or functional [1,3]. Absolute iron deficiency develops as the body's iron stores become depleted to such a low level that not enough iron is available for the production of haemoglobin (Hb) [4,5]. This is usually indicated by a decline in serum ferritin levels to ~<15 μg/l in patients with normal kidney function, but is much higher in patients undergoing HD as a result of chronic inflammation, and is associated with elevated levels of C-reactive protein (CRP) [4,5]. This functional iron deficiency describes the state when iron cannot be mobi-