Hepcidin is not useful as a biomarker for iron needs in haemodialysis patients on maintenance erythropoiesis-stimulating agents

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Abstract

Background. It has been suggested that hepcidin may be useful as a tool for managing iron therapy in haemodialysis (HD) patients on erythropoiesis-stimulating agents (ESA).

Methods. We used SELDI-TOF mass spectrometry assay to measure serum hepcidin-25 (Hep-25) and hepcidin-20 (Hep-20) in 56 adult HD patients on maintenance ESA to assess their ability to predict haemoglobin (Hb) response after 1 g intravenous iron (62.5 mg ferric glucoate at 16 consecutive dialysis sessions) and their relationship with markers of iron status, inflammation and erythropoietic activity.

Results. At multivariate analysis (in a model that also included Hb, reticulocyte, ESA dose, HFE genotype, soluble transferrin receptor [sTfR] and C-reactive protein), Hep-25 independently correlated with ferritin ($\beta = 0.03, P = 0.01$) and the percentage of hypochromic red blood cells [%Hypo] ($\beta = 1.84, P = 0.01$), suggesting that Hep-25 may be a useful biomarker for iron stores and bone marrow iron availability. Hep-20 correlated independently with Hep-25 ($\beta = 0.159, P < 0.001$) and ferritin ($\beta = 0.006, P = 0.05$), suggesting that it may be a useful additional biomarker for iron stores. On receiver operating characteristics curve analysis, neither Hep-25 nor Hep-20 significantly predicted who will increase their Hb after iron loading ($AUC = 0.52 \pm 0.09$ and $0.54 \pm 0.08, P = 0.612$), and the same applied to ferritin and transferrin saturation ($AUC = 0.55 \pm 0.08$ and $0.59 \pm 0.08, P = 0.250$), whereas %Hypo and reticulocyte Hb content were significant predictors ($AUC = 0.84 \pm 0.05$ and $0.70 \pm 0.08, P < 0.01$). At multivariate logistic regression analysis, %Hypo was the only biomarker independently associated with iron responsiveness.

Conclusions. Although our study suggests an important role for hepcidin in regulating iron homeostasis in HD patients on ESA, our findings do not support its utility as a predictor of iron needs, offering no advantage over established markers of iron status.

Keywords: diagnostic accuracy; erythropoiesis-stimulating agents; haemodialysis; hepcidin; iron deficiency

Introduction

Anaemia occurs in the majority of patients with end-stage renal disease (ESRD) requiring dialysis therapy and it can be corrected effectively using erythropoiesis-stimulating agents (ESA) [1]. However, a considerable proportion of patients exhibit a suboptimal response to ESA, and iron deficiency has been identified as the major cause of this hyporesponsiveness [2,3].

Due to the accelerated erythropoiesis driven by ESA treatment (coupled with the ongoing uraemia- and dialysis-related iron losses), ESRD patients on ESA are at high risk of developing iron-restricted erythropoiesis because the rate at which iron is released from stores and delivered to the bone marrow fails to match the increased iron demand. This limited availability of iron to bone marrow can be corrected effectively by intravenous iron therapy, which improves haemoglobin (Hb) response [4]. On the other hand, the inflammation frequently seen in dialysis patients may also contribute to iron-restricted erythropoiesis by reducing the release of stored iron from the reticuloendothelial system to circulating transferrin [5], a condition which—unlike iron depletion—reduces the likelihood and extent of response to intravenous iron administration [5,6].

The accurate identification of patients who would benefit from iron therapy has relevant clinical and economic implications, since it enables a better response to ESA while avoiding the risks associated with overzealous iron therapy [7,8]. Unfortunately, the laboratory tests used to evaluate iron status have revealed a suboptimal accuracy in identifying cases who will or will not respond to intravenous iron [9], since their relationships with iron status tend to be confounded by other factors, such as inflammation (as in the case of ferritin, transferrin saturation [TSAT] and the percentage of hypochromic red blood cells [%Hypo]) [10–12] or erythropoietic activity (as concerns soluble transferrin receptors [sTfR]) [13].

Hepcidin, the recently discovered peptide hormone regarded as the key regulator of iron entry into the plasma [14], is up-regulated by inflammation and increased iron stores and down-regulated by iron depletion [15]. It has
been hypothesized that measuring serum levels of hepcidin may be useful as an additional tool for predicting and monitoring the need for iron supplementation [16–18]. Elevated serum levels of the bioactive 25-amino acid hepcidin isoform, hepcidin-25 (Hep-25), have been consistently reported in dialysis patients [18–25], probably due to the combination of an impaired renal excretion and an increased formation secondary to inflammation and iron overload [24]. Since Hep-25 blocks iron release from the macrophages, its increase may contribute to the disordered iron homeostasis and ESA resistance in uraemia by limiting iron availability for erythropoiesis [14]. So far, all the studies evaluating the role of Hep-25 in the anaemia of dialysis patients have focused on its relationships with the biochemical markers of iron status and virtually all of these studies have reported a strong direct correlation between serum Hep-25 and ferritin levels [18,20,22–26], suggesting that hepcidin regulation by tissue iron is maintained in ESRD. The relationship between Hep-25 and ferritin may be modified by the HFE genotype, as shown by the lower Hep-25 levels and Hep-25/ferritin ratio in haemodialysis (HD) patients carrying the H63D or C282Y mutations [23]. Some studies have also found a direct association between Hep-25 levels and serum iron [24] and/or TSAT [18,22,24], suggesting that Hep-25 levels may also reflect the plasma iron pool and then the availability of iron. Moreover, the evidence that hepcidin may be responsible for iron-restricted erythropoiesis is largely indirect, based on the negative correlation between Hep-25 and Hb and/or reticulocyte (ret) levels [21,23,24].

Fewer data are available on serum levels of the smaller 20-amino acid hepcidin isoform, hepcidin-20 (Hep-20), in HD patients, which was again found increased in one study [25]. Hep-20 also correlated positively with ferritin [25], suggesting that it may also play a part in iron homeostasis.

The purpose of our study was to establish whether or not measuring Hep-25 and Hep-20 might be useful for predicting the Hb response to intravenous iron (a widely accepted reference standard for iron deficiency) [27–30] in stable chronic HD patients on maintenance ESA, as an alternative to measuring conventional markers of iron status. A secondary aim of the study was to test the relationship of these hepcidin isoforms with markers of iron status, inflammation and erythropoietic activity and HFE genotype.

Materials and methods

This study included 56 chronic HD patients treated at the Servizio Emodialisi, Ospedale Policlinico in Verona, Italy between July 2008 and June 2009.

The inclusion criteria were age over 18 years, no residual diuresis, duration of dialysis and time on ESA therapy in excess of 4 months, absence of neoplasia, major infectious diseases, congestive heart failure, hepatic failure and haemoglobinopathies. None of the patients had experienced bleeding, hospitalization or blood transfusions in the previous 3 months. All enrolled patients were on maintenance intravenous ESA (administered at the end of the dialysis session), aiming to maintain Hb within the range of 10.5–12.5 g/dL. Thirty-four patients were on epoetin α (Eprex, Janssen-Cilag, Italy), 22 patients were on darbepoetin α (Aranesp, Amgen, The Netherlands). Prior to enrolment, a majority of patients had been on maintenance low-dose intravenous iron (20–31 mg of iron as sodium ferric gluconate complex in sucrose administered at the end of the dialysis session) aiming to maintain ferritin within 200–600 ng/mL and %Hypo<6%. However, to avoid confounding, none of the patients had received iron within the 10 weeks prior to the baseline evaluation, and there had been no changes in their ESA dosing during the 6 weeks prior to baseline or during the iron challenge period. All subjects were dialysed against standard, not ultrapure, dialysate. Forty-two patients were on bicarbonate HD, 25 with low-flux synthetic membranes (12 polysulphone, Fresenius, Germany; 13 polycryamide, Gambro, Sweden), 11 with high-flux synthetic membranes (6 polysulphone, Fresenius, Germany; 5 polymethylmethacrylate, Toray, Japan) and 6 with high-flux modified cellulose membranes (cellulose triacetate, Nissin-Nipro, Japan). Fourteen patients were on acetate-free biofiltration with AN69 membrane (Hospital, Italy). Dialysers were not reused.

Blood samples for laboratory testing were obtained prior to the first-of-the-week HD sessions. Standard haematology parameters, including ret count and erythrocyte and ret indices, were assessed on the ADVIA 120 haematology analyser (Bayer Diagnostic, Germany), as previously described [28]. Serum ferritin, iron, transferrin, sTfR and C-reactive protein (CRP) levels were measured by commercially available assays. TSAT was calculated as serum iron (in microgrammes per decilitre) × 70.9/serum transferrin (in milligrammes per decilitre). HFE genotyping was done with a reverse-hybridization assay [31].

Fifty-seven controls were enrolled from among healthy volunteers taking part in a phase II trial at the Centre for Clinical Research of the Azienda Ospedaliera, Università di Verona, as described in detail elsewhere [23,32].

Response to intravenous iron

After baseline evaluation, all subjects received a standard dose of 1 g intravenous iron. Iron was administered as a slow bolus injection at the end of 16 consecutive dialysis sessions in the form of sodium ferric gluconate complex in sucrose at a dose of 62.5 mg. The Hb response to the iron challenge at constant ESA doses was used as the reference standard for iron-deficient erythropoiesis [27]. Patients showing an increase in Hb >1 g/dL above the baseline after intravenous iron were considered iron-deficient, while those with a smaller or no change in Hb were considered iron-replete [28,29]. Blood samples for laboratory testing were again obtained prior to the first-of-the-week HD session following the intravenous iron load.

All subjects gave their informed written consent to take part in the study, which was conducted according to the principles of the Helsinki Declaration, and the protocol was approved by the Ethical Committee of the Azienda Ospedaliera di Verona.
Results

The clinical features and baseline laboratory data of the patients included in the study are given in Table 1.

The median Hep-25 and Hep-20 levels were significantly higher in HD patients than in the 57 controls (11.16 nM [0.55–25.04] vs 4.62 nM [1.78–10.26] and 4.00 nM [1.78–8.44] vs 1.93 nM [0.55–7.23], respectively, P < 0.001). Univariate correlations between Hep-25 and Hep-20 levels and clinical and laboratory variables are given in Table 2: the serum levels of the two isoforms were significantly and directly correlated in HD patients, while the only other significantly correlating variables were serum ferritin and sTfR with Hep-25 and ferritin with Hep-20. At multivariate analysis (in a model including as explanatory variables Hep-25 and Hep-20, %Hypo×sTfR, CRP, Hep-25, %Hypo×sTfR, %Hypo×CRP), Hep-25 levels were independently associated with ferritin (β = 0.026 [95% confidence interval (95% CI) = 0.006–0.046], P = 0.01) and %Hypo (β = 1.84 [95% CI = 0.54–3.15], P = 0.01). Overall, the model explained only 37% of the variability in serum Hep-25 levels (R² = 0.371, P = 0.01). At multivariate analysis (including HFE genotype, ESA dose tertiles, Hb, ret, ferritin, %Hypo, sTfR, CRP, Hep-25, %Hypo×sTfR, %Hypo×CRP and ferritin×Hep-25 as explanatory variables), Hep-20 levels were significantly associated with Hep-25 (β = 0.159 [95% CI = 0.101–0.216], P < 0.001) and ferritin (β = 0.006 [95% CI = 0.002–0.010], P = 0.03), and the model accounted for 72% of the variability in serum Hep-20 levels (R² = 0.721, P < 0.001).

Following intravenous iron loading, 21 patients were considered iron-responders (IR) (with an increase in Hb of 1.58 ± 0.59 g/dL) and 35 were non-responders (NR) (their Hb increased by 0.32 ± 0.29 g/dL). Subjects with normal or high CRP values (>6 mg/L) had similar increases in Hb after intravenous iron in both IR (1.50 ± 0.48 vs 1.67 ± 0.82 g/dL, P = 0.62) and NR (0.32 ± 0.30 vs 0.35 ± 0.28 g/dL, P = 0.76). Figure 1 reports the Hb changes during the period before the baseline evaluation and the intravenous iron loading, showing that the Hb levels were stable in both groups before baseline (Week −10 to 0). The baseline laboratory data for the IR and NR patients are given in Table 3, showing that the only significant differences between the two groups concerned Hb, ESA dose, reticulocyte Hb content (CHr) and %Hypo. The lower Hb levels at higher ESA dose in IR patients is consistent with the view that iron-limited erythropoiesis (as indicated by a lower CHr and higher %Hypo) is associated with an impaired erythropoietic response [34].

Changes in Hep-25 and Hep-20 following the intravenous iron load were assessed in 44 patients (Figures 2 and 3) in which the baseline characteristics and prevalence of IR patients (38.6%) resembled that of the total population. Serum Hep-25 response to intravenous iron varied widely, although the median levels did not change significantly: they were 11.84 nM [0.55–26.16] before and 11.70 nM [2.91–29.97] after the iron load (P = 0.50), with no difference in either the IR (Figure 2a) or the NR group (Figure 2b). Conversely, median Hep-20 levels increased.

### Table 1. Clinical features and baseline laboratory data

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Gender (male/female)</th>
<th>Age (years)</th>
<th>Proportion with cardiovascular disease (%)</th>
<th>Proportion with diabetes (%)</th>
<th>Dialysis vintage (months)</th>
<th>HFE genotype</th>
<th>ESA dose</th>
<th>Hep-25</th>
<th>Hep-20</th>
</tr>
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<tbody>
<tr>
<td>56</td>
<td>32/24</td>
<td>67 ± 14</td>
<td>51.8</td>
<td>19.6</td>
<td>42 [5–114]</td>
<td>wt/wt</td>
<td>α</td>
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<td></td>
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<td></td>
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<td>H63D/wt</td>
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<td>H63D and C282Y/wt</td>
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<td></td>
<td>42</td>
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</table>

### Table 2. Correlations of baseline serum Hep-25 and Hep-20 with clinical and laboratory features by Spearman's rho test

<table>
<thead>
<tr>
<th>Hep-25</th>
<th>Hep-20</th>
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<tbody>
<tr>
<td>rho</td>
<td>P-value</td>
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*The lowest tertile includes patients on epoetin α <4000 U/w or darbepoetin α <20 µg/w; the intermediate tertile those on epoetin α dose between 4000 and 12 000 U/w or darbepoetin α between 20 and 40 µg/w; the highest tertile those on epoetin α >12 000 U/w or darbepoetin α >40 µg/w.
significantly after intravenous iron, from 3.73 [1.76–8.52] to 5.51 nM [1.64–14.91] (P = 0.013). The changes in Hep-20 levels after intravenous iron were statistically significant in NR only, however, when the IR (Figure 3a) and NR patients (Figure 3b) were considered separately. Median total hepcidin levels (i.e. the sum of Hep-25 and Hep-20) also increased after the iron load from 17.13 [3.10–33.45] to 21.52 nM [4.71–43.28], although the difference was not statistically significant (P = 0.165). The changes in total hepcidin levels after intravenous iron were not statistically significant also when the IR and NR patients were considered separately (data not shown).

At ROC curve analysis, %Hypo (AUC = 0.844 [95% CI = 0.737–0.950], P <0.001) and CHr (AUC = 0.697 [95% CI = 0.537–0.855], P = 0.01) emerged as significant predictors of IR, while Hep-25 and Hep-20 did not (AUC = 0.517 [95% CI = 0.330–0.672], P = 0.90 and 0.541 [95% CI = 0.373–0.710], P = 0.61, respectively) (Figure 4). TSAT and ferritin could not predict iron status (AUC = 0.593 [95% CI = 0.431–0.754], P = 0.25 and 0.552 [95% CI = 0.391–0.713], P = 0.52), nor could the HFE genotype (AUC = 0.572 [95% CI = 0.433–0.748], P = 0.26). The AUC for %Hypo was significantly greater than the AUC for the other tests (P <0.03), with no significant differences between the latter (P >0.13). At multivariate logistic regression analysis (including HFE genotype, ferritin, TSAT, %Hypo, CHr, Hep-25 and Hep-20), the only biomarker

Table 3. Baseline laboratory data in iron-responsive and iron non-responsive patients

<table>
<thead>
<tr>
<th></th>
<th>Iron-responsive</th>
<th>Non-responsive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>11.0 ± 1.0</td>
<td>11.7 ± 0.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Proportion with HFE mutations (%)</td>
<td>33</td>
<td>20</td>
<td>0.21</td>
</tr>
<tr>
<td>ESA dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epoetin α (U/w)</td>
<td>12 000 [3000–30 000]</td>
<td>6000 [2600–12 000]</td>
<td>0.016</td>
</tr>
<tr>
<td>Darbepoetin α (µg/w)</td>
<td>32.5 [10.0–100.0]</td>
<td>20.0 [10.0–65.0]</td>
<td>0.050</td>
</tr>
<tr>
<td>%Hypo (%)</td>
<td>14.4 [3.8–29.8]</td>
<td>3.6 [0.6–8.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CHr (pg)</td>
<td>31.3 ± 4.3</td>
<td>33.7 ± 2.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>44 ± 20</td>
<td>52 ± 18</td>
<td>0.15</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>18.0 ± 8.7</td>
<td>21.0 ± 8.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>137 [11–547]</td>
<td>174 [46–398]</td>
<td>0.90</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.5 [2.0–17.1]</td>
<td>3.2 [1.6–14.7]</td>
<td>0.30</td>
</tr>
<tr>
<td>Proportion with CRP &gt;6 mg/L (%)</td>
<td>29</td>
<td>33</td>
<td>0.77</td>
</tr>
<tr>
<td>Hep-25 (nM)</td>
<td>11.09 [0.55–38.82]</td>
<td>12.44 [1.38–20.08]</td>
<td>0.45</td>
</tr>
</tbody>
</table>
independently associated with iron responsiveness was %Hypo (OR = 1.60 [95% CI = 1.08–2.39], P = 0.02).

**Discussion**

In agreement with other reports [18,20–25], our study confirms that the mean serum levels of the bioactive isoform Hep-25 are higher in HD patients than in controls. Levels varied widely, however (from undetectable to several times higher than in controls), suggesting that they may be responsive to physiological stimuli. In fact, our finding that Hep-25 significantly and independently correlated with ferritin (the classical marker for iron stores) [9] and %Hypo (an established marker of iron-deficient erythropoiesis in HD patients on ESA treatment) [9,12,28,29] suggests that it maintains a major role in regulating iron homeostasis in these patients.

The results of multivariate analysis are consistent with a model in which circulating levels of Hep-25 may be determined primarily by the body’s iron stores (as suggested by its direct relationship with ferritin) and modulate bone marrow iron availability (as suggested by its direct relationship with %Hypo). Alternatively, Hep-25 may be seen as an important regulator of body iron distribution, any change in Hep-25 levels leading to proportional changes in iron stores and to inversely proportional changes in iron delivery for erythropoiesis. Be that as it may, our findings suggest that Hep-25 may be a crucial determinant of iron-restricted erythropoiesis during ESA treatment.

Like other authors, we found no association between Hep-25 and CRP [20,21,23–25] or between ESA dose and response to ESA [22,23,25] or HFE genotype. These findings probably relate to distinct features of our population, which included stable HD patients with little or no dialysis-related inflammation, stable Hb levels and ESA dose at the baseline and a slightly lower prevalence of HFE mutations than in Valenti et al. [23]. Mean Hep-25 levels did not change after intravenous iron, in agreement with a recent report from Weiss et al. [24] (who detected no change in Hep-25 after a single dose of 100 mg intravenous iron in HD patients), but at variance with the study by Maliszko et al. [35] and the case report by Tomosugi et al. [19] (who found a significant increase of Hep-25 levels after comparable iron load). Their findings are not in conflict with ours, however, since we found a wide variability of response to intravenous iron, many patients showing a significant increase in Hep-25 levels. We also observed an average 26% increase in total hepcidin levels after intravenous iron loading, which is quantitatively similar to what Maliszko et al. observed [35] when they measured hepcidin using a radioimmunoassay that probably detects total hepcidin because of the cross-reactivity of the antibodies with all hepcidin isoforms.

In Hep-20 levels, we observed an average 26% increase in total hepcidin levels after intravenous iron loading, which is quantitatively similar to what Maliszko et al. observed [35] when they measured hepcidin using a radioimmunoassay that probably detects total hepcidin because of the cross-reactivity of the antibodies with all hepcidin isoforms.

Our study also confirmed that the smaller isoform Hep-20 accumulates in HD patients [25] and that its levels correlate significantly, independently and positively with serum Hep-25 and ferritin levels, suggesting that the two hepcidin isoforms share common metabolic and regulatory pathways in uraemia. A regulatory role of Hep-20, however, is unlikely because it has been shown that this isoform does not bind to ferroportin [36].
In short, the relationship between hepcidins and markers of iron status observed in our study are in line with and expand on the reports that hepcidin levels respond to (or modulate) changes in iron status in HD patients on maintenance ESA and suggest that Hep-25 may be a useful new biomarker of iron-deficient erythropoiesis, as envisaged by others [16,17].

To ascertain whether measuring Hep-25 and Hep-20 may help to identify patients who will or will not have an improved Hb response to ESA after standard intravenous iron loading (a widely accepted reference standard for iron needs), we used the classical ROC curve analysis [27–30] and compared their diagnostic accuracy with that of established markers of iron status.

Neither Hep-25 nor Hep-20 proved capable of predicting response to intravenous iron, like the traditional markers of iron status, ferritin and TSAT, whereas %Hypo and CHr were significant predictors of Hb response to intravenous iron, as already demonstrated [28,29].

The best test for predicting iron responsiveness was %Hypo at a threshold of >6%, which achieved a good level of accuracy (84% [95% CI = 72–92%]), thanks to the combination of a fairly good sensitivity (76% [95% CI = 53–92%]) and an excellent false-positive rate (11% [95% CI = 3–27%]). CHr was less accurate (68% [95% CI = 54–80%]) (P = 0.05) due to a poor sensitivity (57% [95% CI = 34–78%]) and only a fair false-positive rate (25% [95% CI = 12–43%]) at its best threshold of <32 pg. The usefulness of %Hypo was confirmed by logistic regression analysis, which identified this test as the only marker associated with iron status.

We are aware that our study has some limitations. First, it is a single-centre study on a small sample of patients, so it may be underpowered for evaluating the role of the different biomarkers in predicting iron status. Our findings nonetheless confirm data from a previous study in a larger population of HD patients on ESA [28], by showing the same hierarchy of the tests for detecting iron-deficient erythropoiesis (%Hypo followed by CHr, TSAT and ferritin), and %Hypo as the only test with independent diagnostic value for iron responsiveness. In addition, the AUC values for Hep-25 and Hep-20 cannot rule out the possibility of them becoming significant predictors of iron status in larger cohorts of patients, but they would be unlikely to perform better than erythrocyte and reticulocyte indices. Second, the end-point we used to separate IR to NR (Hb increase >1 g/dL) may also be open to criticism, given the ample fluctuations in Hb levels often observed in HD patients on ESA [37]. This should not be a concern in our study, however, since the Hb levels prior to the iron loading were stable in both IR and NR patients, supporting the notion that the different Hb responses to the iron load underlie differences in iron needs.

**Conclusion**

In conclusion, our study suggests that Hep-25 and Hep-20 are primarily associated with iron stores and Hep-25 may also be involved in regulating iron availability for erythropoiesis in HD patients on maintenance ESA. In clinical practice, however, measuring their levels does not appear to help predict who will or will not have an improved Hb response to intravenous iron, since neither perform better than the traditional markers of iron status (ferritin and TSAT), and they fare worse than %Hypo, the test revealing the best degree of accuracy in predicting intravenous iron needs in these patients.

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**Conflict of interest statement.** None declared.

**References**


Impact of haemoglobin and erythropoietin dose changes on mortality: a secondary analysis of results from a randomized anaemia management trial

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Abstract

Background. Anaemia is a common complication of chronic kidney disease. A number of studies have identified an adverse association between haemoglobin (Hgb) variability and mortality. To date, no study has evaluated the impact of Hgb variability on mortality in the setting of a uniform Hgb target and erythropoiesis-stimulating agents (ESA) dosing strategy.

Methods. One hundred and fifty-four haemodialysis (HD) patients from a previous randomized anaemia management study were followed up for up to 6 years. The impact of Hgb variability and ESA dosing parameters on subsequent mortality risk were evaluated.

Results. More rapid rises in Hgb (Hgb deflectpos) and ESA dose increases were independently associated with mortality in multivariate analysis, whereas more rapid Hgb declines (Hgb deflectneg) and ESA dose decreases were not. Each gram per litre per week increase in Hgb deflectpos was associated with an adjusted hazard ratio (HR) of 1.23 (1.03–1.48), while for every 1000-unit increase in ESA dose, the adjusted HR was 1.12 (1.01–1.24). Factors associated with positive Hgb deflections...