Low-dose continuous iron therapy leads to a positive iron balance and decreased serum transferrin levels in chronic haemodialysis patients

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Abstract

Background. Iron balance is critical for adequate erythropoiesis, but its optimal therapeutic regimen remains to be defined. Continuous maintenance therapy with iron has been proposed for dialysis patients on recombinant human erythropoietin (rHuEpo) in the hope that the regimen is adequate and safe.

Methods. We determined serum ferritin, transferrin, transferrin saturation (TSAT), serum transferrin receptors, albumin and C-reactive protein (CRP) in a 3-year prospective study in 30 chronic haemodialysis patients on dialysis treatment for 132±111 months (18 males, 12 females; mean age 56±14 years). Beginning in the year 2000, they regularly received low-dose maintenance iron supplementation (i.v. iron gluconate 31.25 mg/week) for 12 months (Period 1 or first treatment phase), followed by a 6-month withdrawal (Period 2 or stop phase) and then by continuous maintenance iron therapy (i.v. iron gluconate 31.25 mg/week) for another 9 months (Period 3 or re-challenge phase).

Results. A significant increase in serum ferritin and TSAT was observed, with values exceeding 500 ng/ml and 50% in 10/30 (33%) and 7/30 (23%) of subjects, respectively, in Period 1, and in 11 and 5% in Period 3. A significant decrease in serum transferrin was documented during Period 1, followed by an increase in Period 2 and a decrease in Period 3. Serum albumin remained stable. Serum transferrin was always negatively correlated with ferritin ($r = -0.41$, $P < 0.001$) and weakly correlated with serum transferrin receptors ($r = 0.178$, $P < 0.05$), but was not correlated with serum albumin or CRP. Regression equations based on pre-treatment serum ferritin values were developed for predicting the value of serum ferritin at any time following the beginning of continuous iron supplementation. They fitted a linear relationship for males ($y = 81 + 21.5 \times \text{time}$) and for females ($y = 65 + 22 \times \text{time}$). Percentile charts for quantitative tracking of serum ferritin increases and decreases in patients have also been developed from values measured at different times. These charts show box-plot distributions of expected ferritin against time.

Conclusions. Even continuous low-dose maintenance iron therapy, with only 31.25 mg weekly over 1 year, cannot prevent the risk of iron overload in patients with moderate anaemia. Furthermore, this treatment is responsible for decreases in serum transferrin, unrelated to changes in serum albumin, possibly of concern for hypo-transferrinaemia as an independent risk factor for iron toxicity.

Keywords: ferritin; iron; peroxidative damage; transferrin; transferrin saturation; uraemia

Introduction

Iron balance is critical for adequate erythropoiesis in dialysis patients during treatment with recombinant human erythropoietin (r-HuEpo), but the optimal schedule of iron supplementation remains to be defined. Several studies have demonstrated that oral iron therapy cannot meet the demands of r-HuEpo-stimulated erythropoiesis in haemodialysis (HD) patients because of poor compliance and poor absorption, and also because of interaction with other drugs used concurrently [1]. Parenteral iron therapy has been demonstrated to be useful in improving r-HuEpo efficacy when administered in an intensive regimen (1000 mg in divided doses over 10 consecutive dialysis sessions) to severely iron-deficient uraemic patients; but repeated, need-based iron ‘pulse’ therapy produced unwanted ‘roller coaster’ changes in iron parameters as
well as in EPO requirements [2,3]. Furthermore, depending on the iron formulation, pulsing with low molecular weight iron salts raises the risk of transferrin oversaturation [4,5]. Current opinion suggests continuous parenteral iron replacement after a course of iron repletion, in the hope of improving anaemia management and saving costs by reducing dosage with recombinant r-HuEpo in uraemic patients on chronic dialysis. However, there is growing concern regarding the short- and long-term side effects and the efficacy and safety of aggressive continuous parenteral iron replacement [2,6].

As to acute iron toxicity, the risk is mainly due to serum transferrin saturation [4], which exhausts the iron-free transferrin fraction and contributes to the appearance of labile non-transferrin-bound iron forms, strongly suspected to release histamine by mast cell degranulation and to enhance peroxidative damage, eventually leading to occasional anaphylactoid reactions and a delayed arthralgia-myalgia syndrome [7]. The three main determinants of transferrin saturation (TSAT) following parenteral iron infusion are: (i) iron dosage, (ii) the parenteral iron formulation used and (iii) serum transferrin concentration. Unfortunately, serum transferrin is reported to be frequently low in uraemic people on chronic dialysis [8], thus increasing the risk of oversaturation. This hypotransferrinaemia is considered to be mainly due to a reduced synthesis, owing to the inflammatory status [9]. As to the risks of chronic iron toxicity, vasculopathy [10] and infections [11,12] are concerns.

The aim of our longitudinal prospective study was to evaluate the safety of a cautious approach to continuous maintenance iron therapy with a low dose of iron gluconate (31.25 mg weekly) in uraemic patients on chronic HD. Our goal was to determine whether such a protocol would achieve and maintain the target values of serum ferritin and TSAT indicated in our National Guidelines [13] of ‘Anaemia in dialysis patients’, which are: (i) iron dosage, (ii) the parenteral iron formulation used and (iii) serum transferrin concentration. Unfortunately, serum transferrin is reported to be frequently low in uraemic people on chronic dialysis [8], thus increasing the risk of oversaturation. This hypotransferrinaemia is considered to be mainly due to a reduced synthesis, owing to the inflammatory status [9]. As to the risks of chronic iron toxicity, vasculopathy [10] and infections [11,12] are concerns.

Subjects and methods

All 40 patients on intermittent HD for at least 6 months at our Self-Care Dialysis Centre were enrolled in the study. Informed consent was obtained from all.

Study design

Of the original cohort, 30 patients completed the 3-year study (18 males, 12 females; mean age 56 ± 5 years, on long-term HD from 132 ± 111 months; mean body weight 64 ± 13 kg); six of the others underwent kidney transplantation and four were transferred to other dialysis centres. Out of the 30 patients, 25 were on bicarbonate HD with low-flux cellulose or synthetic membranes, three on acetate-free biofiltration, and two on haemodiailfiltration (bag infusions 41/h); their dialysis schedules were not changed during the study. Dialysis adequacy was serially measured by Kt/V (calculated by the Casino formula), and was ≥1.2 for all patients for the duration of the study. No subject tested positive for HBsAg; eight tested positive for anti-HCV antibodies. The underlying renal diseases were the following: primary glomerular diseases (n = 15), polycystic kidney diseases (n = 3), vascular nephropathies (n = 3), tubular-interstitial nephropathies (n = 3), congenital malformations (n = 2), diabetic nephropathy (n = 1) and undiagnosed (n = 3).

The initial study protocol consisted of: Period 1 (priming phase), 12 months of continuous low-dose intravenous (i.v.) iron therapy; Period 2 (stop phase), 6 months with no iron supplementation; Period 3 (re-challenge phase), 12 months, resumption of i.v. iron therapy. However, due to the results obtained during Period 1, Period 3 was shortened to 9 months.

Iron therapy. Before the study, the patients had been treated with a need-based intermittent pulse regimen consisting of 625 mg of iron gluconate complex (‘Ferlixit’© 62.5 mg/ampoule; Nattermann and Cie GmbH, Colonia, Germany), administered in divided doses during 10 consecutive dialysis sessions (187.5 mg weekly) and repeated three to four times per year. Beginning in the year 2000, after a wash-out period of 3 months, we treated the subjects with low-dose supplementation by i.v. sodium ferric gluconate complex—31.25 mg dissolved in 20 ml saline infused over 15 min once a week at the end of HD. Patients received iron 1 week before blood was sampled for our assays.

The main aim of the study was to test the hypothesis that 31.25 mg/week of iron was an adequate maintenance iron therapy, able to offset iron losses and maintain target haemoglobin. The secondary aim of this study was to determine if serum transferrin would be decreased by continuous iron supplementation.

Erythropoietin therapy. rHuEPO (epoetin alfa) was administered as subcutaneous injections at the end of HD sessions in doses ranging from 500 to 12 000 U/week (median dose, 4000 U).

Laboratory examinations. Serum ferritin, iron and transferrin concentrations were evaluated by, respectively, immunofluorometric assay, iron FZ assay (based on guanidine hydrochloride/ferrozine reaction) and nephelometric assay. TSAT was calculated by the formula: iron ([µg/dl]/transferrin (mg/dl) × 1.4]. Standard haematologic parameters were measured by an analyzer (ADVIA 120; Bayer Diagnostics, Germany) and serum albumin by an automatic counter. Soluble transferrin receptors (sTfR) were measured by commercially available ELISA (R&D Systems Inc, Minneapolis, MN). C-reactive protein (CRP) was measured by the nephelometric method (Dade Bohringer, Germany).

Statistics

Intra- and inter-assay variation coefficients for all laboratory indices for iron status were <5%. Statistical analyses were performed on data from 30 patients who completed the
study. All data are expressed as mean±standard deviation, unless otherwise indicated. The $x^2$ test, Student's t-test, analysis of variance (ANOVA) and Sheffé tests were conducted to determine associations between variables. The Pearson correlation coefficient was employed when indicated, and regression equations were calculated. A $P$-value <0.05 was considered as statistically significant.

Results

The main biochemical parameters at baseline and during different phases of the study are shown in Table 1.

At the beginning of the study, the patients were in the wash-out phase, lasting 3 months after the final dose of their last pulsed course. All of them had serum ferritin values within the normal range of our laboratory (25–340 ng/ml for men, 15–150 ng/ml for women). The mean values of serum ferritin progressively increased during the 12-month Period 1 from 123±60 ng/ml to 384±200 ng/ml ($P<0.001$; Figure 1A). All patients but one achieved serum ferritins $>$100 ng/ml, but values $>$500 ng/ml were observed in 10/30 (33%) of the patients. TSAT significantly increased from 25±12.1% to 37±19% ($P=0.02$; Figure 2A), and values $>$50% were observed in 7/30 (23%) of the patients.

Table 1. Main biochemical and clinical parameters at baseline and during the different phases of the study of the 30 chronic dialysis patients

<table>
<thead>
<tr>
<th></th>
<th>Start T0</th>
<th>First-treatment Phase end T1</th>
<th>Stop Phase end T2</th>
<th>Re-challenge Phase end T3</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.4±1.1</td>
<td>10.9±1.4</td>
<td>10.9±1.2</td>
<td>10.8±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td>123±109</td>
<td>384±200</td>
<td>190±109</td>
<td>317±325</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum transferrin (mg/dl)</td>
<td>209±39</td>
<td>156±36</td>
<td>181±43</td>
<td>162±29</td>
<td>0.008**</td>
</tr>
<tr>
<td>Serum iron (µg/dl)</td>
<td>61±30</td>
<td>78±36</td>
<td>59±27</td>
<td>64±24</td>
<td>NS</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>25±12</td>
<td>37±19</td>
<td>27±17</td>
<td>29±11</td>
<td>0.02***</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>3.6±0.36</td>
<td>3.7±0.34</td>
<td>3.5±0.32</td>
<td>3.6±0.42</td>
<td>NS</td>
</tr>
<tr>
<td>Patients on r-HuEPO (%)</td>
<td>25 (83%)</td>
<td>25 (83%)</td>
<td>25 (83%)</td>
<td>25 (83%)</td>
<td>NS</td>
</tr>
<tr>
<td>r-HuEPO admin. (UI/week)</td>
<td>3300±1922</td>
<td>3583±2320</td>
<td>3375±1527</td>
<td>3545±1595</td>
<td>NS</td>
</tr>
</tbody>
</table>

Sheffé test identified a statistically significant difference between:
*T0–T1, T1–T2, T0–T3. **T0–T1. ***T0–T1.

Fig. 1. Longitudinal behaviour of serum concentration of ferritin (A, solid circles), transferrin (B, solid circles) and albumin (B, open squares) in a 3-year study in 30 dialysis patients during the following phases of the study: first treatment phase with low-dose (31.25 mg/week) i.v. iron supplementation (Period 1), stop phase (Period 2) and re-challenge phase (Period 3).
During Period 2, serum ferritin and TSAT decreased to 190±109 ng/ml (P < 0.001) and 27±17%, respectively, with values <100 ng/ml found in only two patients, one of them persistently low. Serum ferritin and TSAT increased again to 317±315 ng/ml and to 29±11% during Period 3 (Figures 1A and 2A). Notwithstanding the shortened Period 3, modified because of the unexpectedly high percentage of patients who exceeded the target values for ferritin and TSAT during Period 1, those target values were again exceeded in 4/30 (11%) and in 2/30 (5%) patients, respectively. At baseline, 8/30 (27%) patients had serum transferrins below our normal range (200–300 mg/dl). Serum transferrin concentrations progressively decreased during Period 1 from 209±39 mg/dl to 156±36 mg/dl, increased to a maximum of 181±43 mg/dl during Period 2 and decreased again to 162±29 mg/dl during Period 3 (Figure 1B; P = 0.008). At the peak, 90% (27/30) of patients had serum transferrins below our normal range, but that percentage decreased to 67% (19/30) after iron withdrawal in Period 2.

In contrast, serum albumin concentrations remained stable (3.6±0.36 g/dl to 3.6±0.42 g/dl) throughout the study (Figure 1B). CRP values were determined once a year and did not show significant changes: 6.6±6.4 mg/l to 6.8±8 mg/l (P = 0.098; normal values <3.75 mg/l). Serum iron concentrations did not change significantly: from 61±30 µg/dl to a maximum of 78±36 µg/dl at the end of Period 1, to a minimum of 59±27 µg/dl during Period 2 and to 64±24 µg/dl in Period 3 (Figure 2A). Haemoglobin values remained without significant change (10.4±1.13 to 10.8±1.3 g/dl) throughout the study (Figure 2B), requiring 15% increases of r-HuEpo doses in only three patients. Measurements of sTfR (normal range 8.7–28.2 mmol/l) showed significant changes between the ends of Period 1 (19.4±10.2 mmol/l), Period 2 (25.2±10.2 mmol/l, P < 0.01) and Period 3 (21.9±15.7 mmol/l).

Among the biochemical parameters, serum transferrin was negatively related to serum ferritin concentration (r = -0.409, P < 0.0001) and weakly related to sTfR (r = 0.178, P < 0.05). No significant correlation was found between serum transferrin and serum albumin (r = 0.072, P = 0.194) nor between serum transferrin and CRP (r = -0.110, P = 0.44). No significant correlation was found between serum albumin and CRP (r = 0.027, P = 0.118) or between serum ferritin and CRP (r = -0.229, P = 0.10).

Linear regression was used to predict the value of serum ferritin at any point in time (month) following the start of continuous i.v. iron supplementation given a pre-treatment serum ferritin value. The slope of the increase fitted a linear relationship for males (y = 81 + 21.5 × time) and females (y = 65 + 22 × time). When the increase in serum ferritin was evaluated separately for patients having baseline serum ferritins
below and above 100 ng/ml, the \( \beta \) coefficient was slightly higher for lower baseline values (\( y = 21 + 23.3 \times \text{time} \) and \( y = 130 + 20.1 \times \text{time} \), respectively).

Linear regression analysis was also employed for predicting the value of serum ferritin at a given point in time (month) after withdrawal of iron therapy. The slope of the decrease in serum ferritin values fitted a linear relationship for males (\( y = 370 - 17.6 \times \text{time} \)) and females (\( y = 390 - 15 \times \text{time} \)). When the decrease in serum ferritin was evaluated separately for patients having baseline serum ferritin levels below and above 400 ng/ml, the \( \beta \) coefficient was higher for higher baseline values (\( y = 589 - 37.2 \times \text{time} \), and \( y = 241 - 18 \times \text{time} \), respectively).

We developed percentile charts for quantitative tracking of serum ferritin increases and decreases observed by measuring serum values of patients at different times. These charts show box-plot distributions of expected ferritin values at various points in time. On the basis of the data on our patients, the expected increases in serum ferritin following continuous low-dose i.v. supplementation (31.25 mg/week of iron gluconate) are depicted in Figure 3A. The projected decrease in serum ferritin value following iron withdrawal is depicted in Figure 3B. Both charts have the outer-limits of their curves at the 0 and 100 percentiles, while the 50th percentile line represents the average value for a given point in time.

Discussion

The policy of iron treatment at our Centre has changed over time. Initially, in the 90s, our protocol consisted of intensive, intermittent need-based iron therapy (625 mg i.v. iron gluconate in repeated doses of 62.5 mg over 10 consecutive dialysis sessions), but that has been abandoned because of the unpredictable ‘roller coaster’ changes in iron parameters as well as of EPO doses required [9], and because of the risk of transferrin oversaturation [10]. The second step was chronic maintenance low dose therapy, but in this study we have demonstrated that could still result in excessive and unwanted increases in serum ferritin and TSAT in roughly one-third of our patients on chronic dialysis, although the dosage employed (31.25 mg i.v. iron gluconate/week) was low, and well within the range suggested by the European Best Practice Guidelines [13], National Italian Guidelines [14] and NKF-DOQY guidelines [15].

Therefore, the obvious conclusion of our study is that, even with low dosage, continuous iron supplementation leads to positive iron balance instead of a steady state, in which i.v. replacement would offset external blood loss during HD. The above-mentioned guidelines variously suggest maintenance iron therapy doses ranging from 25 to 100 mg/week [13], 30 mg/week [14] and 25-125 mg/week [15] to offset iron losses common in dialysis patients, including 15–25 ml of whole blood at each dialysis (60 ml/week) due to blood retention in the dialyzer and tubing, blood testing, and gastrointestinal losses. The estimated iron losses in our patients, who had a mean haematocrit value of 33%, were an average of 28 mg iron/week (4 mg/day + 2.8 mg/day for dialysis and blood testing + ~1 mg/day via gastrointestinal routes). Considering other blood losses possible over time, such as vascular access surgery, additional gastrointestinal bleeding, or menstruations in women, it was logical to hypothesize that a continuous infusion of 31.25 mg of iron/week might be adequate to maintain a steady state. However, the results of our studies, which demonstrated that was not the case, and in addition, because more iron (75 mg) had been used during Period 1 to increase haemoglobin values by 0.5 g/dl, forced us to re-evaluate iron losses, which apparently are less than theoretically estimated.

Another important point to be considered is that our patients had mean haemoglobin values at the low end of the therapeutic target range currently recommended.
(10.9 g/dl, with an average hematocrit of 33%). If r-HuEpo had been used to achieve target haematocrit levels of 36%, iron demand would have been higher. In fact, the amount of iron needed to add 1 g/dl to the circulating haemoglobin would have increased by 150 mg. Furthermore, constant blood loss from a higher haematocrit (36 instead of 33%) would have resulted in an increased iron loss, with an average of 31.5 mg/week (4.5 mg/day for dialysis and blood testing + ~1 mg/day for gastrointestinal loss). Over the year in which our treatment schedules resulted in a total iron input of 1.625 mg, iron output would have had to be 1788 mg (1638 mg losses + 150 mg needed to increase haemoglobin from 11 g/dl to 12 g/dl). Therefore, our conclusions only apply to patients with target mean haemoglobin values at 10.9 g/dl.

The second important conclusion to be drawn from our study is that our low-dose, continuous iron therapy was associated with an inhibition of serum transferrin synthesis, which was partially reversible after iron withdrawal. Serum transferrin is a serum β-globulin that binds and transports iron. Serum transferrin and soluble as well as cellular transferrin receptors regulate iron uptake from cells, and their synthesis is up- and down-regulated by the intracellular iron status [16]. Therefore, since it is well known that an inverse relationship exists between ferritin and transferrin (due to the role of iron stores in gene transcription), our finding of a decrease in serum transferrin levels in response to i.v. iron comes as no surprise. Notwithstanding theoretical premises, however, prospective clinical studies confirming these assumptions in patients on chronic dialysis were lacking, and our study first planned a prospective protocol that showed the relationship between iron supplementation, serum ferritin concentration, and transferrin production.

Furthermore, serum transferrin concentration depends on inflammation, hepatic synthesis and nutrition; and transferrin synthesis is inhibited by inflammatory cytokines. Therefore, inflammation has been considered as the main cause of hypo-transferrinaemia in dialysis patients, for uraemia is regarded as a continuous inflammatory state. However, in our patients on chronic dialysis at a Self-Care Centre, who were stable and in good clinical condition with low grade chronic inflammation (as confirmed by the median CRP values of 3.7 mg/gl, the normal value being <3.7 mg/gl), the role of inflammation in inducing hypo-transferrinaemia was not evident, for statistically significant relationships between transferrin and albumin or CRP were lacking, and the only negative relationship of transferrin was with serum ferritin.

Therefore, hypo-transferrinaemia in clinically stable HD patients is not explained only by their inflammatory status, because transferrin values are disproportionately low compared to albumin concentrations and, at least in part, result from the negative feedback provided by continuous iron replacement therapies. However, hypo-transferrinaemia is an independent risk factor for iron overload, because it is responsible for an increased percentage of non-transferrin-bound iron forms. Different iron formulations may have different properties, side effects or labile iron content [17], which could account for the different efficacy/safety ratios of different parenteral iron formulations; but, whichever iron formulation is employed, hypo-transferrinaemia plays a key role in iron toxicity. In fact, only the iron deficient form (apo-transferrin) is the mainstay of the antioxidant power of plasma. An increased oxidative stress has been demonstrated in iron-treated HD patients with elevated baseline serum ferritin levels [18]. Furthermore, it has been demonstrated that apo-ferritin is a potent inhibitor of bacterial adhesion (Staphylococcus epidermidis, Staphylococcus aureus and Pseudomonas aeruginosa) to biomaterials, including polystyrene, polymethylmethacrylate and silicone, thus indicating that hypo-transferrinaemia further increases the risk of infection [19].

Our results should not be taken as providing the wrong message: that i.v. iron administration should be avoided in renal patient receiving r-HuEpo, for several studies have shown that i.v. iron is more effective than oral iron in preventing iron deficiency and allowing lower doses of r-HuEpo to be used for achieving a target haemoglobin. Furthermore, our results do not imply that need-based intermittent schedules with high i.v. doses of iron over short periods are better. Previous ‘roller coaster’ regimens have been shown to be unsafe due to enhanced generation of oxidative stress, as well as being unpractical.

On the contrary, our results confirm the need for an alternative regimen for iron dosing; it has been previously suggested that ‘we should not be hesitant to look for alternative iron dosing regimens’ [2].

To allow patient-tailored regimens, we have developed regression equations and box-plot distributions, with the principal aim of offering a chart-based quantitative system for tracking potential iron overload over time. Our results should be confirmed in larger samples in order to optimize medical surveillance by graphing successive ferritin measurements and in the hope that such an estimation will contribute to improved surveillance efficiency. Optimization would take place in two ways: either by using the regression equations to predict monthly ferritin increases, or the charts to directly look up the expected increase or decrease of serum ferritin in dialysis patients. To exemplify, we can predict that, in the case of a baseline ferritin value of 130 ng/ml, a value of 370 ng/ml will be achieved after 12 months of continuous, low-dosed iron regimen (31.25 mg/week), but the majority of patients with baseline ferritin values of 300 ng/ml will exceed ferritin concentration of 500 ng/ml after 9 months of the same dosing regimen. As to a decrease, we can predict that it will take 6 months of iron withdrawal to decrease serum ferritin from 589 ng/ml to 367 ng/ml.

We conclude that the uninterrupted administration of 31.25 mg/week of iron gluconate over 1 year cannot avoid values of serum ferritin and TSAT above 500 ng/ml and 50%, respectively, in a third of patients on chronic dialysis with mean haemoglobin values of
10.9 g/dl. We also conclude that serum ferritin and TSAT are accompanied by depression in transferrin synthesis and, lastly, that these biochemical values may be looked upon as signs of iron overload, as demonstrated by the increased hepatic iron content [20].

Further studies are needed to clarify which alternative dosing regimens should be used: reducing the amount of a single dose, using longer dosing intervals (e.g. every 2 weeks), adopting ‘periodic’ maintenance instead of ‘low-dose continuous’ maintenance schedules, or taking into account also the need for individualized iron therapy.

Conflict of interest statement. None declared.

References

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