Neutrophil gelatinase-associated lipocalin (NGAL) reflects iron status in haemodialysis patients

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Abstract

Background. An iron deficiency is often present in haemodialysis (HD) patients; however, although transferrin saturation (TSAT) of <20% and/or serum ferritin of <200 ng/mL should express iron scarcity, in HD patients high ferritin levels could be related to inflammation rather than reflecting optimal iron stores.

Methods. The aim of the present study was to evaluate serum levels of neutrophil gelatinase-associated lipocalin (NGAL), a small siderophore-binding protein, in a cohort of 56 chronic HD patients in order to determine its possible relationships with iron status.

Results. NGAL levels were markedly higher in HD patients than in healthy controls; furthermore, HD patients with TSAT <20% had lower NGAL values than healthy controls, whereas the correction of iron deficiency by means of chronic i.v. iron administration significantly increased NGAL values from baseline. Findings from univariate and multivariate analyses demonstrated that NGAL was a significant predictor of hsCRP, spKT/V and TSAT. In ROC analysis, a NGAL cut-off level of ≤473 ng/mL had a greater sensitivity and specificity than a ferritin level of <200 ng/mL in identifying iron deficiency among HD patients.

Conclusions. The findings demonstrated that HD patients have altered NGAL values probably because this protein is involved in the maintenance of iron equilibrium. Finally, NGAL might be proposed as a new tool in the assessment of iron deficiency and in the management of iron therapy for HD patients.

Keywords: haemodialysis; iron deficiency; iron status; neutrophil gelatinase-associated lipocalin

Introduction

Haemodialysis (HD) patients often have iron deficiency as a consequence of frequent blood sampling, gastrointestinal bleeding, dietary restrictions and/or decreased enteral absorption. The Kidney Disease Outcomes Quality Initiative (K/DOQI) anaemia workgroups suggest that serum ferritin and the transferrin saturation (TSAT) should be considered primary tools in the assessment of iron status in nephropathic subjects: in particular, serum ferritin levels of <100 ng/mL in pre-dialysis chronic kidney disease (CKD) patients or <200 ng/mL in chronic HD patients, and/or TSAT values of <20% (in both groups) should reflect an underlying condition of low iron deposits, thus being suggested as cut-off values for deciding upon opportune therapeutic strategies [1].

However, a large body of recently obtained evidence has led to a re-evaluation of the role of serum ferritin as a reliable index of iron storage in HD patients. This protein is an acute-phase reactant, is markedly influenced by malnutrition and has important gender differences, thus making it a less than ideal tool for identifying iron deficiency. For example, the presence of inflammation can explain the apparently paradoxical coexistence of high ferritin (>500 ng/mL) and low TSAT (<25%) levels frequently found in HD patients. This condition, moreover, raises two main clinical dilemmas: whether the patient has iron deficiency true and proper and whether additional iron should be administered or not [2].

Although TSAT may also be influenced by concomitant conditions, such as reduced transferrin synthesis (due to chronic disease or malnutrition), inflammation or daily fluctuations, it appears to indicate iron stores in HD patients more reliably than serum ferritin [3].

Neutrophil gelatinase-associated lipocalin (NGAL) is a small 25 kDa stress protein mainly known for its capacity to bind siderophores, small hydrophobic molecules containing iron, transporting them inside the cells to activate cytoplasmic iron-dependent pathways thus protecting the same cell from oxidative stress [4].

Clinical nephrologists are already well aware of the usefulness of NGAL as a biomarker of acute kidney injury and chronic renal suffering [5], as well as a predictor of the progression of CKD [6]. However, to the best of our knowledge, no study has yet analysed NGAL levels in...
NGAL in HD patients

Table 1. Main clinical and laboratory data of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HD patients (n = 56)</th>
<th>Controls (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>32/24</td>
<td>10/8</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 14</td>
<td>56 ± 9</td>
<td>0.19</td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>43 (6–304)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>spKt/V (weekly mean)</td>
<td>1.37 ± 0.66</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCR (g/kg/day)</td>
<td>1.21 ± 0.40</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EPO dosage (IU/week)</td>
<td>8600 ± 3200</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>10.1 ± 2.2</td>
<td>0.9 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>160.5 ± 45.6</td>
<td>16.3 ± 4.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ca × P product (mg²/dL²)</td>
<td>44.1 ± 14.1</td>
<td>32.3 ± 2.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.3 ± 1.8</td>
<td>14.8 ± 1.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>33.9 ± 5.0</td>
<td>46.5 ± 4.8</td>
<td>0.01</td>
</tr>
<tr>
<td>ALPh (IU/L)</td>
<td>73 (52–157)</td>
<td>88 (42–112)</td>
<td>0.10</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>199 (54–538)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Erythrocytes (n × 10⁵)</td>
<td>3.72 ± 0.63</td>
<td>4.86 ± 0.51</td>
<td>0.01</td>
</tr>
<tr>
<td>White cells (n × 10⁹)</td>
<td>6.72 ± 1.78</td>
<td>7.2 ± 1.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.14 ± 0.25</td>
<td>4.06 ± 0.43</td>
<td>0.44</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>8 (3–52)</td>
<td>3.35 (0.09–0.54)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>β2-microglobulin (mg/dL)</td>
<td>23 (6–42)</td>
<td>0.12 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.92 ± 1.18</td>
<td>5.03 ± 0.77</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum iron (mg/mL)</td>
<td>65.8 ± 25.2</td>
<td>91.5 ± 28.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum transferrin (mg/dL)</td>
<td>187.1 ± 45.0</td>
<td>313.9 ± 74.3</td>
<td>0.01</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>22 (8–46)</td>
<td>37 (24–42)</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum ferritin (ng/mL)</td>
<td>162 (12–1038)</td>
<td>139 (35–190)</td>
<td>0.03</td>
</tr>
<tr>
<td>NGAL (ng/mL)</td>
<td>473 ± 61</td>
<td>42 ± 13</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

PCR, protein catabolic rate; hsCRP, high-sensitivity C-reactive protein; ALPh, alkaline phosphatase; TSAT, transferrin saturation.

Subjects with end-stage renal disease on chronic HD. The aim of the present pilot study was therefore to evaluate NGAL in a small cohort of patients in order to assess any relationships it may have with the iron balance, and its utility as a biomarker of iron deficiency.

Subjects and methods

Study cohort

The study was conducted on 56 chronic HD patients on regular treatment with standard bicarbonate dialysis (138 mmol/L Na, 35 mmol/L HCO3, 1.5 mmol/L K, 1.25 mmol/L Ca and 0.75 mmol/L Mg) by cuprophone or semisynthetic membranes (dialysis filters surface area, 1.1–1.7 m²) with a dialytic rhythm of 4-h sessions three times a week. All subjects had been dry-weight stable for at least 2 months before the study was started and had achieved a normotensive oedema-free state. The causes of end-stage renal disease calling for dialysis were primary: interstitial nephritis (n = 9), polycystic kidney disease (n = 7), glomerulonephritis (n = 14), chronic pyelonephritis (n = 10), diabetic nephropathy (n = 6), unknown (n = 10). All patients had been on recombinant erythropoietin therapy for at least 6 weeks and none had received intravenous iron administration or packed red cell transfusion in the 2 months preceding the start of the study. Exclusion criteria were the presence or a recent history of bleeding, malignancy, liver, thyroid or infectious diseases, alterations in leucocyte count or formula and/or treatment with steroids or immunosuppressors. The study was approved by the local ethics committee, and fully informed consent was obtained from all participants.

Laboratory measurements

Peripheral venous blood samples were taken in the mid-week interdialytic day. Biochemical parameters were measured in all patients, according to standard methods used in the routine clinical laboratory. Iron balance was assessed by measuring total serum iron, serum transferrin, serum ferritin and transferrin saturation (TSAT), calculated according to the following formula: (serum iron/serum transferrin) × 70.9. Neutrophil gelatinase-associated lipocalin (NGAL) was measured in the blood using the ELISA commercially available kit (Biopoint, Gentofte, Denmark), according to the manufacturer's instructions. The enzymatic reactions were quantified in an automatic microplate photometer. All measurements were made, in triplicate. NGAL levels were expressed as ng/mL. NGAL was also measured in a small group of 18 healthy subjects matched with HD patients for age and gender.

Statistical analysis

The statistical analysis was performed using the MedCalc (version 8.0) software and the GraphPad Prism (version 4.0) package. Data were presented as mean ± SD or median (IQR range) as appropriate. Differences between groups were determined using the unpaired t-test for normally distributed values and Kruskal–Wallis analysis followed by Dunn’s test for nonparametric values. The Pearson correlation coefficient was employed to test correlations between NGAL and the other variables considered in the study. Before testing correlations, all non-normally distributed values were log transformed to better approximate normal distributions. Multiple regression analyses were performed by constructing a model including all univariate correlates of NGAL in order to assess independent relationships. Data were expressed as partial correlation coefficients (β) and P-value. Receiver operating characteristics (ROC) analysis was employed to calculate the area under the curve (AUC) for NGAL and serum ferritin, and to find the best cut-off values for identifying a status of iron deficiency (TSAT<20%). The results were considered significant if the P-value was <0.05.

Results

HD patients and controls characteristics

The main characteristics of the study cohort are summarized in Table 1. The mean age of patients was 54 ± 14 years. The median value for dialysis vintage was 43 months (range 6–304) and the mean weekly spKt/V was 1.37 ± 0.66. Surrogate indexes of nutritional status yielded satisfactory values (protein catabolic rate: 1.21 ± 0.40 g/kg/day; albumin: 4.14 ± 0.25 g/dL). Inflammatory markers, such as β2-microglobulin and hsCRP, as well as uric acid and serum ferritin, were higher than in healthy controls, whereas the
Table 2. Differences in main clinical and laboratory parameters between patients with (TSAT <20%) or without (TSAT >20%) iron deficiency

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TSAT &lt;20% (n = 23)</th>
<th>TSAT ≥20% (n = 33)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>10/13</td>
<td>22/11</td>
<td>−</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 ± 15</td>
<td>54 ± 14</td>
<td>0.56</td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>42 (6–236)</td>
<td>50 (9–304)</td>
<td>0.03</td>
</tr>
<tr>
<td>PCR (g/kg/day)</td>
<td>1.24 ± 0.20</td>
<td>1.29 ± 0.17</td>
<td>0.23</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>163.5 ± 46.2</td>
<td>166.6 ± 44.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Ca × P product (mg²/dL²)</td>
<td>43.3 ± 14.6</td>
<td>42.2 ± 13.6</td>
<td>0.13</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.8 ± 1.2</td>
<td>11.6 ± 1.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>33.4 ± 4.1</td>
<td>34.4 ± 4.6</td>
<td>0.08</td>
</tr>
<tr>
<td>ALPh (IU/L)</td>
<td>69 (54–107)</td>
<td>80 (59–288)</td>
<td>0.03</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>199 (46–499)</td>
<td>228 (43–538)</td>
<td>0.04</td>
</tr>
<tr>
<td>Erythrocytes (n × 10⁶)</td>
<td>3.66 ± 0.65</td>
<td>3.77 ± 0.62</td>
<td>0.06</td>
</tr>
<tr>
<td>White cells (n × 10⁶)</td>
<td>6.72 ± 1.48</td>
<td>6.75 ± 1.93</td>
<td>0.13</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.14 ± 0.17</td>
<td>4.15 ± 0.22</td>
<td>0.42</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>11 (4–52)</td>
<td>8 (3–17)</td>
<td>0.04</td>
</tr>
<tr>
<td>β2-microglobulin (mg/dL)</td>
<td>28 (8–42)</td>
<td>19 (7–36)</td>
<td>0.03</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>6.24 ± 1.27</td>
<td>5.65 ± 1.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum iron (mcg/mL)</td>
<td>45.9 ± 10.5</td>
<td>79.6 ± 23.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum transferrin (mg/dL)</td>
<td>173.2 ± 30.1</td>
<td>207.4 ± 55.2</td>
<td>0.02</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>17 (9–18)</td>
<td>29 (22–46)</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum ferritin (ng/mL)</td>
<td>61 (15–377)</td>
<td>276 (21–1025)</td>
<td>0.02</td>
</tr>
<tr>
<td>NGAL (ng/mL)</td>
<td>458 ± 58</td>
<td>491 ± 80</td>
<td>0.04</td>
</tr>
</tbody>
</table>

PCR, protein catabolic rate; hsCRP, high-sensitivity C-reactive protein; ALPh, alkaline phosphatase; TSAT, transferrin saturation.

Fig. 1. Scatterplot of NGAL values in HD patients with (TSAT <20%) or without (TSAT >20%) iron deficiency (P = 0.04)

NGAL levels and iron treatment

The patients with TSAT <20% underwent chronic intravenous administration of sodium ferric gluconate (62.5 mg) 10 min after the end of the dialysis session (mean, twice per week), until adequate TSAT values (>20%) were reached. The correction of iron status increased serum iron, serum ferritin and TSAT values. Furthermore, a slight reduction in serum transferrin levels was observed, although this change was not statistically significant (P = 0.14). Finally, the normalization of iron status led to a statistically significant increase in serum NGAL values from baseline (458 ± 58 versus 491 ± 80 ng/mL; P = 0.04, see Figure 1). Table 2 shows the main differences between the two groups.

Iron balance in HD patients

HD patients were sub-divided into two different groups on the basis of the presence or absence of iron deficiency, defined as a TSAT <20%. According to this classification, 23 patients (41%) belonged to the low-TSAT group, whereas the remaining 33 (49%) presented an apparently good iron status (TSAT >20%). Interestingly, among HD patients with low TSAT, only 15 subjects (65%) simultaneously fulfilled the serum ferritin criteria for low iron deposits (<200 ng/mL).

Univariate correlations of NGAL and multiple regression analysis

In univariate analysis, NGAL was directly correlated with hsCRP (R = 0.34; P = 0.01), serum ferritin (R = 0.30; P = 0.02), TSAT (R = 0.29; P = 0.04), spKt/V (R = 0.41; P = 0.04) and dialysis vintage (R = 0.30; P = 0.02), whereas a significant inverse correlation was found with serum transferrin (R = −0.31; P = 0.02). In contrast, no significant correlation was found between NGAL and the
Fig. 2. Univariate relationships (Pearson’s coefficient) of NGAL in HD patients. Significant correlations were shown with hsCRP (A), serum ferritin (B), TSAT (C), serum transferrin (D), dialysis vintage (E) and spKt/V (F).

Table 3. Changes in main iron parameters of HD patients with TSAT <20% after correction of iron deficiency

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After iron correction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (mcg/mL)</td>
<td>45.9 ± 10.5</td>
<td>70.4 ± 11.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum transferrin (mg/dL)</td>
<td>173.2 ± 30.1</td>
<td>159.5 ± 32.2</td>
<td>0.14</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>17 (9–18)</td>
<td>24 (21–26)</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum ferritin (mg/dL)</td>
<td>61 (15–377)</td>
<td>110 (80–566)</td>
<td>0.02</td>
</tr>
<tr>
<td>NGAL (ng/mL)</td>
<td>458 ± 58</td>
<td>501 ± 62</td>
<td>0.04</td>
</tr>
</tbody>
</table>

TSAT, transferrin saturation.

Other clinical and laboratory parameters such as age, gender, haemoglobin, haematocrit and white or red cells (R range = 0.06–0.18, P > 0.06). Figure 2 summarizes these findings

All variables found to be significantly related to NGAL in univariate analysis were introduced in a multivariate model using NGAL as a dependent variable. As TSAT represents a function of serum transferrin, this last variable was not included in order to avoid collinearity.

After adjustment for other factors, significance was maintained for the correlation between NGAL and TSAT (β = 0.29; P = 0.04), hsCRP (β = 0.39; P = 0.005) and spKt/V (β = 0.41; P = 0.01). In contrast, the correlations with dialysis vintage and serum ferritin, found in univariate analysis, were lost. Interestingly if hsCRP had not been included in the model, serum ferritin would have again become a significant predictor of NGAL levels (β = 0.20; P = 0.04), thus suggesting that the univariate correlation between these two parameters was strictly influenced by inflammation, rather than by iron status. Of note, the multivariate model explained ~27% of the total variance of
ROC analysis

Considering the presence of iron deficiency (TSAT <20%) as a status variable, ROC analysis was employed to assess and compare the diagnostic potentials of NGAL and serum ferritin in identifying this condition among HD patients. The areas under the curve (AUC) for NGAL and serum ferritin were respectively 0.685 (95% CI 0.528–0.817) and 0.707 (95% CI 0.561–0.840); these areas were not significantly different ($P = 0.66$). The best NGAL cut-off value was $\leq 473$ ng/mL with a sensitivity of 66.5% (95% CI 44.5–86.8) and a specificity of 85.7% (95% CI 63.6–96.8). For serum ferritin, the best cut-off level was $\leq 254$ ng/mL with a sensitivity of 81.3% (95% CI 61.9–88.7) and a specificity of 47.6% (95% CI 25.7–70.2).

**Table 4.** Univariate and multiple regression analysis of NGAL in HD patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial $R$</th>
<th>$\beta$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Log) TSAT</td>
<td>0.29 ($P = 0.04$)</td>
<td>0.29</td>
<td>0.04</td>
</tr>
<tr>
<td>(Log) hsCRP</td>
<td>0.34 ($P = 0.01$)</td>
<td>0.39</td>
<td>0.005</td>
</tr>
<tr>
<td>spKt/V</td>
<td>0.41 ($P = 0.04$)</td>
<td>0.41</td>
<td>0.01</td>
</tr>
<tr>
<td>(Log) serum ferritin</td>
<td>0.30 ($P = 0.02$)</td>
<td>0.08</td>
<td>0.51</td>
</tr>
<tr>
<td>(Log) dialysis vintage</td>
<td>0.30 ($P = 0.02$)</td>
<td>0.13</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Dependent variable: NGAL; multiple $R = 0.52$, $R^2 = 27%$; $P = 0.01$. $\beta$, standardized coefficient of correlation.

Discussion

The findings made in the present study raise at least two points of discussion.

First, HD patients presented increased circulating NGAL levels with respect to healthy subjects. Although the main physiologic font of NGAL is represented by neutrophils (in accordance with its function as an innate anti-bacterial factor), it is now widely accepted that this protein is a true acute-phase factor that can be released by virtually almost every injured tissue, often becoming a marker of disease severity [7]. For instance, in subjects with CKD, increased serum and urinary NGAL levels correlate with residual renal function [8] and a single measurement of NGAL after treatments potentially detrimental to the kidney (e.g. contrast administration, cardiac surgery) becomes useful in the early prediction of incipient acute kidney injury [5,9]. In our HD patients, we can speculate that, overall, the up-regulation of NGAL belongs to a wide panel of responses to the systemic inflammation associated with chronic HD treatment; this would be supported by the strict, independent correlation found between NGAL and hsCRP levels. As reported for several other cytokines, extracorporeal treatments can induce the release of NGAL, probably as a stress response [10]. In our study, however, the influence of HD on NGAL balance seems to go beyond this simple aspect. Because a direct independent correlation was observed between NGAL and mean weekly spKt/V, the efficacy of HD treatment may significantly influence per se NGAL values. As this unexpected finding goes beyond the initial purpose of the present study, future specific examinations are eagerly awaited to clarify whether NGAL measurement may somehow be useful in predicting the adequacy of dialysis and, perhaps, in guiding the management of dialysis prescriptions.

In a recent murine model [11], NGAL tissue levels were shown to be markedly increased after different experimental models of induced anaemia had been achieved with, for example, phlebotomy, iron deprivation or phenylhydrazine administration; this protein may therefore play a physiological role during increased iron utilization and mobilization from stores. These observations prompted us to test the intriguing hypothesis that NGAL might also be involved in the maintenance of the iron balance in HD patients.

Findings reported effectively confirmed the opening hypothesis, as NGAL levels were found to be closely correlated with both TSAT and serum ferritin values, the two main laboratory references of iron stores proposed in the KDOQI guidelines [1]. However, if the relationship with TSAT remained significant also after multivariate corrections, the introduction of hsCRP in the model completely erased the correlation with serum ferritin; this could be indicative of a strong confounding effect played by inflammation, which supports the recent criticism concerning the effective utility of serum ferritin measurement in correctly evaluating iron status in HD patients with inflammation [12]. The direct involvement of NGAL in iron balance is further supported by other important observations. First, patients with TSAT levels below the optimal ‘suggested’ value of 20%, thus with presumed iron scarcity, presented significantly reduced NGAL levels compared to
NGAL in HD patients

undoubtedly of great potential, but the findings made in the present study are only preliminary. Therefore, no clinical application can be contemplated without, for instance, specifically evaluating the real effect of chronic inflammation on circulating NGAL levels, and making an effective cost-to-benefits analysis since rapid NGAL measurement incurs considerable economic costs. Likewise, further in-depth examinations are also required to ascertain whether NGAL measurement could be useful in guiding the management of iron therapy, as previously reported for other treatments [16].

Conflict of interest statement. None declared.

References


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others. Secondly, in these subjects, the correction of iron deficiency with chronic i.v. iron supplements induced a statistically significant increase in NGAL values, which reached levels very similar to those reported in HD patients with optimal initial iron storage. Thirdly, NGAL showed a good diagnostic power in identifying a status of iron deficiency (TSAT <20%) among all HD patients, as shown by ROC analysis. In this context, the discriminatory capacity of NGAL (using the best cut-off value of ≤473 ng/mL) was superior also to that of serum ferritin (assuming the cut-off as the ‘suggested’ value of <200 ng/mL) in terms of both sensitivity and specificity.

Recently, several new biomarkers have been investigated as potential tools in evaluating iron balance and deciding upon iron therapy in HD patients. Cullen et al. [13], for instance, observed that the diagnostic potential of the reticuloocyte haemoglobin content (CHR) and the percentage of hypochromic red cells (PHRC) were promising in the identification of low TSAT levels in 36 chronic HD patients. On considering our findings in relation to these parameters, NGAL was found to have a slightly greater sensitivity (66.5 versus 64%) and specificity (85.7 versus 77%) than PHRC, whereas the measurement of CHr appeared to be more accurate than NGAL in relation to both parameters.

As shown by Fernandez-Rodriguez et al. [14], the measurement of circulating levels of soluble transferrin receptor (TfR) seems to have a diagnostic ability similar to that of NGAL in identifying a status of iron deficiency (albeit with a lower specificity). However, unlike NGAL, in this study at its best cut-off value (2.6 mg/L), TfR was found to be less accurate than serum ferritin in diagnosing iron scarcity.

Serum pro-hepcidin is another promising factor directly involved in the maintenance of iron balance in HD and healthy subjects. In a recent study [15], in line with our findings for NGAL, it was found that HD patients with iron deficiency presented significantly lower pro-hepcidin levels than subjects with normal iron status, even if, overall, these levels did not differ from those of controls. Furthermore, similar to our findings, pro-hepcidin values increased significantly after iron deficiency had been treated with chronic i.v. therapy; this suggests that pro-hepcidin is also a potentially useful biomarker for the management and guidance of iron therapy in HD patients.

Indeed our study has some limitations. First, the study cohort was relatively small: our findings should be validated by studies on larger populations. Secondly, the patients enrolled were homogeneous, in particular with respect to dialysis prescription and clinical parameters, and this may have caused a bias, particularly in explaining the correlations between NGAL and adequacy of dialysis, and the inflammatory and metabolic state. Thirdly, the patients enrolled had a good overall nutritional status: as malnutrition is known to potentially alter TSAT values, as well as serum ferritin levels, the presence of this condition may have influenced the correlations between NGAL and iron parameters.

In conclusion, the potential use of NGAL measurement in the assessment of iron status among HD patients is undoubtedly of great potential, but the findings made in the present study are only preliminary. Therefore, no clinical application can be contemplated without, for instance, specifically evaluating the real effect of chronic inflammation on circulating NGAL levels, and making an effective cost-to-benefits analysis since rapid NGAL measurement incurs considerable economic costs. Likewise, further in-depth examinations are also required to ascertain whether NGAL measurement could be useful in guiding the management of iron therapy, as previously reported for other treatments [16].