Original Research Article

Hepcidin serum levels and resistance to recombinant human erythropoietin therapy in hemodialysis patients

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A B S T R A C T

Objective: The aim of this study was to analyze the factors that are associated with the response to erythropoiesis-stimulating agents (ESAs) and its association with hospitalization and mortality rates; to evaluate the serum hepcidin level and its associations with iron profile, inflammatory markers, ESA responsiveness, and mortality; and to determine independent factors affecting ERI and hepcidin.

Materials and methods: To evaluate a dose-response effect of ESAs we used the erythropoietin resistance index (ERI). Patients were stratified in two groups: nonresponders and responders (ERI > 15, n = 20, and ERI ≤ 15 U/kg/week/g per 100 mL, n = 153, respectively). Hematological data, hepcidin levels, iron parameters, inflammatory markers, hospitalization and mortality rates were compared between the groups. Multiple regression analysis was used to determine independent factors affecting ERI and hepcidin.

Results: C-reactive protein (CRP) (β = 0.078, P = 0.007), albumin (β = −0.436, P = 0.004), body mass index (β = −0.374, P < 0.001), and hospitalization rate per year (β = 3.017, P < 0.001) were found to be significant determinants of ERI in maintenance hemodialysis (MHD) patients. Inadequate dialysis was associated with higher ERI. Patients with concomitant oncological diseases had higher ERI (31.2 ± 12.4 vs 9.7 ± 8.1 U/kg/week/g per 100 mL, P = 0.002). The hepcidin level was 158.51 ± 162.57 and 120.65 ± 67.28 ng/mL in nonresponders and responders, respectively (P = 0.33). Hepcidin correlated directly with ERI, dose of ESAs, ferritin and inversely with Hb, transferrin saturation, and albumin. ERI (β = 4.869, P = 0.002) and ferritin (β = 0.242, P = 0.003) were found to be significant determinants of hepcidin in MHD patients. The hospitalization rate per year was 2.35 ± 1.8 and 1.04 ± 1.04 in nonresponders and responders, respectively (P = 0.011). The mean length of one hospitalization was 25.12 ± 21.26 and 10.82 ± 17.25 days, respectively (P = 0.012). Death occurred in 30% of the patients from the responders’ group and in 50% from the nonresponders’ group (P = 0.289). The mean hepcidin concentration of patients who died was 141.9 ± 129.62 ng/mL and who survived, 132.98 ± 109.27 ng/mL (P = 0.797).

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Conclusions: CRP, albumin, BMI, and hospitalization rate per year were found to be significant determinants of ERI in MHD patients. Inadequate dialysis was associated with higher epoetin requirements. There were no difference in patient mortality by ERI, but a significant difference in hospitalization rates and mean length of one hospitalization was revealed. A significant positive relation between hepcidin and ERI was revealed. ERI and ferritin were found to be significant determinants of hepcidin in MHD patients. Hepcidin was not related to mortality.

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1. Introduction

Resistance to erythropoiesis-stimulating agents (ESAs) has been observed in a considerable proportion of patients with chronic kidney disease (CKD) and it is associated with increased cardiovascular (CV) morbidity and all-cause mortality. ESAs improve the quality of life and reduce transfusion requirements; however, they have not been demonstrated to improve other adverse outcomes associated with anemia of CKD, such as CV disease and mortality. Conversely, recent clinical trials have raised important safety concerns about using ESAs, including an increased risk of death, CV events and stroke, particularly when ESAs are used in higher doses to target higher hemoglobin (Hb) and in hypo-responsive patients [1,2]. Besides, ESA therapy is expensive and leads to enormous costs for the health care system. Approximately 50% of total ESA costs are spent on 15% of patients requiring the highest dosage [3]. Therefore, strategies to reduce ESA resistance and to avoid unnecessary ESA usage are required.

Iron deficiency is an important cause of anemia and resistance to ESAs in CKD patients. The causes of iron deficiency are multifactorial. Some patients with CKD have true iron deficiency, other patients have “functional” iron deficiency, which results in ESA resistance in 10%–20% of cases and can occur even in the context of normal or increased body iron stores [4,5]. Hepcidin, a small antimicrobial peptide discovered in 2001, has turned out to be a key regulator of iron homeostasis [5-7]. Excess levels of hepcidin in patients with CKD especially in those receiving maintenance hemodialysis (MHD) are thought to contribute to anemia by decreasing iron availability from the diet and body stores. Hepcidin induces internalization and degradation of ferroportin-1 (Fp-1), which is a cellular iron exporter on enterocytes, macrophages, and hepatocytes. Many studies have reported a strong relationship between hepcidin and iron profile in MHD patients [8–10], whereas data on the relationship with the maintenance dose of ESAs and ESA resistance is controversial [11,12].

Recently, the role of hepcidin as a cardiovascular marker has gained considerable attention in the CKD population [5,13,14]. New data support the hypothesis that hepcidin may be involved in the progression of atherosclerosis, and its measurement may help to stratify individual risk of patients. It may be associated with the high rate of mortality and CV disease in MHD patients [15,16]. An increasing understanding about the molecular mechanisms governing iron homeostasis regulation and its disturbance in CKD may lead to improved diagnosis and therapeutic strategies for management of this patient population [17].

The aims of this study were to analyze the factors that are associated with the response to ESAs in MHD patients and its association with hospitalization rates and mortality; to evaluate serum hepcidin levels in MHD patients and to observe the correlation of serum hepcidin with conventional iron, inflammatory markers and ESA responsiveness; to determine independent factors affecting ERI and hepcidin; and to study whether hepcidin is related to all-cause mortality in MHD patients.

2. Materials and methods

The study was initiated on January 1, 2010, and included 173 adult patients who received MHD at least for 6 months from 5 MHD units. All patients used bicarbonate dialysate and had been subjected to regular MHD procedures for 3–4 h 3 times per week. Routine patient care and prescription of medications were practiced according to the local MHD patients care guidelines. Patients received ESAs at a dose aimed to maintain Hb between 100 and 105 g/L, according to our local renal anemia management algorithm at that time, which defined a target range of Hb 100–105 g/L. Iron was administered IV according to the local algorithm (Table 1) and suspended when the ferritin level was above 500 μg/L. However, transferrin

| Table 1 – A local algorithm for IV iron administration in MHD patients. |
|-----------------|-----------------|
| Ferritin (μg/L or ng/mL) | IV iron therapy |
| <100 | 100–500 mg per day, not exceed 1000 mg |
| 100–300 | Repeat ferritin test one week after the last dose of IV iron, if it is still less 100 mg/L, repeat the same treatment course |
| >300–500 | 100 mg of iron per week. Measure ferritin concentration every 3 months |
| >500 | 100 mg of iron every second week. Measure ferritin concentration every 3 months |
| | Suspend IV iron therapy. Measure ferritin concentration after 1–3 months |
saturation (TSAT) was not routinely performed in all MHD patients, so we determined a dose of iron according to ferritin concentration only. Data on demography, causes of renal failure, history of CV disease, diabetes mellitus, echocardiographic parameters, type of vascular access, dialysis vintage and treatment parameters, prescription of medications were collected. Precise documentation of the administered doses of ESAs and iron was available in all 5 dialysis facilities. The dose of ESAs (IU/kg/week) among all MHD patients was calculated, and laboratory values of follow-up patients were included each month for 12 months until December 31, 2010. For those patients receiving darbepoetin alfa, a conversion scale was applied (1 μg = 200 IU) to transform the dose into international units (IU). Predialysis blood samples were taken and routine laboratory assessments were performed in the local hospitals by standard laboratory techniques. Venous blood samples for complete blood count, iron parameters, hepcidin and markers of inflammation were collected in the morning before hemodialysis at standardized times after the last administration of therapies potentially altering iron status and hepcidin release: one week after the last dose of IV iron. To evaluate the dose-response effect of ESA therapy in order to normalize the amount of ESAs required depending on the severity of anemia we used the erythropoietin resistance index (ERI), calculated as weekly weight-adjusted dose of ESAs divided by the Hb level (grams per deciliter). The ERI was measured each month of 2010 and finally the mean value of 12 months was calculated. ERI ranging between 5 and 15 was considered to be acceptable while ERI of more than 15 was considered high [18,19]. We stratified patients in two groups according to the mean ERI value: nonresponders’ group (ERI > 15 U/kg/week/g per 100 mL, n = 20) and responders’ group (ERI ≤ 15 U/kg/week/g per 100 mL, n = 153).

We measured serum hepcidin levels in 50 MHD patients in January 2011. We decided to compare hepcidin levels in responders and nonresponders groups so the patients were selected randomly to the control group from the responders group (n = 30) and two groups of patients were matched for age and gender. Serum hepcidin measurements were performed for each patient (n = 50) by a mass spectrometry method (SELDI-TOF-MS) at the University of Birmingham, UK. Predialysis blood samples from participating patients were drawn, centrifuged at 2000 × g for 10 min at room temperature and stored at –80 °C before sending to the laboratory of the University of Birmingham. Concentrations of serum hepcidin were expressed as ng/mL. Correlation of serum hepcidin with conventional markers of anemia, iron metabolism, inflammatory and nutritional status, calcium-phosphorus metabolism, hepatic enzymes were evaluated. We measured hospitalization rate during the year 2010 and evaluated its correlation with ERI and hepcidin. The follow-up of outcomes included 48 months until December 31, 2013.

For statistical analysis we used Statistical Package for Social Science, version 20.0. For evaluation of continuous variables the statistical mean and standard deviation were used. Kolmogorov–Smirnov statistics were used to evaluate sample normality distribution. Comparison between groups was performed using the Student t test and Mann–Whitney U test. Spearman rank correlation was used to evaluate relationship between sets of data. Multiple regression analysis was used to determine independent factors affecting ERI and hepcidin. Significant values were considered when P < 0.05.

3. Results

The study included 173 MHD patients. The mean time from the start of dialysis to inclusion in the study was 4.6 ± 3.8 years. The cause of CKD was chronic interstitial nephritis in 36% of patients, glomerulonephritis in 18%, renal vascular disease in 12%, polycystic kidney disease in 12%, diabetes mellitus in 10%, systemic diseases in 6%, and other in 6%. As much as 11.6% (n = 20) of patients had resistance to ESA therapy (ERI > 15 U/kg/week/g per 100 mL). General characteristics of the patients can be seen in Table 2. When comparing the two groups of MHD patients, we found that nonresponders presented a significantly lower single pool Kt/V. Although they got significantly higher doses of ESAs and cumulative iron dose, these patients were more anemic, as shown by a significant decrease in erythrocytes, Hb, and Ht. Nonresponders also presented a decrease in albumin concentration and an increase in CRP. They had higher ferritin and hepcidin concentrations and lower body mass index although without statistical significance. As the significant difference in ferritin concentration was very expected, we divided patients into three groups on the basis of ERI and found a significant difference in ferritin concentrations. The highest serum ferritin levels were found in the nonresponders’ group (Fig. 1). However TSAT were not routinely performed in all MHD patients in 2010, so that variable could not be included in the analysis. Non responders group had significantly more patients with malignancy (26.1% vs. 5.8%, P = 0.003) and patients with diagnosed oncological disease, regardless of its localization and clinical course, had a higher ERI than the rest of the patients (31.2 ± 12.4 U/kg/week/g per 100 mL vs. 9.7 ± 8.1 U/kg/week/g per 100 mL, P = 0.002). Table 3 shows correlation of ERI with demographic and dialysis-related data, hematological parameters, iron metabolism, inflammatory and nutritional status, bone mineral status, hepatic enzymes and hospitalization parameters. While ERI was not correlated with iron parameters, such as MCV, MCH and ferritin in MHD patients, it showed a significant positive correlation with hepcidin (r = 0.349, P = 0.017) (Fig. 2). Among the inflammatory and nutritional status parameters, we found a significant correlation between BMI, CRB, albumin and ERI (Fig. 3). Multivariate analysis was performed to investigate the predictors of ERI in MHD patients among the variables that correlated with ERI in simple regression analysis. Among these variables, CRP, albumin, BMI, hospitalization rate per year were found to be significant independent determinants of ERI (Table 4).

Hepcidin serum concentration was evaluated in 50 patients: 20 nonresponders and 30 responders. The hepcidin concentration of the total population (n = 50) was 135.79 ± 115.52 ng/mL; 158.51 ± 162.57 ng/mL in the nonresponders’ group and 120.65 ± 67.28 ng/mL in the responders’ group. Hepcidin correlated directly with weekly dose of ESAs, ERI and ferritin, inversely with Hb, Ht, erythrocytes, TSAT and albumin concentrations (Table 5). Multivariate linear regression analysis of factors influencing hepcidin in MHD patients was
Table 2 - Demographic and dialysis-related data, treatment characteristics, cardiovascular and hematological parameters, iron metabolism, inflammatory and nutritional status, bone mineral status and hepatic enzymes in MHD responders and nonresponders.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total population (n = 173)</th>
<th>Nonresponders, ERI &gt; 15 (n = 20)</th>
<th>Responders, ERI ≤ 15 (n = 153)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic and dialysis-related data</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>63.19 ± 15.60</td>
<td>62.19 ± 15.73</td>
<td>63.5 ± 15.59</td>
<td>0.082</td>
</tr>
<tr>
<td>Males, %</td>
<td>51.2</td>
<td>43.2</td>
<td>53.7</td>
<td>0.475</td>
</tr>
<tr>
<td>Time on dialysis, min per week</td>
<td>682.2 ± 79.66</td>
<td>667.50 ± 118.44</td>
<td>685.63 ± 67.92</td>
<td>0.668</td>
</tr>
<tr>
<td>UF, kg</td>
<td>2.17 ± 0.84</td>
<td>2.04 ± 0.73</td>
<td>2.21 ± 0.87</td>
<td>0.404</td>
</tr>
<tr>
<td>Kt/V (single pool)</td>
<td>1.42 ± 0.18</td>
<td>1.35 ± 0.17</td>
<td>1.43 ± 0.17</td>
<td>0.039</td>
</tr>
<tr>
<td>CVC versus fistula, %</td>
<td>14.3</td>
<td>25</td>
<td>11.8</td>
<td>0.095</td>
</tr>
<tr>
<td>Diabetic patients, %</td>
<td>22.2</td>
<td>13</td>
<td>24.3</td>
<td>0.242</td>
</tr>
<tr>
<td>Malignancy, %</td>
<td>9.5</td>
<td>26.1</td>
<td>5.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Treatment characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESA dose, IU/week</td>
<td>6210.59 ± 5276.89</td>
<td>14,973.25 ± 4902.54</td>
<td>4168.80 ± 2588.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESA dose, IU/kg/week per 100 mL</td>
<td>88.65 ± 77.31</td>
<td>216.75 ± 72.89</td>
<td>58.81 ± 37.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular parameters</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>140.1 ± 17.06</td>
<td>141.10 ± 11.09</td>
<td>139.13 ± 17.65</td>
<td>0.617</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>76.34 ± 11.02</td>
<td>77.25 ± 10.18</td>
<td>75.05 ± 11.17</td>
<td>0.399</td>
</tr>
<tr>
<td>LVMM, g</td>
<td>260.36 ± 92.19</td>
<td>239.40 ± 50.37</td>
<td>264.47 ± 98.19</td>
<td>0.461</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>143.28 ± 51.57</td>
<td>141.51 ± 29.36</td>
<td>143.62 ± 55.11</td>
<td>0.912</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>49.60 ± 9.16</td>
<td>51.79 ± 9.58</td>
<td>49.16 ± 9.06</td>
<td>0.194</td>
</tr>
<tr>
<td>No. of antihypertensive medications</td>
<td>2.09 ± 1.71</td>
<td>2.13 ± 1.56</td>
<td>2.08 ± 1.75</td>
<td>0.901</td>
</tr>
<tr>
<td>Prescription of RAS inhibitors, %</td>
<td>48.2</td>
<td>53.8</td>
<td>47.1</td>
<td>0.657</td>
</tr>
<tr>
<td>Hematological data</td>
<td></td>
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<tr>
<td>Erythrocytes, ×10¹²/L</td>
<td>3.31 ± 0.32</td>
<td>3.0 ± 0.38</td>
<td>3.37 ± 0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>98.8 ± 9.8</td>
<td>91.1 ± 9.8</td>
<td>103.9 ± 5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>30.32 ± 3.23</td>
<td>28.24 ± 2.72</td>
<td>32.01 ± 2.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>94.86 ± 5.71</td>
<td>95.6 ± 5.9</td>
<td>94.34 ± 5.6</td>
<td>0.479</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>30.87 ± 2.2</td>
<td>31.11 ± 2.27</td>
<td>30.70 ± 2.21</td>
<td>0.556</td>
</tr>
<tr>
<td>Platelets, ×10⁹/L</td>
<td>223.66 ± 94.06</td>
<td>235.43 ± 81.82</td>
<td>220.95 ± 96.83</td>
<td>0.230</td>
</tr>
<tr>
<td>Iron metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative iron dose, mg per year</td>
<td>2668.04 ± 1300.69</td>
<td>3560.00 ± 1576.98</td>
<td>2504.88 ± 1138.62</td>
<td>0.003</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>283.1 ± 139.8</td>
<td>318.87 ± 168.8</td>
<td>260.16 ± 114.98</td>
<td>0.206</td>
</tr>
<tr>
<td>Hepcidin-25, ng/mL</td>
<td>135.79 ± 115.52</td>
<td>158.51 ± 162.57</td>
<td>120.65 ± 67.28</td>
<td>0.330</td>
</tr>
<tr>
<td>Inflammatory and nutritional status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.53 ± 6.37</td>
<td>24.83 ± 4.89</td>
<td>26.93 ± 6.62</td>
<td>0.067</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>36.39 ± 4.13</td>
<td>33.91 ± 4.12</td>
<td>37.98 ± 3.33</td>
<td>0.001</td>
</tr>
<tr>
<td>Protein, g/L</td>
<td>66.10 ± 4.25</td>
<td>64.64 ± 5.19</td>
<td>66.44 ± 3.97</td>
<td>0.130</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>18.17 ± 21.67</td>
<td>27.16 ± 29.24</td>
<td>12.39 ± 12.50</td>
<td>0.048</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.89 ± 1.12</td>
<td>4.78 ± 1.17</td>
<td>4.95 ± 1.10</td>
<td>0.638</td>
</tr>
<tr>
<td>Calcium-phosphorus metabolism</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Phosphorus, mmol/L</td>
<td>1.78 ± 0.38</td>
<td>1.82 ± 0.41</td>
<td>1.76 ± 0.38</td>
<td>0.640</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.26 ± 0.17</td>
<td>2.29 ± 0.13</td>
<td>2.22 ± 0.22</td>
<td>0.270</td>
</tr>
<tr>
<td>PTH, pmol/L</td>
<td>29.22 ± 25.67</td>
<td>25.44 ± 22.93</td>
<td>31.65 ± 27.41</td>
<td>0.412</td>
</tr>
<tr>
<td>Hepatic enzymes</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AST, U/L</td>
<td>20.15 ± 10.38</td>
<td>20.31 ± 11.96</td>
<td>20.12 ± 10.06</td>
<td>0.982</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>19.89 ± 12.49</td>
<td>19.51 ± 12.16</td>
<td>19.99 ± 12.51</td>
<td>0.770</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>97.08 ± 55.85</td>
<td>93.32 ± 43.99</td>
<td>97.94 ± 58.39</td>
<td>0.647</td>
</tr>
<tr>
<td>Hospitalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization rate per year</td>
<td>1.53 ± 1.5</td>
<td>2.35 ± 1.8</td>
<td>1.04 ± 1.04</td>
<td>0.011</td>
</tr>
<tr>
<td>Mean length of one hospitalization</td>
<td>16.22 ± 19.90</td>
<td>25.12 ± 21.26</td>
<td>10.82 ± 17.25</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values are mean ± SD, unless otherwise stated. UF, ultrafiltration; CVC, central venous catheter; LVMM, left ventricular myocardium mass; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; RAS, renin angiotensin aldosterone system; CRP, C-reactive protein; PTH, parathyroid hormone; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
performed. ERI and ferritin were found to be significant determinants of hepcidin in MHD patients (Table 6).

The hospitalization rate for the entire study population was 1.53 ± 1.5 hospitalizations per year. We found a significant difference in hospitalization rates according to ERI: 2.35 ± 1.8 hospitalizations per year in the nonresponders’ group and 1.04 ± 1.04 in responders (P = 0.011). The mean length of one hospitalization was 16.22 ± 19.90 days (range, 1–77), 25.12 ± 21.26 days in the nonresponders’ group and 10.82 ± 17.25 days in the responders’ group (P = 0.012).

The follow-up of outcomes (n = 50) included 48 months until December 31, 2013. There were no differences in mortality of MHD patients according to ERI and hepcidin concentration during this period. Death occurred in 30% (n = 9) of the patients from the responders’ group and in 50% (n = 10) of the nonresponders’ group (P = 0.289). The mean serum hepcidin concentration of the patients who died was 141.9 ± 129.62 ng/mL, and the mean serum hepcidin concentration of the alive patients was 132.983 ± 109.27 ng/mL (P = 0.797).

4. Discussion

Anemia is caused by a variety of mechanisms in CKD, including erythropoietin deficiency, resistance to ESAs, impaired iron metabolism and its clinical management remains challenging.

Data about anemia control in Lithuania has been collected since 1996. Changes of anemia control depending on local conditions and protocols were evaluated till nowadays and published by Žiginskiené et al. [20]. Improvement of anemia control during this period was associated with the improvement of the quality of MHD in Lithuania. The mean Hb concentration increased from 92 ± 15.4 to 107 ± 13.6 g/L, and the percentage of patients with Hb > 100 g/L increased from 27.5% in 1997 to 68.2% in 2010. However the percentage of hyporesponsiveness to ESAs in Lithuanian MHD patients and the reasons of that were not evaluated. So the aim of our recent study was to analyze the main factors which are associated with the response to ESAs in patients on MHD, its influence on hospitalization rates and mortality.

A chronic inflammatory state has been shown to be involved, in part, as a cause of these processes [4,5,21–23] and response to ESAs was related to CRP and albumin as the markers of inflammation in our study. A decrease in albumin and an increase in CRP levels was accompanied by an increase in ERI. Patients with the highest ERI had the highest ferritin values in part as the marker of inflammation status as well. Chronic inflammation and cytokines can worsen anemia by shortening erythrocyte life span, inducing apoptosis of erythroid precursors and directly inhibiting erythroid progenitor proliferation [4,5]. As far as we know, we are the first in Lithuania to show an association between hepcidin and renal anemia in MHD patients. Hepcidin levels in CKD patients have the strongest correlation with serum ferritin [5,9,12,17,24,25], but are also influenced by inflammation, iron administration, estimated glomerular filtration rate, dialysis clearance, ESA dose and Hb [5,8,12,17,24] which explains the interest about hepcidin in nephrology and especially in the setting of renal anemia. Hepcidin is the key regulator of iron

![Fig. 1 - Differences in ferritin concentrations according to erythropoietin resistance index groups in 2010. Boxes show range of ferritin and horizontal line inside the boxes indicates mean value. Higher serum ferritin levels are shown in nonresponders group. ^ ^ P < 0.05.](image)
Table 3 – Correlation of ERI with demographic and dialysis-related data, hematological parameters, iron metabolism, inflammatory and nutritional status, bone mineral status, hepatic enzymes and hospitalization parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r</th>
<th>P</th>
<th>Unstandardized B coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic and dialysis-related data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>−0.061</td>
<td>0.495</td>
<td></td>
</tr>
<tr>
<td>Time on dialysis, min per week</td>
<td>−0.067</td>
<td>0.451</td>
<td></td>
</tr>
<tr>
<td>Kt/V (single pool)</td>
<td>−0.048</td>
<td>0.598</td>
<td></td>
</tr>
<tr>
<td>UF, kg</td>
<td>−0.102</td>
<td>0.284</td>
<td></td>
</tr>
<tr>
<td><strong>Hematological data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes, ×10¹²/L</td>
<td>−0.444</td>
<td>&lt;0.001</td>
<td>−12.84 (−16.98; −8.701)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>−0.537</td>
<td>&lt;0.001</td>
<td>−1.29 (−1.704; −0.876)</td>
</tr>
<tr>
<td>Platelets, ×10⁹/L</td>
<td>−0.031</td>
<td>0.732</td>
<td></td>
</tr>
<tr>
<td><strong>Iron metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV, fl</td>
<td>0.028</td>
<td>0.779</td>
<td></td>
</tr>
<tr>
<td>MCH, pg</td>
<td>−0.102</td>
<td>0.303</td>
<td></td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>0.057</td>
<td>0.524</td>
<td></td>
</tr>
<tr>
<td>Hepcidin, ng/mL</td>
<td>0.349</td>
<td>0.017</td>
<td>0.032 (0.006; 0.059)</td>
</tr>
<tr>
<td>Cumulative iron dose, mg per year</td>
<td>0.100</td>
<td>0.442</td>
<td></td>
</tr>
<tr>
<td><strong>Inflammatory and nutritional status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>−0.344</td>
<td>&lt;0.001</td>
<td>−0.368 (−0.598; −0.137)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.217</td>
<td>0.015</td>
<td>0.148 (0.083; 0.213)</td>
</tr>
<tr>
<td>Protein, g/L</td>
<td>−0.09</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>−0.181</td>
<td>0.044</td>
<td>−0.640 (−0.994; −0.286)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>−0.04</td>
<td>0.661</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium-phosphorus metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>−0.026</td>
<td>0.776</td>
<td></td>
</tr>
<tr>
<td>Phosphorus, mmol/L</td>
<td>0.144</td>
<td>0.109</td>
<td></td>
</tr>
<tr>
<td>PTH, pmol/L</td>
<td>−0.077</td>
<td>0.398</td>
<td></td>
</tr>
<tr>
<td><strong>Hepatic enzymes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, U/L</td>
<td>−0.067</td>
<td>0.461</td>
<td></td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>−0.06</td>
<td>0.508</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>−0.057</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td><strong>Hospitalization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization rate per year</td>
<td>0.459</td>
<td>&lt;0.001</td>
<td>3.725 (2.73; 4.74)</td>
</tr>
<tr>
<td>Mean length of one hospitalization</td>
<td>0.406</td>
<td>&lt;0.001</td>
<td>0.25 (0.17; 0.327)</td>
</tr>
</tbody>
</table>

UF, ultrafiltration; CRP, C-reactive protein; PTH, parathyroid hormone; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Fig. 2 – Relationship between erythropoietin resistance index and serum hepcidin.
transport, responsible for intestinal absorption as well as iron release from the reticuloendothelial system, by its action on Fp-1, which is the only known mammalian iron export protein responsible for iron entry into the bloodstream [17]. The assessment of iron needs for erythropoiesis in MHD patients is cumbersome. Ferritin levels and TSAT are widely used to assess iron status, but have limited sensitivity and specificity in the MHD patients’ population. Determination of serum ferritin is a good marker for body iron status in the absence of inflammation. However, a low-grade inflammation is found in most dialysis patients, so ferritin levels reflect a combination of inflammatory activity and iron availability [10]. TSAT is not routinely performed in Lithuanian MHD centers. We determine a dose of iron according to ferritin concentration only, so we cannot accurately evaluate iron stores and it is difficult to prescribe an appropriate iron dose. There are several studies supporting an important role of hepcidin in the control of iron metabolism in MHD patients [5,21,26–28]. Recent data based on magnetic resonance imaging (MRI) suggest that a substantial fraction of MHD patients receiving ESAs and IV iron supplementation have hepatic iron overload [29]. This illustrates the need for updating guidelines on the amount of iron infused in these patients which may promote not only organ damage, but also a vicious cycle because of increased hepcidin production and ensuing iron maldistribution, without improving anemia [5]. Although cumulative iron dose was significantly higher in our nonresponders’ group, they were more anemic. They also had a significantly higher degree of inflammation, higher ferritin and hepcidin levels although without statistical significance. This corresponds to the results of other authors [30] comparing ESA responders’ and nonresponders’ groups. The features of our nonresponders refer to “reticuloendothelial blockage,” which can be considered as an extreme form of functional iron deficiency – iron stores are locked partly because of high hepcidin levels and there is no release of iron to transferrin. Whether or not evaluation of hepcidin levels may help in driving iron supplementation therapy in MHD patients is still matter of debate. The study by Tessitore et al. [31] did not show hepcidin as a useful predictor of hemoglobin response to IV

**Table 4 – Multivariate linear regression analysis of factors influencing ERI in MHD patients.**

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Unstandardized B coefficient (95% CI)</th>
<th>Standardized B coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>0.078 (0.021; 0.134)</td>
<td>0.198</td>
<td>0.007</td>
</tr>
<tr>
<td>Albumin</td>
<td>−0.436 (−0.73; −0.142)</td>
<td>−0.209</td>
<td>0.004</td>
</tr>
<tr>
<td>Body mass index</td>
<td>−0.374 (−0.553; −0.195)</td>
<td>−0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hospitalization rate per year</td>
<td>3.017 (2.077; 3.957)</td>
<td>0.446</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>30.959 (18.099; 43.818)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$r^2 = 0.46$.  

**Fig. 3 – Relationship between erythropoietin resistance index and inflammatory – nutritional status parameters.** BMI ($r = -0.344, P < 0.001$), CRP ($r = 0.217, P = 0.015$), and albumin ($r = -0.181, P = 0.044$).
Table 5 - Correlation of serum hepcidin with conventional markers of anemia and ESA dose, iron metabolism, inflammation and nutritional status, calcium-phosphorus metabolism, hepatic enzymes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>P</th>
<th>Unstandardized B coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematological data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>-0.4</td>
<td>0.013</td>
<td>-3.404 (-5.554; -1.255)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>-0.382</td>
<td>0.008</td>
<td>-11.976 (-18.945; -5.008)</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>-0.3</td>
<td>0.041</td>
<td>-78.895 (-136.14; -21.647)</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.015</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>0.036</td>
<td>0.816</td>
<td></td>
</tr>
<tr>
<td>MCH</td>
<td>0.084</td>
<td>0.587</td>
<td></td>
</tr>
<tr>
<td><strong>ESA therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESA dose, IU/week</td>
<td>0.4</td>
<td>0.008</td>
<td>0.006 (0.002; 0.011)</td>
</tr>
<tr>
<td>ERI, IU/kg/week per 100 mL</td>
<td>0.349</td>
<td>0.017</td>
<td>3.756 (0.691; 6.821)</td>
</tr>
<tr>
<td><strong>Iron metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>0.5</td>
<td>&lt;0.001</td>
<td>0.334 (0.212; 0.457)</td>
</tr>
<tr>
<td>TSAT</td>
<td>-0.43</td>
<td>0.025</td>
<td>-4.642 (-9.101; -0.183)</td>
</tr>
<tr>
<td>Cumulative iron dose, mg per month</td>
<td>-0.096</td>
<td>0.485</td>
<td></td>
</tr>
<tr>
<td><strong>Inflammatory and nutritional status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.11</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.4</td>
<td>0.013</td>
<td>-8.34 (-14.846; -1.833)</td>
</tr>
<tr>
<td>Protein</td>
<td>0.097</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.11</td>
<td>0.607</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>0.213</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium-phosphorus metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>-0.179</td>
<td>0.223</td>
<td></td>
</tr>
<tr>
<td>Calcium corrected</td>
<td>0.147</td>
<td>0.138</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>-0.138</td>
<td>0.351</td>
<td></td>
</tr>
<tr>
<td>CaxP</td>
<td>-0.24</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>0.113</td>
<td>0.444</td>
<td></td>
</tr>
<tr>
<td><strong>Hepatic enzymes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>0.118</td>
<td>0.261</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>0.14</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>-0.034</td>
<td>0.745</td>
<td></td>
</tr>
</tbody>
</table>

ESAs, erythropoiesis-stimulating agents; ERI, ESA resistance index; TSAT, transferrin saturation; CRP, C-reactive protein; PTH, parathyroid hormone; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Iron. However, this study had some limitations like possible underpowering and lack of a control group, so larger trials comparing hepcidin-driven to standard therapeutic approaches are awaited [5].

Data about association of ERI with hepcidin are still controversial. Although in some studies authors present a positive correlation between ERI and hepcidin that correspond to our data, other authors present opposite results. For example, Costa et al. and do Sameiro-Faria et al. found that hepcidin serum levels among nonresponders were significantly lower than among responders [28,32]. As nonresponders present high inflammatory markers, hepcidin levels are expected to be increased in nonresponders, but it has been demonstrated that erythropoietin downregulates liver hepcidin expression, acting, therefore, as a hepcidin inhibitory hormone. Since nonresponders were treated with much higher doses of ESAs compared with responders, the lower hepcidin levels among nonresponders could be explained by this mechanism [28,32]. We found a significant positive correlation between serum hepcidin and ERI in our study. Correlation between a ESA weekly dose and hepcidin was significant as well; however, it was not a significant difference in hepcidin concentration in ESA responders and nonresponders’ groups, possibly because of a small sample size. Hepcidin levels have been described in association with markers of anemia and most strongly with iron status [8,21,27,33,34] that correspond to our data. However, hepcidin did not relate to CRP as a marker of inflammation, iPTH, Ca, P, CaxP, hepatic enzymes in our study. It is known that hepcidin synthesis is induced by inflammation, so positive correlation between

Table 6 - Multivariate linear regression analysis of factors influencing hepcidin in MHD patients.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Unstandardized B coefficient (95% CI)</th>
<th>Standardized B coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERI, IU/kg/week per 100 mL</td>
<td>4.889 (1.844;7.895)</td>
<td>0.439</td>
<td>0.002</td>
</tr>
<tr>
<td>Ferritin</td>
<td>0.242 (0.086;0.399)</td>
<td>0.422</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\[ r^2 = 0.56. \]
haptoglobin and CRP was very expected. The lack of correlation may be explained on the basis of differences in the half-lives of CRP and haptoglobin [35], besides it may be due to the fact that we could not measure hsCRP. That corresponds to the data of Kali et al. [36].

Nutritional status plays an important role in the clinical course of MHD patients and the BMI has shown to be a factor influencing the body response to ESAs [19,23,32,37]. Malnutrition is closely related to inflammation and arteriosclerosis and through mediators such as IL-6, TNF-α, it may play a relevant role in ESA resistance. Our data corroborate these findings as ERI was inversely related to BMI. A lower uremic load, relative and absolute, in larger individuals may contribute to this observation [37]. An alternative hypothesis is that the fat tissue may autonomously modulate the response to ESAs via autocrine regulation. Leptin, higher in overweight patients, has shown to stimulate erythropoiesis [19]. The underweight patients present a decreased nutritional reserve and a reduced protein-calorie intake reducing the capacity to overcome inflammation and chronic acidosis; hospitalization and failure of vascular access are also more frequent in these patients. These findings are usually related with the enhancement of the inflammatory state, which is a hallmark of MHD patients and one of the most important factors associated with anemia and resistance to ESAs therapy [32].

Not all comorbidity factors had the same effect on the response to ESAs. Ischemic heart disease, arrhythmia, liver disease, chronic obstructive lung disease or stroke were not related with ERI in our study, whereas oncological disease clearly increased resistance to ESAs. Malignant tumors decrease concentration of endogenous erythropoietin and increase levels of pro-inflammatory cytokines such as IL-6, which is able to impair erythropoiesis [18]. Although we did not measure tumor activity levels or record the type of treatment, our findings confirmed that patients with a history of cancer had lower Hb levels and a higher ERI.

Our results have shown that lower Kt/V values are associated with higher doses of ESAs and that correspond to the results of other authors [38,39], although not all authors confirm that [18]. Movilli et al. also showed that inadequate dialysis was associated with higher erythropoietin requirements, but increasing Kt/V values above 1.33, had no further effect on erythropoietin responsiveness in iron-replete MHD patients [40].

Despite advances in renal replacement therapy, mortality of MHD patients is still excessive and CV disease accounts for about 50% of all deaths. In CKD, cardiovascular mortality is 20–40 times higher than in the general population and the lifetime risk of sudden cardiac death in dialysis patients with normal left ventricular ejection fraction is 28%, more than double that seen in the general population (11–12%) [36,41]. It is well known that anemia and ESA resistance are associated with an adverse outcome of MHD patients [18,42–44]. Annual data collection allowed us to analyze association between anemia and mortality in incident MHD patients in Lithuania in 1998–2005 [20]. Multivariate Cox proportional hazards analysis revealed that anemia was an independent risk factor of death. Relative risk of mortality was 5% lower for every 1 g/L greater Hb concentration used as continuous variable and adjusted for age, sex and primary kidney disease. The Hb concentration below 100 g/L was associated with a 2.5-fold increased relative risk of death [20]. There were no difference in mortality of patients according to ERI and haptoglobin concentration in our recent study, however we found a significant difference in hospitalization rates and the mean length of one hospitalization according to ERI. That corresponds to the data of the study by Vaiciumiene, who showed that risk of total hospitalizations of MHD patients in Lithuania was increased when lower level of Hb was detected [45]. Disordered iron transport caused by inflammation and increased haptoglobin concentration may accelerate arterial disease, for example by increasing arterial stiffness, thus further enhancing the risk for CV events in CKD patients [21]. In the study of 405 MHD patients with a median follow-up of 3 years, haptoglobin levels were associated with the incidence of cardiovascular events [15]. Their results implicated that haptoglobin plays a role in the pathophysiology of arteriosclerosis and CV disease. It was hypothesized that haptoglobin promotes the progression of atherosclerotic plaques by slowing or preventing the mobilization of iron from macrophages resulting in enhanced oxidative stress and increased atherogenicity [46]. Several reports have demonstrated a relationship between iron accumulation and arterial alteration, including generation of oxidized low-density lipoproteins, endothelial cell dysfunction and arterial smooth muscle proliferation. They suggest that elevated levels of serum haptoglobin may be closely associated with arterial stiffness and possibly the development of arteriosclerosis in MHD patients [33,47]. Serum levels of haptoglobin are usually higher in MHD patients, therefore controlling this risk factor may provide the significant benefits in helping to prevent CV disease in these patients. We found a significant correlation between haptoglobin and iron profile, but there were no difference in mortality of patients according to haptoglobin concentration in our study, possibly because of a small sample size.

Since management of renal anemia remains challenging, more research is needed to better understand the efficacy, long-term safety and targets of current iron therapies as well as novel haptoglobin-lowering approaches in large prospective randomized controlled trials [48]. The understanding that haptoglobin excess contribute to iron-restricted erythropoiesis in CKD patients has generated interest in developing new therapies that target the haptoglobin–ferroportin axis [49–51]. Haptoglobin antagonists and inhibitors of haptoglobin production may find utility in the treatment of iron-restricted anemias, alone or in combination with ESAs.

A potential limitation of our study is that we did not measure TSAT so that variable could not be included in the multivariate analysis of factors influencing ERI in MHD patients. Furthermore, nutritional deficiencies which can be limiting factors in the production of erythrocytes, such as vitamin B12 and folate were not evaluated. Another limitation of this study is possible underpowering because of a small sample size, so we can only talk about a relationship based on the data but not a causal relationship and interpretations should be made carefully. We are planning to continue the study involving a larger number of patients hoping for more precise results.
5. Conclusions

C-reactive protein, albumin, body mass index and hospitalization rate per year were found to be significant determinants of erythropoietin resistance index in MHD patients. Inadequate dialysis was associated with higher epoetin requirements.

A significant positive relation between serum hepcidin and erythropoietin resistance index was revealed. Erythropoietin resistance index and ferritin were found to be significant determinants of hepcidin in MHD patients.

Not all comorbidity factors had the same effect on the response to ESAs: patients diagnosed as having oncological disease, regardless of its localization and clinical course, had a higher erythropoietin resistance index than the rest of the patients.

There were no difference in mortality of patients according to erythropoietin resistance index, but significant difference in hospitalization rates and the mean length of one hospitalization according to erythropoietin resistance index was revealed (nonresponders had a higher hospitalization rate and their mean length of one hospitalization was longer).

Hepcidin was not related to all-cause mortality in our MHD patients.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors’ contributions


All authors read and approved the final manuscript.

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REFERENCES


