Neutrophil Impairment Associated with Iron Therapy in Hemodialysis Patients with Functional Iron Deficiency

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Abstract. Hemodialysis patients treated with recombinant human erythropoietin (rHEPO) need adequate iron supplementation to avoid rHEPO hyporesponsiveness due to iron deficiency. Low serum ferritin reflects absolute iron deficiency, whereas normal or high ferritin values in combination with low transferrin saturation (<20%) indicate functional iron deficiency. In this study, healthy subjects (group I) were compared with intravenous (i.v.) rHEPO-treated and i.v. iron-saccharate-treated regular hemodialysis patients that were subdivided into three groups as follows: patients with serum ferritin >100 and <350 μg/L (group II), patients with ferritin <60 μg/L (group III), and patients with ferritin >650 μg/L but transferrin saturation <20% (group IV). Polymorphonuclear leukocyte (PMNL) parameters (phagocytosis, intracellular killing of bacteria, oxidative metabolism, glucose uptake, intracellular calcium) for each group were compared with those of multitransfused, iron-overloaded primary hematologic patients (group V) and those of patients suffering from hereditary hemochromatosis (group VI). Compared with PMNL obtained from healthy subjects (group I), group II hemodialysis patients showed mild inhibition of phagocytosis but significant inhibition of intracellular killing of bacteria. Oxidative burst of PMNL from group II patients was also significantly reduced after stimulation in vitro. These dysfunctions were not affected by absolute iron deficiency (comparable data in group III patients). However, impairment of PMNL was markedly aggravated in group IV patients. Intracellular calcium concentration under basal conditions and after stimulation was not different. These data suggest that iron is responsible for the PMNL dysfunctions observed in group IV patients. The PMNL defect of group IV patients was comparable to group V and group VI patients with normal renal function, suggesting again a direct inhibitory effect of iron. It is concluded that hemodialysis patients with high ferritin but low serum iron and low transferrin saturation ("functional iron deficiency") display a significant impairment of fundamental PMNL functions during i.v. iron and rHEPO therapy. This may result in increased risk of infectious complications. Therefore, overtreatment of hemodialysis patients with i.v. iron should be avoided. (J Am Soc Nephrol 9: 655-663, 1998)

Iron deficiency is the most common cause of rHEPO hyporesponsiveness in patients with end-stage renal disease. Although low ferritin values indicate absolute iron deficiency, normal (between >100 μg/L and <350 μg/L) or even high (>650 μg/L) ferritin values do not exclude iron deficiency (1). Functional iron deficiency is characterized by normal or elevated ferritin in combination with low transferrin saturation (<20%).

To maximize the effects of rHEPO, parenteral iron should be also administered to the majority of the patients. Depending on the degree of preexisting iron deficiency, a mean rHEPO dosage reduction of 42.3% has been observed (2-7) after intravenous (i.v.) iron supplementation. We have shown that high-dose i.v. iron therapy with a mean iron saccharate dosage of 2523 ± 810 mg reduced the rHEPO requirement from 217 ± 179 U/kg per wk to 62.6 ± 70.2 U/kg per wk. This effective iron substitution was accompanied by an increase of serum ferritin from 52.4 ± 92.5 to 534 ± 329 μg/L, of transferrin saturation from 12.5 ± 5.9 to 24.3 ± 12.9%, and of blood hemoglobin from 9.4 ± 1.2 to 11.1 ± 1.1 g/dl (8).

However, high-dose iron therapy may enhance the risk of iron overload associated with a higher frequency of infections, cardiovascular complications, or cancer (9). Recent consensus recommendations of the workshop for iron supplementation during rHEPO therapy were as follows: Serum ferritin should be maintained at >100 μg/L (no upper limit was set), and transferrin saturation should be maintained at >20% (10). More than 10% of the patients undergoing regular hemodialysis therapy in our unit display the constellation of serum ferritin >650 μg/L and transferrin saturation <20%. Similar observations were made by Kaufman et al. (11). Thirty-two (20%) of 163 patients with transferrin saturation <20% had a serum ferritin >400 μg/L. Ten years ago, Eschbach et al. (12) reported one patient who developed functional iron deficiency (transferrin saturation 13%, serum ferritin 578 μg/L) with poor response to rHEPO. In this patient, erythropoiesis improved markedly after a short course of high-dose i.v. iron therapy. Tsobanelis et al. (13) studied hemodialysis patients with a mean serum ferritin of 325 μg/L. Of these 33 patients, 30 displayed a transferrin saturation of less than 20%. Iron overload in rHEPO-treated patients was ascribed to ferritin levels of more than 500 μg/L (14) or more than 1000 μg/L (15).
According to Eschbach (16), serum ferritin should be maintained above 300 μg/L but not exceed 1000 μg/L. End-stage renal disease patients may benefit from continuation of iron therapy by reduction of their maintenance rhEPO dose, even in the presence of elevated ferritin levels. They may, however, have a higher risk of infectious complications (17,18). Multiple dysfunctions of the PMNL have been described in iron-overloaded patients (19–22).

Therefore, the aims of the present study were to investigate functional and metabolic PMNL parameters of neutrophils isolated from three different groups of regular hemodialysis patients treated with rhEPO (Erypo®, Janssen & Cilag, Vienna, Austria): (1) patients with serum ferritin >100 μg/L and <350 μg/L; (2) patients with serum ferritin <60 μg/L; and (3) patients with serum ferritin >650 μg/L but transferrin saturation <20%.

The results were compared with the identical PMNL parameters of healthy subjects; multitransfused, iron-overloaded primary hematologic patients; and patients with hereditary hemochromatosis.

Materials and Methods

Patients

All clinical investigations described in this article were conducted in accordance with the guidelines proposed in the Declaration of Helsinki. Informed consent was obtained from all patients studied. We investigated 38 patients and nine healthy subjects. None of the patients had clinical signs or symptoms of infection and, except for the hematologic patients, no evidence of malignancy. Patients and their controls were subdivided into several groups.

Group I consisted of nine healthy subjects (five women, four men) ranging in age from 37 to 63 yr, with a mean of 46.2 ± 3.1 yr (mean values ± SEM). Mean serum creatinine was 1.00 ± 0.04 mg/dl (range, 0.74 to 1.18 mg/dl). This group acted as control subjects for groups II through VI.

Group II consisted of 10 chronically uremic patients (three women, seven men) aged 51.0 ± 6.1 yr (range, 16 to 86 yr), undergoing regular bicarbonate hemodialysis for 13.8 ± 2.5 mo (range, 5 to 28 mo). This group of patients displayed neither evidence for absolute iron deficiency (serum ferritin >100 μg/L) nor iron overload (serum ferritin <350 μg/L). Mean Kt/V was 1.35 ± 0.04 (range, 1.18 to 1.56). These patients received between 10 and 20 mg of iron saccharate i.v. after hemodialysis treatment.

Group III consisted of eight chronically uremic patients (six women, two men) aged 52.0 ± 5.7 yr (range, 26 to 81 yr), undergoing regular bicarbonate hemodialysis treatment for 32.6 ± 11.2 mo (range, 4 to 92 mo). All patients had a serum ferritin <60 μg/L. Mean Kt/V was 1.38 ± 0.07 (range, 1.13 to 1.63). These patients did not receive i.v. iron for at least 3 mo.

Group IV consisted of eight chronically uremic patients (four women, four men) aged 69.2 ± 5.1 yr (range, 44 to 83 yr), undergoing regular bicarbonate hemodialysis treatment for 47.9 ± 5.2 mo (range, 30 to 65 mo). All patients had “functional iron deficiency” with low transferrin saturation (<20%) and low serum iron (<60 μg/dl) despite high serum ferritin values (>650 μg/L). These patients received 10 mg of iron saccharate i.v. after hemodialysis treatments. Mean Kt/V was 1.24 ± 0.10 (range, 0.96 to 1.67).

None of the hemodialysis patients of groups II through IV had any clinical signs of infection. Twenty-one patients had plasma levels of C-reactive protein (CRP) lower than the detection limit (<0.5 mg/dl).

One patient had a CRP value within the normal range (<1.0 mg/dl) and four patients showed slightly elevated CRP values (between 1.18 and 3.0 mg/dl). One of these patients belonged to group II, one to group III, and two to group IV. Three times weekly, hemodialysis therapy was performed using biocompatible membrane materials made of polysulfone (Fresenius, Oberursel, Germany; n = 13) or cellulose triacetate (Nissho Corp., Osaka, Japan; n = 13). Each of the patients had been dialyzed more than 3 mo with the membranes they were using during the study. None of the dialysis patients was transfused within 3 mo of the study. Blood samples were obtained predialysis. All measurements and PMNL isolation were immediately performed.

Group V consisted of six multitransfused, iron-overloaded primary hematologic patients (one woman, five men) aged 65.7 ± 6.8 yr (range, 38 to 85 yr). Five of these patients suffered from myelodysplastic syndrome and one from acute myeloid leukemia. Mean serum creatinine was 1.1 ± 0.2 mg/dl (range, 0.89 to 1.92 mg/dl). Mean number of blood units transfused was 116 ± 36 (range, 48 to 290) over 31.5 ± 8.8 mo (10 to 66 mo).

Group VI consisted of six patients (four women, two men) suffering from hereditary hemochromatosis. Their mean age was 62.0 ± 5.7 yr (range, 39 to 78 yr). Mean serum creatinine was 1.1 ± 0.1 mg/dl (range, 0.8 to 1.5 mg/dl). Diagnosis of hereditary hemochromatosis was established by measurement of serum transferrin saturation, serum ferritin concentrations, histologic analysis of liver biopsy specimens for iron content in parenchymal cells and Kupffer cells (23), and by genetic testing for the presence of the Cys282Tyr mutation in the gene coding for HFE (designation according to the World Health Organization committee on HLA nomenclature) as described by Feder et al. (24). A mean of 20.5 ± 5.2 (range, 2 to 41) U of blood (300 to 500 ml each) was collected from this patient group. No phlebotomy was performed at least 4 wk before collection of data. This group of patients was subdivided as follows: Data of group VIA represent the time when hereditary hemochromatosis was diagnosed, and data of group VIB were collected at the time when PMNL isolation was performed.

PMNL Isolation

PMNL were isolated from the venous blood of healthy subjects according to Nauseef et al. (25) and Metcalf et al. (26). Ten milliliters of Ficoll-Paque (Pharmacia LKB Biotechnology, Uppsala, Sweden) were overlaid by 10 ml of whole blood anticoagulated with 10 ml of heparin sodium (Liquemine Roche iv; F. Hoffmann-La Roche, Basel, Switzerland). After approximately 45 min, most of the erythrocytes sedimented into the Ficoll layer. Granulocytes, monocytes, lymphocytes, and thrombocytes in the plasma supernatant were separated on a Percoll (Pharmacia) density gradient. After isolation of the PMNL band, the PMNL were washed twice with the buffer used in the in vitro test and counted under the microscope. The viability of the PMNL population obtained by this procedure was greater than 95% as determined by the trypan blue (Sigma, St. Louis, MO) exclusion test.

PMNL Function Tests

2-Deoxyglucose. Hexose uptake was determined as described by McCall et al. (27). Isolated PMNL were resuspended in phosphate-buffered saline (PBS) with Ca²⁺ and Mg²⁺ at a concentration of 1 × 10⁶ cells/ml. Ten microliters of PBS or 10 μl of N-formylmethionyl-leucylphenylalanine methyl ester in a final concentration of 10⁻⁷ M was added to 200 μl of cell suspension and incubated for 15 min at 37°C in a shaking water bath. After addition of 100 μl of 2-deoxy-
and further incubated for 30 min at 37°C in a shaking water bath. The tubes were centrifuged at 300 × g for 15 min at 4°C. The supernatant was decanted and read at 550 nm. For basal levels, Hank’s buffer was used instead of cell suspension. All tests were performed in duplicate. Data are expressed as nmol O2− produced by 2 × 10^5 cells. The calculation was made using the molar extinction coefficient of 29.9 × 10^3 mol/L.

**Intracellular Calcium Concentration.** The basal levels of intracellular calcium concentration ([Ca2+]i) in PMNL were measured with Fura-2 AM, using a Perkin-Elmer fluorometer model LS 5B (Perkin-Elmer, Norwalk, CT). The details of this methodology have been reported by Alexiewicz et al. (29) and Kiersztein et al. (30). The dissociation constant for Ca2+-Fura-2 was assumed to be 225 mM, and the calculation of [Ca2+]i was made using the Grykiewicz equation (31). All tests were performed in duplicate. Data are expressed as nmol/L. Plasma intact parathyroid hormone (iPTH) levels of control subjects and groups II through IV patients were measured using the immunoradiometric assay made by CIS Biointernational (Gif-Sur-Yvette Cedex, France).

**Statistical Analyses**

Data are given as means ± SEM. Statistical comparisons were performed using one-way ANOVA and the Student–Newman–Keuls test for post hoc analysis (32). A P value <0.05 was considered statistically significant. All data analyses were computed using commercially available software (Statistical Package for Social Sciences® software, version 6.1).

**Results**

Table 1 shows red blood cell parameters of the different groups investigated. Dialyzed patients of groups II through IV had significantly lower numbers of erythrocytes than healthy subjects. The lowest erythrocyte number was found in the primary hematologic patient group. Similarly, hemoglobin concentration and hematocrit levels were lower in dialysis patients than in healthy subjects. Both values were lowest in the group V patients. The red blood cell parameters were initially (group VIa) not different between patients with hereditary hemochromatosis and healthy subjects but decreased after therapeutic phlebotomy (group VIb). Leukocyte and neutrophil

Table 1. Red and white blood cell parameters of healthy subjects (group I); different groups of hemodialysis patients (groups II through IV), multiransfused, iron-overloaded primary hematologic patients with secondary hemochromatosis (group V); and patients with hereditary hemochromatosis at the time of diagnosis (group VIa) and after repeated phlebotomy at the time of performing the leukocyte function tests (group VIb)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n = 9)</th>
<th>Group II (n = 10)</th>
<th>Group III (n = 8)</th>
<th>Group IV (n = 8)</th>
<th>Group V (n = 6)</th>
<th>Group VIa (n = 6)</th>
<th>Group VIb (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (×10^6/μL)</td>
<td>4.8 ± 0.1</td>
<td>3.4 ± 0.1b</td>
<td>3.6 ± 0.1b</td>
<td>3.5 ± 0.1b</td>
<td>2.6 ± 0.1c</td>
<td>4.6 ± 0.2</td>
<td>3.8 ± 0.4b</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.1 ± 0.5</td>
<td>10.5 ± 0.3b</td>
<td>10.3 ± 0.5b</td>
<td>10.7 ± 0.4b</td>
<td>7.9 ± 0.3c</td>
<td>14.5 ± 0.4</td>
<td>11.9 ± 1.1b</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41.6 ± 1.3</td>
<td>31.6 ± 0.8b</td>
<td>32.1 ± 1.6b</td>
<td>33.1 ± 1.0b</td>
<td>23.3 ± 1.0c</td>
<td>43.0 ± 0.6</td>
<td>35.3 ± 2.9b</td>
</tr>
<tr>
<td>Leucocytes (×10^3/μL)</td>
<td>7.3 ± 0.7</td>
<td>6.1 ± 0.7</td>
<td>5.5 ± 0.5</td>
<td>6.2 ± 0.6</td>
<td>4.8 ± 0.8</td>
<td>5.8 ± 1.1</td>
<td>5.1 ± 1.5</td>
</tr>
<tr>
<td>PMNL (×10^3/μL)</td>
<td>4.2 ± 0.6</td>
<td>4.2 ± 0.5</td>
<td>4.1 ± 0.4</td>
<td>4.9 ± 0.7</td>
<td>2.7 ± 0.4</td>
<td>3.6 ± 0.8</td>
<td>3.4 ± 1.1</td>
</tr>
</tbody>
</table>

* Mean values ± SEM. PMNL, polymorphonuclear leukocyte.
  b P < 0.05 versus groups I and VIa.
  c P < 0.05 versus all other groups.
Table 2 shows the iron status of healthy subjects and the hemodialysis patients. Serum ferritin was 125.9 ± 60.3 µg/L in healthy subjects and 229.0 ± 21.8 µg/L in group II patients. Group III patients had the lowest serum ferritin (37.3 ± 11.1 µg/L) and transferrin saturation (15.6 ± 3.7%), whereas group IV patients displayed a serum ferritin of 911 ± 68.7 µg/L but a transferrin saturation of 16.5 ± 1.2%. In contrast, group V patients showed a mean serum ferritin of 2597 ± 319 µg/L and a transferrin saturation of 69.1 ± 10.0%. Group VI patients had a serum ferritin of 1459 ± 410 µg/L at the time of diagnosis, which decreased significantly after repeated phlebotomy.

The serum transferrin level was 325.6 ± 23.4 mg/dl in healthy subjects (group I) but was significantly lower in group II patients. The group IV patients had the lowest transferrin level within the dialysis patient groups. A further decrease of serum transferrin was found in group V patients. Serum iron was lower in all groups of hemodialysis patients compared with healthy subjects. On the other hand, serum iron was significantly higher in patients of groups V and VI.

Table 2. Iron status of healthy subjects (group I); different groups of hemodialysis patients (groups II through IV), multitransfused, iron-overloaded primary hematologic patients with secondary hemochromatosis (group V); and patients with hereditary hemochromatosis at the time of diagnosis (group VI) and after repeated phlebotomy at the time of performing the leukocyte function tests (group Vb).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n = 6)</th>
<th>Group II (n = 10)</th>
<th>Group III (n = 8)</th>
<th>Group IV (n = 6)</th>
<th>Group V (n = 9)</th>
<th>Group VI (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (µg/L)</td>
<td>125.9 ± 60.3</td>
<td>229.0 ± 21.8</td>
<td>911 ± 68.7</td>
<td>2597.3 ± 319.4</td>
<td>1459.8 ± 410.8</td>
<td>1459.8 ± 410.8</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>19.2 ± 21.8</td>
<td>15.6 ± 3.7</td>
<td>16.5 ± 1.2</td>
<td>15.6 ± 3.7</td>
<td>37.3 ± 11.1</td>
<td>229.0 ± 21.8</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>325.6 ± 23.4</td>
<td>60.5 ± 6.7</td>
<td>180.4 ± 10.6</td>
<td>157.8 ± 12.9</td>
<td>180.4 ± 10.6</td>
<td>180.4 ± 10.6</td>
</tr>
<tr>
<td>Iron (µg/dl)</td>
<td>83.9 ± 5.9</td>
<td>43.4 ± 4.5</td>
<td>43.4 ± 4.5</td>
<td>155.7 ± 23.6</td>
<td>155.7 ± 23.6</td>
<td>155.7 ± 23.6</td>
</tr>
</tbody>
</table>

Basal and stimulated PMNL glucose uptake, important for adequate ATP formation, was significantly enhanced in patients of group II (hemodialysis patients with iron deficiency) compared with healthy subjects. Group III and group IV patients had glucose uptake closer to healthy subjects than group II did (Table 3).

Table 3. Plasma levels of iPTH were not significantly different between the three groups of hemodialysis patients and were in a range two- to threefold higher than those of healthy subjects. The iPTH values were 134.3 ± 17.2 pg/ml for group II.
Figure 1. Polymorphonuclear leukocyte (PMNL) phagocytosis and intracellular killing of bacteria (Escherichia coli) by isolated PMNL obtained from healthy subjects (group I, □), patients undergoing regular hemodialysis treatment with serum ferritin >100 μg/L and <350 μg/L (group II, ▼), hemodialysis patients with serum ferritin <60 μg/L (group III, □), hemodialysis patients with serum ferritin >650 μg/L and transferrin saturation <20% (group IV, ▼), multitransfused, iron-overloaded hematologic patients (group V, □), and patients with hereditary hemochromatosis (group VI, □). *P < 0.05 versus healthy subjects; **P < 0.01 versus healthy subjects.

Figure 2. PMNL oxidative burst under basal conditions and stimulation (% increase) of isolated PMNL obtained from healthy subjects (group I, □), patients undergoing regular hemodialysis treatment with serum ferritin >100 μg/L and <350 μg/L (group II, ▼), hemodialysis patients with serum ferritin <60 μg/L (group III, □), hemodialysis patients with serum ferritin >650 μg/L and transferrin saturation <20% (group IV, ▼), multitransfused, iron-overloaded hematologic patients (group V, □), and patients with hereditary hemochromatosis (group VI, □). *P < 0.05 versus healthy subjects.

patients, 212.0 ± 48.0 pg/ml for group III, and 143.8 ± 51.5 pg/ml for group IV.

The rhEPO dosage necessary to keep target hemoglobin concentration constant tended to be higher (264.4 ± 87.3 U/kg body wt per wk) in patients with severe iron deficiency (serum ferritin <60 μg/L) than in hemodialysis patients with "normal" iron status receiving 170.8 ± 49.8 U/kg per wk or dialysis patients with high ferritin but low transferrin saturation receiving 145.8 ± 21.6 U/kg per wk. Statistical comparison among the groups was without any significance.
Table 3. PMNL glucose uptake and intracellular calcium concentration ([Ca2+]i) of isolated PMNL of healthy subjects (group I) and different groups of hemodialysis patients (groups II through IV)∗

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n = 9)</th>
<th>Group II (n = 10)</th>
<th>Group III (n = 8)</th>
<th>Group IV (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose uptake (103 cpm/2 × 105 PMNL)</td>
<td>13.5 ± 1.7b</td>
<td>19.4 ± 1.8</td>
<td>14.0 ± 1.5b</td>
<td>13.4 ± 1.4</td>
</tr>
<tr>
<td>Stimulated glucose uptake (103 cpm/2 × 105 PMNL)</td>
<td>29.1 ± 3.4b</td>
<td>44.6 ± 2.5</td>
<td>35.7 ± 4.4</td>
<td>39.7 ± 3.5</td>
</tr>
<tr>
<td>Stimulated increase (%)</td>
<td>224 ± 17</td>
<td>240 ± 15</td>
<td>269 ± 35</td>
<td>313 ± 41</td>
</tr>
<tr>
<td>Basal ([Ca2+]i) (nmol/L)</td>
<td>38.4 ± 1.8</td>
<td>35.4 ± 3.7</td>
<td>46.3 ± 9.3</td>
<td>43.7 ± 6.4</td>
</tr>
<tr>
<td>Stimulated ([Ca2+]i) (nmol/L)</td>
<td>484 ± 32</td>
<td>588 ± 70</td>
<td>532 ± 48</td>
<td>490 ± 48</td>
</tr>
<tr>
<td>Stimulated increase (%)</td>
<td>1293 ± 118</td>
<td>1696 ± 155</td>
<td>1314 ± 161</td>
<td>1187 ± 106</td>
</tr>
</tbody>
</table>

∗Mean values ± SEM.

b P < 0.05 versus group II.

Discussion

The present study demonstrates comparable blood hemoglobin levels independent of the iron status in three groups of patients undergoing regular hemodialysis treatment and rEPO therapy. There was, however, a tendency for higher rEPO need in iron-deficient patients compared with the patient groups with normal or high ferritin. Besarab (33) defined patients responding to 105 to 450 U/kg per wk as average rEPO responders. Absolute iron deficiency in uremia is controversially discussed. According to the classical definition, a serum ferritin <30 μg/L indicates absolute iron deficiency in patients with intact kidney function (34). However, several authors recommended a higher threshold for iron deficiency in uremic patients than in healthy subjects. Cutoffs of 50 μg/L (35), 70 μg/L (36), 80 μg/L (37), or 100 μg/L (38) have been suggested. Normal subjects of this study had relatively low hematocrit (41.6 ± 1.3%; normal value, 45%). This group (five women, four men) had normal ferritin, transferrin, and plasma iron levels (Table 2).

As described in the literature (39,40), hemodialysis patients with normal iron status had significantly lower serum transferrin concentration than healthy subjects. A tendency toward a further reduction was seen in the group IV hemodialysis patients with the high serum ferritin. A significant reduction of serum transferrin was found in the group IV of iron-overloaded primary hematologic patients, whereas a significant increase of transferrin was found in group III (iron-deficient) hemodialysis patients (Table 2). Several studies have shown an inverse correlation between serum transferrin and ferritin (41,42). Interestingly, hemodialysis patients with absolute (group III) and functional (group IV) iron deficiency have comparably low serum iron despite differences in serum ferritin up to 30-fold (Table 2). The relative macrocytosis of groups II and IV relative to group III may be due to a more effective response to rEPO.

Hemodialysis patients tended to have a lower phagocytosis of their PMNL (Figure 1) compared with healthy subjects. Iida et al. (43) found intact phagocytic activities of polymorphonuclear cells in chronic hemodialysis patients, whereas several other studies have shown significantly reduced phagocytosis (44–47). Data of Alexiewicz et al. (29) indicate that phagocytosis of PMNL obtained from hemodialysis patients is impaired if [Ca2+]i is elevated. Normalization of intracellular calcium by calcium channel blocker therapy results in normalization of impaired PMNL phagocytosis (29). Lida et al. (43) observed impaired bactericidal activities of polymorphonuclear cells in chronic hemodialysis patients. Significant inhibition of intracellular killing of bacteria was observed in PMNL of group II patients, which was aggravated in patients with high ferritin but low transferrin saturation and low serum iron (group IV) (Figure 1). These results suggest that the dysfunction is related to iron, over and above the dysfunctions seen in uremia alone. The degree of inhibition was not different from the cells obtained from multitransfused, iron-overloaded primary hematologic patients (group V). It could be argued that the group of iron-overloaded patients with hematologic disease is a poor choice for comparison, because patients with myelodysplastic syndromes have dysfunctional neutrophils. Therefore, we selected as a further nonuremic group patients with hereditary hemochromatosis to compare PMNL function of group VI patients with either healthy subjects or subjects on dialysis. As demonstrated, group IV and group VI patients have comparable inhibition of fundamental PMNL functions (Figures 1 and 2). Our data suggest that group IV patients (serum ferritin >650 μg/L) should not receive further i.v. iron therapy despite low transferrin saturation or low serum iron (“functional” iron deficiency). It may be argued that the changes seen and attributed to iron-overload are relatively minor, and that the physiologic or clinical relevance of these small differences are unclear. Although group IV hemodialysis patients have a 12% lower degree of phagocytosis, there is a 16% reduction of killing. Continuing i.v. iron therapy may further aggravate the degree of PMNL inhibition. Other studies have shown that a similar PMNL impairment during iron overload is associated with a higher incidence of bacterial infections (17–22).

In agreement with Ward and McLeish (48), we also observed higher basal oxidative burst of groups II and III hemodialysis patients compared with healthy subjects, indicating "priming" of PMNL by uremia. Disturbed intracellular killing of bacteria by PMNL is due to reduced oxidative metabolism. After stimulation of PMNL, in vitro oxidative metabolism is
significantly reduced in PMNL of group II and III patients. A further 50% reduction is observed by “overtreatment” with iron (group IV) or iron-overload (group V and group VI patients). The decrease of PMNL oxidative burst after stimulation is of potential clinical importance, simulating the situation of PMNL in the presence of bacterial or viral antigens. These data also suggest impaired inactivation of invading micro-organisms in iron-overdosed uremic patients. It could be argued that the absolute oxidative burst is more important than the percentage increase. Ward and McLeish (48) suggested that the primed state of uremic PMNL indicates that any increase in susceptibility to infection is likely due to other alterations in the immune response to bacteria. However, intracellular killing of bacteria by isolated PMNL was markedly reduced (group IV and V patients) despite significant priming expressed by stimulated basal oxidative burst (Figures 1 and 2).

It could be argued that alterations of different PMNL functions in hemodialyzed patients may be caused by accumulation of intracellular calcium via secondary hyperparathyroidism rather than by iron overload. It has been shown that increased [Ca\(^{2+}\)]\(_i\) inhibits fundamental functions and metabolism of PMNL in uremia (25,26,49,50). Our data demonstrate unchanged [Ca\(^{2+}\)]\(_i\) under basal conditions and after stimulation between dialysis patient groups (Table 3). There is also no difference between plasma levels of iPTH between the three groups of hemodialysis patients investigated. Iron deficiency caused significantly higher PMNL glucose uptake necessary for ATP synthesis under basal conditions and after in stimulation \textit{in vitro}. This phenomenon was not observed in hemodialysis patients with normal or high plasma ferritin (Table 3). We have not measured intracellular iron. However, our data show no relationship between plasma iron or ferritin and intracellular calcium.

Recent guidelines for the management of iron deficiency in erythropoietin-treated renal failure patients recommended the use of a combination of serum ferritin levels <100 \(\mu\)g/L, transferrin saturation <20%, and/or percentage of hypochromic red blood cells >10% for the diagnosis of absolute or functional iron deficiency. A patient fulfilling any one of these criteria was considered to require iron therapy (51). According to these guidelines, iron supplementation should aim to achieve and maintain serum ferritin levels and a transferrin saturation of 25 to 35%. It was also outlined that problems with iron toxicity should be avoided, and iron therapy should be used or continued with extreme caution in patients whose serum ferritin is \(\geq 1000 \mu\)g/L or the transferrin saturation is \(\geq 50\%\) (51). Therefore, we selected in this study a key group of patients with serum ferritin of 9110.0 \(\pm\) 68.7 \(\mu\)g/L but transferrin saturation of 16.5 \(\pm\) 1.2% (“functional iron deficiency”). We observed dysfunctions of PMNL in this patient group. It is our position that these patients should not receive more iron, because to do so might further impair their leukocyte function. None of these patients investigated had an underlying inflammatory disease, and there was no evidence that their leukocyte dysfunction was due to the underlying inflammatory disease instead of iron overload. Leukocyte function has not been investigated in such a group of end-stage renal disease patients before. The results obtained are comparable with earlier publications on patients with iron overload (17–22). Although it is clear that iron overloaded patients should not receive any iron supplementation, iron therapy was recommended for rhEPO-treated end-stage renal disease patients with low transferrin saturation despite high ferritin levels. Therefore, iron therapy was discontinued in our group IV patients to avoid further impairment of PMNL function.

In conclusion, uremia impairs the function of PMNL, which may be aggravated by intensive i.v. iron therapy. Hemodialysis patients showing the constellation of high ferritin (>650 \(\mu\)g/L), low transferrin saturation (<20%), and low serum iron, known as functional iron deficiency, display significant impairment of fundamental PMNL functions. Inhibition of PMNL, intracellular killing of bacteria, and oxidative metabolism after stimulation \textit{in vitro} are comparable to multitransfused, iron-overloaded primary hematologic patients and also to patients with hereditary hemochromatosis. Our data are consistent with the assumption that there is a risk for infection in rhEPO-treated patients supplemented with i.v. iron if serum ferritin is higher than 650 \(\mu\)g/L, even in the presence of low transferrin saturation. However, a multicenter study is necessary to confirm the clinical importance of our observation made in the investigation presented here.

References


