Intra- and post-dialytic changes of haemoglobin concentrations in non-anaemic haemodialysis patients

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Abstract

Background. Non-anaemic haemodialysis (HD) patients are potentially more prone to the adverse effects of ultrafiltration-induced haemoconcentration. No study, however, has assessed the effects of dialytic session on haemoglobin (Hb) levels in these patients.

Methods. The levels of Hb and total protein before, at the end (T0) and up to 120 min (T120) after the third HD session of the week were compared in non-anaemic (Hb >13 g/dl, n=14, NOR) and anaemic (Hb=11–12 g/dl, n=18, LOW) HD patients.

Results. The intradialytic weight loss was similar in the two groups (4.0 ± 0.9 and 4.1 ± 0.9% body weight). During the treatment, Hb levels increased to the same extent in both groups (from 14.4 ± 1.2 to 16.3 ± 1.9 g/dl in NOR, and from 11.4 ± 0.8 to 12.7 ± 0.9 g/dl in LOW) in the presence, presumably, of a smaller plasma volume in NOR, whereas the increment of total protein was greater in NOR (from 7.1 ± 0.2 to 9.6 ± 0.5 g/dl) than in LOW (from 7.3 ± 0.6 to 8.7 ± 0.8 g/dl) (P<0.0001). At T120, the Hb decline in NOR was almost double that measured in LOW (−9.2 ± 3.0 vs −4.7 ± 2.4%, P<0.001). Consequently, Hb concentration did not differ from the pre-dialytic value in NOR (P=0.10), but persisted higher in LOW (P<0.005). The extent of the post-dialytic decrement of Hb was inversely related to the total protein values at T0 (r = −0.547, P=0.0012).

Conclusions. This study indicates that in NOR: (i) the extent of intradialytic increment of Hb is limited by a greater intradialytic plasma refilling; (ii) the greater plasma refilling persists after the end of dialysis, with the restoration of pre-dialytic Hb levels within the initial 2h; and (iii) the force driving this phenomenon resides mainly in the larger changes of total protein concentration.

Keywords: anaemia; haematocrit; haemodialysis; haemoglobin; plasma refilling; serum protein

Introduction

The complete correction of anaemia in uraemic haemodialysis (HD) patients is a major topic of debate. Several studies have suggested that higher haemoglobin (Hb) levels are associated with an improved outcome in terms of quality of life, physical activity, brain function, cardiac function and reduced risk of hospitalization [1–6]. On the other hand, concerns about potential side effects, such as development or worsening of hypertension and increased clotting tendency, have been raised as barriers against the normalization of Hb levels [7–10]; indeed, the ambitions of most nephrologists for the eradication of anaemia in HD patients have been tempered further by the unfavourable results of the Hematocrit Normalization Trial [11].

In particular, it has been hypothesized that in HD patients with normal Hb levels, the risk of intravascular thrombosis is greater at the end of dialytic treatment, i.e. when the ultrafiltration (UF)-induced haemoconcentration is maximal [7,8,12,13]. It is important to note, however, that the primary process that limits these events is plasma refilling, i.e. the shift of fluid from the extra- to the intravascular compartment, which is driven mainly by the interaction between hydrostatic and oncotic forces across the vessel wall [14], and which helps preserve blood pressure and tissue perfusion during and immediately after the end of UF-induced haemoconcentration [15–17]. In this regard, we have demonstrated recently that in HD patients with Hb levels in the target range of 11–12 g/dl, plasma refilling is not limited to the dialysis session but persists in the immediate post-dialysis period [18].
The haemodilution that results from plasma refilling becomes certainly more critical in non-anaemic HD patients; in fact, Hb and haematocrit (Hct) values higher than usual theoretically make them more vulnerable to the adverse effects of UF-induced haemoconcentration in comparison with anaemic patients. Nevertheless, no study has evaluated the entity of plasma refilling in these patients. Previous studies, in fact, addressed only the protective effects of plasma refilling on blood volume in anaemic HD patients, and their authors did not report on the dialytic changes of Hb or Hct [19–23].

The current study therefore was aimed at comparing the effects of both intra- and post-dialytic fluid redistribution on Hb levels in uraemic patients who were either non-anaemic (Hb > 13 g/dl) or anaemic (Hb = 11–12 g/dl).

Subjects and methods

Patients

The study included maintenance HD patients with spontaneous or therapeutically induced levels of Hb > 13 g/dl (NOR group), and for control, patients treated with erythropoietin to obtain Hb levels within the 11–12 g/dl range (LOW group). In order to select patients with stable clinical conditions, the following inclusion criteria were applied: adult anuric patients (urine output ≤200 ml in the long interdialytic interval), standard HD for at least 12 months, stability of Hb values and constant achievement of dry weight in the last 3 months. Patients with co-morbidities associated with altered fluid redistribution, such as advanced heart or liver failure and peripheral or pulmonary oedema, were excluded. From a potential pool of 42 patients, we enrolled 14 patients in the NOR group and 18 in the LOW group. The remaining 10 patients refused to give their consent to the study, because the protocol required some extra time for them. All patients gave their informed consent to the study.

Study protocol

Prior to the study, all patients underwent a run-in period of 3 months to verify their achievement of dry body weight. During this period, dry weight was assessed monthly by means of clinical evaluation, measurements of the inferior vena cava diameter and bioelectrical impedance assessment (BIA) [18,24]. At the end of the run-in period, patients had the experimental HD session. The study was performed in the third session of the week in order to minimize the influence of fluid overload on the measured parameters [18]. Patients were dialysed while supine. Their body weight and blood pressure were measured and blood samples were obtained before and at the end (T0) of the dialysis session, and in the following 2 h (at 15, 30, 60, 90 and 120 min after the end of the session), through the dialysis needles remaining in place, and the patients remaining supine. In those patients receiving erythropoietin, the drug was administered subcutaneously.

Haemodialysis procedure

Standard bicarbonate HD was done with artificial systems equipped with automatic devices regulating UF rates. UF was kept constant throughout the dialysis session, which lasted 4 h. In order to avoid any exogenous factor interfering with the haemoconcentration during the session, and in the 2 h following the end of HD, patients were not allowed to have any food, drink or i.v. infusion of saline or plasma expanders. Dialysate temperature and sodium concentration were also kept constant (36.0°C and 142 mmol/l) since these two parameters can influence the plasma refilling rate. Blood and dialysate flow rates were 300–350 and 500 ml/min, respectively.

Measurements

At each experimental time, we measured Hb, Hct, total protein concentration and plasma sodium, as previously described [18,25]. Serum urea nitrogen was measured to calculate Kt/V according to the second generation equation of Daugirdas [26]. The pre-dialysis samples were obtained before starting the blood pump, while the post-dialysis samples (T0) were drawn after maintaining a low blood flow rate (50 ml/min) for 2 min in the absence of dialysate flow to minimize cardio-pulmonary blood recirculation. Blood pressure was measured with a mercury sphygmomanometer, with the first and fifth Korotkoff sounds being used to identify systolic and diastolic blood pressure, respectively. The mean of three consecutive measurements of blood pressure was calculated and used for statistical analysis. Body weight was recorded before and after HD by a bed balance with gradations of 0.05 kg (Tassinari, Cento-Italy). Serum urea nitrogen and total protein were measured using an autoanalyzer (Olympus AU 560, Olympus Italia, Segrate, Italy). Hb was measured by a Coulter counter (Coulter Electric, Hialeah, FL). To determine Hct, we used heparinized microtubes and a microcentrifuge (Bicasa, Sesto San Giovanni-Italy).

To evaluate the stability of the analytic method, three different standard samples of Hb (8.2, 12.4 and 17.2 g/dl) were analysed in triplicate during the analytical session, the analysis of the Hb standards being performed at the beginning, middle or end of each analytical session. Results are expressed as coefficients of variation: 1.0, 0.9 and 1.1% for the 8.2, 12.4 and 17.2 g/dl standards, respectively. A similar evaluation was performed for total protein concentration by using the following control samples: 4.2, 6.7 and 9.6 g/dl; their respective coefficients of variation were 1.1, 1.2 and 1.1%.

Statistics

All the values are reported as means ± SD. A paired Student t-test was used for intra-group comparisons between pre-and post-dialytic measurements. Analysis of variance with repeated measures followed by the Newman–Keuls as a post hoc test was used for the statistical analysis of post-dialytic changes. Unpaired Student t-test was used for inter-group comparisons. Correlations between individual parameters were evaluated using linear regression analysis. A two-tailed P-value < 0.05 was considered significant.
Results

The clinical characteristics of the patients are presented in Table 1. The two groups had the same body mass index, age, dialytic age and gender distribution. The achievement of dry weight, presumed on a clinical basis, was testified by the values of ICVD (8.6 ± 2.1 and 8.9 ± 2.4 mm/m² in NOR and LOW, respectively) and the BIA-derived total body water (54.4 ± 4.1 and 56.2 ± 6.4% of body weight in NOR and LOW, respectively) obtained at the end of the last HD session of the run-in period.

The values are means ± SD. BMI, body mass index; BW, body weight; EPO, erythropoietin.

Table 1. Baseline characteristics of patients with normal (NOR) and low (LOW) Hb levels

<table>
<thead>
<tr>
<th></th>
<th>NOR (n=14)</th>
<th>LOW (n=18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>8 (57)</td>
<td>11 (61)</td>
<td>1.00</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.4 ± 18.2</td>
<td>52.9 ± 13.4</td>
<td>0.67</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
<td>96 ± 68</td>
<td>82 ± 45</td>
<td>0.48</td>
</tr>
<tr>
<td>Primary renal disease n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>6 (43)</td>
<td>0 (0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2 (14)</td>
<td>5 (28)</td>
<td>0.43</td>
</tr>
<tr>
<td>Hypertensive nephropathy</td>
<td>3 (21)</td>
<td>5 (28)</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>1 (7)</td>
<td>2 (11)</td>
<td>1.00</td>
</tr>
<tr>
<td>Obstructive nephropathy</td>
<td>0 (0)</td>
<td>3 (17)</td>
<td>0.24</td>
</tr>
<tr>
<td>Tubulointerstitial</td>
<td>0 (0)</td>
<td>2 (11)</td>
<td>0.49</td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
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</table>

The values are means ± SD. BMI, body mass index; BW, body weight; EPO, erythropoietin.

Table 2. Main laboratory parameters and blood pressure values at the beginning (pre-HD) and from the end (T0) of the third HD session of the week up to the following 120 min in patients with normal (NOR) and low (LOW) Hb levels

<table>
<thead>
<tr>
<th></th>
<th>Pre-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>14.4 ± 1.2*</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>[Na⁺]p (mmol/l)</td>
<td>140 ± 3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>138 ± 23</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86 ± 9</td>
</tr>
<tr>
<td>LOW</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.4 ± 0.8</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>[Na⁺]p (mmol/l)</td>
<td>139 ± 4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>142 ± 12</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>88 ± 9</td>
</tr>
</tbody>
</table>

The values are means ± SD. Hb, haemoglobin; TP, total protein concentration; [Na⁺]p plasma sodium concentration; SBP, systolic blood pressure; DBP, diastolic blood pressure. To convert g/dl into g/l multiply by 10.

Post-dialytic changes

Body weight remained constant up to 120 min, since, according to the protocol, patients were anuric and did not receive food, drink or i.v. infusions during the post-dialytic period.

A progressive decrease of Hb was observed in both groups (Table 2). However, in LOW, the decrement

Intradialytic changes

The intradialytic changes of the main parameters are presented in Table 2. The duration of dialysis sessions was the same in NOR and LOW (239 ± 30 and 240 ± 24 min, respectively); UF rates (10.0 ± 1.7 and 10.3 ± 2.8 ml/h/kg) and Kt/V (1.28 ± 0.09 and 1.27 ± 0.12) also did not differ. As depicted in Figure 1, the intradialytic weight loss was comparable, being 4.0 ± 0.9 and 4.1 ± 0.9% of body weight in NOR and LOW, respectively. Hb increased from 14.4 ± 1.2 g/dl (range: 13.3–16.4 g/dl) to 16.3 ± 1.9 g/dl (range: 14.1–20.1 g/dl) in NOR patients, and from 11.3 ± 0.8 g/dl (range: 10.0–12.8 g/dl) to 12.7 ± 0.9 g/dl (range: 11.0–14.2 g/dl) in the LOW group (Table 2). This corresponded to a similar UF-induced haemocencentration (Figure 1). Similarly, during the session, Hct increased from 43.3 ± 3.6 to 49.0 ± 5.6% in NOR and from 34.1 ± 2.4 to 38.2 ± 2.7% in LOW.

The values of total protein were analogous at the beginning of HD (Table 2). At variance with Hb, the extent of the increase of total protein during dialysis was strikingly greater in NOR (34.6 ± 7.1%) than in LOW (19.6 ± 7.3%) (P < 0.0001 (Figure 1)).

At the start of the session, plasma sodium concentrations were similar in NOR and LOW patients. The intradialytic alterations of plasma sodium did not differ between the two groups (Table 2). Similarly, both systolic and diastolic blood pressures did not differ before and after HD in either group (Table 2).
reached a plateau within the first post-dialytic hour while it continued to decrease in NOR. Indeed, at T90 and T120, the decline was significantly larger in NOR (−7.9 ± 2.4 and −9.2 ± 3.0%) than in LOW (−4.8 ± 2.3 and −4.7 ± 2.4%) (P < 0.001) (Figure 2). As a consequence, the Hb concentration at the end of the observation period (T120) did not differ from its pre-dialytic value in NOR (P = 0.10), while it persisted higher (P = 0.004) in LOW (Table 2), with the intradialytic Hb increase being completely reversed in NOR (−93 ± 40%) but not in LOW (−49 ± 29%, P < 0.001). A similar pattern of changes was observed for Hct.

In NOR, a prompt and progressive reduction of total protein was detected in the post-dialytic period. The magnitude of this decrement was greater in NOR than in LOW, reaching pre-dialytic levels within 120 min in NOR, as observed for the Hb concentrations (Table 2). In contrast, the decrease was minor and over a shorter duration in LOW, with the plateau being reached within the first hour (Table 2).

It is noteworthy that the post-dialytic changes of Hb were inversely correlated with total protein values measured at the end of the dialytic session (r = −0.547, P = 0.0012, Figure 3).

Discussion

This study evaluates for the first time the effects of intra- and post-dialytic fluid redistribution on Hb levels in non-anaemic uraemic patients. Our report presents novel information, as no previous work has assessed the haemodiluting effect of plasma refilling in these patients. Our protocol allows us to assess the isolated effect of this phenomenon since all the factors that could cause haemodilution and are independent from plasma refilling, such as food or drink intake, i.v. saline infusion, or variations of plasma tonicity [24] and dialysate temperature [27], were avoided.


During HD, Hb levels increased by the same extent (+13% and +12%) in NOR and LOW patients (Figure 1). This increment is exclusively dependent on the entity of plasma refilling, since UF volume did not differ in the two groups. Plasma refilling can be calculated as the difference between the amount of UF and the intradialytic change of plasma volume [21]. Although plasma volume was not directly measured, it is likely that the NOR patients had a smaller plasma volume than anaemic subjects, because of their higher Hcts. Under these conditions, and in the presence of identical UF volumes, the similar intradialytic Hb changes in the two groups may actually reflect a smaller absolute variation of the plasma compartment in NOR. These data therefore suggest greater plasma refilling during dialysis in this group.

Interestingly, during HD, the increment of total protein concentration was greater in NOR than in LOW patients (Figure 1). This phenomenon certainly is unrelated to a different degree of volume depletion since the changes of Hb and Hct were analogous in the two groups. On the contrary, the difference of the magnitude of the increase of total protein between NOR and LOW is attributable to greater protein back-flow from the interstitial space toward the vascular compartment in NOR patients. Other authors have hypothesized that the intradialytic shift of proteins into the refilling fluid is a result of solvent drag [22,28]. The reason for the dissimilar extent of protein back-flow between NOR and LOW is not readily apparent; however, different sizes of the plasma compartment may play a role. Indeed, since plasma volume is smaller in NOR with respect to LOW, and UF subtracts water mainly from this compartment, the contraction of plasma volume and the ensuing increase of plasma total protein concentration are more rapid and larger in NOR. The same also holds true for the changes of total protein in the interstitial space, since protein concentrations in plasma and the interstitium are strictly correlated [21,22]. Therefore, it is reasonable to hypothesize that during the dialytic session in NOR, the greater increment of total protein concentration was dependent on the higher protein content of the fluid refilled from the interstitium. It is worth noting that these data also indicate that the assumption of the single pool compartment for total protein is incorrect and, consequently, that this parameter is not adequate to calculate the relative changes of plasma volume during HD.

The crucial finding of our study is the major protective role of post-dialytic plasma refilling in non-anaemic patients. Other authors have documented the persistence of plasma refilling upon termination of fluid removal in anaemic patients [19–23]. Those studies, however, were specifically aimed at assessing the protective effect of plasma refilling against HD-related alterations of systemic haemodynamics. In fact, in those studies, the mean Hb was not even mentioned [19–23]. In addition, their experimental conditions were considerably different from those of a standard HD; instead, post-dialytic plasma refilling was evaluated with blood circulation ongoing, in the absence of UF or dialysate flow [19], or after treatment with aggressive and very short UF profiles (15–120 min) [20–23]. Finally in one of those studies, eight of the 15 patients studied were on conservative treatment rather than on maintenance HD [20]. More importantly, no information on the refilling-induced changes of Hb or Hct was given. In the current study, we provide first-time evidence that Hb levels are restored to their predialytic values in a very short time (2 h from the end of the session) in NOR but not in LOW patients. Specifically, in both groups, the decrease of Hb occurred immediately after the end of the session (15 min) and continued up to 60 min. However, at T60, the Hb reduction was complete in LOW while it continued in NOR patients. In fact, in NOR patients, additional Hb decreases were detected at T90 and T120.

Interestingly, we recently have demonstrated that in HD patients, the post-dialytic fluid shift from the intracellular compartment toward the intravascular
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restoration of pre-dialytic Hb levels within the initial in- tradialytic plasma refilling; (ii) the larger plasma increase of Hb concentration is limited by greater anaemic HD patients: (i) the extent of intradialytic oncotonic pressure. We cannot rule out that in NOR, the major dialytic increment of oncotonic pressure may also have contributed to the development of a larger intradialytic plasma refilling. It is intriguing to argue that the rapid restoration of Hb and Hct to basal values in NOR, but not in LOW, could represent a mechanism of defence against excessive haemoconcentration in this high risk group of patients. Indeed, previous studies have reported a significant increase of blood viscosity during HD in non-anaemic patients compared with anaemic ones [12,13]. The increase of blood viscosity, however, was not properly quantified since UF was not applied to all patients and saline was infused during the treatment. Furthermore, no information on blood viscosity in the immediate post-dialytic period was provided. On the basis of the Hb and Hct values that we obtained in this study, it is possible to postulate that the rheological properties of blood were completely restored in NOR patients within 2h after the end of the session. Whether or not this phenomenon is protective against thrombotic events in patients with normal Hb levels cannot be established by this short-term study. Nevertheless, previous prospective trials in larger HD populations have demonstrated that the complete correction of anaemia is not invariably coupled with increased thrombosis in the vascular access [1,5]; these results could be explained by the presence of greater plasma refilling in patients with higher Hb levels. The limitation of our study is that the documentation of the Hb and Hct rebound in the immediate post-dialytic period was obtained in the course of conventional HD with an UF volume < 5 l. Caution is necessary in extrapolating these results to broader dialysis populations. Similarly, whether or not the same phenomenon also occurs in high efficiency dialysis or haemodiafiltration needs to be investigated.

In conclusion, this study indicates that in non-anaemic HD patients: (i) the extent of intradialytic increase of Hb concentration is limited by greater intradialytic plasma refilling; (ii) the larger plasma refilling persists after the end of the session, with the restoration of pre-dialytic Hb levels within the initial 2h; and (iii) the force driving this phenomenon is mainly the larger changes of total protein concentration. The rapid correction of HD-induced haemoglobin-concentration in NOR may provide experimental support to the proposal to correct anaemia completely, at least in patients treated with conventional HD.

Conflict of interest statement. None declared.

References


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