Dialysis: its role in optimizing recombinant erythropoietin treatment

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Abstract

Although iron deficiency is probably the most important factor affecting response to recombinant erythropoietin (Epo, epoetin), other factors are of significance, including dialysis adequacy. Additionally, water treatment and distribution, sterilizants and the quality of the dialysate in terms of trace elements (particularly chloramine) are of importance in relation to erythropoiesis inhibition. Microbiological or pyrogenic contamination can cause or aggravate anaemia in haemodialysis patients, and the impact of enhanced production of cytokines should be taken into consideration. By removing small and (possibly) medium/large molecules, adequate dialysis is of paramount importance in correcting anaemia and optimizing epoetin therapy. The biocompatibility of dialysis membranes and flux are other important factors. As yet unknown uraemic toxins may suppress erythropoiesis and contribute towards the development of anaemia. It is reasonable to hypothesize that, because anaemia improves after the start of dialysis with cellulose membranes, low molecular weight erythropoiesis inhibitors are involved, as well as medium/large molecular weight inhibitors, which are removed by more permeable membranes. However, in highly selected, adequately dialysed patients without iron or vitamin depletion, the effects of dialysis membrane type on haematological parameters and epoetin efficacy are smaller than might be expected from the results of uncontrolled studies. Improvement in anaemia has been observed using on-line haemofiltration, haemodiafiltration, and sterile dialysate. The results of prospective, randomized trials examining the impact of these factors on anaemia and the effectiveness of epoetin treatment are eagerly awaited.

Keywords: anaemia; dialysate; dialysis dose; erythropoietin; membrane; on-line treatments

Introduction

Anaemia is one of the major negative clinical characteristics of patients with chronic renal failure (CRF) on renal replacement therapy and, together with hypertension, is the leading cause of cardiac hypertrophy and subsequent dilatation. Given that cardiovascular disease is the leading cause of morbidity and mortality in both dialysed and transplanted patients [1], as much as possible should be done to prevent, reverse or at least reduce this complication.

Due to the availability of recombinant human erythropoietin (Epo, epoetin) therapy, severe anaemia of end-stage renal disease (ESRD) among patients requiring repeated blood transfusions has almost completely disappeared over the last 10 years [2]. It has also reduced left ventricular hypertrophy [3,4] and led to a direct improvement in myocardial function. However, despite an increase in the use and average dose of epoetin, a substantial proportion of patients do not achieve a haematocrit level of >30% [5,6], which is well below the target range (33–36%) proposed by The National Kidney Foundation—Dialysis Outcomes Quality Initiative (NKF-DOQI) guidelines and the European Best Practice Guidelines for the treatment of anaemia in CRF [7,8].

Anaemia in haemodialysis (HD) patients is a complex syndrome and many factors other than absolute or relative erythropoietin deficiency may contribute to it. Although iron deficiency is probably the most important factor affecting response to epoetin in most patients, occult blood loss, infection, inflammation, and dialysis adequacy are also important [9]. Bone marrow suppression may also be present [10], probably induced by the retention of toxic metabolites. Less frequent causes are hyperparathyroidism with narrow fibrosis, aluminium toxicity, vitamin B12 and folic acid deficiencies, carnitine deficiency (absolute or dialysis related) [11], bone marrow disorders, and
haemoglobinopathies. ACE inhibitors and angiotensin II receptor antagonists may also play a role.

This review will focus on the effect of dialysis on anaemia and epoetin treatment, analysing the main factors that are directly related to dialysis treatment.

Water quality and dialysate

The quality of the dialysate, in terms of chemical (trace element), microbiological and pyrogenic contamination, can cause or aggravate anaemia in HD patients. While some contaminants are present in the water at source, others are added as part of the treatment process for the production of safe drinking water or leach from the water piping system. Official national healthcare committees, i.e. the Association for the Advancement of Medical Instrumentation in the US and the European Pharmacopoeia in Europe, set upper limits for chemical and microbiological contaminants of dialysis water. However, even if manufacturers are obliged to specify the chemical composition and microbiological purity of their concentrates, no such stipulations are made on the final dialysis fluid produced by adding water in the clinical setting. Here, chemical contamination is mainly the consequence of using inadequately processed water. HD patients come into blood contact with approximately 120 litres per dialysis session, i.e. 19 800 litres each year. In these patients, chronic exposure to even low concentrations of toxic substances can produce a number of complications, including the development or worsening of anaemia.

In this regard, it is well known that the aluminium concentration in water supplies, in addition to aggravating osteopathy and dementia, can aggravate bone marrow suppression and cause anaemia. Water contains many other potential contaminants that can induce anaemia, such as nitrates, copper, fluorine, arsenic, and zinc. Chloramines, which are added to water as disinfectants, can also cause haemolytic anaemia secondary to oxidative damage [12,13]. The introduction of reverse osmosis treatment, which removes aluminium and many other substances from water, and activated carbon filters, which remove chloramines, have partially solved these problems.

Superimposed contamination of water with bacteria or toxins may also be dangerous and even potentially life threatening in HD patients. After a drought in 1996, all 126 patients in a HD unit in Brazil developed signs and symptoms of acute neurotoxicity and subacute hepatotoxicity following the use of water from a lake that had a massive growth of cyanobacteria, a blue-green algae. Sixty patients died [14]. It is also worth remembering that microbial growth was observed in up to 18% of commercially available liquid bicarbonate concentrates tested in one study [15]. This level of contamination is certainly reduced in the currently available dry bicarbonate concentrates.

Given the importance of dialysate quality and purity, not only in anaemia correction, but also in reducing patient morbidity, stringent control of each component of the water treatment system and of both the chemical and microbial purity of water and final dialysis fluid are mandatory. Regular contact with local water suppliers is also recommended for early recognition of changes in water quality.

Extracorporeal circuit

Anaemia in HD can occur as a result of haemolysis due to mechanical injury of erythrocytes from connectors, and inlet and outlet chambers of the dialyser [16], occluded or kinked HD blood lines [17], or from faulty blood tubing sets [18,19]. Although these events are rare, in 1996, two patients died because of haemolysis caused by a faulty tubing lot [17]. The negative pressure in the extracorporeal circulation does not seem to be a significant haemolytic factor in flowing blood. However, defects in the blood pump system can cause haemolysis. Also, the highest shear stresses in a dialysis system can be expected to be found within the needle, where the largest velocity : diameter ratio appears. Haemolysis can thus occur when using small ago-cannulae at high blood flow rates [20]. Large-diameter needles are thus recommended, and single-need HD should be avoided as much as possible.

Dialysis dose and frequency

Adequate dialysis is of paramount importance in correcting anaemia by removing small, and possibly medium/large, molecules that may inhibit erythropoiesis. In the early 1980s, Radtke et al. [21] demonstrated the relationship between anaemia and endogenous serum erythropoietin level in 42 ESRD patients beginning HD. After the start of dialysis, an increase in haematocrit levels was observed, in spite of a decrease in erythropoietin levels. It was hypothesized that the improvement in anaemia was not a consequence of increased erythropoietin production, but was probably due to the haemodialytic elimination of a bone marrow inhibitor. However, given the great efficacy of epoetin in correcting anaemia, and the prominent role of iron deficiency on epoetin resistance, the role of dialysis dose per se on response to epoetin has been largely underestimated in recent years. In a prospective, randomized study of 135 patients, Ifudu et al. [22] found that an increased dialysis dose in patients receiving inadequate dialysis led to a significantly increased response to epoetin. However, as this result was achieved using a highly permeable and biocompatible membrane (high-flux polysulphone), it is at least possible that biocompatibility and/or permeability had an additive effect on increased dialysis dose and the correction of anaemia [23].
Recently, the same authors analysed this effect further in a retrospective study of 309 HD patients [24]. Mean haematocrit differed significantly between quartiles of urea reduction rate (URR), with patients with URR > 70% being 2.6 times more likely to have haematocrit levels > 33%. The same held true not only in individual patients, but also at dialysis facility level. Ifudu et al. [24] found that the mean haematocrits of 141 dialysis facilities correlated with mean URRs ($r = 0.32, P < 0.001$). Unfortunately, no information was given on type of dialyser membrane used and dialysis modality. Data from the US Renal Data System also indicate a correlation between dialysis dose and haematocrit level in HD patients treated with epoetin [25]. However, in this observational study, haematocrit was also higher in patients treated with synthetic compared with non-synthetic membranes, making it very difficult to distinguish the effect of dialysis adequacy from that of membrane biocompatibility.

Similarly, recent preliminary results from a cross-sectional study of a random sample of 7092 ESRD Medicare patients receiving HD indicate that URR is independently associated with both epoetin weekly dose and haematocrit level [26]. This association was stronger for patients with a URR < 70%.

Additional data on the possible role of dialysis dose on anaemia correction came from the dialysis centre in Tassin, France [27], which uses long HD (8 h). Fifty-nine patients from the Tassin centre were compared with 53 patients recruited from three dialysis centres in Sweden, where treatment times were considerably shorter (3–5 h). Mean haematocrits were similar in the two groups, but the proportion of patients treated with epoetin was much higher in the Swedish than in the Tassin group. The mean dose of HD ($K_d/V$) was significantly higher for Tassin patients than for Swedish patients ($P < 0.0005$). It is difficult to distinguish whether the control of anaemia observed in the Tassin group was due mainly to a higher depuration rate or to an effect associated with dialysis time per se, independent of dialysis adequacy.

In order to separate the direct effect of dialysis adequacy per se from the effects of dialysis modality and membrane biocompatibility, Movilli et al. [28] investigated the relationship between epoetin and dialysis doses in 68 patients on conventional HD. While haematocrit did not correlate with $K_d/V$, epoetin dose and $K_d/V$ were inversely correlated. In a multivariate regression analysis with epoetin as dependent variable, $K_d/V$ was confirmed as the only significant variable that contributed independently to epoetin dose ($P < 0.002$). Additionally, division of the patients into two groups according to $K_d/V$ showed that weekly epoetin dose was significantly lower in the group with $K_d/V > 1.4$ than in the group with $K_d/V \leq 1.2$ ($P < 0.0001$).

As well as dialysis dose, dialysis frequency could have an effect on anaemia. Some preliminary observations suggest that a more frequent schedule, such as daily HD (2 h, six times a week), could enable better control of anaemia [29,30].

Altogether, these results stress the importance of adequate dialysis, not only as a tool for reducing the morbidity and mortality of HD, but also for optimizing epoetin responsiveness and achieving anaemia correction in a higher percentage of patients.

Membranes and convective treatments

A number of experimental and clinical studies suggest that biocompatibility of dialysis membranes and convective treatments may have a role in anaemia correction and epoetin responsiveness [22], independent of dialysis adequacy. As yet unknown uraemic toxins may suppress erythropoiesis and contribute towards the development of anaemia [31,32]. Conventional HD may have a significant role in removing low molecular weight erythropoiesis inhibitors. However, medium molecular weight (around 10 kDa) inhibitors and high molecular weight (around 1000 kDa: the KR4-0 peak fraction) inhibitors have also been found in uraemic serum [33]. These inhibitors can only be removed by more permeable membranes (medium molecular weight) or highly porous membranes (high molecular weight inhibitors).

Kobayashi et al. [33] reported clinical results obtained in eight HD patients treated with a large-pore membrane (BK-F polymethylmethacrylate) and suggested there was a major effect in two patients as they achieved a haematocrit of 25% after 11 months from baseline values of approximately 21%. However, a wide range of haematocrit levels was reported and no information was given concerning iron status. Furthermore, the study was not randomized, and had a very small sample size and no control group.

Villaverde et al. [34] found that a switch from cellulose to high-flux polysulphone membrane, without a change in dialysis dose, improved response to epoetin by about 14% in 31 HD patients with a target haematocrit level of 35%. However, this study was not randomized, making it difficult to draw definite conclusions from the data. Similar considerations apply to the study of Kawano et al. [35], who reported results from 10 HD patients treated with a high-flux dialyser (BK-F). The epoetin dose was considerably reduced, but only three of the patients had baseline haematocrit values of 30% or more, and none of them experienced an increase in baseline haematocrit level of > 10%.

With the aim of analysing the same hypothesis further, Locatelli et al. [36] performed a multicentre, controlled, randomized study of 84 HD patients. The efficacy of HD with a high-flux membrane (BK-F polymethylmethacrylate) was compared with conventional HD using a cellulose membrane, in terms of improving anaemia. The calculated sample size and length of follow-up (12 weeks) were based on data generated by Ifudu et al. [22]. An increase in haemoglobin (Hb) level
was observed in the population as a whole, but this trend was not significantly different between treatment groups [36] (Figure 1). In the experimental group, the tendency of Hb level to increase was present at each month during follow-up, suggesting that the period of observation was too short [36]. However, four out of six patients in the study by Kobayashi et al. [33] had improved haematocrit levels from baseline after as little as 4 months, despite much lower starting haematocrit values, and Ifudu et al. [22] reported increases in haematocrit values as early as 6 weeks after increasing the dialysis dose (URR increased from 60 to 72%). However, as the patients in this study had received a very inadequate dialysis dose at baseline, it is possible that the increased haematocrit values observed were due mainly to the effect of increasing the dialysis dose rather than the use of a high-flux biocompatible membrane.

The lack of effect on anaemia correction with the high-flux membrane in the study by Locatelli et al. [36] may have a number of other possible explanations. Certainly, the selection of only well-nourished patients, without other known factors affecting uraemic anaemia and receiving adequate dialysis \((K_t/V = 1.29 \pm 0.22)\), may have reduced the power of the study. Another possible explanation is that the patients (with haematocrit values of \(28.3 \pm 2.3\%\)) were not anaemic enough, in comparison with the general dialysis population. Data from the Lombardy Registry give a mean haematocrit value for the general dialysis population of \(30 \pm 4\%\), regardless of epoetin treatment [5], similar to that found in other dialysis populations (Canadian Erythropoietin Study Group). Also, a nearly statistically significant imbalance in the percentage of patients receiving iron therapy (higher in the conventional group) may have favoured anaemia correction in patients on conventional HD. Although not statistically significant, the differential distribution of underlying disease (polycystic kidney and diabetes mellitus), possibly favouring conventional treatment, should also be taken into account.

Another important factor to consider is the endogenous erythropoietin concentration. Kobayashi et al. [33] and Kawano et al. [35] showed that high plasma erythropoietin concentrations overcame the inhibitory effect of the KR4-0 peak fraction on erythropoiesis. Locatelli et al. [36] could not exclude this possibility in their patients because the median baseline endogenous erythropoietin concentrations were only in the normal range \((5.4–19.4, \text{and } 5.7–22.6 \text{ in the conventional and experimental groups, respectively})\). The absence of a correlation between endogenous erythropoietin and haematocrit levels highlights the fact that, in addition to erythropoietin, other growth factors (such as insulin-like growth factors I and II, growth hormone, colony-stimulating factors, and several interleukins [ILs]) may play a role in erythropoiesis.

The results obtained by Locatelli et al. [36] also illustrate the need for a control group in order to obtain an unbiased estimate of treatment effect. In any case, the negative results of this study strongly suggest that, when patients are highly selected, adequately dialysed and have no iron or vitamin depletion, the effect of high-flux membrane is much less than might be expected from the results of uncontrolled studies. Similar negative findings were obtained from the secondary analysis of a multicentre trial comparing biocompatible and traditional membranes, and convective and diffuse treatment modalities in 380 patients followed for 24 months [37]. Haematocrit levels increased during the course of the study in the overall trial population, probably as a consequence of a trial effect, but did not differ significantly among the four treatment modalities: cuprophane HD, low-flux polysulphone HD, high-flux polysulphone HD, and high-flux polysulphone haemodiafiltration (HDF) (unpublished data) (Figure 2). However, a significant increase in haematocrit levels was observed in the combined groups of patients on high-flux vs those on low-flux treatments (Figure 3). It is possible that a difference in dialysis dose, which was higher in the HDF group, may partially explain this observation.

**On-line treatments**

It has recently been suggested that on-line treatments have a stronger effect on anaemia correction than conventional treatments or standard HDF techniques. On-line HDF is a technique that combines diffusion with higher convection than standard HDF and in which the dialysis liquid, free of toxins and pyrogens, is used as substitution fluid. It is possible that this technique is more effective in achieving higher...
haematocrit levels as a result of two mechanisms: higher removal of medium and large molecules (possibly including bone marrow inhibitors) and reduced microbiological and pyrogenic contamination of the dialysate.

Maduell et al. [38] studied 37 patients who were switched from conventional HDF to on-line HDF and followed up for 1 year. Interestingly, significant increases in Hb (from $10.7 \pm 1.1$ to $11.4 \pm 1.5$ g/dl) and haematocrit (from $32.2 \pm 2.9$ to $34.0 \pm 4.4\%$) were observed during the on-line HDF period. This allowed a significant decrease in the epoetin dose (from $3861 \pm 2446$ to $3232 \pm 2492$ IU/week). It should be noted that the patients also experienced an increased dialysis dose (15% increase in $K_d/V$) during the on-line HDF period, which could have partially favoured the observed increase in Hb and haematocrit levels. Moreover, this study was not randomized. Similarly, Grillo et al. [39] studied 31 patients, who were treated by on-line HDF for at least 9 months. They found a significant increase in Hb levels (higher in polycystic kidney patients) and a consequent reduction in epoetin consumption, though not statistically significant. The $K_d/V$ remained constant during the course of the study. However, other studies could not confirm these observations [40,41].

Ward et al. [40] prospectively compared on-line HDF with high-flux HD in 44 patients who were followed up for 1 year. Although anaemia control was not a primary outcome, there was no change in haematocrit or Hb over the course of the study and the average weekly dose of epoetin increased only slightly in both groups. Wizemann et al. [41] also failed to confirm a possible effect of on-line HDF on anaemia correction. In this prospective, controlled study, 44 patients were randomized to undergo either low-flux HD or on-line HDF. To eliminate confounding factors, low molecular dialysis dose was matched ($K_d/V$, 1.8), and the same treatment duration (4.5 h) and membrane (polysulphone) were used in each group. After 24 months of follow-up, haematocrit levels and epoetin dose did not differ between the two groups. Given that the same ultrapure dialysate was used in both groups in this study, it is possible to hypothesize that the main factor accounting for anaemia correction reported by Maduell et al. [38] may have been a reduced inflammatory stimulus from the dialysate. Wizemann et al. [41] found a significant decrease in C-reactive protein (CRP) level during HDF in comparison with the levels during low-flux dialysis. However, the acute effect of on-line HDF on CRP needs further elucidation. Favouring the importance of dialysate sterility on anaemia correction in HD patients, Sitter et al. [42] reported a significant and sustained reduction of epoetin dose in patients switched from conventional bicarbonate HD, with potentially microbiologically contaminated dialysate, to a similar treatment modality using online-produced, ultrapure dialysate. The switch resulted in lower bacterial contamination with a significant decrease in CRP and IL-6 levels. Interestingly, by multivariate analysis, IL-6 levels were shown to have a strong predictive value for epoetin dose in both groups.

Altogether, the possibility that on-line HDF/haemofiltration may achieve a better control of anaemia is intriguing. However, the available results are conflicting, mainly because of differences in treatment modalities in control groups, frequent lack of randomization, small numbers of enrolled patients and because anaemia was not the primary outcome in any of the studies. Furthermore, in many cases, on-line HDF resulted in a higher dialysis dose than control treatments, further complicating the interpretation of any observations. The results of prospective, randomized trials aimed at better testing this hypothesis are awaited.

Conclusions

Given that epoetin resistance is a clinically and economically relevant problem, it is important to understand...
the extent to which the mode and/or dose of dialysis can influence anaemia and epoetin efficacy. Adequate dialysis may contribute to the correction of anaemia by removing erythropoiesis inhibitors. Furthermore, the biocompatibility and/or permeability of membranes could have an additive effect to increased dialysis dose in relation to the correction of anaemia.

However, in highly selected, adequately dialysed patients without iron or vitamin depletion, the effect of dialysis membrane type seems to be smaller than might be expected from the results of uncontrolled studies. The role of on-line treatments is still controversial, mainly because anaemia was not the primary outcome in any of the studies performed so far and because of the difficulty in discriminating between the effects of on-line HDF per se from that of an increased dialysis dose. Dialysate quality is also important in reducing haemolysis and pyrogen production. On-line-produced ultrapure dialysate will probably be a quality target in future years, in order to reduce bacterial contamination and the consequent chronic inflammatory response. This will probably improve anaemia correction, reduce epoetin doses and partially compensate for the extra cost of convective treatments.

References


