Dialysis adequacy and response to erythropoietic agents: what is the evidence base?

Francesco Locatelli and Lucia Del Vecchio

Department of Nephrology and Dialysis, Ospedale A. Manzoni, Lecco, Italy

Abstract
Anaemia secondary to chronic kidney disease is a complex syndrome. Adequate dialysis can contribute to its correction by removing small, and possibly middle/large molecules, that may inhibit erythropoiesis. A clear relationship between higher haemoglobin or haematocrit levels, lower recombinant human erythropoietin (epoetin) dose and increase in dialysis dose has been reported in a number of prospective and retrospective studies. This is particularly true in patients receiving inadequate dialysis. Increased attention has also been paid to the relationship between dialysis, increased inflammatory stimulus and response to erythropoietic therapy, as dialysate contamination and low-compatible treatments may increase the production of cytokines and consequently inhibit erythropoiesis. As middle-/large-molecular-weight inhibitors can only be adsorbed or removed by more permeable membranes, the biocompatibility of dialysis membranes and flux are also important factors. In highly selected, adequately dialysed patients without iron or vitamin depletion, however, the effect of these treatment modalities on anaemia appears to be smaller than expected. The role of on-line treatments is still controversial; moreover, it is still difficult to discriminate between the effects of on-line haemodiafiltration per se (use of high-flux biocompatible membranes and pyrogen-free dialysate) from that of an increased dialysis dose. Prospective, randomized, adequately sized studies on this topic are still needed. Results, albeit very preliminary, obtained with short or long nocturnal daily haemodialysis on anaemia correction are encouraging. Adequate dialysis is not only a tool for reducing morbidity and mortality of haemodialysis patients, but is also a way of optimizing responsiveness to erythropoietic therapy, allowing easier achievement of anaemia correction in a higher percentage of patients.

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

Introduction
Anaemia secondary to chronic kidney disease (CKD) is a complex syndrome and there are many factors that contribute to its cause, the main ones being erythropoietin (EPO) deficiency, hypoproliferative bone marrow function and reduced survival of erythrocytes. Although iron deficiency is probably the most important factor affecting the response to erythropoietic therapy (epoetin alfa, epoetin beta or darbepoetin alfa) in most patients, occult blood loss, infection and inflammation are also important [1]. Less frequent causes of anaemia are hyperparathyroidism with marrow fibrosis, aluminium toxicity, vitamin B_{12} and folate deficiencies, carnitine deficiency [2], bone marrow disorders and haemoglobinopathies. Angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists may also play a role in the development of anaemia. In addition, pure red cell aplasia secondary to epoetin therapy has recently emerged as a rare cause of profound resistance to treatment [3].

Adequate dialysis can play a role in anaemia correction by removing small, and possibly middle/large molecules, that may inhibit erythropoiesis. The role of dialysis dose per se on the response to erythropoietic therapy has been largely underestimated in the past and, only recently, more interest has been focused on this matter. Furthermore, there has been an increase in the attention paid, not only to risks related to water and dialysate impurities and pump or line defects, but also to the relationship between dialysis, increased inflammatory stimulus and response to EPO therapy. In addition, it has been hypothesized that a certain amount of administered erythropoietic treatment could adhere to a stagnant layer at the top of the
bloodline drip chamber, thus causing a potential loss of the drug when injected into the venous bloodline.

Uraemic toxins and their role in the suppression of erythropoiesis

The observation that the start of dialytic treatment and the administration of androgens and various growth factors are capable of increasing the concentration of haemoglobin independently suggests that erythropoiesis in patients with CKD is largely influenced by the retention of toxic metabolites. A number of metabolites have been implicated as potential EPO toxins, including various amines such as spermine [4] and parathyroid hormone [5]. These substances are, however, general bone marrow toxins and not specific suppressors of erythropoiesis [6]. As anaemia improves after the start of dialysis with cellulose membranes, these toxic inhibitors are thought to be of low molecular weight. However, high-molecular-weight inhibitors, removed only by the use of highly porous membranes, have also been found to be suppressors of erythropoiesis [7].

In recent years, evidence has accumulated for the role of inflammatory cytokines in the inhibition of erythropoiesis in CKD-related anaemia. Signs of inflammation are seen in 35–65% of patients with end-stage renal disease (ESRD) on haemodialysis. Several factors, such as impaired clearance of cytokines, accumulation of advanced glycation end-products, atherosclerosis per se and other inflammatory diseases, and unrecognized persistent infections, have been implicated. In addition, the dialysis procedure itself has been linked to an increased risk of inflammation. Indeed, the prevalence of elevated serum levels of C-reactive protein (CRP) is higher after the start of dialysis [8].

The most important mechanism for cytokine-induced anaemia is the suppression of bone marrow erythropoiesis, but the extent to which elevated levels of cytokines and the acute-phase response may contribute to resistance to epoetin treatment is still not clear. Allen et al. [9] investigated the effect of sera from ESRD patients with and without infection or inflammatory disease on colony-forming unit erythroid (CFU-E) colony formation in vitro. Colony formation was suppressed by soluble factors in the sera of uraemic patients with or without inflammation causing stimulation of the production of interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α), which then inhibited erythropoiesis further.

Interestingly, TNF-α was a significant individual predictor of epoetin requirements in 34 patients on haemodialysis [10]. Conversely, the patients who required more epoetin had lower levels of IFN-γ and interleukin-12 (IL-12) [11]. IL-6 is a pro-inflammatory cytokine, which is 8–10-fold higher in patients receiving haemodialysis and has been related to poor outcome [10]. IL-6 was found to antagonize the effect of EPO on bone marrow proliferation [12] and its levels were directly related to the dose of epoetin in patients receiving haemodialysis [11]. Of interest, IL-6 levels were significantly higher in the patients treated with less-biocompatible membranes [11]. The possibility that toxic metabolites and depressive cytokines may play a role in reducing EPO synthesis cannot be ruled out, but intense dialysis does not modify levels of EPO appreciably [13].

Dialysate fluid contamination and potential consequences on erythropoiesis

Blood of patients receiving haemodialysis is in contact with ~120 litres of water per dialysis session, i.e. 19 800 litres each year. Chronic exposure to even low concentrations of toxic substances can produce a number of complications including the development or the deterioration of anaemia. Some contaminants are present in the water at its source, while others are added as part of the treatment process for the production of safe drinking water or are leached from the water piping system. Mains water contains many potential contaminants that can induce anaemia, such as aluminium, nitrates, copper, fluorine, arsenic and zinc. Chloramines, which are added to water as disinfectants, can also cause haemolytic anaemia secondary to oxidative damage [13,14]. The introduction of reverse osmosis treatment, which effectively removes aluminium and many other substances from water, and activated carbon filters, which remove chloramines, have partially solved these problems.

Dialysis fluid does not have to be sterile, but higher levels than the accepted standards of bacteria and endotoxin may be found in the water supplied to dialysis centres, even after adequate treatment [15]. These organisms can multiply in dialysis fluids to achieve a level of contamination sufficient to cause bacteraemia or pyrogenic reactions. Intact dialysis membranes should prevent bacterial passage, but the presence of defects in the membrane integrity or a high level of bacterial contamination can allow bacteria to ‘grow through’. Bacteria-derived substances can cross the dialytic membrane very easily as demonstrated by the presence of antibodies against endotoxins in patients during both low-flux cuprophane and high-flux polymethylmethacrylate dialysis [16]. This inflammatory stimulus, often sub-clinical, can contribute to monocyte activation, cytokine production and subsequent inhibition of erythropoiesis. Given the importance of dialysate quality and purity, not only in anaemia correction, but also in reducing patient morbidity, stringent controls of the function of each component of the water treatment system and of both the chemical and microbial purity of the water and final dialysis fluid are essential.

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. [17]
had already observed a relationship between anaemia and serum levels of EPO in 42 patients with ESRD starting haemodialysis. After the start of dialysis, an increase in haematocrit levels was observed (from 21.7 to 28.6%, \( P < 0.001 \)), in spite of a decrease in endogenous serum EPO levels (from 509 to 182 mIU/ml; \( P < 0.001 \)). It was hypothesized that the improvement in anaemia was probably caused by the haemodialytic elimination of a bone marrow inhibitor. The role of dialysis dose itself on anaemia and epoetin response has progressively become known, even though its significance may be underestimated. In a prospective study of 135 patients, Ifudu et al. [18] found a direct relationship between haematocrit and urea reduction rate (URR) after adjustment for other factors; at logistic regression analysis, an 11% increase in URR double the probability of a patient having a haematocrit level higher than 30%. Twenty consecutive patients on inadequate dialysis (baseline URR < 65%) received an increase in dialytic dose and were compared with 20 other consecutive patients on inadequate dialysis in whom the dialysis schedule was not modified [18]. After 6 weeks, in parallel with an increase of mean URR to 72%, haematocrit levels rose from 28.4 ± 0.78 to 32.3 ± 0.71% (\( P = 0.002 \)), but remained unmodified in the control group. The mean weekly dose of EPO was similar in the two groups. Surprisingly, however, the standard deviation of the URR values obtained after the increase in the dialysis dose in this study on intensity of haemodialysis, was not available in the paper [18]. As a result, this lack of information does not allow the reader to estimate the degree of variability in dialysis adequacy and if this could have influenced the data. Furthermore, as these results were achieved using a highly permeable and biocompatible membrane (high-flux polysulphone), it is possible that the biocompatibility or permeability, or both, have an additive effect to the increased dialysis dose on the correction of anaemia. More recently, Ifudu et al. [19] further analysed the effect of dialysis adequacy on anaemia in a retrospective study of 309 patients on haemodialysis. Mean haematocrit levels differed significantly between quartiles of URR, with patients having a URR > 70% being 2.6 times more likely to have haematocrit levels > 33%. Unfortunately, no information on the dialyser membranes and dialysis modality in these patients was given.

Large cohort studies have also found a clear relationship between the degree of anaemia and dialysis dose. None of these studies, however, have been able to discriminate the role of different dialysis modalities in addition to that of adequacy. From data collected from 21 899 patients on haemodialysis in the USA in 1993, Madore et al. [20] found that the dose of dialysis (URR) was significantly and independently associated with the haemoglobin concentration (\( P < 0.001 \)). Similarly, results from a study of large cohorts of nearly 7600 haemodialysis patients in Medicare on dialysis from 1994 to 1998 showed that URR was an independent predictor of higher haematocrit levels and lower prescribed epoetin dose [21]. It is interesting to note that despite the progressive increase in dialysis dose over the last few years in the USA, the relationship between dialysis dose and anaemia has remained. This could suggest that dialysis dose can also be of importance for anaemia correction in patients receiving more adequate dialysis.

Data on the possible role of dialysis dose in anaemia correction has also been collected from the dialysis centre in Tassin, France. It is well known that patients from this facility are treated with haemodialysis for relatively long sessions (8 h). Fifty-nine patients from this facility were compared with 53 patients from Sweden receiving conventional haemodialysis lasting 3–5 h [22]. The mean haematocrit level was similar in the two groups, but the proportion of patients receiving erythropoietic treatment was much higher and mean urea clearance ratio (Kt/V) was significantly lower in the shorter-treated group than in the longer-treated group. Nevertheless, it is difficult to determine whether the better control of anaemia observed in the patients on longer haemodialysis mainly resulted from a higher epuration rate or was a possible effect of the length of dialysis, independent of dialysis adequacy.

In order to separate the direct effect of dialysis adequacy from the effects of dialysis modality and membrane biocompatibility, Movilli et al. [23] investigated the relationship between epoetin and dialysis dose in 68 patients receiving conventional haemodialysis. Haematocrit levels did not correlate with Kt/V, but epoetin dose and Kt/V were inversely correlated. Using multivariate regression analysis with epoetin as the dependent variable, Kt/V was the only significant variable contributing independently to the dose of epoetin. The influence of dialysis adequacy on the epoetin dose required to maintain the target haematocrit was also evident in patients with Kt/V of 1.4 or more.

Interestingly, we were unable to demonstrate an association between dialysis adequacy and haemoglobin levels in 197 patients on long-term haemodialysis regularly followed at our centre (unpublished data) (Figure 1). Furthermore, Kt/V urea levels were not associated with the need for EPO in these patients. A number of confounding factors, however, have probably biased these results obtained in an unselected population.

Membranes and convective treatments

Conventional haemodialysis may have a significant role in removing low-molecular-weight erythropoietin inhibitors, but not in removing middle-/large-molecular-weight inhibitors as these can only be removed using more permeable membranes. Using this hypothesis, Kobayashi et al. [24] found a significant increase in haematocrit levels in two out of eight patients receiving haemodialysis treated with a large-pore membrane (BK-F polymethylmethacrylate). This small, non-randomized study, however, had a number of
drawbacks. In particular, patients had a wide range of haematocrit levels and no information was provided on their iron status. Similarly, Villaverde et al. [25] found that the switch from cellulosic to high-flux polysulphone membrane, without any change in the dialysis dose, improved the response to epoetin by $\sim14\%$ in 31 haemodialysis patients with a target haematocrit level of 35%. Interesting results also came from a study of 10 patients receiving haemodialysis who experienced a considerable reduction in epoetin dose when treated with a high-flux dialyser (BK-F) [26]. Only three of the patients, however, had baseline haematocrit levels of 30% or more, and none of them experienced an increase of $>10\%$ in baseline haematocrit levels.

The secondary analysis of a multicentre trial [27], comparing biocompatible with traditional membranes, and convective with diffuse treatment modalities in 380 patients followed for 24 months, was in disagreement with the former positive results. Haematocrit levels increased during the course of the study in the overall trial population (probably as a consequence of a trial effect), but this did not differ significantly in the four treatment modalities, i.e. cuprophane haemodialysis, low-flux polysulphone haemodialysis, high-flux polysulphone haemodialysis and high-flux polysulphone haemodiafiltration (HDF) [28]. A significant increase in haematocrit levels was observed, however, in patients receiving high-flux compared with those receiving low-flux treatments; a difference in dialysis dose (higher in the HDF group) may partially explain this observation.

Following the preliminary data, Locatelli et al. [29] performed a multicentre, controlled, randomized study of 84 patients receiving haemodialysis to test whether haemodialysis with high-flux membrane (BK-F poly-methylmethacrylate) improved anaemia in comparison with conventional haemodialysis using a cellulose membrane. An increase in haemoglobin levels was observed in the population as a whole, but this trend was not significantly different between the conventional and high-flux membrane groups [29]. In the high-flux membrane group, the tendency of haemoglobin levels to increase was present at each month during the trial follow-up, possibly suggesting that the period of observation was insufficient. As the investigators took particular care to select only well-nourished patients with haematocrit levels of $<30\%$, without having other known factors affecting uraemic anaemia and receiving adequate dialysis ($\text{Kt/V} = 1.3$), this may have reduced the power of the study [29]. The negative results of this study therefore 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 would be expected from the results of uncontrolled studies. It is worth noting that median serum ferritin levels tended to increase in the conventional group, but tended to decrease in the high-flux membrane group, as observed previously by Fourtounas et al. [30]. Although this difference was apparently not statistically significant, the decrease could have been caused by the more intensive iron administration in the conventional group (as these patients are more likely to experience platelet aggregation in the extracorporeal circuit than the patients receiving high-flux membrane haemodialysis). The percentage of transferrin saturation did not show the same pattern as ferritin, however, and increased significantly during follow-up in a similar fashion in both groups (from 25 to 28% in the conventional treatment group; from 24 to 28% in the high-flux membrane group) making the hypothesis of higher blood loss in patients undergoing standard haemodialysis unlikely.

Similarly to Locatelli et al. [29], a small, prospective, cross-over study comparing acetate-free biofiltration with a high-flux biocompatible membrane vs standard bicarbonate haemodialysis with a low-flux cellulosic membrane was not able to show any improvement in anaemia when treating a highly selected patient group not receiving epoetin therapy [31].

**On-line treatments**

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 may be more effective in achieving higher haematocrit levels via three mechanisms: higher dialysis dose, higher removal of middle-/large-sized molecules (possibly containing bone marrow inhibitors) and reduced microbiological and pyrogenic contamination of the dialysate, which can also be important in causing or aggravating anaemia in patients receiving haemodialysis through an enhanced production of cytokines. (On-line HDF achieves a greater filtration of middle-/large-sized molecules through an increase in convection.)
Maeuget al. [32] studied 37 patients who were switched from conventional HDF to on-line HDF and followed for 1 year. Significant increases in haemoglobin (from 10.66 ± 1.1 to 11.4 ± 1.5 g/dl) and haematocrit levels (from 32.2 ± 2.9 to 34.0 ± 4.4%) were observed during the on-line HDF period, together with a significant decrease in epoetin doses (from 3861 ± 2446 to 3232 ± 2492 IU/week). It should be noted that the patients also experienced an increase in dialysis dose (a 15% increase in Kt/V) during the on-line HDF period; this could have partially favoured the observed increase in haemoglobin and haematocrit levels. Furthermore, the lack of a control group does not exclude the possibility of a trial effect on anaemia correction.

Bonforte et al. [33] studied 32 patients treated with on-line HDF for at least 9 months. A significant increase in haemoglobin levels (from 11.0 ± 1.7 to 12.0 ± 1.8 g/dl after 6 months and to 12.0 ± 1.6 g/dl after 9 months) was found in the nine patients who were not treated with epoetin; patients who were receiving epoetin maintained stable haemoglobin levels despite a significant reduction in epoetin dose. As Kt/V urea remained constant during the course of the study, these findings cannot be explained by an increase in dialysis dose. In addition, an increase in dialysis dose cannot explain the results from another prospective, uncontrolled study of 92 patients on haemodialysis, who were switched from conventional haemodialysis to on-line HDF [34]. During the on-line HDF period, these patients experienced a significant increase in mean haematocrit levels (from 29.5 ± 3.9 to 31.8 ± 4.4%) together with a significant reduction of epoetin dose (from 3.913 ± 8154 to 8662 ± 9021 IU/month). On-line HDF, however, also caused a significant rise in Kt/V urea (from 1.28 ± 0.99 to 1.63 ± 0.26; P < 0.01), possibly influencing epoetin response.

Other researchers could not confirm these observations [35,36]. Ward et al. [35] conducted a prospective study comparing on-line HDF with high-flux haemodialysis in 44 patients, who were followed for 1 year. Although anaemia control was not a primary outcome, there was no change in haematocrit or haemoglobin levels over the course of the study; overall, the average weekly dose of epoetin increased slightly during the study, but this modification was independent of treatment modality. Wizemann et al. [36] also failed to confirm the possible effect of on-line HDF in anaemia correction. In this prospective, controlled study, 44 patients were randomized to undergo either low-flux haemodialysis or on-line HDF. To eliminate confounding factors, low-molecular efficacy was matched (Kt/V = 1.8) and the same treatment duration (4.5 h) and membrane (high-flux polysulphone) were used in each group. After 24 months of follow-up, haematocrit levels and epoetin dose did not differ between the two groups. The same ultra-pure dialysate was used in both groups, and so a possible hypothesis may be that a reduced inflammatory stimulus from the dialysate could be an important factor influencing the effect of on-line HDF on anaemia correction. A sustained reduction of epoetin dose was found when bicarbonate haemodialysis was performed using online-produced ultra-pure dialysate, which supports this hypothesis [37,38]. The switch from a potentially microbiologically contaminated dialysate also resulted in a significant decrease in CRP and IL-6 levels [37,38], with IL-6 levels displaying a strong predictive value for epoetin dose by multivariate analysis [37].

Altogether, the possibility that on-line HDF/haemofiltration may achieve a better control of anaemia and reduce epoetin expenditure is intriguing. The available results are conflicting, however, mainly because of differences in treatment modalities or membranes, lack of control groups, and small numbers of enrolled patients. Furthermore, on-line HDF achieved higher dialysis doses than control treatments in many cases, which adds further complications regarding the interpretation of these observations. The results of prospective, randomized, adequately sized trials aimed at testing this hypothesis better are awaited.

**Short or nocturnal daily haemodialysis**

Dialysis adequacy could have an effect on anaemia not only in terms of dialysis dose, but also in terms of dialysis frequency. Some preliminary observations suggest that a more frequent schedule, short daily (2 h, 6 times/week) or long nocturnal haemodialysis, could enable better control of anaemia and a 20–50% reduction in the dose of erythropoietic treatment [39–42]. These positive findings were, however, invariably accompanied by an increase in dialysis dose, making it impossible to discriminate a possible role of dialysis frequency per se. Furthermore, most studies do not have an adequate control group, patient populations are often different from the standard haemodialysis population, and many studies have very small numbers that preclude statistical significance; non-uniformity of patient selection and study design prevents accurate comparison and pooling of data [43]. It should also be noted that these patients experienced lower interdialytic gains in body weight, and lower haemodilution may partially account for the observed improvement of anaemia by these treatment schedules.

**Conclusions**

Adequate dialysis contributes to anaemia correction and allows for a significant reduction in expenditure on erythropoietic therapy. Biocompatibility or permeability of membranes, or both, could have an additive effect to increased dialysis dose on the correction of anaemia, but in highly selected, adequately dialysed patients without iron or vitamin depletion, this effect seems to be smaller than expected. The role of on-line treatments is still controversial. In particular, it is difficult to discriminate between the effect of on-line HDF and that of an increased dialysis dose. Dialysate quality
could also be of importance. On-line-produced ultra-pure dialysate is probably a quality target to be reached in the next few years, in order to reduce bacterial contamination, pyrogen production and the consequent chronic inflammatory response. It will probably improve anaemia correction, reduce the need for erythropoietic therapy and partially compensate for the extra costs of convective treatments. Finally, the preliminary results obtained with short or long nocturnal daily haemodialysis are remarkable. However, only the realization of a dialysis machine with a nearly complete reduction of initial preparation and final sterilization, the so-called ‘one button machine’, together with the theoretical possibility of automating vascular accesses and avoiding repeated injections, will allow a much more widespread use of these treatment modalities, which are the most physiological and rehabilitating for patients.

References

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