Chapter 12
Effects of Haemodiafiltration of Anemia Control

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Abstract Anaemia secondary to chronic kidney disease is a complex syndrome. Adequate dialysis can contribute to its correction by removing small, and possibly medium/large toxins that inhibit erythropoiesis. Accordingly, a positive relationship between anaemia improvement and dialysis dose has already been observed in the 1980s. Dialysate contamination and low-compatible treatments may also increase cytokine production and consequently inhibit erythropoiesis. Convective treatments and, particularly, on-line haemodiafiltration, could theoretically improve anaemia correction by two mechanisms: higher removal of medium and large solutes (possibly containing bone marrow inhibitors) and reduced microbiological and pyrogenic contamination of the dialysate. Unfortunately, available results are conflicting, mainly because of differences in treatment modalities or membranes, and lack of control groups. Patient selection and higher achieved dialysis dose with online hemodiafiltration may also have complicated interpretation. Increasing treatment time (nocturnal dialysis) and/or frequency (daily dialysis) may diminish rebound from the extravascular space of middle- large molecules and thus possibly improve anaemia. Again, available studies are conflicting.

Keywords Chronic kidney disease • Anaemia • Dialysis • Erythropoiesis stimulating agents • Inflammation • On-line haemodiafiltration • Adequacy • Iron • Hepcidin

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Introduction

Anaemia is a common complication of chronic kidney disease (CKD), affecting the majority of patients on dialysis. Traditionally, renal anaemia is treated by the administration of erythropoiesis stimulating agents (ESA), iron administration and blood transfusions in selected cases. Despite the fact that these strategies are available for decades, there are still many "grey areas", such as the optimal haemoglobin (Hb) target concentration and concerns about the general safety of ESA and iron supplementation. In particular, clinical trials investigating the role of complete anaemia correction with ESA have shown an increased risk of thromboembolic events (in particular stroke) and cancer-related death in the higher Hb target groups [1]. Currently, however, it is still unclear whether patient characteristics, such as comorbidities and inflammatory status, or treatment-related factors, including high ESA doses, expose patients to a higher risk of adverse events [2]. ESA hyporesponsiveness appears important, since for every range of achieved Hb levels, a higher ESA dose [erythropoiesis resistance index (ERI)] was associated with an unfavourable outcome [3, 4]. Moreover, irrespective of the achieved Hb concentration, those treated with the highest ESA doses had a significantly higher relative risk for the primary end point [5], indicating that the ESA dose and not the achieved Hb was the principal determinant of clinical outcome. However, ESA dose may be a marker of a higher comorbidity burden as well. Overall, any effort to reduce ESA requirements for a given Hb target could be of potential benefit.

Why Do Patients with Chronic Kidney Disease Develop Anaemia?

In CKD patients, the main factor causing anaemia is a reduced renal production of erythropoietin (EPO) by the failing kidneys, together with a resistance of the bone marrow cells to this hormone. The balance between the two conditions determines the severity of anaemia in the individual patient. Accordingly, some CKD patients may have near-to normal erythropoietin levels, which, however, are inadequate for the severity of anaemia. The presence of relative high endogenous EPO levels despite persisting anaemia is a marker of poor outcome [6], underlying the relative importance of factors that depress erythropoiesis.

The reduced erythropoiesis of CKD patients has several causes, including iron deficiency, chronic inflammation and oxidative stress. Causes of anaemia in CKD patients are summarised in Table 12.1.

Research from the 1960s already showed that toxic substances inhibiting erythropoiesis could be found in the serum of nephrectomised rabbits [7]. A number of metabolites or substances are potential uremic toxins, including various polyamines, such as spermine, spermidine, putrescine [8], cadaverine. High levels of parathyroid hormone can also worsen anaemia, although it may not specifically

Table 12.1 Factors contributing to anaemia in patients with chronic kidney disease

Low erythropoietin production (relative)
Absolute or functional iron deficiency
Vitamin B12/folate defiency
Shorter erythrocyte survival
Severe secondary hyperparathyroidism
Infections/chronic inflammation
Bleeding
Inadequate dialysis
Malnutrition
Frequent blood sampling
Blood loss during dialysis

suppress erythropoiesis, but rather cause a fibrotic transformation of the bone marrow [9]. Polymeric polyamine-protein conjugates are more selective and accumulate during dialysis, suggesting a possible causative role of dialysis treatment per se [10].

Inflammatory cytokines can also inhibit erythropoiesis. Although available data are not unequivocal, interleukin-6 (IL-6) has been found to antagonize the effect of EPO on bone marrow proliferation [11]. Actually, IL-6 levels were directly related to the ESA dose [12] and significantly higher in patients treated with less compatible membranes [13]. Together with C-reactive protein (CRP), IL-6 appeared to be a strong and independent predictor of ERI in HD patients [14]. An inverse correlation between IL-6 and anaemia was observed also in CKD patients not yet on dialysis [15]. Other pro-inflammatory cytokines, including Interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and interferon-ϒ (IFN-ϒ) have been related as well to EPO resistance [16, 17]. The latter substances, however, act by different mechanisms, such as inducing a shortened red blood cell survival, abnormal mobilization of reticuloendothelial iron stores, blunted EPO response and impaired erythroid colony formation in response to EPO.

Uremic toxins originating from the gut may also negatively influence EPO synthesis. Quinolinic acid, which is the product of tryptophan oxidation by intestinal bacteria, can both suppress erythroid colony formation [18] and inhibit EPO production [19]. Indoxylsulfate, which accumulates early in CKD and exerts detrimental effects on the cardiovascular system, increases oxygen consumption in tubules and aggravates hypoxia in the kidney. Several data showed that indoxylsulfate suppresses EPO expression, which is partially mediated by a reduced induction of hypoxia-inducible factor (HIF)-1 target genes in the presence of hypoxia [20].

A concomitant shortened red blood cell survival may also play a role. Inflammation, increased oxidative stress and uremic toxins induce premature changes in the erythrocyte membrane and cytoskeleton, leading to exposition of phosphatidylserine at the cell surface and accelerated phagocytosis by macrophages. This phenomenon, called programmed cell death or eryptosis, is enhanced in CKD. Other uremic toxins, such as vanadate [21], acrolein [22], methylglyoxal [22], and indoxylsulphate [23] have been shown to trigger eryptosis as well.

Anaemia and Dialysis Dose

In 1980 it was already found that anaemia improved after the start of HD [24], which was then attributed to the removal of small molecules, which may inhibit erythropoiesis. If so, a higher dialysis dose, as indicated by Kt/V_{urea}, would improve anaemia. In the 1990s, Ifudu et al. [25] found a direct relationship between haematocrit and dialysis dose, which, however, could have been influenced by the concomitant shift from modified cellulose to high permeable and more biocompatible membranes in the patients previously receiving inadequate dialysis [26]. Later, in large cohort studies, a clear relationship between the degree of anaemia and dialysis dose was found [26, 27], although it was not completely clear whether anaemia control improved by the application of a different dialysis modality and/or by an improved dialysis dose. More recently, Movilli et al. [28] found an inverse relationship between ESA dose and Kt/V_{urea} in 68 patients on conventional HD, independent of membrane permeability and biocompatibility. In a larger cohort, this correlation was significant only in patients with Kt/V_{urea} below 1.33 [29]. Gaweda et al. [30] confirmed the observation that the relationship between dialysis dose and anaemia is not linear and vanishes when "adequate dialysis" (i.e. $Kt/V_{urea} > 1.4$) is obtained.

Convective Treatments

Convective treatments combine large pore membranes with a high trans-membrane flux. Thanks to these properties, theoretically, middle molecular weight (MMW) inhibitors of erythropoiesis are more easily removed. However, due to obligate back filtration in high-flux HD the amount of convective transport is unpredictable, immeasurable and fluctuates per treatment. Nevertheless, anaemia improvement had been observed after switching from standard HD to HD with high permeable and biocompatible membranes in several small and uncontrolled studies from the 1990s [31–34], and more recently in a small randomised study as well [35]. The Italian Cooperative Dialysis Study compared biocompatible and traditional dialyzers as well as convective and diffuse treatment modalities in 380 patients [36]. A secondary analysis showed a significant increase in haematocrit levels in patients on high-flux polysulphone (PS) HD and high-flux PS haemodiafiltration (HDF) compared to those on low-flux treatments (cuprophane HD, low-flux PS HD), but did not find any difference when all four groups were analysed separately. Hence, it is highly doubtful whether an increase in convective transport in the HDF group has influenced these findings [37]. Finally, one large, observational, cohort study from the Japanese phase II DOPPS [38], as well as two randomised, controlled trials [39, 40] failed to demonstrate an effect of high-flux HD on anaemia. Unfortunately, neither the Hemo-dialysis (HEMO) Study [41] nor the Membrane Permeability Outcome Study (MPO) study [42], which are the largest randomized clinical trials (RCT) that examined the effect of membrane flux and dialysis dose on clinical

outcomes, published data on anaemia control (although unpublished data of the MPO study do not suggest that high-flux was superior to low-flux in this respect).

Online Haemodiafiltration

Online HDF is probably the most efficient technique of removing solutes up to 50 kD. Since the dialysis fluid in this modality is obligatory ultrapure (bacterial contamination <0.1 CFU/ml, LAL <0.025 IU/ml), both high convection coupled with purity may constitute the rationale for an improvement of anaemia. Unfortunately, studies investigating the effects of HDF on anaemia and iron parameters, as well as on ESA dosing and iron supplementation differ considerably in design, patient numbers, control group and endpoints (Table 12.2). In most publications, anaemia management was a secondary endpoint, without providing information on iron parameters and iron supplementation. Lin et al. [43] switched 92 patients from conventional HD to on-line HDF and found a significant decrease of the median ESA/haematocrit ratio (from 504.6 ± 310.1 to 307.6 ± 334.4), which might, however, be also the result of a marked increase in Kt/V in this group. Bonforte et al. [44] studied 32 patients treated by on-line HDF for at least 9 months in whom the dialysis dose was kept constant. Despite the small sample size and the lack of a control group, a significant increase in Hb levels was found in patients without ESA therapy and stable Hb values and lower ESA doses in those patients who already received ESA therapy. Vaslaki et al. [45] performed a randomised, cross-over study in 70 patients receiving either HDF or conventional HD for 6 months. Overall, a higher haematocrit at a lower ESA dose was found during the HDF period, although these data were less distinct when considering separately the two groups undergoing HDF. Finally, two small, RCTs [46, 47] failed to demonstrate an effect of HDF on anaemia control, which may be due to an inadequate statistical power (a relative small sample size to test the difference between two efficient dialysis techniques).

To overcome the issue of small sample size, in 2013 a meta-analysis of 65 studies was performed (12,182 patients, only studies published before December 2012 included) comparing convective therapies (including high-flux HD, hemofiltration [HF] and HDF) with low-flux HD, which did not show an improved anaemia control nor a decreased ESA dose or improved iron parameters in patients treated with convective therapies [48]. After publication of this meta-analysis, several larger randomised studies have been published on this topic.

In 2012 a pre-specified secondary analysis of a multicentre, open-label, RCT of 146 CKD patients, who were randomized to standard HD (70 patients) or convective treatments (online pre-dilution hemofiltration [n = 36] and online pre-dilution HDF [n = 40]) was published by Locatelli et al. [49]. In comparison with low-flux HD, neither HF nor HDF significantly improved Hb levels or ESA requirements. The randomised CONvective TRAnsport STudy (CONTRAST) [50] compared low-flux HD with online HDF on survival in 714 participants. The effect of online HDF on

Table 12.2 Studies on the effect of HDF on anemia control and iron parameters

Author and year of publication	Design	N	HDF method, convection volume per session	Control group/ treatment at baseline	HDF effect on anemia/ESA	HDF effect on iron
Maduell 1999 [78]	Observational	37	Post-dilution, 24 L	Low-volume HDF, 4.1 L	Hb/Hct ↑; ESA ↓	Ferritin/TSAT=
Ward 2000 [46]	RCT	44	Post-dilution, 21 L	High-flux HD	Hb/Hct=; ESA=	NA
Wizeman 2000 [47]	RCT	44	Mid-dilution, 60 L (target)	Low-flux HD	Hct=; ESA=	NA
Bonforte 2002 [44]	Observational	32	Post-dilution 19.5 L	Low-flux HD	ESA +: Hb/ Hct=, ESA ↓; No ESA: Hb/ Hct ↑	Ferritin/TSAT=
Lin 2002 [43]	Cross-over	92	Post-dilution, 25.7 L	High-flux HD	Hct ↑; ESA ↓; ERI ↓	Ferritin/TSAT ↓
Vaslaki 2006 [45]	Randomized cross-over	129 (70 analyzed)	Post-dilution, 20.3 L (substitution)	Low-flux HD	ERI ↓	NA (iron policy changed during study)
Schiffl 2007 [59]	Randomized cross-over	76	Post-dilution, 19.1 L (substitution)	High-Flux HD (with starting phase on low-flux HD)	Hb/ESA = (but ESA ↓ compared to low-flux HD)	NA
Vilar 2009 [54]	Retrospective observational	858 (232 HDF)	Post-dilution 14.9 L (range 5.8–33.2)	High-flux HD	Hb ↑; ESA ↑; ERI =	NA
Pedrini 2011 [79]	Randomized cross-over	69	Pre-, mid- or postdilution; 48.5, 39.9, 22.0 L respectively	Low-flux HD	Hb=; ESA ↓; ERI =	NA

Oates 2011 [80]	Observational/semi-random	78 (34 HDF)	Post-dilution; 16.2–21.2 L (substitution)	High-flux HD	Hb=; ESA=	Iron dose ↑
Stefánsson 2012 [61]	Randomized cross-over	20	Post-dilution; 24.5 L	Low-flux HD	Hb=; ESA ↑	Ferritin ↓; hepcidin ↓
Ok 2013 (Turkish HDF study) [52]	RCT	782	Post-dilution; 17.2 L (substitution)	High-flux HD	Hb=; ESA ↓; ERI ↓	Ferritin/TSAT=
Maduell 2013 (ESHOL) [53]	RCT	906	Post-dilution; 22.9–23.9 L	High-flux HD	Hb=; ESA=	Ferritin/TSAT=; iron dose=
Van der Weerd 2014 (CONTRAST) [51]	RCT	714	Post-dilution; 20.7 L	Low-flux HD	Hb=; ESA=; ERI=	Ferritin=; TSAT ↓; iron dose=
Panichi 2014 [55]	Randomized cross-over (primary endpoint)	40	Post-dilution, 23.8 L	Low-flux HD	Hb=; ESA ↓; ERI ↓	Ferritin/TSAT=; iron dose =; hepcidin ↓

Hb/Hct hemoglobin/hematocrit, *ESA* erythropoiesis stimulating agents, *ERI* ESA resistance index (weight-adjusted ESA dose per hemoglobin unit), *TSAT* transferin saturation, *NA* not available

ESA resistance and iron parameters was a pre-specified secondary endpoint of this RCT [51]. After 12 months, ERI was not different between patients treated with HDF or HD. Even in the highest third of convection volume (>22 L), which was associated with a beneficial effect of HDF on mortality, there was no effect on ESA resistance. In these individuals only a trend towards a lower transferrin saturation ratio and lower ferritin levels was found, despite slightly more iron supplementation. The Turkish Online Haemodiafiltration Study [52] randomised 782 HD patients to either post-dilution online HDF (mean convection volume 19.6 L/session) or high-flux HD. Despite a similar clinical outcome in the two groups, the mean ESA dosage was significantly lower in the HDF group than in the HD patients (2282 ± 2121 versus 2852 ± 2702 U/week, respectively, P = 0.001). The On-Line Hemodiafiltration Survival Study (ESHOL) was a large, multicenter, open-label, RCT in which 906 chronic HD patients were randomised to continue standard HD (n = 450) or to switch to high-efficiency post-dilution online HDF (n = 456) [53]. Despite a significant reduction in all-cause and cardiovascular mortality, which were the primary endpoints, Hb levels and ESA dose did not differ between groups. Finally, it should be mentioned that increased ESA requirements in patients treated with HDF were reported as well in some studies [54, 61]. Apart from an inadequate study design, repeated blood loss due to recurring clotting in the extracorporeal circuit as a result of increased pro-coagulatory activity during HDF and increased post filter Ht levels in post-dilution HDF may play a role in this respect (see also Chap. 15).

Stimulated by the favourable results of ESHOL on clinical outcome, the REDERT study was designed to test the effect of high-volume (>20 L/session) HDF on ERI and hepcidin levels. In this two-arm, multicentre, crossover study, 40 stable HD patients were randomised to either online HDF or standard low-flux HD [55]. Interestingly, ERI was significantly reduced during the HDF period, while it increased during standard HD. Actually, Hb levels remained stable, while the total amount of ESAs administered during HD was considerably higher (HD 192,444 ± 131,341 versus HDF 135,955 ± 96,070 UI/6 months, respectively; p < 0.001). Hepcidin levels were also lower in HDF compared to standard HD.

Several factors could well explain the different results of the various trials. First, patient selection might play an important role, as stable patients without comorbidity or intercurrent illness, such as infections, may profit less from the beneficial effects of HDF on clinical outcome. Second, anaemia management is target driven and treating physicians may not necessarily adhere to the same guidelines because of cultural, geographical or economical reasons. Indeed, in multivariate analysis Locatelli et al. [49] found that the participating centre was the most significant predictor of Hb levels and ESA resistance, suggesting a large degree of heterogeneity among individual centres in treating anaemia. Third, the improved anaemia control in patients treated with HDF may not be caused by the effect of convective transport, but by the use of ultra pure dialysate. Many studies have shown that the use of ultrapure dialysis fluid results in increased Hb levels and diminished ESA requirements [56–58]. In this respect it should be mentioned that occasionally a beneficial effect of HDF on anaemia control was found when ultrapure dialysis fluid was not used in the control group (or at least dialysis fluid of inferior quality com-

pared to the fluid used for HDF) [52, 53, 56, 59]. Another factor that may contribute to the conflicting findings of HDF on anaemia control is the magnitude of the convection volume, which, as aforementioned, has been related to survival [60]. At present it is unclear whether there is also a dose-response effect on anaemia control. Finally, HDF may increase the clearance of erythropoiesis inhibiting toxins, although the concomitant removal of essential substances needed for erythropoiesis may occur as well. Examples of the first category are inflammatory toxins and hepcidin. Two studies showed a decrease in hepcidin levels in patients treated with HDF [55, 61], but only one of them showed improved ESA responsiveness [55]. In this respect, however, the treatment period of only 2 months may have been too short to obtain significant and reliable findings. Others showed an increased clearance of hepcidin with HDF as well [62], but also a substantial rebound of hepcidin levels already 1 h after a conventional HD session [63]. Since hepcidin is highly protein bound, its removal may differ according to dialysis the modality or membranes used [64]. Nevertheless, the reduced ferritin levels in patients treated with HDF in two studies may indicate an improved iron utilization, which may be mediated by reduced hepcidin levels because of a decrease in inflammation [44, 61]. No data are available on loss of substances, which are essential for erythropoiesis by HDF, except for vitamin C, which enhances iron availability [65].

Intensified Extracorporeal Dialysis Strategies

The clearance of MMW toxins by dialysis techniques is limited by their high intracorporeal mass transfer resistance [66]. As a result, optimal removal of these solutes by dialysis requires both enhanced convective clearance, such as with HDF, and increasing treatment time and/or frequency, which may diminish rebound from the extravascular space [67]. In this respect, increasing treatment frequency (short daily dialysis) or time (nocturnal dialysis or long dialysis), or both (daily nocturnal dialysis), may be interesting treatment options to improve ESA response, given their capacity of better removing MMW toxins [68]. The experience of the Tassin Centre in France showed good anaemia control in patients treated with low-flux HD three times per week for 8 h [69]. This effect is most likely mediated by the removal of small molecules, but also to a certain extent of MMW toxins, thanks to the very long dialysis time. Unfortunately, in this study no control group was included.

In 2008 a systematic review of small studies on the effects of short daily HD on various clinical parameters was published [70]. In six studies, the ESA dose was reduced, whereas in two studies no difference with standard HD was observed. The Frequent Hemodialysis Network (FHN) performed a trial on 245 patients who were randomized to either short daily or conventional HD [71]. The ESA dose was not different between both treatment arms, as were Hb levels [72]. Similarly, studies on the effect of increasing both treatment frequency *and* time, as in nocturnal HD, have shown mixed results [73, 74]. In a retrospective Canadian study, 63 patients treated with nocturnal HD had a rise in Hb levels and a fall in ESA requirements, whereas

iron saturation was lower compared to conventional dialysis [75]. In the FHN Nocturnal Trial, 87 patients were randomized to receive either nocturnal HD (six times/week for more than 6 h) or conventional HD [76]. In this study, ESA dose was not different between groups, despite lower doses of IV iron in those treated with nocturnal HD [72]. Finally, in a cross-over study 26 patients were switched from 4 to 5 h thrice-weekly HDF to 7–8 h nocturnal every-other-day HDF with the same (20–30 L) or higher (35–50 L) convective volume [77]. While nutritional status, phosphate and hypertension control improved and left ventricular mass (LVM) decreased over 12 months' of follow-up, in the higher convective volume group neither Hb levels, nor ESA index or iron parameters changed. In this respect it should be noted that more frequent and/or longer exposure to blood tubes, dialyzers and dialysis fluid, as well as repeated cannulations, may result in an enhanced inflammatory state and increased blood loss [67, 68], although in the FHN trial patients on nocturnal dialysis needed less iron supplementation than those treated with conventional HD [72].

Conclusion

Improving anaemia control in dialysis patients by removing erythropoiesis inhibiting toxins or substances involved in ESA responsiveness seems a logical and desired treatment option, since treatment with high ESA doses, especially in patients with a marked ERI, may be associated with detrimental effects. Over the past decades, many toxins that inhibit erythropoiesis and decrease red blood cell lifespan have been identified. Removal of these substances might have a beneficial effect on anaemia control. Treatment with HDF not only enhances the clearance of small and MMW uremic toxins, but also induces less inflammation than standard HD because of the ultrapure dialysis fluid applied. Therefore, HDF may, at least theoretically, have a beneficial effect on anaemia control and ESA resistance. However, results of clinical studies on this topic are conflicting and differ substantially with respect to the treatment protocol (including dialyzers and use of ultrapure dialysis fluid), control group and the treatment dose (i.e. applied convection volume). As for the effect of HDF on iron supplementation, data are even more limited. Considering alternative extracorporeal dialysis modalities, such as short daily HD or long/nocturnal HD, results of available studies are conflicting as well. Hence, when looking exclusively at anaemia control, no single treatment modality seems to be really preferable over the other.

Teaching Points

- Renal anemia is multifactorial and results from a decreased EPO production and responsiness, a shortened red blood cell survival (eryptosis) and/or shortage of essential nutritients
- ESA hypo-responsiness plays an important role in the risk of adverse effects

- Therefore, at present, the optimal Hb target concentration is unclear
- Uremic toxins contributing to ESA hypo-responsiness include toxic metabolites, inflammatory cytokines and gut derived protein-bound degradation products
- In ESKD patients the degree of anemia is correlated with Kt/V_{urea} up to 1.4. Higher dialysis doses do not improve anemia any further.
- Addition of a limited amount of convection (10–12 L/session), as occurs in high-flux HD, does not improve anemia control
- Conflicting reports have been published whether anemia control is improved by high volume HDF (convection volume >21 L/session)
- Available evidence suggest better iron utilization in high volume HDF

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