38. Salas M, Hofman A, Stricker BH. Confounding by indication: an example of variation in the use of epidemiologic terminology. *Am J Epidemiol* 1999; 149: 981–983.

39. Cantor T. Parathyroid hormone assay drift: an unappreciated problem in dialysis patient management. *Semin Dial* 2005; 18: 359–364.

40. Sturgeon CM, Sprague SM, Metcalfe W. Variation in parathyroid hormone immunoassay results—a critical governance issue in the management of chronic kidney disease. *Nephrol Dial Transplant* 2011; 26: 3440–3445.

Predictors of haemoglobin levels and resistance to erythropoiesis-stimulating agents in patients treated with low-flux haemodialysis, haemofiltration and haemodiafiltration: results of a multicentre randomized and controlled trial

Francesco Locatelli, Paolo Altieri, Simeone Andrulli, Giovanna Sau, Piergiorgio Bolasco, Luciano A. Pedrini, Carlo Basile, Salvatore David, Mariano Feriani, Pier Eugenio Nebiolo, Rocco Ferrara, Domenica Casu, Francesco Logias, Renzo Tarchini, Francesco Cadinu, Mario Passaghe, Gianfranco Fundoni, Giuseppe Villa, Biagio Raffaele Di Iorio and Carmine Zoccali

[1]Department of Nephrology and Dialysis, Azienda Ospedaliera della Provincia di Lecco, Ospedale Alessandro Manzoni, Lecco, Italy, [2]Department of Nephrology and Dialysis, Azienda Ospedaliera G. Brotzu, Cagliari, Italy, [3]Department of Nephrology and Dialysis, Dipartimento territoriale ASL 8, Cagliari, Italy, [4]Department of Nephrology and Dialysis, Ospedale Bolognini, Seriate, Italy, [5]Department of Nephrology and Dialysis, Ospedale F. Miulli, Acquaviva delle Fonti, Italy, [6]Department of Nephrology and Dialysis, Ospedale Maggiore, Parma, Italy, [7]Department of Nephrology and Dialysis, Ospedale dell'Angelo, Mestre, Italy, [8]Department of Nephrology and Dialysis, Ospedale regionale, Aosta, Italy, [9]Department of Nephrology and Dialysis, Ospedale SS. Trinità ASL 8, Cagliari, Italy, [10]Department of Nephrology and Dialysis, Ospedale Civile, Alghero, Italy, [11]Department of Nephrology and Dialysis, Ospedale San Camillo, Sorgono, Italy, [12]Department of Nephrology and Dialysis, Azienda Ospedaliera Carlo Poma, Mantova, Italy, [13]Department of Nephrology and Dialysis, Ospedale "S. Francesco", Nuoro, Italy, [14]Department of Nephrology and Dialysis, ASL 2 Olbia - P.O. "P. Dettori", Tempio Pausania, Italy, [15]Department of Nephrology and Dialysis, Ospedale S. Giovanni di Dio, Olbia, Italy, [16]Department of Nephrology and Dialysis, Fondazione Maugeri IRCCS, Pavia, Italy, [17]Department of Nephrology and Dialysis, Ospedale Agostino Landolfi, Solofra, Italy and [18]Department of Nephrology and Dialysis, Azienda Ospedaliera "Bianchi Melacrino Morelli," Reggio Calabria, Italy

Correspondence and offprint requests to: Francesco Locatelli; E-mail: f.locatelli@ospedale.lecco.it

Abstract

Background. Predictors of haemoglobin (Hb) levels and resistance to erythropoiesis-stimulating agents (ESAs) in dialysis patients have not yet been clearly defined. Some mainly uncontrolled studies suggest that online haemodiafiltration (HDF) may have a beneficial effect on Hb, whereas no data are available concerning online haemofiltration (HF). The objectives of this study were to evaluate the effects of convective treatments (CTs) on Hb levels and ESA resistance in comparison with low-flux haemodialysis (HD) and to evaluate the predictors of these outcomes.

Methods. Primary multivariate analysis was made of a pre-specified secondary outcome of a multicentre, open-label, randomized controlled study in which 146 chronic HD patients from 27 Italian centres were randomly assigned to HD (70 patients) or CTs: online pre-dilution HF (36 patients) or online pre-dilution HDF (40 patients).

Results. CTs did not affect Hb levels (P = 0.596) or ESA resistance (P = 0.984). Hb correlated with polycystic kidney disease (P = 0.001), C-reactive protein (P = 0.025), ferritin (P = 0.018), ESA dose (P < 0.001) and total cholesterol (P = 0.021). The participating centres were the main source of Hb variability (partial eta^2 0.313, P < 0.001). ESA resistance directly correlated with serum ferritin (P = 0.030) and beta2 microglobulin (P = 0.065); participating centres were again a major source of variance (partial eta^2 0.367, P < 0.001). Transferrin saturation did not predict either outcome variables (P = 0.277 and P = 0.170).

Conclusions. In comparison with low-flux HD, CTs did not significantly improve Hb levels or ESA resistance.

The main sources of variability were participating centres, ESA dose and the underlying disease.

Introduction

According to the current guidelines, optimizing anaemia treatment in haemodialysis (HD) patients remains a priority worldwide as it has significant health and financial implications. However, one of the main factors hindering the achievement of this therapeutic goal is the variability of individual responses to erythropoiesis-stimulating agents (ESAs), which has been attributed to patient-related factors, such as iron deficiency, malnutrition and inflammation [1–4], hyperparathyroidism and the accumulation of uraemic toxins inhibiting erythropoiesis [5, 6], as well as to factors relating to HD technique, such as dialysis adequacy [7] and the microbiological purity of dialysis fluid [8–11].

In terms of dialysis technique, convective treatments (CTs) are purportedly more effective in correcting anaemia than standard HD [12] because they may allow better removal of substances with molecular weights of 5–50 kDa: i.e. within the range that typically includes erythropoiesis inhibitors [13]. However, studies of the effects of CTs on ESA resistance have produced conflicting results: the findings of observational and non-randomized studies suggest that CTs have a more beneficial effect on erythropoiesis than other treatments [13–16], whereas two small randomized trials involving a total of 88 patients failed to confirm this [17, 18]. Moreover, no data are available concerning the effect of online pre-dilution haemofiltration (HF), a technique that may theoretically have a better impact than other types of extracorporeal treatment. Discrepancies between observational and experimental studies may be due to known and unknown confounding factors, such as water and dialysate quality and the related cytokine production, inflammation, infection, iron deficiency, the biocompatibility of dialysis membranes, dialyser flux and the adequacy of dialysis in terms of small and medium molecular weight toxins, all of which are notoriously difficult to control in observational settings.

Within the framework of the CONVESTUDY [19], a randomized trial comparing standard low-flux HD with two convective techniques [online pre-dilution HF and online pre-dilution haemodiafiltration (HDF)] in terms of haemodynamic stability and various clinical end points, we specifically investigated whether these techniques have a differential effect on haemoglobin (Hb) levels and sensitivity to ESAs. This paper describes the effects of these techniques on the study's haematological outcomes and the results of a secondary analysis aimed at identifying predictors of Hb levels and ESA resistance in the setting of the trial (i.e. a well-standardized context with accurate data collection and systematic external quality control).

Materials and methods

Study design

This is a primary analysis of a pre-specified secondary outcome of a multicentre, open-label, randomized trial involving 27 Italian dialysis centres and comparing low-flux HD with online pre-dilution HF and/or online pre-dilution HDF (CONVESTUDY) [19]. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of each centre. All of the patients gave their written informed consent before enrolment.

Details of the study protocol have been published elsewhere [20]. Eligible patients were randomly assigned by e-mail to receive low-flux HD or CTs (1:1 to online pre-dilution HF or online pre-dilution HDF) using a central computer-generated randomization list stratified by centre. After a 2-month run-in period, the planned 2 years of the experimental phase was divided into two periods: a fixed 3-month 'adaptation period' and a subsequent 21-month 'evaluation period'. In accordance with the protocol, the patients who did not complete the adaptation period were excluded from the intention-to-treat analysis.

Participants

Patients aged 18–80 years were considered eligible if they had been undergoing thrice-weekly HD or HDF for at least 6 months, had a body weight of ≤90 kg and were in stable clinical condition.

Patients with clinically relevant infections, malignancies, active systemic diseases, active hepatitis or cirrhosis, unstable diabetes, diuresis >200 mL/24 h or a dysfunctioning vascular access with a blood flow rate of <300 mL/min were excluded from the study.

Treatment parameters valid for all patients

The HD, HF and HDF machines were all equipped with a dialysis fluid ultrafiltration system for the production of ultrapure dialysate [with each millilitre containing <0.1 colony-forming units (CFU) and <0.03 endotoxin units (EU)] and sterile non-pyrogen substitution fluid (<0.001 CFU/L and <0.03 EU/mL), which were checked at monthly intervals. Blood flow rate was 300–400 mL/min and treatment time 3–4.5 h per session.

Specific characteristics of the three treatments

HD was performed using a low-flux membrane and a dialysate flow rate of 500 mL/min; HF using a synthetic high-flux membrane and an infusate/blood flow ratio of one and HDF using a synthetic high-flux membrane with an infusate/blood flow ratio of 0.6 and a dialysate plus infusate rate of 700 mL/min.

Clinical data

At baseline, co-morbidities (hypertension, diabetes, ischaemic cardiopathy, peripheral arteriopathy and a previous transient ischaemic attack) were recorded using a detailed form.

Pre- and post-session body weight, blood pressure and heart rate were recorded together with the dialysis parameters, including filter type, blood flow, dialysis time and total infusion (in the case of CT).

Dialysis schedules

All of the data related to the dialysis prescription (dry body weight, dialysis time, dialysis modality, dialysate/infusate composition and heparin dose) were evaluated by the attending physician and recorded monthly.

Therapy

The following therapies were recorded for each session: quality and quantity of saline infusions, ESA treatments, iron and any other drug administered orally or intravenously during or at the end of the session and all inter-dialysis therapies. Routine patient care and the prescription of medications were decided by the attending nephrologists on the basis of the European Best Practice [21] and the Kidney Disease Outcomes Quality Initiative guidelines [22, 23]. ESAs and iron supplements were administered through the venous blood line at the end of the dialysis sessions. The HDF and HD patients were treated with ultrapure dialysis fluids containing <0.1 CFU/mL and <0.03 EU/mL. The quality of the dialysis solutions was monitored monthly.

Laboratory data

Pre-dialysis levels of Hb, serum electrolytes (including sodium, potassium, bicarbonate, calcium and phosphate), urea and creatinine were checked monthly; urea and sodium were also evaluated monthly at the end of the session. Iron status and the levels of C-reactive protein (CRP) and albumin were checked every 3 months. Serum cholesterol, triglycerides, beta2 microglobulin and intact parathyroid hormone were checked every 6 months. All of the laboratory samples were analysed in the hospitals using standard laboratory techniques. Ferritin levels (ng/mL) were used as an index of iron stores or inflammation, and per cent transferrin saturation (TS) was used as an index of iron stores. TS was calculated as serum iron divided by the product of serum transferrin multiplied by a conversion factor of 1.25.

Dialysis dose

Equilibrated Kt/V and nPCR values were calculated monthly using the procedures and simplified equations of Daugirdas [24].

ESA and iron therapy

ESA was prescribed as epoetin α (Eprex®, international unit) or β (NeoRecormon®, international unit) or darbepoetin α (Aranesp® or Nespo®, microgram) and expressed as dose per week. To compare the different types of ESA, the prescribed doses of darbepoietin α in microgram per week were converted to international unit per week by means of multiplication by the European label conversion factor of 200. ESA resistance was expressed as the weekly patient body weight-adjusted ESA dose divided by Hb level. Iron supplements were prescribed as iron gluconate (Ferlixit®, milligram per week) or, less frequently, as oral iron sulphate (Ferrograd®, milligram per week).

Statistical analysis

The descriptive analysis was based on the median values and interquartile ranges (IQRs) or mean values and SDs of the normally distributed continuous variables and counts and percentages of the categorical variables. Baseline differences in clinical and laboratory variables between the three groups were tested using Mann–Whitney *U*-test for continuous variables and the chi-squared test for categorical variables. A separate analysis was made for Hb levels and erythropoietin resistance. The general linear model for repeated measures of analysis of variance was used to test the effect of the experimental treatments (HF and HDF) in comparison with the reference treatment (HD) and to identify predictors related to Hb levels and ESA resistance. The tested predictors were the participating centre, ESA dose (international unit/kilogram × week), polycystic kidney disease (categorical 0/1), ferritin (log scale), CRP (milligram per decilitre), TS (percentage), iron therapy (categorical 0/1), total cholesterol (milligram per decilitre), dialysis dose as equilibrated Kt/V (eKt/V) according to Daugirdas [24], dialysis time (hours) and pre-dialysis beta2 microglobulin levels (milligram per litre). The major inter-subject factor was the randomly assigned group. The group effect was tested using the group-by-time interaction, with the HD group being considered the reference. The effect size was estimated by means of the partial eta-squared (eta^2) value associated to each predictor. All of the statistical analyses were made using SPSS for Windows, Release 18.0.

Outcome measures

The primary outcome of the study was the possible beneficial effect of pure (HF) and/or mixed convection (HDF) in comparison with diffusion (HD) on chronic kidney disease 5D patient anaemia, as estimated by the changes in Hb levels and ESA resistance between the 2-month run-in period and the evaluation period, adjusted for the relevant associated covariates.

Results

Baseline characteristics

A total of 146 patients were enrolled, centrally randomized to HD (70 patients), HF (36 patients) or HDF (40 patients), and followed up for a median of 1.5 years (IQR 0.8–2.2).

Table 1 summarizes the baseline clinical and laboratory characteristics of the three groups, which were similar in terms of gender, body weight, co-morbidities, dialysis vintage and dialysis treatment time, whereas there were some marginal differences among groups for age, proportion of diabetes and the distribution of TS values. There were no between-group differences in the biochemical variables related to the dialysis dose for small molecular weight solutes (estimated by means of equilibrated Kt/V), Hb, CRP, albumin, total cholesterol or triglycerides.

Fifteen patients (10.3%) died during the study, with no difference between the groups (P = 0.403). The causes of death were infection (4), acute myocardial infarction (3), cachexia (2), pulmonary embolism (2) and post-operative complications, cardiovascular disease, acute pulmonary oedema and acute cerebral bleeding (1 each). Thirteen patients (8.9%) received a transplanted cadaveric kidney with no difference between the groups (P = 0.273). In accordance with the protocol [20], although there were 48 dropouts (32.9%), only 10 patients (6.8%: 4 in the HD, 1 in the HDF and 5 in the HF group) (P = 0.127) were not included in the final analysis as they dropped out during the 3-month adaptation period. The main analysis therefore involved 136 patients (93.2%), 66 on HD, 39 on HDF and 31 on HF.

Total median re-infusion in pre-dilution mode was equal to 60.4 L per HF session (IQR 50.2–69.9), 106% of dry body weight and 39.9 L per HDF session (IQR 28.2–51.0), 64% of dry body weight.

Follow-up data

Baseline Hb values were slightly different in the three groups (11.5 ± 1.3 g/dL in HD group, 11.3 ± 1.2 g/dL in HF and 11.8 ± 1.2 in HDF, P = 0.135), being a little higher in the HDF than the HD group (difference of 0.3 g/dL; P = 0.160). Between baseline and follow-up, the values remained stable in the population as a whole (11.5 ± 1.3 versus 11.5 ± 1.0 g/dL; P = 0.748) and in the HD and HDF groups, but tended to increase in the HF group (11.3 ± 1.1 versus 11.6 ± 1.0 g/dL) although the difference was not statistically significant (P = 0.509) (Figure 1).

Baseline ESA resistance was more balanced in the three groups (P = 0.505) and remained stable during follow-up in the population as a whole (P = 0.718), although there was a non-significant decrease in the HDF group (from 8.7 to 7.9 IU/kg/week; P = 0.188) (Figure 2).

At baseline, the iron was given intravenously in 78 of 146 patients (53.4%) and without differences among groups (P = 0.619) whereas only four patients (2.7%) received oral iron. During the follow-up, the proportion of patients who received iron therapy was almost significantly reduced in the HD group (from 60 to 44%, P = 0.061) while it remained stable in the other two groups (from 56 to 63% in the HF group, P = 0.561 and from 48 to 46% in HDF group, P = 0.905). Moreover, at baseline, 8% of the patients were iron depleted at baseline with TS < 20% and ferritin values of < 100 μg/mL; the percentage was slightly higher in the HDF group than in the HD and HF groups (15% versus 5 and 6%; P = 0.130). During the follow-up, the percentage of iron depleted patients

Table 1. Clinical and laboratory characteristics of enrolled patients at baseline[a]

	Total	HD	Pre-HF	Pre-HDF	P-value
No. of patients	146	70	36	40	
Gender					0.646
Male, No. (%)	84 (57.5)	43 (61.4)	19 (52.8)	22 (55.0)	
Female, No. (%)	62 (42.5)	27 (38.6)	17 (47.2)	18 (45.0)	
Age (years)	63.9 ± 11.9	63.0 ± 10.7	66.8 ± 12.1	62.8 ± 13.4	0.086
Body weight (kg)	64.1 ± 10.9	64.7 ± 9.7	60.9 ± 9.6	66.0 ± 13.2	0.115
Hypertension, No. (%)	83 (56.8)	39 (55.7)	20 (55.6)	24 (60)	0.894
Diabetes, No. (%)	26 (17.8)	12 (17.1)	3 (8.3)	11 (27.5)	0.091
Dialysis vintage (years)	3.0 (1.4–7.7)	2.5 (1.2–9.0)	4.1 (1.4–7.7)	3.1 (1.5–6.1)	0.636
Dialysis time (min)	240 (210–240)	240 (210–240)	240 (210–240)	240 (221–240)	0.650
Urea at start of session (mg/dL)	169 ± 36	170 ± 37	166 ± 41	169 ± 29	0.851
Haemoglobin (g/dL)	11.5 ± 1.3	11.5 ± 1.3	11.3 ± 1.2	11.8 ± 1.2	0.135
TS (%)	27.5 (20.1–36.1)	27.3 (20.3–39.7)	30.3 (23.2–39.0)	24.2 (17.1–32.8)	0.055
Ferritin (ng/mL)	382 (199–619)	394 (197–626)	386 (236–611)	344 (185–618)	0.674
CRP (mg/dL)	0.8 (0.3–2.3)	0.7 (0.3–2.2)	0.7 (0.3–2.4)	0.9 (0.3–2.6)	0.935
Plasma albumin (g/dL)	3.95 ± 0.42	3.93 ± 0.46	3.94 ± 0.37	3.98 ± 0.40	0.958
Total cholesterol (mg/dL)	167 ± 40	167 ± 39	168 ± 39	166 ± 43	0.988
Triglycerides (mg/dL)	154 (100–217)	162 (115–255)	138 (92–200)	154 (94–196)	0.381
eKt/V	1.28 ± 0.20	1.31 ± 0.20	1.27 ± 0.15	1.23 ± 0.24	0.317
ePCRn (g/kg/day)	1.12 ± 0.22	1.15 ± 0.23	1.09 ± 0.22	1.11 ± 0.19	0.425
ESA dosage (IU/week)	6000 (4000–9250)	6000 (4000–12000)	6000 (4000–9625)	6000 (4000–8000)	0.491
ESA resistance (IU/week/kg × g/dL of Hb)	8.15 (4.67–13.71)	8.13 (4.67–16.05)	9.22 (4.89–14.49)	6.60 (3.94–12.49)	0.287

[a]Some continuous variables, such as dialysis vintage, ferritin, CRP, ESA dosage and ESA resistance, are skewed to the right and thus represented by median and IQR. P-values were obtained from chi-square or Mann–Whitney U-test, for categorical and continuous variables, respectively.
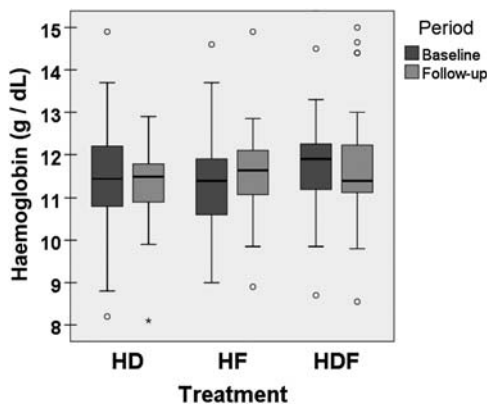

Fig. 1. Baseline haemoglobin levels were slightly different in the three groups, with numerically higher values in the HDF group (P = 0.135). There were very limited and non-significant variations between groups during the follow-up (P = 0.509).
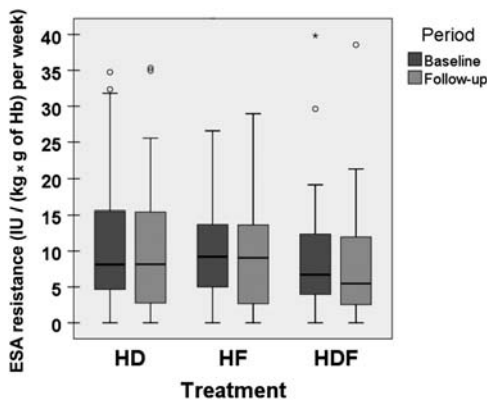

Fig. 2. ESA resistance in the three groups was neither different at baseline (P = 0.505) nor during the follow-up (P = 0.188).

increased in the HF group (from 6 to 13%; P = 0.336) and decreased in the HDF group (from 15 to 5%; P = 0.131).

At baseline, treatment with rhEPO was performed in 89.7% of patients without statistical differences among groups (P = 0.794), using more frequently the IV route of administration (80%) without statistical differences among groups (P = 0.769) and using more frequently the alfa or beta rhEPO (72.5%) compared to darbopoietin (27.5%) again without statistical differences among groups (P = 0.142). The type of rhEPO and the route of administration remained roughly constant for each patient throughout the follow-up: only four patients (2.7%) changed the type of rhEPO and height patients (5.5%) changed the route of rhEPO administration, without statistical differences among groups (P = 0.44 and P = 0.66, respectively).

Predictors of haemoglobin levels and ESA resistance

Tables 2 and 3 summarize the results of the multivariate analysis of the predictors of Hb levels and ESA resistance and the net effect of pre-dilution HF and HDF on them (a list of the investigated covariates is given in the Materials and methods section). The Hb model reached a higher adjusted R^2 than the ESA model (0.35 versus 0.28) and was based on a larger number of covariates. The most relevant predictors of Hb levels were the participating centres (partial eta^2: 0.313), ESA dose (partial eta^2: 0.094) and a diagnosis of polycystic kidney disease (partial eta^2: 0.051) (Table 2). The role of the participating centres as a source of variance in Hb levels is shown in Figure 3; the ESA dose inversely correlated with Hb levels (beta coefficient: −0.003 g/dL per each international unit per kilogram per week increase), and polycystic kidney disease was associated with an increase in Hb of 0.82 g/dL. Ferritin (in log scale) and serum CRP and total cholesterol levels also inversely correlated with Hb levels, although the association was weaker.

**Table 2.** Predictors associated with haemoglobin levels in dialysis patients[a]

Variable	P-value	Partial eta^2
Period effect	0.900	0.000
Group difference at baseline	0.079	0.026
Convective Therapy	0.596	0.005
Participating centre	<0.001	0.313
ESA dose [IU/(kg × week)]	<0.001	0.094
Polycystic kidney disease (0/1)	0.001	0.051
Ferritin (ln ng/mL)	0.018	0.029
C reactive protein (mg/dL)	0.025	0.025
Total cholesterol (mg/dL)	0.021	0.027
Iron therapy (0/1)	0.077	0.016
Iron saturation (%)	0.277	0.006

[a]The most relevant were the participating centres, ESA dose and the underlying disease; ferritin in log scale, C reactive protein and total cholesterol levels inversely correlated with haemoglobin levels. There was a borderline between-group variability at baseline (P = 0.079) and no Convective therapy (P = 0.596). This model was highly significant (P < 0.001) and has an adjusted R^2 of 0.35. CRP.

Table 3. Predictors associated with ESA resistance in dialysis patients[a]

Variable	P-value	Partial eta^2
Period effect	0.515	0.002
Group difference at baseline	0.492	0.007
Convective Therapy	0.984	0.000
Participating centre	<0.001	0.367
Ferritin (ln ng/mL)	0.030	0.025
Beta2 microglobulin (mg/L)	0.065	0.018
Transferrin Saturation (%)	0.170	0.010
Total cholesterol (mg/dL)	0.216	0.008
Iron therapy (0/1)	0.307	0.005
C Reactive Protein (mg/dL)	0.616	0.001
Polycystic kidney disease (0/1)	0.996	0.000

[a]The relevant predictors were participating centre and the levels of ferritin (in log scale) and beta2 microglobulin (both directly correlated). The groups were well balanced at baseline (P = 0.492). There was no CT effect (P = 0.984). This model was highly significant (P < 0.001) and has few predictors and an adjusted R^2 of 0.28.
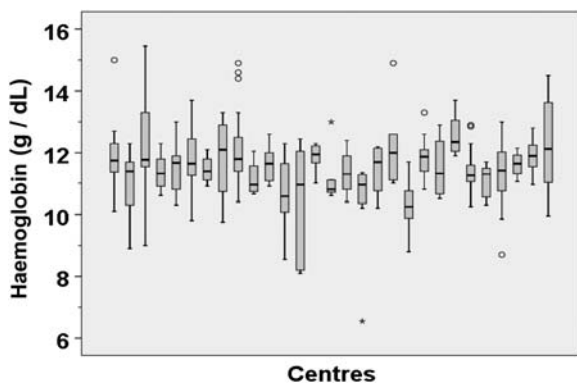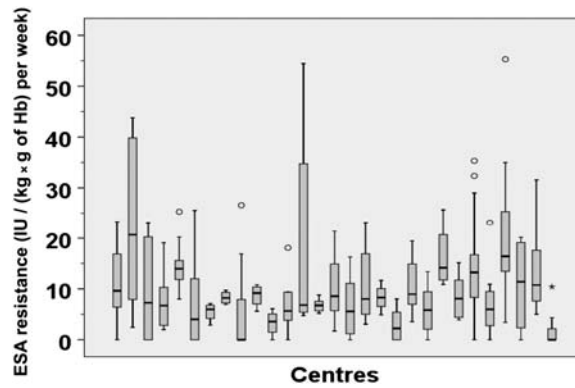
Fig. 4. ESA resistance varied widely among the participating centres.

beta coefficient: 0.25 g/dL). The overall adjusted R^2 of this model was highly significant (P < 0.001) and equal to 0.35. The condition of iron depletion (defined from TS < 20% and ferritin levels < 100 ng/mL) was not a significant and independent predictor of the haemoglobin levels (P = 0.830) and also did not influence the action of convective therapies. This last point was tested by means of the interaction between the iron depletion condition and the convective therapies and resulted not significant (P = 0.251). Removing the small proportion of patients that during follow-up changed the type of rhEPO (2.7%) or the route of rhEPO administration (5.5%), the results of multivariate analysis did not change.

The multivariate model for ESA resistance showed that relevant predictors were the participating centres and ferritin levels (in log scale); the statistical significance of beta2 microglobulin levels was borderline (P = 0.065) (Table 3). The role of participant centres as a source of variance in ESA resistance levels is shown in Figure 4. Transferrin saturation was not a relevant predictor (P = 0.170), nor were serum CRP levels (P = 0.616). CT had no independent effect (P = 0.984).

Discussion

The lack of prospective trials and the contradictory nature of the available data concerning HDF means that the effect of CTs (pure convection as in HF or convection mixed with diffusion as in HDF) on Hb levels and ESA resistance has not yet been established. The aim of this study was to make a primary analysis of the pre-defined secondary outcome data about the predictors of Hb levels and ESA resistance, that were prospectively recorded in a multicentre, randomized and controlled trial comparing cardiovascular stability during the course of treatments using different levels of convection and diffusion [20].

Unlike the findings of observational studies [13–16], but in line with those of two small randomized trials of HDF [17, 18], our results show that, in comparison with standard low-flux HD, neither HDF nor HF played a significant role in increasing Hb levels or decreasing ESA resistance. However, one interesting finding was that many predictors were associated with Hb levels and ESA

Fig. 3. Haemoglobin levels varied widely among the participating centres.

CT had no independent effect on Hb levels (P = 0.596), and iron saturation had no significant predictive power (P = 0.277); however, there was a borderline significant direct relationship between Hb levels and iron therapy (P = 0.077;

resistance, the most relevant of which was the participating centre, a variable that influences both dialysis adequacy and mortality [25, 26]. Consequently, despite the guidelines [21–23], it seems that there is still a large degree of heterogeneity among centres in treating the anaemia of HD patients and therefore considerable room for improvement [27, 28].

As expected, one of the predictors of Hb levels was polycystic kidney disease, which was associated with higher Hb levels (+0.82 g/dL) than other underlying kidney diseases. However, strangely enough, this expected predictor has not been included in most previous analyses.

In our study, the relationship between Hb levels and ESA doses had a negative beta coefficient, thus confirming the finding of previous randomized trials that higher ESA doses are associated with lower Hb levels. This is an apparent paradox because higher ESA doses should increase Hb levels and the true relationship of ESA with Hb levels should be direct; however, the effect of ESA is confounded by physicians who increase the ESA dose when Hb levels are low, as well as by other confounders such as inflammation. As expected, the inflammation indices of serum CRP and ferritin levels were inversely associated with Hb levels, but the fact that per cent TS was only weakly predictive suggests that there is still a need for a simple and reliable index of iron stores. The inverse relationship between total cholesterol and Hb levels was an unexpected finding that requires confirmation by other studies.

The participating centre was also a major predictor of ESA resistance, with the highest partial eta^2 (0.367). We interpret this finding in the light of poor implementation of the guidelines concerning anaemia treatment in HD patients because it is difficult to believe that ESA resistance was primarily different among centres, whereas it is very likely that there were between-centre differences in the intensity of ESA and iron treatments. Strangely enough, CRP levels lost their predictive power, whereas that of beta2 microglobulin emerged, albeit at borderline significance (P = 0.065). The absence of a correlation between CRP levels and ESA resistance may be explained by the fact that the model only used the median CRP levels of the tested periods (run-in and experimental), whereas the effect of inflammation (CRP) on ESA resistance may be more complex, including peak CRP values. We therefore also tested the 'peak CRP' hypothesis, but the results did not change. The relationship between ESA and beta2 microglobulin levels is intriguing and merits further investigation.

This study has both weaknesses and strengths. Its main weakness is that the ESA therapy was not randomly controlled, as was also the case in previous randomized trials [17, 18]. One of its strengths is that it made a primary analysis of pre-specified secondary outcome variables and so the related information was prospectively recorded, something that is not true of previous observational studies [13–16]. Another strength is the prospective 2-month run-in period before the experimental phase, as this allowed the use of a within-patient comparison of Hb levels and ESA resistance between the two periods and gave more power to the findings. We would like to underline that despite the relative small number of the groups, the power of this study, mainly dependent on the size of the population as a whole, is highly valuable as can be appreciated from the two final models showed in Tables 2 and 3. Moreover, the fact that our concurrent control group consisted of well-dialysed patients with a low level of inflammation who were treated with a relatively pure dialysate should be taken into account when interpreting the study finding because of the well-known difficulty of demonstrating the positive effect of any kind of experimental treatment under optimal conditions of the control group.

In conclusion, in comparison with low-flux HD, it seems that convective therapies do not significantly improve Hb levels and ESA resistance. The major sources of variability in Hb levels were the participating centres, the ESA dose and the underlying kidney disease. A centre effect was also a relevant source of the variance in ESA resistance.

References

1. Agarwal R, Davis JL, Smith L. Serum albumin is strongly associated with erythropoietin sensitivity in hemodialysis patients. *Clin J Am Soc Nephrol* 2008; 3: 98–104.

2. Kalantar-Zadeh K, McAllister CJ, Lehn RS *et al*. Effect of malnutrition-inflammation complex syndrome on EPO hyporesponsiveness in maintenance hemodialysis patients. *Am J Kidney Dis* 2003; 42: 761–773.

3. Locatelli F, Andrulli S, Memoli B *et al*. Nutritional-inflammation status and resistance to erythropoietin therapy in haemodialysis patients. *Nephrol Dial Transplant* 2006; 21: 991–998.

4. Lopez-Gomez JM, Portoles JM, Aljama P. Factors that condition the response to erythropoietin in patients on hemodialysis and their relation to mortality. *Kidney Int Suppl* 2008; 111: S75–S81.

5. Ifudu O, Feldman J, Friedman EA. The intensity of hemodialysis and the response to erythropoietin in patients with end-stage renal disease. *New Engl J Med* 1996; 334: 420–425.

6. Lee CT, Chou FF, Chang HW *et al*. Effects of parathyroidectomy on iron homeostasis and erythropoiesis in hemodialysis patients with severe hyperparathyroidism. *Blood Purif* 2003; 21: 369–375.

7. Movilli E, Cancarini GC, Zani R *et al*. Adequacy of dialysis reduces the doses of recombinant erythropoietin independently from the use of biocompatible membranes in haemodialysis patients. *Nephrol Dial Transplant* 2001; 16: 111–114.

8. Hsu PY, Lin CL, Yu CC *et al*. Ultrapure dialysate improves iron utilization and erythropoietin response in chronic hemodialysis patients —a prospective cross-over study. *J Nephrol* 2004; 17: 693–700.

9. Matsuhashi N, Yoshioka T. Endotoxin-free dialysate improves response to erythropoietin in hemodialysis patients. *Nephron* 2002; 92: 601–604.

10. Schiffl H, Lang SM, Bergner A. Ultrapure dialysate reduces dose of recombinant human erythropoietin. *Nephron* 1999; 83: 278–279.

11. Sitter T, Bergner A, Schiffl H. Dialysate related cytokine induction and response to recombinant human erythropoietin in haemodialysis patients. *Nephrol Dial Transplant* 2000; 15: 1207–1211.

12. Locatelli F, Manzoni C, Del Vecchio L *et al*. Management of anaemia by convective treatments. *Contrib Nephrol, Basel Karger* 2011; 168: 162–172.

13. Lin CL, Huang CC, Yu CC *et al*. Improved iron utilization and reduced erythropoietin resistance by on-line Hemodiafiltration. *Blood Purif* 2002; 20: 349–356.

14. Bonforte G, Grillo P, Zerbi S *et al*. Improvement of anemia in hemodialysis patients treated by hemodiafiltration with high-volume on-line-prepared substitution fluid. *Blood Purif* 2002; 20: 357–363.

15. Vaslaki L, Major L, Berta K *et al*. On-line haemodiafiltration versus haemodialysis: stable haematocrit with less erythropoietin and improvement of other relevant blood parameters. *Blood Purif* 2006; 24: 163–173.

16. Panichi V, Rosati A, Bigazzi R *et al*. Anaemia and resistance to erythropoiesis-stimulating agents as prognostic factors in haemodialysis patients: results from the RISCAVID study. *Nephrol Dial Transplant* 2011; 26: 2641–2648.

17. Ward RA, Schmidt B, Hullin J *et al*. A comparison of on-line hemodiafiltration and high-flux hemodialysis: a prospective clinical study. *J Am Soc Nephrol* 2000; 11: 2344–2350.

18. Wizemann V, Lotz C, Techert F *et al*. On-line haemodiafiltration versus low-flux haemodialysis. A prospective randomized study. *Nephrol Dial Transplant* 2000; 15 (Suppl 1): 43–48.

19. Locatelli F, Altieri P, Andrulli S *et al*. Hemofiltration, hemodiafiltration reduce intradialytic hypotension in ESRD. *J Am Soc Nephrol* 2010; 21: 1798–1807.

20. Bolasco P, Altieri P, Andrulli S *et al*. Convection versus diffusion in dialysis: an Italian prospective multicentre study. *Nephrol Dial Transplant* 2003; 18 (Suppl 7): 50–54.

21. Locatelli F, Aljama P, Barany P *et al*. Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure. *Nephrol Dial Transplant* 2004; 19 (Suppl 2): ii1–ii47.

22. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. *Am J Kidney Dis* 2006; 47: S11–S145.

23. KDOQI clinical practice guideline and clinical practice recommendations for anemia in chronic kidney disease: 2007 update of hemoglobin target. *Am J Kidney Dis* 2007; 50: 471–530.

24. Daugirdas JT. Simplified equations for monitoring Kt/V, PCRn, eKt/V and ePCRn. *Adv Ren Replace Ther* 1995; 2: 295–304.

25. Fink JC, Zhan M, Blahut SA *et al*. Measuring the efficacy of a quality improvement program in dialysis adequacy with changes in center effects. *J Am Soc Nephrol* 2002; 13: 2338–2344.

26. Spiegel B, Bolus R, Desai AA *et al*. Dialysis practices that distinguish facilities with below- versus above-expected mortality. *Clin J Am Soc Nephrol* 2010; 5: 2024–2033.

27. Locatelli F, Andrulli S, Del Vecchio L. Difficulties of implementing clinical guidelines in medical practice. *Nephrol Dial Transplant* 2000; 15: 1284–1287.

28. Spiegel BM, Bolus R, Desai AA *et al*. Dialysis practices that distinguish top- versus bottom-performing facilities by hemoglobin outcomes. *Am J Kidney Dis* 2010; 56: 86–94.