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Original Article

Effect of high-flux dialysis on the anaemia of haemodialysis patients

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Abstract

Background. Anaemia is one of the major clinical characteristics of patients with chronic renal failure, and has a considerable effect on morbidity and mortality. Adequate dialysis is of paramount importance in correcting anaemia by removing small and medium-sized molecules, which may inhibit erythropoiesis. However, high-molecular-weight inhibitors cleared only by means of highly porous membranes have also been found in uraemic serum and it has been claimed from uncontrolled studies that high-flux dialysis could improve anaemia in haemodialysis patients.

Methods. We therefore planned this multicentre randomized controlled trial with the aim of testing whether the use of a large-pore biocompatible membrane for a fixed 12-week follow-up improves anaemia in haemodialysis patients in comparison with the use of a conventional cellulose membrane. Eighty-four (5.3%) of a total of 1576 adult haemodialysed patients attending 13 Dialysis Units fulfilled the entry criteria and were randomly assigned to the experimental treatment (42 patients) or conventional treatment (42 patients).

Results. Haemoglobin levels increased non-significantly from 9.5 ± 0.8 to 9.8 ± 1.3 g/dl ($P = 0.069$) in the population as a whole, with no significant difference between the two groups ($P = 0.485$). Erythropoietin therapy was given to 32/39 patients (82%) in the conventional group, and 26/35 (74%) in the experimental group ($P = 0.783$) with subcutaneous administration to 26/32 patients in conventional and to 23/26 patients in experimental group, $P = 0.495$. Dialysis dose (Kt/V) remained constant in both groups (from 1.30 ± 0.17 to 1.33 ± 0.20 in the conventional group and from 1.28 ± 0.26 to 1.26 ± 0.21 in the experimental group, $P = 0.242$). Median pre- and post-dialysis β_2-microglobulin levels remained constant in the conventional group (31.9 and 34.1 mg/dl at baseline) and decreased in the experimental group (pre-dialysis values from 31.1 to 24.7 mg/dl, $P = 0.004$ and post-dialysis values from 24.8 to 20.8 mg/dl, $P = 0.002$). Median erythropoietin doses were not different at baseline (70 IU/kg/week in conventional treatment and 90 IU/kg/week in experimental treatment, $P = 0.628$) and remained constant during follow-up (from 70 to 69 IU/kg/week in the conventional group and from 90 to 91 IU/kg/week in the experimental group, $P = 0.410$). Median erythropoietin plasma levels were in the normal range and remained constant (from 12.1 to 12.9 mU/ml in the conventional group and from 13.2 to 14.0 mU/ml in the experimental group, $P = 0.550$).

Conclusions. This study showed no difference in haemoglobin level increase between patients treated for 3 months with a high-flux biocompatible membrane in comparison with those treated with a standard membrane. When patients are highly selected, adequately dialysed, and have no iron or vitamin depletion, the effect of a high-flux membrane is much less than might be expected from the results of uncontrolled studies.

Keywords: anaemia; biocompatibility; biocompatible membrane; cellulose membrane; high-flux haemodialysis; β_2-microglobulin

Introduction

Anaemia is one of the major negative clinical characteristics of patients with chronic renal failure (CRF) on

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substitutive therapy and, together with hypertension, is the leading cause of cardiac hypertrophy and subsequent dilation. 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.

Over the last 10 years, the availability of recombinant human erythropoietin (rHuEpo) therapy has led to the almost complete disappearance of the severe anaemia of ESRD patients requiring repeated blood transfusions [2]; it has also reduced left ventricular hypertrophy [3,4] and led to a direct improvement in myocardial function. Although the results of long-term studies are still awaited, these effects may delay the progression of cardiac disease and have already been associated with less overall and cardiovascular mortality and morbidity [5–7]. The National Kidney Foundation—Dialysis Outcomes Quality Initiative (NKF-DOQI) guidelines for the treatment of anaemia in chronic renal insufficiency suggest that the target range for haematocrit should be 33–36% [8]; however, despite an increase in the use and average dose of rHuEpo, a substantial percentage of patients do not achieve a haematocrit level of more than 30% [2,5–7], although other factors should be considered as reimbursement policy.

CRF is a complex syndrome in which many factors other than absolute or relative Erythropoietin deficiency may contribute towards causing anaemia, such as the blood loss related to the dialytic procedure itself, and the haemolysis and bone-marrow suppression [9] probably induced by the retention of toxic metabolites. Given the current availability of rHuEpo, the other factors increasing the severity of anaemia (and sometimes leading to rHuEpo resistance) in chronic renal insufficiency need to be considered.

Adequate dialysis is of paramount importance in correcting anaemia by removing small and possibly medium/large molecules, as the blood of CRF patients contains some substances whose molecular weight of 10 000 Daltons inhibits erythropoiesis. The biocompatibility of dialysis membranes is also probably involved [10]. In a prospective randomized study of 135 patients, Ifudu *et al.* [11] found that an increased dialytic dose in patients receiving inadequate dialysis led to a significant increase in their response to rHuEpo; however, as this result was achieved using a highly permeable and biocompatible membrane (high-flux polysulphone), it is at least possible that biocompatibility or permeability, or both, have an additive effect to increased dialysis dose on the correction of anaemia. The results of Villaverde *et al.* [12] support this hypothesis.

Thus, a still unknown uraemic toxin may suppress erythropoiesis and contribute towards the development of anaemia [13,14]. It is reasonable to postulate low-molecular-weight erythroid inhibitors (because anaemia improves after the start of dialysis with cellulose membranes) and possibly medium- to large-molecular-weight inhibitors of 10 000 Daltons only removed by more permeable membranes. It is well known that the addition of uraemic serum to c.f.u.-E (erythroid colony-forming unit) and b.f.u.-E (erythroid burst-forming unit) cell cultures inhibits their reproduction and differentiation, as well as haeme synthesis. High-molecular-weight inhibitors of one million Daltons of molecular weight (KR4–0 peak fraction) that can only be cleared by means of highly porous membranes have also been found in uraemic serum [15].

Radtke *et al.* [16] investigated the relationship between anaemia and erythropoietin serum levels in 42 patients with terminal renal failure beginning haemodialysis, and concluded that endogenous serum erythropoietin levels decreased and haematocrit increased with the start of dialysis; the improvement in anaemia is therefore not a consequence of increased erythropoietin production but probably due to the haemodialytic elimination of a bone-marrow inhibitor.

Kobayashi *et al.* [15] have reported the clinical results obtained in eight haemodialysis patients treated with a large-pore membrane (BK-F polymethylmethacrylate) but, although they suggest a major effect in two patients with baseline haematocrit levels of approximately 21 and 22% (reaching only 25% at 11 months), these results cannot be considered conclusive for a number of reasons: the patients had a wide range of haematocrit levels and no information was given concerning their iron status; overall, the study was not randomized, the sample size was too small, there was no control group, and the eligibility criteria were unclear. The same considerations apply to the study of Kawano *et al.* [17], and the study of Villaverde *et al.* [12] was not randomized.

We therefore planned a multicentre, controlled, and randomized study with the aim of testing whether erythroid suppression due to a still unknown large-molecular-weight uraemic toxin may play a role in the anaemia of haemodialysed patients. The aim of this study was to test whether haemodialysis with high-flux membrane improves the anaemia in haemodialysis patients in comparison with conventional haemodialysis using a cellulose membrane.

Subjects and methods

A 12-month recruitment period was estimated; the fixed-duration follow-up for each patient was 12 weeks. The inclusion criteria were: conventional thrice-weekly, 180–270-min haemodialysis with a cellulose dialyser for at least 6 months; haemodialysis patients aged more than 18 years; negligible residual renal function (average urea and creatinine clearance <1 ml/min/1.73 m^2); pre-dialysis haematocrit $<30\%$ without any trend or only negligible variations ($<2.5\%$) between successive evaluations during the previous 3 months; no change in rHuEpo, iron, folic acid, vitamin B_{12}, or ACE-inhibitor therapies [18] in the previous 3 months; dialysis dosage >1.2 or >1.4 (respectively estimated by means of equilibrated or non-equilibrated Kt/V)[19]. Exclusion criteria were: patients whose clinical condition had been unstable during the previous 3 months; patients on treatment with erythropoiesis-related drugs (steroids, cyclo-

phosphamide, azathioprine, or androgen hormones); severe cardiac, hepatic, bone marrow, connective tissue, or neoplastic diseases [20,21]; percent transferrin saturation $<25\%$ or serum ferritin <200 ng/ml, or a mean corpuscular volume of less than 85 μm^3 (to exclude iron depletion) [22] or a mean corpuscular volume >100 μm^3 (to exclude B_{12} or folic acid depletion) [23]; hyperparathyroidism [24] defined as NH_2-amino terminal parathyroid hormone (PTH) >500 mU/ml, with ionized blood calcium levels of 1.15–1.37 mmol/l; use of aluminium hydroxide-based binders at a dose of more than 2 g/day for at least 1 year before study entry, or clinical or laboratory findings of aluminium intoxication; use of a dialyser with a synthetic membrane or use of a convective dialysis technique in the previous 6 months.

Definition and assignment of treatments

The compared treatments were haemodialysis with a cellulose membrane (conventional treatment) *vs* haemodialysis with a high-flux membrane (BK-F PMMA membrane, Hoechst, Toray, Japan) (experimental treatment) having the same surface area as the cellulose dialyser used before study entry.

The patients satisfying the entry criteria were centrally randomized to one of the two treatment groups. The investigators were advised to avoid changes in drug dosage as iron and rHuEpo therapy or changes in dialysis schedule during the study.

Timing of clinical and laboratory measurements

The patients considered eligible for the trial on the basis of the entry criteria were fully informed about the study and asked to give their written consent. Their date of birth, sex, underlying nephropathy, time on dialysis, dialysis duration, the type and surface area of the dialyser, residual renal function and the randomly assigned treatment were recorded on *ad hoc* clinical record forms.

Every 4 weeks throughout the 12-week study period, the following clinical and laboratory variables were recorded: the type of dialyser and membrane, filter surface area, dialysis duration, blood and dialysate flow rate, Kt/V according to Daugirdas [25] and normalized protein catabolic rate (nPCR) [26]; pre- and post-dialysis body weight, systolic and diastolic blood pressure, blood urea nitrogen, serum creatinine and β_2-microglobulin (β_2-M); pre-dialysis blood sodium, potassium, bicarbonate, pH, phosphorus, haematocrit, haemoglobin values, erythrocyte, reticulocyte, and leukocyte cell counts, and mean globular volume; as were the drug therapy data, concentrating on iron, rHuEpo, vitamins (folic acid, B_{12}, multivitamin preparations, and vitamin D), sodium bicarbonate, calcium salts, aluminium-containing phosphorus binders, anti-H_2 receptors, and antihypertensive drugs.

At the beginning and end of the 12-week study period, the following pre-dialysis blood levels were checked and recorded: total serum proteins, serum albumin, C3 and C4 complement factors, serum iron, total iron-binding capacity, serum ferritin, and serum erythropoietin.

Statistical analysis

On the basis of the data provided by Ifudu *et al.* [11], the sample size was calculated by considering the between-treatment difference in the variation of haemoglobin levels during the study as the main response variable. The calculated sample size of 74 patients (37 patients per group) was based on a significance level (α error) of 0.05, a β error of 0.1, a power (1-β) of 0.9, an increase of 0.83 g/dl (corresponding to 2.5% of haematocrit) in haemoglobin levels (SD 1.16) during the experimental treatment in comparison with the conventional treatment, a two-tailed independent *t*-test for both α and β errors, and a drop-out rate of 30%.

The descriptive analysis was based on the mean values and standard deviations of continuous normally distributed variables (such as haematocrit and haemoglobin levels); the percentile distribution with the median as central tendency statistic was used for continuous not normally distributed variables (such as plasma ferritin levels). Imbalances between the two groups in terms of relevant clinical and laboratory variables at baseline were tested using the non-parametric Mann–Whitney test for continuous variables and Fisher's exact test for categorical variables.

The main statistical analysis was made by means of the general linear model for repeated measures of analysis of variance for two parallel groups using the conventional treatment as the reference, the type III sum of squares, and the multivariate tests of Pillais, Hotellings, and Wilks. The main between-subject factor was the randomly assigned conventional or experimental treatment. Using this general linear model procedure, we tested the null hypotheses concerning the effects of both between-and within-subject factors (such as the duration of follow-up) and their interactions. The natural logarithm transformation was used when the assumption of an equal error variance in the dependent variable required by the model was violated, and this was tested by means of the Levene's test. The assumption of the normality of the error term required by this model was tested using the analysis of residuals. The statistical analysis was made considering the variation in haemoglobin levels during follow-up as the main response variable. The effect of the experimental treatment was tested using the group-by-time interaction and is labelled in Tables 3, 4 and 5 as 'treatment effect'. Baseline blood levels of erythropoietin, haemoglobin, and ferritin, the percent transferrin saturation, the rHuEpo dose, and Kt/V were considered covariates in the model. A probability value of <0.05 was considered statistically significant. The statistical analyses were made using SPSS for Windows, Release 8.0.

Informed consent and ethical surveillance

Before starting the study, the patients were informed about its aims, the expected benefits to them and/or others, the risks and inconveniences involved, and their right to refuse to participate or to withdraw from the study at any time without sanction. Their written consent was obtained. The study was carried out in accordance with the Declaration of Helsinki and its subsequent modifications, and was approved by the local Ethics Committee of the co-ordinating centre (Lecco Hospital).

Results

Baseline characteristics

Between 28 March 1997 and 31 March 1998, 84 (5.3%) of a total of 1576 adult haemodialysed patients attending 13 Italian dialysis units were randomly assigned to the conventional treatment (42 patients) or experimental treatment (42 patients) (Figure 1).

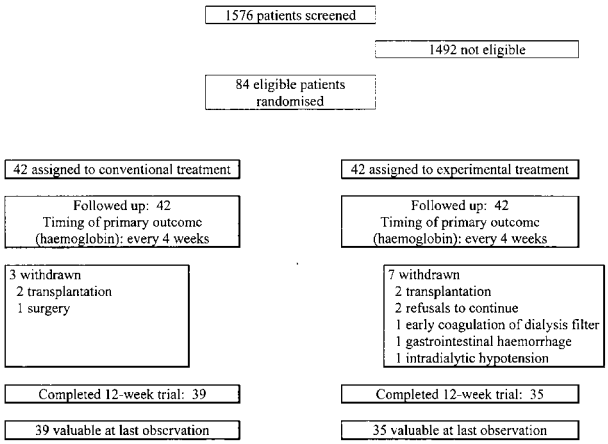

Fig. 1. Trial profile.

Ten of the 84 randomized patients dropped out of the study (three in the conventional group and seven in the experimental group): the causes were transplantation (two patients in each group), refusal to continue experimental treatment (one patient because of intradialytic hypertension, and one because of the bitter taste related to the treatment), and clinical reasons (one patient in the conventional group because of surgery due to an unrecognized diagnosis of cardiac aneurysm of Valsalva sinus, and three in the experimental group: one case each of early dialysis filter coagulation, gastrointestinal haemorrhage, and intradialytic hypotension).

The final analysis therefore involved 74/84 patients (88%): 39 on conventional and 35 on experimental treatment (Figure 1). The baseline characteristics of these patients by group are shown in Table 1. The two groups were similar in age, sex, duration of dialysis, time on dialysis, filter surface area, previous filter membrane type, prevalence and dosage of rHuEpo treatment, body weight, and pre-dialysis blood pressure values.

rHuEpo treatment was used in 32 of 39 patients (82%) on conventional treatment and in 26 of 35 patients (74%) on experimental treatment ($P=0.783$). Subcutaneous administration was used in 26 of 32 patients (81%) on conventional treatment and in 23 of 26 patients (88%) on experimental treatment ($P=0.495$). The median rHuEpo dosage was 70 IU/kg/week (interquartile range 54–120) in conventional treatment and 90 IU/kg/week (interquartile range 66–116) in experimental treatment ($P=0.628$) and the median rHuEpo resistance index (IU/kg/week/g of haemoglobin) was 7.5 (interquartile range 5.3–12.7) in conventional treatment and 9.2 (interquartile range 6.1–11.9) in experimental treatment ($P=0.586$). The median number of administrations per week was three (interquartile range 2–3) in conventional treatment and two (interquartile range 2–3) in experimental treatment ($P=0.440$). Therefore all these differences were not statistically significant.

Iron therapy was given to 30/39 patients (77%) in the conventional group, and to 19/35 patients (54%) in the experimental group, and this imbalance was near to statistical significance ($P=0.051$). A large percentage of the patients used iron intravenously (29/30 patients in conventional and 18/19 patients in experimental group) and the median intravenous iron dosage was similar in the two groups (62 mg/week with interquartile range of 50–62 mg/week in the conventional group and 62 mg/week with interquartile range of 40–62 mg/week in the experimental group, $P=0.903$).

The distribution of the cause of chronic renal failure was unbalanced (but not significantly so) for two underlying diseases: polycystic kidney disease was more prevalent in the conventional treatment arm (20 *vs* 6%, $P=0.065$) and diabetes mellitus was less frequent (3% *vs* 11%, $P=0.132$).

As shown in Table 2, the baseline biochemical data regarding dose dialysis indices, anaemic status variables, electrolyte and acid–base metabolism, and the biochemical nutritional indexes were similar in the two groups; only baseline total protein levels were higher in the patients randomized to the experimental treatment (6.7 ± 0.5 *vs* 6.9 ± 0.4 g/dl, $P=0.04$).

Table 1. Baseline characteristics of 74 patients by conventional (39 patients) or experimental (35 patients) treatment group

Characteristic	Conventional treatment (39 patients)	Experimental treatment (35 patients)	*P* value[a]
Age (years)	65 (59–71)	64 (56–71)	0.880
Sex (male/female)	20/19	19/16	0.797
Duration of dialysis (min)	240 (210–240)	240 (210–240)	0.574
Time on dialysis (years)	3.4 (1.6–7.3)	4.1 (1.9–6.9)	0.534
Filter surface area (m^2)	1.5 (1.3–1.7)	1.5 (1.3–1.6)	0.552
Iron treatment	30	19	0.051
Intravenous administration	29	18	0.054
Iron dosage i.v. (mg/week)	62 (50–62)	62 (40–62)	0.903
Erythropoietin treatment	32	26	0.783
Subcutaneous administration	26	23	0.495
Erythropoietin dosage (IU/kg/week)[b]	70 (54–120)	90 (66–116)	0.628
Erythropoietin resistance index (IU/kg/week/g of Hb[c])	7.5 (5.3–12.7)	9.2 (6.1–11.9)	0.584
Number of administrations/week	3 (2–3)	2 (2–3)	0.440
Pre-dialysis body weight (kg)	65.8 ± 12.8	66.4 ± 13.6	0.713
Post-dialysis body weight (kg)	63.0 ± 12.5	63.9 ± 13.1	0.657
Pre-dialysis systolic blood pressure (mmHg)	150 ± 24	157 ± 18	0.099
Pre-dialysis diastolic blood pressure (mmHg)	86 ± 12	84 ± 9	0.588

Data are median and interquartile range (IQR) or number of patients. Plus–minus values are means $\pm$ SD. [a]Mann–Whitney test for continuous variables and Fisher's exact test for categorical variables. [b]Intravenous or subcutaneous administration. [c]Hb, haemoglobin.

Table 2. Baseline biochemical data concerning dose dialysis indices, anaemia-related variables, nutritional status, electrolyte and acid–base metabolism of 74 patients by conventional or experimental treatment group

Biochemical data	Treatment				*P* value[a]
	Conventional (39 patients)		Experimental (35 patients)		
	Mean	SD	Mean	SD	
Pre-dialysis plasma creatinine (mg/dl)	11.26	2.87	10.31	2.02	0.108
Post-dialysis plasma creatinine (mg/dl)	4.74	1.37	4.20	1.09	0.051
Pre-dialysis plasma urea (mg/dl)	172	64	150	51	0.110
Post-dialysis plasma urea (mg/dl)	53	24	46	21	0.150
Intradialytic urea reduction (%)	70	5	69	7	0.705
Pre-dialysis β_2-M (mg/dl)	35.3	14.1	31.9	13.1	0.317
Kt/V	1.30	0.17	1.28	0.26	0.548
nPCR (g/kg/day)	1.23	0.38	1.09	0.35	0.077
Pre-dialysis haematocrit (%)	28.3	1.9	28.3	2.7	0.721
Pre-dialysis haemoglobin (g/dl)	9.5	0.8	9.4	0.9	0.377
Erythrocytes ($n \times 1000$/mm^3)	3076	265	3068	398	0.386
Leukocytes (n/mm^3)	6596	2249	6786	1410	0.309
Thrombocytes ($n \times 1000$/mm^3)	194	60	201	45	0.176
Mean globular volume (μm^3)	93	5	93	5	0.850
Reticulocytes (n/1000 erythrocytes)	11	6	11	9	0.590
Pre-dialysis plasma erythropoietin levels (mU/ml)	19.2	25.1	15.4	12.0	0.970
Total proteins (g/dl)	6.7	0.5	6.9	0.4	0.040
Albumin (g/dl)	3.73	0.59	3.88	0.55	0.312
C3 (mg/dl)	86	23	83	18	0.584
C4 (mg/dl)	29	8	29	9	0.881
Transferrin (mg/dl)	218	60	214	62	0.766
Transferrin saturation (%)	25	11	24	11	0.481
Ferritin (ng/ml)	349	419	385	281	0.171
Na (mEq/l)	139.5	3.4	139.0	2.7	0.450
K (mEq/l)	5.38	0.66	5.25	0.74	0.401
Phosphorus (mg/dl)	4.9	1.3	4.9	1.4	0.566
HCO_3 (mEq/l)	20.8	3.4	21.2	3.0	0.461
pH	7.358	0.045	7.358	0.040	0.987

[a]Mann–Whitney test.

All 74 patients were followed up for a fixed 12-week period.

Follow-up results

Anaemia

The evolution of the variables concerning anaemic status is shown in Table 3. Between the beginning and end of the 12-week study period, blood haemoglobin levels (the primary response variable of the study) increased in the study population as a whole from 9.5 ± 0.8 to 9.8 ± 1.3 g/dl ($P = 0.069$), and the haematocrit and erythrocyte counts significantly increased from 28.3 ± 2.3 to 29.7 ± 4.2 ($P = 0.003$) and from 3.072 ± 332 to 3180 ± 474 units $\times 1000$/mm^3 ($P = 0.01$) respectively. This increase had a linear and quadratic pattern for haematocrit but only linear for the erythrocyte count. Transferrin saturation also increased (from $25 \pm 11\%$ to $28 \pm 14\%$, $P = 0.016$). There were no significant differences between the beginning and end of the study in terms of mean globular volume (from 93 ± 5 to 94 ± 6 μm^3, $P = 0.069$), the number of reticulocytes (from 11 ± 8 n/1000 erythrocytes to 12 ± 10 n/1000 erythrocytes, $P = 0.202$), ferritin (from a median of 237 ng/ml to 233 ng/ml, $P = 0.121$), or plasma erythropoietin levels (from a median of 13.1 mU/ml to 12.5 mU/ml, $P = 0.457$). No significant correlation was found between plasma erythropoietin levels and haemoglobin levels at baseline (Pearson correlation -0.124, $P = 0.317$) and at the end of the study (Pearson correlation -0.051, $P = 0.710$).

Between the conventional and experimental treatments, there were no significant differences (Table 3) in the pre- and post-treatment values of haematocrit ($P = 0.303$), haemoglobin ($P = 0.485$) (Figure 2), erythrocyte counts ($P = 0.171$), mean globular volume ($P = 0.217$), the number of reticulocytes ($P = 0.334$), ferritin ($P = 0.164$), and plasma erythropoietin levels ($P = 0.550$).

Iron and rHuEpo therapy

As expected from the recommendations included in the protocol, no significant changes were made in the iron and rHuEpo therapy throughout the study.

The percentage of patients with iron therapy in the conventional group was higher in comparison with the experimental group at baseline (30/39 patients 77% *vs* 19/35 patients 54%, $P = 0.051$ respectively) and this imbalance remained constant through the study. However, in the patients who received iron the dosage (median of 62 mg/week) and the route of administration (almost all i.v.) were not different in the two groups (Table 1) and did not change during follow-up.

rHuEpo therapy was given to 32/39 patients (82%) in the conventional group, and 26/35 (74%) in the experimental group ($P = 0.783$) at baseline and did not change during follow-up. Subcutaneous administration was more frequent in both groups (26/32 patients, 81% in the conventional and 23/26 patients, 88% in the experimental group, $P = 0.495$) and did not change during follow-up. Median rHuEpo doses remained constant in the two groups during follow-up (from 70 to 69 IU/kg/week in the conventional group and from 90 to 91 IU/kg/week in the experimental group, $P = 0.410$) (Table 3).

Nutritional status

In the study population as a whole, the pre-dialysis body weight (66.1 ± 13.1 kg at baseline) did not change during follow-up ($P = 0.163$) and the post-dialysis body weight decreased from 63.4 ± 12.7 to 63.2 ± 12.9 kg

Table 3. Follow-up data concerning the anaemia-related variables of 74 patients by conventional or experimental treatment group. There was a small but significant increase in haematocrit and erythrocyte values during follow-up in the population as a whole ($P=0.003$ and 0.01 respectively). There was no significant effect on any variable of the experimental in comparison with the conventional treatment (see the statistical significance in the treatment effect column)

Parameter		Conventional treatment (39 patients) Follow up (weeks)				Experimental treatment (35 patients) Follow up (weeks)				*P* value[a]	
		0	4	8	12	0	4	8	12	Period effect	Treatment effect
Haematocrit (%)	Mean	28.3	29.4	29.5	29.6	28.3	29.1	30.0	29.9	0.003[b,c]	0.303
	SD	1.9	3.6	4.0	4.8	2.7	3.0	4.2	3.5		
Haemoglobin (g/dl)	Mean	9.5	9.7	9.7	9.7	9.4	9.5	9.6	9.8	0.069	0.485
	SD	0.8	1.2	1.2	1.4	0.9	1.0	1.2	1.1		
Erythrocytes ($n \times 1000/mm^3$)	Mean	3076	3139	3149	3138	3068	3121	3189	3227	0.01[b]	0.171
	SD	265	390	426	450	398	415	448	502		
Leukocytes (n/mm^3)	Mean	6596	6177	6446	6637	6786	6641	6601	6690	0.452	0.708
	SD	2249	1814	1847	1809	1410	1708	1494	1799		
Thrombocytes ($n \times 1000/mm^3$)	Mean	194	196	193	196	201	217	211	218	0.069	0.270
	SD	60	54	50	43	45	50	48	53		
Mean globular volume (μm^3)	Mean	93	94	94	95	93	93	94	93	0.069	0.217
	SD	5	5	6	5	5	5	6	6		
Reticulocytes (n/1000 erythrocytes)	Mean	11	11	11	12	11	11	12	12	0.202	0.334
	SD	6	10	5	8	9	10	8	12		
Ferritin (ng/ml)[d]	25%	164			200	203			153		
	Median	222			246	255			226	0.121	0.164
	75%	365			372	587			541		
Erythropoietin levels (mU/ml)	25%	5.4			5.6	5.7			6.6		
	Median	12.1			12.9	13.2			14.0	0.457	0.550
	75%	19.4			22.6	22.6			26.3		
rHuEpo dosage (IU/kg/week)	25%	54			48	56			61		
	Median	70			69	90			91	0.794	0.410
	75%	120			114	116			113		

[a]From the multivariate test of repeated measure analysis of variance. When the multivariate test is significant, [b]indicates a significant (<0.05) *P* value for the linear effect and [c]a significant (<0.05) *P* value for the quadratic effect. [d]Ferritin, erythropoietin levels and rHuEpo dosage are indicated as percentiles because of their skewed, abnormal distribution. For these variables, the multivariate test was used after natural logarithm transformation allowed a normal distribution to be obtained.

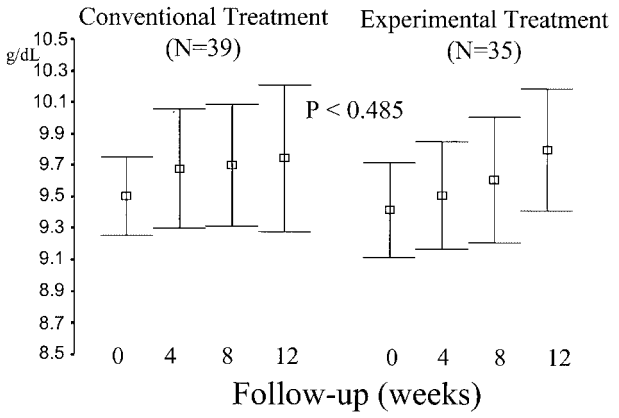

Fig. 2. Haemoglobin levels (g/dl) during follow-up of 74 patients by conventional (39 patients) or experimental (35 patients) treatment group. Data are means and confidence intervals of the mean.

($P=0.020$). There was no difference in the body weight patterns of the two groups (data not shown).

The biochemical nutritional status indices recorded in the two groups are shown in Table 4. In the study population as a whole, there was a significant decrease in plasma total protein, albumin and transferrin levels (*P* values of respectively 0.014, 0.030 and 0.004); the reduction in transferrin during follow-up was associated with an overall increase in transferrin saturation (from 25 ± 11 to $28\pm14\%$, $P=0.016$). Total protein levels decreased only in the experimental group (from 6.9 ± 0.4 to 6.7 ± 0.5 g/dl *vs* 6.7 ± 0.5 to 6.7 ± 0.5 g/dl, $P=0.043$) but were higher than in the conventional group at baseline (6.9 ± 0.4 *vs* 6.7 ± 0.5 g/dl, $P=0.04$, Table 2). There was no difference in the patterns of albumin, transferrin, C3, and C4 complement factors between the two groups (Table 4).

Dialysis dose and nPCR

The pre- and post-dialysis urea and creatinine levels at baseline and during follow-up in the two groups are shown in Table 5. In the study population as a whole, the pre-dialysis plasma urea levels decreased from 162 ± 59 to 158 ± 64 mg/dl ($P=0.031$) following a quadratic curvilinear U pattern (i.e. the lowest values were observed during the middle of the follow-up period), whereas plasma creatinine levels decreased from 10.81 ± 2.53 to 10.25 ± 3.12 mg/dl ($P=0.015$) following a linear pattern; there was no change in post-dialysis plasma urea and creatinine levels during the follow-up ($P=0.557$ and $P=0.502$ respectively).

There was no difference between the groups in terms of either pre- or post-dialysis plasma urea and creatinine levels (Table 5).

Table 4. Follow-up data of the biochemical indices of nutritional status of 74 patients by conventional or experimental treatment group. There was a decrease in total proteins, albumin and transferrin from the start to the end of follow-up in the population as a whole (see the statistical significance in the period effect column). Total proteins decreased only in the experimental group, but the baseline values were higher than in the conventional group. During follow-up, a reduction in transferrin values ($P=0.004$) was observed together with an increase in transferrin saturation ($P=0.016$)

Parameter		Conventional treatment (39 patients) Follow-up (weeks)		Experimental treatment (35 patients) Follow-up (weeks)		*P* value[a]	
		0	12	0	12	Period effect	Treatment effect
Total proteins (g/dl)	Mean	6.7	6.7	6.9	6.7	0.014	0.043
	SD	0.5	0.5	0.4	0.5		
Albumin (g/dl)	Mean	3.73	3.69	3.88	3.64	0.030	0.098
	SD	0.59	0.64	0.55	0.55		
Transferrin (μg/dl)	Mean	218	209	214	196	0.004	0.324
	SD	60	60	62	60		
C3 (mg/dl)	Mean	86	84	83	86	0.852	0.315
	SD	23	28	18	22		
C4 (mg/dl)	Mean	29	29	29	29	0.562	0.845
	SD	8	9	9	10		
Transferrin saturation (%)	Mean	25	28	24	28	0.016	0.757
	SD	11	12	11	16		

[a]From the multivariate test of repeated measure analysis of variance.

Table 5. Follow-up data concerning the depurative indices of 74 patients by conventional or experimental treatment group. The pre- and post-dialysis β_2-microglobulin levels remained constant in the conventional group and decreased in the experimental group (from a median of 31.1 mg/dl at baseline to a median of 24.7 mg/dl at the end of follow-up, $P=0.004$) with a linear and quadratic curvilinear U pattern. Similarly, the median post-dialysis β_2-microglobulin levels remained constant in the conventional group and decreased in the experimental group (from a median of 24.8 mg/dl at baseline to a median of 20.8 mg/dl at the end of follow-up, $P=0.002$). There was no treatment effect for the other variables

Parameter		Conventional treatment (39 patients) Follow up (weeks)				Experimental treatment (35 patients) Follow up (weeks)				*P* value[a]	
		0	4	8	12	0	4	8	12	Period effect	Treatment effect
pre-HD urea (mg/dl)	Mean	172	161	167	173	150	147	140	141	0.031[c]	0.099
	SD	64	66	68	70	51	54	50	52		
post-HD urea (mg/dl)	Mean	53	48	49	51	46	47	45	46	0.557	0.315
	SD	24	24	23	24	21	21	21	23		
pre-HD creatinine (mg/dl)	Mean	11.26	11.45	10.89	11.06	10.31	10.22	9.94	9.36	0.015[b]	0.491
	SD	2.87	2.64	2.95	3.14	2.02	2.08	2.08	2.89		
post-HD creatinine (mg/dl)	Mean	4.74	4.52	4.47	4.50	4.20	4.32	4.16	4.43	0.502	0.498
	SD	1.37	1.28	1.24	1.18	1.09	0.98	1.02	1.33		
pre-HD β_2 (mg/dl)	25%	26.7	28.1	28.1	28.7	22.6	19.3	20.0	20.2		
	Median	31.9	34.7	35.6	32.6	31.1	26.3	24.2	24.7	0.264	0.004[b,c]
	75%	46.8	50.0	48.5	45.4	39.4	30.8	28.7	30.5		
post-HD β_2 (mg/dl)	25%	25.9	27.8	27.5	25.8	18.7	15.4	15.0	13.5		
	Median	34.1	40.0	36.1	36.2	24.8	20.0	20.5	20.8	0.054	0.002[b,c]
	75%	51.3	58.0	60.5	53.0	31.0	25.5	23.0	25.8		
Equilibrated Kt/V[d]	Mean	1.30	1.32	1.34	1.33	1.28	1.22	1.24	1.26	0.362	0.242
	SD	0.17	0.18	0.20	0.20	0.26	0.20	0.19	0.21		
nPCR (g/kg/day)[e]	Mean	1.23	1.16	1.22	1.26	1.09	1.04	1.02	1.02	0.022[b]	0.168
	SD	0.38	0.40	0.42	0.45	0.35	0.33	0.31	0.31		

[a]From the multivariate test of repeated measure analysis of variance. When the multivariate test is significant, [b]indicates a significant (<0.05) *P* value for the linear effect and [c]a significant (<0.05) *P* value for the quadratic effect. [d]According to Daugirdas (see reference [25]), [e]According to Garred *et al.* (see reference [26]). HD, haemodialysis.

The baseline dialysis dose estimated by means of body fractional clearance Kt/V according to Daugirdas [25] was 1.29 ± 0.22 in the study population as a whole: no period or treatment effect was observed ($P=0.362$ and 0.242 respectively).

The nPCR calculated according to Garred *et al.* [26] varied significantly ($P=0.022$) during the study period in the population as a whole from 1.17 ± 0.37 to 1.15 ± 0.41 g/kg/day, following a quadratic U pattern similar to that of the pre-dialysis plasma urea levels.

No differences were detected between the two-treatment groups ($P=0.128$).

β_2-*M*

Table 5 shows the percentiles of the β_2-M data because these were not normally distributed and had a right-skewed tail.

Between baseline and the end of follow-up, pre-dialysis (from a median of 31.1 to 24.7 mg/l *vs* 31.9 to 32.6 mg/l, $P=0.004$ with a quadratic curvilinear decreasing pattern) and post-dialysis β_2-M levels (from a median of 24.8 to 20.8 mg/l *vs* 34.1 to 36.2 mg/l, $P=0.002$ with a similar quadratic curvilinear decreasing pattern) significantly decreased only in the experimental group (-20.6 *vs* $+2\%$ for pre-dialysis values and -16.1 *vs* $+6.2\%$ for non-equilibrated post-dialysis values) (Table 5). Moreover, the error variance of these two variables in the two groups was not the same when checked by the Levene test ($P=0.04$). The natural logarithm transformation satisfies this assumption, making it possible to use the powerful technique of repeated analysis of variance measures.

The pre-dialysis β_2-M levels remained constant in the conventional group (a median of 31.9 mg/dl at baseline and 32.6 mg/dl at the end of follow-up) and decreased in the experimental group (from a median of 31.1 mg/dl to a median of 24.7 mg/dl, $P=0.004$) (Table 5, Figure 3) with a linear and quadratic curvilinear U-shaped pattern. The median post-dialysis β_2-M levels also remained constant in the conventional group (34.1 mg/dl at baseline and 36.2 mg/dl at the end of follow-up) and decreased in the experimental group (from a median of 24.8 mg/dl to a median of 20.8 mg/dl, $P=0.002$) with a similar linear and quadratic curvilinear U-shaped pattern. After correcting the post-dialysis levels for haemoconcentration, a similar profile at a lower level was observed in both groups (data not shown).

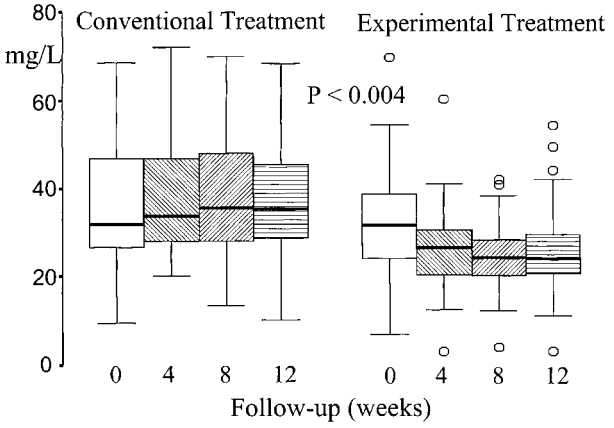

Fig. 3. Pre-dialysis β_2-M levels during follow-up by conventional or experimental treatment group. Lines crossing boxes, median; boxes, interquartile range; whiskers show largest and smallest observed values that are less than 1.5 box lengths from the 25th or 75th centile.

Safety

All the 74 evaluated patients completed the 12-week follow-up without experiencing any major side-effects. Neither pre- nor post-dialysis systolic (154 ± 21 and 143 ± 28 mmHg respectively) and diastolic (85 ± 11 and 80 ± 11 mmHg respectively) blood pressure values changed during follow-up, nor was there any difference in pattern between the two groups (data not shown).

Discussion

Anaemia is certainly a major symptom of CRF, and despite an increase in the use and average dose of rHuEpo, a substantial percentage of patients do not achieve a haematocrit level of more than 30% [2,5]. This is of particular concern as it has been found that there is an inverse relationship between haematocrit levels and mortality and days spent in hospital [5–7]. It has also been shown that cardiac hypertrophy can be reduced in dialysis patients not only by controlling hypertension [27] but also by correcting anaemia [3,4], thus possibly improving the high cardiovascular morbidity and mortality associated with end-stage renal failure.

Although iron deficiency is probably the most important factor affecting the response to rHuEpo in most patients, occult blood loss, infection, and inflammation are also important [28]. Less frequent are problems of hyperparathyroidism with marrow fibrosis, aluminium toxicity, vitamin B_{12} and folic deficiency, haemolysis, bone marrow disorders, haemoglobinopathies, dialysis, and carnitine deficiency [29]. ACE inhibitors [18] and angiotensin II receptor antagonists may also play a role.

We therefore used very selective criteria when admitting patients to this trial (5.3% of the total screened number) designed to compare the effect of high-flux synthetic and low-flux cellulose membrane on the anaemia affecting chronic haemodialysis.

The results of this trial show that blood haemoglobin levels increased (although not significantly) in the population as a whole; the trend was not significantly different between the conventional and experimental groups. However, in the experimental group the tendency of haemoglobin concentrations to increase was present at each month during the trial follow up (Figure 2), possibly suggesting too short a period of observation. The sample size and the length of follow-up of our trial were calculated on the basis of the data of Ifudu *et al.* [11], who found an increase in haematocrit values as early as 6 weeks after increased dialysis dose (urea percentage reduction during dialysis from 60 to 72%). In our study the main response variable was considered to be an increase of 0.83 g/dl in haemoglobin levels in the experimental group in comparison with the control group. Inadequate dialysis in the Ifudu study [11] was defined as a reduction in blood urea nitrogen concentration of less than 65%, but this does not take into account intradialytic urea generation,

variations in urea distribution volume, or urea rebound; therefore we used the more accurate parameter of Kt/V. Our mean level of Kt/V (1.3) was adequate, and did not change during follow-up in the population as a whole or in the two patient groups. nPCR statistically decreased during follow-up in the population as a whole, but this had no concomitant clinical relevance and was the same in the two groups. Metabolic acidosis was well corrected, with no difference being observed in either group at baseline or during the trial follow-up.

Given the results of our study, we suggest that the increase in haematocrit values observed by Ifudu *et al.* [11] was due mainly to the increased dialysis dose (from very inadequate dialysis dose) rather than to the use of a high-flux biocompatible membrane.

Our results are in contrast with previous reports [12,15,17] insofar as there was only a non-significant difference in haemoglobin levels between the patients treated with a high-flux membrane and those receiving standard dialysis. There may be various reasons for this, perhaps the most important being our selection criteria. We selected patients with haematocrit levels of less than 30% without other known factors affecting uraemic anaemia. Particular care was taken to admit only well-nourished patients without iron depletion. We also tried to exclude vitamin B_{12} and folic acid deficiencies, aluminium intoxication, and PTH toxic effects on erythropoiesis. Finally, the dialysis dose was adequate (Kt/V 1.3).

Another possible explanation for the lack of a significant difference between the study groups in terms of the main response variable (i.e. haemoglobin values) could be that our patients (with haematocrit level values of $28.3 \pm 2.3\%$) were not too anaemic in comparison with the general dialytic population. Data from the Lombardy Registry [5] give a mean haematocrit value for the general dialysis population of $30 \pm 4\%$ (regardless of rHuEpo treatment), similar to that found in other dialysis populations [2].

Although not statistically significant, the different distribution of underlying disease (polycystic kidney and diabetes mellitus) possibly favouring the conventional treatment should be also taken into consideration.

Kobayashi *et al.* [15] reported positive results using the same membrane, but this study has some major drawbacks: the sample size was very small (eight patients) and the eligibility criteria unclear; the study was not randomized and there was no control group; no information was given concerning iron or vitamin status of the patients; the effect was particularly positive in patients starting high-flux treatment with very low haematocrit levels that only reached 25% at the end of 11 months of observation. Villaverde *et al.* [12] found that the switch from cellulosic to high-flux polysulphone membrane, without any change in the dialysis dose, improved the response to rHuEpo by about 14% in 31 haemodialysis patients with a target haematocrit level of 35%. In our population as a whole, we found a nearly significant increase in haemoglobin values (possibly as a trial effect), without any changes in plasma erythropoietin levels or the dose of rHuEpo. This underlines the need for a control group in order to obtain an unbiased estimate of the treatment effect. It is worth noting that Kobayashi *et al.* themselves [15] point out that they are not suggesting that the use of this membrane can increase haematocrit levels indefinitely, but rather stabilize them at 25–30%.

In the study of Kawano *et al.* [17], only three of the 10 evaluated patients had baseline haematocrit values of 30% or more, and none of the patients experienced a more than 10% increase in their baseline haematocrit levels (but the rHuEpo dose was considerably reduced).

Another important factor is native plasma erythropoietin concentrations. Kobayashi [15] and Kawano [17] underlined the fact that high plasma erythropoietin concentrations overcame the inhibitory effect of BF4–0 on erythropoiesis, and we cannot exclude this possibility in our patients because the median baseline plasma erythropoietin concentrations were in the normal range: 12.1 mU/ml (interquartile range 5.4–19.4) and 13.2 (interquartile range 5.7–22.6) in the conventional and experimental groups respectively. The absence of a correlation between plasma erythropoietin and haematocrit levels is intriguing, and 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) play a role in erythropoiesis.

As expected, there was a significant reduction in serum β_2-M concentration only in the experimental group. Both the pre-and post-dialysis values confirmed the effectiveness of this high-flux membrane in reducing the plasma levels of possible toxins with a molecular weight in the range of that of β_2-M. It can thus be suggested that at this dialysis dose and haemoglobin levels the removal of toxins in this molecular weight range has no effect on anaemia in these very selected patients. The reported effect of high-flux treatment on improving survival, reducing the need for carpal-tunnel syndrome surgery and reducing mortality of haemodialysis patients [30,31] are beyond the aim of this study.

The possibility that metabolites and depressive cytokines may play a role in reducing erythropoietin synthesis cannot be ruled out. The quality of the dialysate in terms of chemical (trace elements), microbiological and pyrogenic contamination is also extremely important in causing or aggravating anaemia in haemodialysis patients; the enhanced production of cytokines should be taken into particular consideration. In any case, the water treatment and distribution, sterilizants, and controls were the same in the two groups, and the participating centres were asked to take great care to prevent clinical manifestations in the patients and misleading results for the study.

Another reason for the non-striking results of our study could be that the period of treatment was too short. However, four of six patients in the Kobayashi study [15] improved their baseline haematocrit levels after as little as 4 months (despite starting with much

lower haematocrit values), and Ifudu *et al.* [11] found an increase in haematocrit values as early as 6 weeks after the dialysis dose was increased (urea percentage reduction from 60 to 72%). Interestingly, the patients in the experimental group showed a tendency to increase haemoglobin level (although not statistical significant), possibly suggesting the need for a larger follow up.

The last important factor is the safety of the procedure. We did not find any difference between the control and experimental groups in terms of intradialytic tolerance. Hypotension, emesis, and fever were not a problem during follow-up, thus supporting the lack in this study of a significant effect of dialysate in producing some cytokines with an inhibitory effect on myelopoiesis. The fact that the isolated toxin possibly responsible for inhibiting erythropoiesis was in the high-molecular-weight range (10^6 Daltons) raises some concern. However, it has been reported [15] that this membrane has a broader molecular weight fractional curve designed to allow the removal of high-molecular-weight substances at a level that poses no danger (sieving coefficient for albumin is approximately 0.03). Looking at our results, plasma protein levels decreased only in the experimental group ($P=0.043$) (in which the baseline levels were higher than in the conventional group, $P=0.04$), and albumin levels were lower (although not significantly so) in patients treated with BK-F.

In conclusion, this study showed an improvement of anaemia (although not significant) in the population as a whole (thus underlining the importance of a control group to avoid biased results), but no greater effect in patients treated for 3 months with a high-flux biocompatible membrane than in those treated with standard membrane (although the possibility of too short a follow-up cannot be ruled out). The level of plasma erythropoietin was in the normal range at baseline and during the follow-up with no differences between the two groups. When patients are well selected, adequately dialysed, and suffer from no iron or vitamin depletion, the effect of a high-flux membrane is less than might be expected from the results of uncontrolled studies.

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Appendix

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Editor's note

Please see also Editorial Comment by K.U. Eckardt, pp. 1278–1280.