On-Line Haemodiafiltration versus Haemodialysis: Stable Haematocrit with Less Erythropoietin and Improvement of Other Relevant Blood Parameters

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Key Words

Haemodialysis · On-line haemodiafiltration · oHDF and high-flux HD, comparison · Anaemia · Erythropoietin · Haematocrit · Pentosidine · Phosphate

Abstract

Background: Controlled randomised studies to prove improved cardiovascular stability and improved anaemia management during on-line haemodiafiltration (oHDF) are scarce. **Methods:** 70 patients were treated with both haemodialysis (HD) and oHDF in a cross-over design during 2 × 24 weeks at a dialysis dose of $eKt/V \geq 1.2$. Patients randomised into group A started on HD and switched over to oHDF, whereas patients in group B began with oHDF and were treated with HD afterwards. Intradialytic morbid events (IME), such as symptomatic hypotension or muscle cramps, were noted in case of appearance. Blood parameters reflecting anaemic status, phosphate status, lipid metabolism, oxidative stress, and accumulation of advanced glycation end products were recorded either monthly or at the end of each study

phase. **Results:** The mean incidence of IME was 0.15 IME per treatment, and there was no statistical difference between oHDF and HD. A higher haematocrit (oHDF 31.5% vs. HD 30.5%, $p < 0.01$) at a lower erythropoietin dose (oHDF 4,913 vs. HD 5,492 IU/week, $p = 0.02$) was found during oHDF, when the sequence of HD and oHDF had not been taken into account. For the study groups, the results were less distinct: in group A, a higher haematocrit (HD 30.4% vs. oHDF 32.0%, $p < 0.01$) at a comparable erythropoietin dose (HD 5,421 vs. oHDF 5,187 IU/week, ns) was observed during oHDF, whereas in group B an identical haematocrit (oHDF 30.8% vs. HD 30.7%, ns) was achieved at a reduced erythropoietin dose (oHDF 4,622 vs. HD 5,568 IU/week, $p < 0.01$). During oHDF, lower levels of free and protein-bound pentosidine and of serum phosphate were found. **Conclusion:** In contrast to other studies, no benefit regarding cardiovascular stability for oHDF was found, but oHDF could well offer a potential benefit regarding anaemia correction, inflammation, oxidative stress, lipid profiles, and calcium-phosphate product.

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Introduction

In chronic kidney disease patients, haemodialysis (HD) does not provide sufficient clearance for the removal of middle and high molecular uraemic toxins such as β_2 -microglobulin; the diffusive transport becomes ineffective with increasing molecular weight of the solute. In comparison to standard HD, the combination of diffusion and convection by on-line haemodiafiltration (oHDF) is characterised by high elimination rates for the middle molecular weight solutes [1, 2]. This is suggested to be associated with the observed clinical benefit of oHDF [3–7]. Moreover, a better intradialytic cardiovascular stability was reported [8], but never in a controlled randomised trial. This was therefore the primary objective of this investigation.

Anaemia in HD patients is a complex syndrome, and many other factors besides relative or absolute erythropoietin deficiency may contribute to its pathogenesis. Microbiological and pyrogenic contamination of dialysate also plays a role in aggravating inflammation and thereby anaemia [9]. With double stage filtration, oHDF provides microbiologically safe substitution fluid by efficient retention of microorganisms, endotoxins and other cytokine-inducing substances compared to conventional HD carried out without ultrapure dialysate [10, 11]. Whereas in one study an increase of the haemoglobin levels was observed after the switch from HD (with cuprophane dialysers) to oHDF (with polysulfone and polyamide dialysers) [7], other studies could not confirm the positive impact of oHDF on anaemia [6, 12]. These results are conflicting, but the possibility that oHDF may achieve a better control in anaemia is intriguing. Therefore, the effect of oHDF on anaemia was investigated as a major secondary objective in a cross-over study with identical membranes, taking into consideration further factors potentially affecting erythropoiesis.

In HD with low-flux membranes the diffusive transport is nearly identical to the total transport, whereas in HD with high-flux membranes additionally convective transport takes place due to internal filtration over the membrane [13]. In oHDF, the convective transport is increased with the infusion rate of the on-line prepared substitution fluid [14]. Therefore, a study on the influence of convection during dialysis should include the comparison between HD with a low-flux membrane (representing predominantly diffusive solute transport) and oHDF with a high-flux membrane and a high substitution rate (representing a highly convective solute transport additionally to the diffusive solute transport). For this comparison the

membrane material should be identical to eliminate interfering effects. Compared to HD, the dialysis dose of oHDF is usually higher due to its additional convective clearance. Thus, the benefit of oHDF compared to HD reported in most of the studies might at least partly be a consequence of the higher dialysis dose of oHDF. To minimise such a bias, the aim was to keep treatment dose sufficiently high in both treatment modes ($\text{eKt/V} \geq 1.2$) and as close as possible to each other.

Subjects and Methods

Study Design and Patients

The study was designed as an open, randomised, prospective multi-centre trial with cross-over design. Seven dialysis units in Hungary participated in the study. Local legal and administrative regulations were followed, and approval of the respective Ethics Committees was obtained.

All adult patients with chronic kidney failure in the participating centres were asked to participate if no exclusion criteria (pregnancy, lactation, infectious diseases, simultaneous participation in another clinical trial) applied and they had been on HD treatment for at least 3 months. All patients gave their written informed consent. After a pre-phase of 3 months with low-flux HD using cuprophane membranes, all patients were randomised to group A or B. Randomisation was centrally performed by an independent institute. A random code was used, with a separate list for each study centre. Patients who dropped out of the study were not replaced.

Patients in group A started with HD using low-flux polysulfone membranes, patients in group B started with oHDF using high-flux polysulfone membranes. After 24 weeks, both groups switched over to the other treatment mode for further 24 weeks. Following the study protocol, the same minimum dialysis dose ($\text{eKt/V} \geq 1.2$) was required in each study phase; necessary adaptations, e.g. changing blood flow and/or size of the dialyser, were made during the first 3 weeks of each study phase, if patients were repeatedly below 1.2, they had to be withdrawn from the study.

Study Treatment

HD and oHDF were performed with 4008 HD machines from Fresenius Medical Care. In the pre-phase, low-flux cuprophane membranes (Focus 120 R, National Medical Care) were used, in the HD phase low-flux polysulfone dialysers (HPS series, Fresenius Medical Care), and in the oHDF phase high-flux polysulfone dialysers (Fresenius Medical Care). Dialysate filters (Diasafe®, Fresenius Medical Care) were used for both modes and exchanged on a regular basis as described by the manufacturer. The microbiological quality was monitored and documented according to Hungarian law on a quarterly basis with the requirements of CFU in the dialysate $<100/\text{ml}$. Specific data on the inflammatory response from 27 patients have been analysed in more depth and are published in a separate publication [15]. The set dialysate temperature at the HD machines was identical in HD and oHDF. The dialysate composition was identical in HD and oHDF; the calcium concentration in the dialysate was 1.75 mmol/l.

Study Parameters

Patient characteristics (such as age, gender, height, dry weight), their medical history and co-morbid diseases were recorded.

For each dialysis session the treatment parameters and the occurrence of intradialytic morbid events (IMEs) were noted. An IME (e.g. symptomatic hypotension, muscle cramps, dizziness, nausea, headache) was defined by a typical symptom, severe enough for the nurse to counteract. Thus, neither a single symptom (without necessity of a counter-measure), nor a therapeutic intervention (e.g. a transient reduction of the ultrafiltration rate to prevent the symptom) was counted as IME. Intradialytic hypotension was not defined in advance as a fixed decrease of the blood pressure in time, but assessed by the nurse.

The following parameters were monitored and documented monthly: treatment dose eKt/V , haematocrit, haemoglobin, serum ferritin, transferrin, C-reactive protein, serum calcium and serum phosphate, moreover weekly erythropoietin and iron dose. At the end of each study phase, the following blood parameters were measured mid-week, pre-dialysis: interleukin-6 (IL-6), myeloperoxidase (MPO), fibrinogen, albumin, lipoproteins, triglycerides, pentosidines (free and protein-bound), carboxy-methyl-lysine (CML), advanced oxidation protein products (AOPP), homocysteine, and parathyroid hormone (PTH). β_2 -Microglobulin (β_2m) was only determined in a smaller subgroup of patients.

IL-6 was determined by an immunoassay (Quantikine, R&D British Biotechnology Ltd, UK). High-performance liquid chromatography (HPLC) with fluorescence detection [16] has been utilised for the rapid determination of total homocysteine. AOPP were determined by a spectrophotometric assay. CML was quantified by an enzyme-linked immunosorbent assay (ELISA) using the CML-specific monoclonal antibody 4G9 (research assay provided by Roche Diagnostics GmbH, Penzberg, Germany) [17]. Free and protein-bound pentosidine were determined by an HPLC assay [18].

Statistical Evaluation

The primary outcome parameter was cardiovascular stability (a comparison of the number of treatments with IME during the different treatment modalities). Sample size estimation was based on results of Pizzarelli et al. [8] and used the statistical method of Hills and Armitage [19] for cross-over trials. A significance level of 5%, a power of 80%, and a drop-out rate of 30% per year was assumed. Based on these assumptions, a minimum sample size of 64 patients was fixed in the study protocol. The influence of HD and oHDF on anaemic status was a secondary parameter.

All data was entered into an electronic database; statistical analysis was performed using SPSS for Windows, version 10.0. Analyses were carried out by χ^2 test, Student's t-test for paired samples, Mann-Whitney test, and Wilcoxon signed rank test, where appropriate. Results are presented as mean $\pm$ SD.

According to a recently published paper, haematocrit values were, in addition to the planned evaluation of data, checked for seasonal variations and adjusted accordingly [20]. The seasonal variation for haematocrit is taken into account by the formula $HCT_{corrected} = HCT_{measured} - \pi/2 \Delta_{mean} \sin(\pi(4-m)/6)$, with m indicating the month (e.g. $m = 1$ for January, $m = 2$ for February). Seasonal variations in clinical and laboratory variables among HD patients were found, in some cases probably due to outdoor temperatures, but in most cases without apparent reason. The authors recommended considering these seasonal variations to avoid bias in the interpretation of clinical studies [20]. Pre-dialysis haemato-

crit values were found to be at minimum in January and at maximum in July. Due to the start of our study and enrolment of all patients in October, the first study phase was in the cold season, whereas the second phase of the study was in the warm season.

Results

Study Population

129 patients were enrolled and randomised into group A or B. In the first 3 weeks of each study phase, attempts were made to adjust treatment parameters to deliver a treatment dose $eKt/V \geq 1.2$. During these periods Kt/V was measured weekly, thereafter monthly. Patients with a repeatedly lower treatment dose were withdrawn from the trial. The withdrawn patients (39 in total, thereof 27 male) were considerably heavier than the patients with a sufficient dose (table 1). Largely due to the withdrawal of heavier male patients, the gender imbalance aggravated. For online HDF and HD a comparable percentage of patients were withdrawn. The overall mean blood flow rate during all treatments was 294 ml/min (table 2). However, those patients withdrawn from the study due to low dose during on-line HDF had a lower mean blood flow rate of 270 ml/min, increasing the difficulty to reach the goal of $eKt/V \geq 1.2$. The combination of both, heavy weight and limited blood flow, might have made it difficult to reach the goal of $eKt/V \geq 1.2$.

In addition to this, 20 patients dropped out during the study (mainly due to transplantation (11) and death (4)), with comparable drop-out rates between male and female patients. The total drop-out rate over 1 year has been 16%.

Only the patients who completed the study were taken into account for evaluation (cohort of study completers). The patient characteristics of these 70 patients are presented in table 3.

The majority of the patients suffered from hypertension (table 3). Antihypertensive medication was prescribed in 86% of the patients with 2.1 ± 1.4 different antihypertensives (range 0–6). Moreover, in 74% of the patients drugs having an indirect influence on blood pressure such as nitrates or diuretics were prescribed (number of different medications 1.7 ± 0.8 , range 0–4).

Differences of the presented patient characteristics between both study groups were not statistically significant. Only the imbalance of gender showed a trend: the percentage of male patients was lower in group B than in group A ($p = 0.081$).

Table 1. Data of the intent-to-treat cohort, the patients who were withdrawn or dropped out, and the cohort of study completers

a Distribution of male and female patients in the study groups

Patients	Group A		Group B	
	male/female	all	male/female	all
Intent-to-treat cohort	37/28	65	22/42	64
Withdrawn patients (eKt/V<1.2)	16/3	19	11/9	20
Drop-out patients (transplantation 11, death 4, other 5)	5/5	10	3/7	10
Cohort of study completers	16/20	36	8/26	34

b Body weight in withdrawn patients and in the cohort of study completers

Patients	Withdrawn patients (eKt/V<1.2)		Cohort of study completers (eKt/V ≥ 1.2)		p*
	n	body weight, kg	n	body weight, kg	
Male	27	76.6 ± 13.1	24	68.9 ± 10.5	0.030
Female	12	73.1 ± 13.4	46	62.1 ± 11.4	0.009
All	39	75.5 ± 13.1	70	64.4 ± 11.4	<0.001

* Mann-Whitney test.

Table 2. Measured treatment parameters for both study groups of the cohort of study completers (mean ± SD values)

	Group A			Group B		
	phase 1: HD	phase 2: oHDF	p	phase 1: oHDF	phase 2: HD	p
Session length, min	250 ± 19	251 ± 18	ns	244 ± 15	246 ± 16	ns
Blood flow rate, ml/min	306 ± 32	311 ± 31	ns	269 ± 37	289 ± 38	*
Treatment dose, eKt/V	1.33 ± 0.09	1.39 ± 0.12	*	1.42 ± 0.14	1.35 ± 0.12	*
Number of IMEs per treatment	0.16 ± 0.19	0.13 ± 0.20	ns	0.18 ± 0.20	0.15 ± 0.15	ns

IMEs = Intradialytic morbid events. Significance of the intra-individual comparison within the group by Wilcoxon signed rank test is indicated by ns for $p \geq 0.05$ and by * for $p < 0.01$. Between the groups no significant difference concerning treatment parameters was found.

Treatment

During the study, in total 10,144 treatments of 70 patients completing the study were documented. The mean volume of the on-line prepared substitution fluid during oHDF was 20.3 ± 3.0 litres. Details of the measured treatment parameters are listed in table 2.

No statistically significant difference in session length was observed for HD and oHDF. The mean blood flow rate of 294 ml/min was low for post-dilutional oHDF. According to protocol it was tried to minimise the difference in dialysis dose between HD and oHDF (e.g. by in-

crease of blood flow during HD), nevertheless, oHDF still presented with a significantly higher dialysis dose.

For the incidence of IMEs (the primary outcome parameter) no statistically significant difference between both treatment modalities was found: in pooled data 0.15 IME per session for HD and 0.16 IME per session for oHDF (see table 2 for the results per group).

Control of Anaemic Status

A higher haematocrit (oHDF $31.5 \pm 3.2\%$ vs. HD $30.5 \pm 2.9\%$, $p < 0.01$) at a lower erythropoietin dose (oHDF

Table 3. Baseline data in both study groups of the cohort of study completers

	All patients	Group A	Group B
<i>Age, gender, body weight</i>			
Number	70	36	34
Gender, % male	34.3	44.4	23.5
Age, years	62.3 ± 12.4	62.1 ± 14.6	62.6 ± 9.6
Body weight, kg	64.4 ± 11.4	67.0 ± 10.0	61.7 ± 12.2
<i>Underlying renal diseases, prevalence in %</i>			
Diabetic nephropathy	21.4	22.2	20.6
Glomerulonephritis	14.3	13.9	14.7
Pyelonephritis	14.3	13.9	14.7
Vascular nephropathy	14.3	11.1	17.6
Polycystic kidney disease	12.9	11.1	14.7
Analgetic nephropathy	5.7	5.6	5.9
Other, unknown	18.6	25.0	11.8
<i>Comorbid diseases, prevalence in %</i>			
Hypertension	85.7	91.7	79.4
Diabetes mellitus	24.3	25.0	23.5
<i>Residual renal function</i>			
Anuric patients, %*	37.1	41.7	32.4
Residual diuresis, ml/day**	740 ± 385	714 ± 269	763 ± 472

Patients with a diuresis ≤ 100 ml/day are defined as anuric (*), residual diuresis is calculated only for non-anuric patients (**).

Table 4. Parameters concerning anaemia management in the pre-phase and the study phases (mean ± SD values)

	Group A				Group B			
	pre-phase	phase 1 HD	phase 2 oHDF	p HD vs. oHDF	pre-phase	phase 1 oHDF	phase 2 HD	p HD vs. oHDF
Intravenous iron dose, mg/week	46 ± 57	72 ± 53	53 ± 38 ^s	ns	48 ± 61	77 ± 52 ^s	56 ± 37	**
Erythropoietin dose, IU/week	5,059 ± 2,335	5,421 ± 2,152	5,187 ± 2,318	ns	5,601 ± 2,207	4,622 ± 1,462	5,568 ± 2,264	**
Ferritin, ng/ml	211 ± 186	241 ± 189	170 ± 183 ^{ss}	**	197 ± 146	250 ± 154 ^{ss}	211 ± 227	**
Transferrin, mg/dl	196 ± 60	159 ± 63	158 ± 803	ns	194 ± 50	168 ± 60	167 ± 59	ns
Transferrin saturation, %	26.9 ± 10.0	27.4 ± 13.3	26.9 ± 14.3	ns	28.9 ± 15.1	26.8 ± 14.6	29.9 ± 14.7	ns
Haemoglobin, g/dl	9.9 ± 1.2	10.2 ± 1.0	10.7 ± 1.0	**	9.8 ± 1.0	10.2 ± 1.2	10.1 ± 1.1	ns
Haematocrit measured value, %	30.2 ± 3.6	30.4 ± 2.7	32.0 ± 3.3	**	29.9 ± 2.9	30.8 ± 3.0	30.7 ± 3.1	ns
Haematocrit corrected for seasonal variation, %	30.1 ± 3.6	30.8 ± 2.7	31.6 ± 3.2	*	29.8 ± 2.9	31.2 ± 2.9	30.3 ± 3.1	**
Epo resistance index, IU/week/kg/g/dl	8.7 ± 5.0	8.1 ± 3.8	7.3 ± 5.1	ns	8.5 ± 3.9	7.6 ± 4.1	9.3 ± 5.8	*

Epo resistance index was calculated as weekly erythropoietin dose per kilogram body weight divided by haemoglobin. Significance of the intra-individual comparison between the study phases by Wilcoxon signed rank test is indicated by ns for $p \geq 0.05$, by * for $p < 0.05$ and by ** for $p < 0.01$. For the oHDF treatment phases a comparison between groups was made for intravenous iron dosage and ferritin: Significance of this comparison by Mann-Whitney test is indicated by ^s for $p < 0.05$ and by ^{ss} for $p < 0.01$.

4,913 ± 1,957 vs. HD 5,492 ± 2,192 IU/week, $p = 0.02$) was found during oHDF compared to HD, when the sequence of HD and oHDF was not taken into account (pooled data). In the study groups, the results were less distinct.

Parameters reflecting the anaemic status per group of patients are presented in table 4, with the baseline values (pre-phase) and the mean values over both study phases. Most patients obtained iron intravenously (A: 94%, B: 88%). The mean weekly iron dose was reduced in the sec-

Fig. 1. Time course of erythropoietin dose (■) and measured haemoglobin (○) (a in group A, b in group B). Each data point covers the mean value of 8 weeks. Significance of the intra-individual comparison by Wilcoxon signed rank test is indicated by ns for $p \geq 0.05$, and by * for $p < 0.05$.

ond study phase, independently of the treatment mode HD or oHDF (change of reimbursement policy in Hungary). Only a minority of patients was supplemented orally with iron (A: 8%, B: 12%, ns). Erythropoietin dose was less during oHDF in both groups, however not statistically significant in group A. In spite of this, a statistically significant increase in haematocrit and haemoglobin was found during oHDF, $\approx 5\%$ in group A ($p < 0.023$).

Details within the study phases concerning the delivered erythropoietin dose and the measured haemoglobin values are given in figure 1. The course of both parameters during the study phases are characterised by the displayed mean values which cover 8-week intervals within each study phase.

Serum ferritin levels were lower in the second than in the first study phase, independently of the treatment mode due to reduced iron supply. As a result, the erythropoietin resistance index (weekly erythropoietin dose per kilogram body weight divided by haemoglobin) was higher for HD than for oHDF only in group B (table 4).

Seasonal variations were found for the mean pre-dialysis haematocrit values, with an amplitude between minimum (in January) and maximum (in July) of 0.6% [20]. Seasonal variation analysis in our patients revealed a difference of $\Delta_{\text{mean}} = 0.67\%$, which was statistically significant ($p = 0.03$). The start of our study and the enrolment of all patients was in October, in the cold season, whereas the second phase of the study was in the warm season. The mean haematocrit of all patients irrespective

of group allocation was $30.7 \pm 2.8\%$ for weeks 1–24 and $31.3 \pm 3.2\%$ for weeks 25–48. The amplitude of this sine-shaped variation was calculated to be 1.05% ($\Delta_{\text{ampl}} = \pi/2 \Delta_{\text{mean}}$). Using the formula described in the Methods, the haematocrit without seasonal variation was calculated to be $30.8 \pm 2.7\%$ (for HD) vs. $31.6 \pm 3.2\%$ (for oHDF) in group A, and $31.2 \pm 2.9\%$ (for oHDF) vs. $30.3 \pm 3.1\%$ (for HD) in group B, with a significant higher level of haematocrit during oHDF (A: $p = 0.023$, B: $p = 0.006$) (table 4). Originally, the mean differences between HD and oHDF were not balanced in both groups for the measured haematocrit values: $\Delta\text{HCT}_{\text{meas}} = \text{HCT}_{\text{oHDF}} - \text{HCT}_{\text{HD}} = 1.6\%$ in group A, but only $\Delta\text{HCT}_{\text{meas}} = 0.1\%$ in group B. After correction for seasonal variation these mean differences between HD and oHDF became nearly identical: $\Delta\text{HCT}_{\text{corr}} = 0.89\%$ in A, and $\Delta\text{HCT}_{\text{corr}} = 0.91\%$ in B.

Blood Parameters

The mean values of the measured biochemical parameters of the blood are given in table 5. Whereas MPO increased and fibrinogen decreased significantly during the second treatment phase independently of the treatment mode, serum albumin presented with nearly identical values. In contrast, IL-6 decreased during oHDF in parallel with CRP and fibrinogen, but only in group A.

Whereas CML was not different between treatment modes or phases, free pentosidine and protein-bound pentosidine significantly decreased in both groups (see fig. 2) during oHDF; AOPP, however, only in group A.

Fig. 2. Plasma pentosidine (**a** in group A, **b** in group B): (I) free pentosidine (mean $\pm$ SD), and (II) protein-bound pentosidine (mean $\pm$ SD).

Table 5. Biochemical parameters in the pre-phase and the study phases measured once at the end of the phase ([#]) or monthly (^{##}) (mean $\pm$ SD values)

	Group A				Group B			
	pre-phase	phase 1 HD	phase 2 oHDF	p HD vs. oHDF	pre-phase	phase 1 oHDF	phase 2 HD	p HD vs. oHDF
IL-6, pg/ml [#]	7.7 $\pm$ 8.9	6.1 $\pm$ 5.6	4.0 $\pm$ 2.6	**	10.6 $\pm$ 13.0	6.7 $\pm$ 4.2	7.0 $\pm$ 8.5	ns
CRP, mg/l ^{##}	19.0 $\pm$ 19.8	19.6 $\pm$ 13.0	13.9 $\pm$ 15.3	**	27.9 $\pm$ 37.4	22.0 $\pm$ 17.2	19.0 $\pm$ 13.9	ns
MPO, μ mol/l [#]	2.84 $\pm$ 1.03	4.27 $\pm$ 1.13	5.09 $\pm$ 1.16	**	2.68 $\pm$ 0.83	4.04 $\pm$ 1.20	4.68 $\pm$ 1.24	*
Fb, g/l [#]	4.92 $\pm$ 1.13	3.42 $\pm$ 0.76	2.59 $\pm$ 0.75	**	5.30 $\pm$ 1.70	3.88 $\pm$ 1.11	2.86 $\pm$ 0.65	**
Alb, g/l [#]	38.6 $\pm$ 4.2	41.2 $\pm$ 4.2	41.1 $\pm$ 4.5	ns	39.7 $\pm$ 4.0	39.7 $\pm$ 4.3	39.9 $\pm$ 3.1	ns
HDL, mmol/l [#]	1.15 $\pm$ 0.43	1.00 $\pm$ 0.41	1.16 $\pm$ 0.54	*	1.23 $\pm$ 0.36	1.16 $\pm$ 0.38	1.08 $\pm$ 0.38	ns
LDL, mmol/l [#]	3.09 $\pm$ 0.76	3.39 $\pm$ 1.12	3.36 $\pm$ 1.32	ns	3.33 $\pm$ 1.31	3.68 $\pm$ 1.10	3.45 $\pm$ 1.68	ns
TG, mmol/l [#]	2.32 $\pm$ 1.27	2.13 $\pm$ 1.51	2.28 $\pm$ 1.55	ns	2.06 $\pm$ 1.61	1.85 $\pm$ 1.67	2.18 $\pm$ 2.20	*
CML, ng/ml [#]	539 $\pm$ 210	510 $\pm$ 224	463 $\pm$ 235	ns	504 $\pm$ 191	441 $\pm$ 210	436 $\pm$ 186	ns
f-Pe, pmol/ml [#]	57.4 $\pm$ 31.0	57.4 $\pm$ 32.2	38.5 $\pm$ 52.7	**	45.8 $\pm$ 28.5	34.0 $\pm$ 9.6	49.2 $\pm$ 5.9	**
pb-Pe, μ mol/mg [#]	26.0 $\pm$ 14.3	27.0 $\pm$ 12.6	21.3 $\pm$ 13.5	**	22.3 $\pm$ 10.5	18.5 $\pm$ 9.8	24.3 $\pm$ 11.7	**
AOPP, μ mol/l [#]	154 $\pm$ 54	165 $\pm$ 82	133 $\pm$ 56	**	120 $\pm$ 53	150 $\pm$ 94	150 $\pm$ 92	ns
Hcy, μ mol/l [#]	37.8 $\pm$ 279	48.2 $\pm$ 55.3	40.2 $\pm$ 31.5	ns	29.8 $\pm$ 17.3	32.3 $\pm$ 20.9	28.2 $\pm$ 14.4	ns
PTH, pmol/l [#]	15.7 $\pm$ 18.8	10.7 $\pm$ 9.6	8.2 $\pm$ 9.4	**	14.4 $\pm$ 20.2	13.1 $\pm$ 14.4	8.9 $\pm$ 9.9	*
Ca, mmol/l ^{##}	2.36 $\pm$ 0.19	2.33 $\pm$ 0.16	2.34 $\pm$ 0.12	ns	2.37 $\pm$ 0.22	2.36 $\pm$ 0.12	2.35 $\pm$ 0.16	ns
Pi, mmol/l ^{##}	1.84 $\pm$ 0.76	1.63 $\pm$ 0.40	1.49 $\pm$ 0.46	*	1.75 $\pm$ 0.49	1.59 $\pm$ 0.47	1.85 $\pm$ 0.52	**
CaxP, mmol/l ^{2##}	4.45 $\pm$ 2.03	3.83 $\pm$ 1.03	3.52 $\pm$ 1.20	*	4.16 $\pm$ 1.12	3.77 $\pm$ 1.14	4.37 $\pm$ 1.39	**
β 2m, mg/l [#]	37.8 $\pm$ 14.8	29.0 $\pm$ 12.3	28.1 $\pm$ 11.3	ns	36.9 $\pm$ 17.6	23.7 $\pm$ 11.2	42.3 $\pm$ 15.5	**

The parameters are interleukin-6 (IL-6), C-reactive protein (CRP), myeloperoxidase (MPO), fibrinogen (Fb), albumin (Alb), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), carboxymethyllysine (CML), free pentosidine (f-Pe), protein-bound pentosidine (pb-Pe), advanced oxidation protein products (AOPP), homocysteine (Hcy), parathyroid hormone (PTH), calcium (Ca), phosphate (Pi), calcium phosphate product (CaxP), and β ₂-microglobulin (β 2m). Significance of the intra-individual comparison between the study phases by Wilcoxon signed rank test is indicated by ns for $p \geq 0.05$, by * for $p < 0.05$ and by ** for $p < 0.01$.

Fig. 3. Serum phosphate levels (**a** in group A, **b** in group B): mean $\pm$ SD.

As bone metabolism may also influence anaemia correction, these parameters were also investigated. Vitamin D (calcitriol) was supplemented in 19 patients (A: 8, B: 11), with a mean value of 0.8 ± 0.7 μ g/week. PTH was lower in the second study phase, independent from the treatment mode. Serum calcium level did not change, whereas serum phosphate was observed to be significantly lower during oHDF in both groups (A: -9%, B: -14%, see fig. 3), as was the calcium-phosphate product.

β 2m was measured only in a subgroup of patients: 21 out of 36 patients could be analysed in group A and 22 out of 34 patients in group B. In group A, β 2m was found to be only marginally lower for oHDF than for low-flux HD (median for HD and oHDF: 28.0 and 25.9 mg/l, respectively, for mean values see table 5). In group B, significantly lower values of β 2m were observed during oHDF than during low-flux HD (median for oHDF and HD: 28.2 and 41.2 mg/l, respectively, for mean values see table 5). The reason for the different effects in groups A and B remain unclear.

Discussion

129 patients started this prospective study, of which 70 patients completed the planned 24 weeks each on HD and oHDF in randomised order. 39 patients were withdrawn from the study due to the study-specific require-

ment of the minimum equilibrated dialysis dose eKt/V of at least 1.2, and another 20 patients dropped out due to common reasons such as transplantation and death. The required minimum dialysis dose turned out to aggravate an imbalance of gender between both groups. Even though there was no statistically significant difference between the two patient groups completing the study, with $p = 0.08$ a trend to fewer male patients in group B was observed. Other differences concerning patient characteristics such as the prevalence of hypertension, the number of anuric patients, and the mean residual renal function are also mentionable. Although not statistically significant, these differences between the two study groups may contribute to the different observations for some parameters.

An identical membrane material (polysulfone) was applied in both treatment modes, but low-flux dialysers were used during HD whereas high-flux dialysers were applied during oHDF. Although a direct comparison of oHDF and high-flux HD demonstrated an advantage of oHDF in small and middle molecular clearance [12], some of the benefit found in the oHDF phase could have been due to the use of high-flux dialysers, rather than to oHDF.

For many investigated parameters the results were comparable for oHDF and for HD, and for many parameters better results were found for oHDF than for HD; no result in favour of HD was observed.

During oHDF we observed an improved anaemia control, either with increase of haematocrit at the same erythropoietin dose (group A), or with comparable haematocrit at reduced erythropoietin dose (group B).

As availability of erythropoietin was limited the mean erythropoietin dose was not prescribed according to an optimal target level. In addition, iron reimbursement changed during the second half of the study and patients had to substantially contribute to the payment, so that the overall dose decreased. As a consequence, most haematocrit and haemoglobin values were below targets (33% and 11 g/dl, respectively) recommended in guidelines on anaemia management [21]. The intravenous iron dose was in the lower half of the recommended range [21], even more so in the second study phase. In group A, less intravenous iron was supplied during oHDF than during HD (-26%, $p < 0.01$), at the same erythropoietin dose (-4%, ns), nevertheless, a higher haematocrit was observed (5%, $p < 0.01$). In group B, during oHDF more intravenous iron was supplied (27%, $p < 0.01$) but less erythropoietin (-20%, $p < 0.01$) and the haematocrit was found to be 1% higher (ns). Whether this nearly identical value for hae-

matocrit at a lower erythropoietin dosage during oHDF in group B is the consequence of a higher intravenous iron dose, or the consequence of the oHDF treatment mode, remains open. The increased erythropoietin resistance for female patients compared to male patients is most pronounced in young patients and decreases with age [22]. For our intra-individual comparison, this gender-associated difference between male and female patients is less important. We found a lower erythropoietin resistance index for online HDF compared to HD in both study groups, but statistically significant only in group B with a predominantly female population.

Seasonal variations in clinical and laboratory variables among 1,445 chronic HD patients were found, in some cases probably due to outdoor temperatures, but in most cases without apparent reason. The authors recommended to consider these seasonal variations to avoid bias in the interpretation of clinical studies [20]. Due to the start of our study and enrolment of all patients in October, the first study phase was in the cold season, whereas the second phase of the study was in the warm season. Another recent study among 34 HD patients reported of seasonal variations of several blood parameters, but did not find an impact on haemoglobin levels [23]. The amplitude of the seasonal variation of haematocrit was with a value of 1.05% even more pronounced in the Hungarian study population than the value of 0.6% found in the American population. An explanation for the weaker effect observed in US patients might be the heterogeneous climate in the area of the participating dialysis centres in between 30' and 50' latitude in the northern hemisphere, and from west to east coast of the United States. In contrast to this, less distinct climatic differences are expected between the various locations in Hungary. In our patient population the positive impact of oHDF in the treatment of anaemia is statistically significant in both study groups, if the effect of seasonal variation is taken into account (table 4). The neglected superposition of the seasonal variation may have covered the underlying increase of haematocrit due to oHDF in other studies.

The role of ultrapure water for dialysis and thereby less inflammatory stimulus with the consequence of less advanced glycation end product (AGE) formation and less oxidation was considered to be an important aspect of better results with oHDF treatment in other investigations [24–26]. In our study a dialysate filter (Diasafe®) was used for low-flux dialysis HD as well as for oHDF. The markers for inflammation such as CRP and IL-6 were lower in group A after oHDF, whereas there was no difference in group B. A detailed analysis covering this item

in a subpopulation of this study (27 patients) did not show any difference in plasma cytokine levels between the different treatment modalities, the substitution fluid did not provoke an additional inflammatory stimulus [15]. The difference in erythropoietic response cannot be explained by a difference based on water or dialysate quality, but may be associated to increased convective flux, possibly supporting recently published mechanisms of anaemia improvement by elimination of phosphatidylserine [27].

AOPP, formed during oxidative stress [26, 28], and AGEs were effectively reduced during oHDF not only in the free form of pentosidine, but also in its protein-bound form, accounting for more than 90% of its total amount. This suggests that indeed less oxidation was responsible for the lowered protein-bound fraction. Other studies have shown that by convective transport such as high-flux dialysis [18, 29], several of these AGE compounds may be markedly reduced. However, in other investigations a constant lowering of pre-dialysis levels with oHDF or HF was only found when ultrapure water was used [24–26].

Recently a correlation was found between AGEs, AOPP and triglycerides (e.g. $r = 0.68$ for linear correlation between triglycerides and AGEs) [30]. During oHDF we observed a 15% lower mean value of triglycerides in one group and a 16% increase of HDL in the other group, whereas LDL did not change significantly.

Increase of MPO and decrease of fibrinogen did not change treatment-dependent, just phase- or season-dependent. Albumin remained unchanged. There is no obvious explanation for these findings, and possibly the sample size and the length of the investigation do not allow a meaningful interpretation.

Hyperphosphataemia is a major determinant of morbidity and mortality in HD patients [31]. It was recently reported that even a mild hyperphosphataemia (>1.61 mmol/l) might be independently associated with an increased risk for death in HD patients [32]. Dialysis centres with increased frequency of dialysis treatment or prolonged duration of the treatment report successful prevention of hyperphosphataemia; for oHDF also increased removal compared to HD has been demonstrated [33, 34]. In our study a constant lowering of the calcium-phosphate product was found only due to the lowering of phosphate. With a mean value of 1.80 mmol/l in the pre-phase our patients had a mild hyperphosphataemia, and mean phosphate values were 1.49 mmol/l (vs. 1.62) for group A and 1.57 mmol/l (vs. 1.85) for group B during oHDF. In this study convective solute transport in oHDF improved the elimination of phosphate such that a constant

lowering of serum phosphate levels was possible, which has been not described before to our knowledge.

The incidence of IME was low in our patient population (mean 0.154 IME per session). After covering 10,144 treatments, we did not find a decrease of frequency of symptomatic hypotension during oHDF. The better intradialytic cardiovascular stability of oHDF compared to HD found by others [8] is probably due to cooling of the blood via enhanced thermal energy losses within the extracorporeal circuit in case of oHDF. A comparison of the effect of oHDF with HD and temperature-controlled HD on the haemodynamic stability of hypotension-prone patients showed no difference in the rate of hypotensive episodes, if the energy transfer rates from the dialysate to the blood were identical in oHDF and HD [35]. In our study the identical set temperature at the HD machines in oHDF and HD should result in a slightly lower temperature of the returning blood in the venous line during oHDF compared to HD. This cooling should result in a better cardiovascular stability. On the other hand, with an infusion of 20 litres of on-line prepared substitution fluid, a larger amount of bicarbonate is given to the patient during oHDF than during HD. Recently, an increase of hypotensive episodes has been demonstrated as a result of a transient mild metabolic alkalosis induced

by bicarbonate transferred from the dialysis fluid [36]. During oHDF the superposition of the effects of bicarbonate load (inducing peripheral vasodilation) and of cooling (inducing peripheral vasoconstriction) may have led to a cardiovascular stability which is not different from that observed during HD.

In summary, even though many unforeseen circumstances prevented an ideal course of the study, our results, in accordance with others [5, 7], demonstrate that on-line HDF is of clinical and practical importance in the management of anaemia in HD patients, and may have a beneficial effect on inflammation, oxidative stress, lipid profiles, and calcium-phosphate product, i.e. cardiovascular risk profiles. Larger trials with longer treatment periods will help to answer remaining questions.

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