Online hemodiafiltration: treatment optimization and effects on biochemical parameters

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Online hemodiafiltration: treatment optimization and effects on biochemical parameters

Online hemodiafiltratie: optimalisatie van de behandeling en effecten op biochemische parameters
(met een samenvatting in het Nederlands)

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Contents

Preface

Online hemodiafiltration is a promising dialysis technique with superior removal of uremic toxins as compared to conventional hemodialysis. Whether hemodiafiltration leads to improved clinical outcome is currently under investigation in the Dutch CONvective TRAnsport STudy (CONTRAST). In Chapter 1.1 and 1.2 technical principles and current evidence on the effects of online hemodiafiltration are reviewed. In Chapter 1.3 the design of the CONTRAST study is described extensively. All data presented in this thesis originates from the CONTRAST study.

State of the art treatment with online hemodiafiltration requires high convective volumes and sustained high microbiological quality of dialysis solutions. To establish the highest possible convective volume, factors determining the convective volume in clinical practice should be identified, which was the aim of Chapter 2.1. The microbiological quality of dialysis solutions was evaluated in Chapter 2.2.

Effects of hemodiafiltration have mostly been evaluated in small and/or observational studies. Hence, results of these studies should be interpreted with caution. The CONTRAST study has provided the ideal opportunity to analyze effects of hemodiafiltration on biochemical parameters in a large and randomized clinical trial. In Chapter 3.2 and Chapter 3.3 the effects of six months treatment with online hemodiafiltration on β_2 microglobulin and phosphate removal were evaluated and compared with conventional hemodialysis. Residual kidney function appeared an important influencing factor of these parameters and was therefore evaluated in more detail in Chapter 3.1.

Chapter 4 comprises the overall discussion and perspectives of this thesis. Chapter 4.1 focuses on the optimal convective dose that should be delivered during hemodiafiltration and provides practical recommendations to optimize convective volumes in clinical practice. In Chapter 4.2 benefits and potential drawbacks of hemodiafiltration are discussed with special attention to the role of residual kidney function and the quality of dialysis solutions.

Chapter 1

Introduction

Chapter 1.1

Resolving controversies regarding hemodiafiltration versus hemodialysis: the Dutch CONvective TRAnsport STudy

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Abstract

Hemodialysis patients suffer from a high incidence of cardiovascular disease. Among the many predisposing factors, such as high blood pressure, dyslipidemia, and fluid overload, the accumulation of high molecular weight uremic toxins, the so-called middle molecules, may play an important role. Since convective therapies such as online hemodiafiltration have a better clearance profile for these compounds than standard hemodialysis, it has been suggested that these dialysis strategies may reduce cardiovascular morbidity and mortality. Since conclusive evidence on this topic is not yet available, the Dutch Convective Transport Study (CONTRAST) was recently initiated. This prospective randomized trial was designed to compare online hemodiafiltration with low-flux hemodialysis with respect to cardiovascular morbidity and mortality.

Chronic hemodialysis (HD) patients suffer from a high burden of cardiovascular disease, which is the major cause of death in this patient group. Even in the absence of clinically evident atherosclerotic disease, target organ damage is often present. Seventy-four percent of the patients starting dialysis show left ventricular hypertrophy on echocardiography [1]. Furthermore, increased arterial stiffness and carotid intima media thickness, both associated with cardiovascular disease and mortality, are common features in the majority of these individuals [2,3]. Traditional risk factors can only partially explain these observations. Therefore other factors, such as the retention of uremic toxins, increased oxidative stress, and microinflammation, have been implicated in the development of accelerated atherosclerosis and cardiovascular disease [4]. In addition, dialysis-related factors such as fluid overload, bioincompatibility of the dialysis membrane, and contaminated dialysate may play an important role. The retention of uremic toxins, especially so-called middle molecules (MMs; molecular weight [MW] greater than 500 Da), has been associated with the extremely high cardiovascular risk in chronic HD patients [5,6]. Since MMs are almost exclusively removed by convection, patients with end-stage renal disease (ESRD) are increasingly being treated with online hemodiafiltration (HDF). However, as yet, it is unknown whether improved correction of the uremic environment by online HDF ultimately leads to a better clinical outcome. Therefore the Dutch Convective Transport Study (CONTRAST) was designed to investigate the effect of increased convective transport by online HDF on all-cause and cardiovascular mortality in chronic HD patients. In the first part of this article we will discuss theoretical and technical principles of online HDF. Thereafter, data from clinical studies on the efficacy of online HDF will be reviewed. Finally, we will present a summary of the study design of CONTRAST.

Technical Differences

During low-flux HD, small MW substances are cleared by diffusion, driven by the concentration gradient between the blood and dialysate. In contrast, middle and large MW substances are largely cleared by convection, occurring passively with the flux of water through the dialyzer membrane. During low-flux HD, convective transport is minimal, since ultrafiltration is restricted to the required fluid removal and the membrane limits the sieving of larger solutes. In high-flux HD, solutes are cleared by both diffusion and convection. Total. ultrafiltration usually exceeds the required weight loss due to internal filtration; this increases convective transport up to 8 .10 L per session [7]. Lastly, removal of MMs occurs to a variable extent by adsorption onto the dialyzer membrane [8]. In HDF, the characteristics of diffusive and convective transport are combined. During this treatment modality,

convective transport is increased by excess ultrafiltration, whereas fluid balance is maintained by the infusion of a pyrogen-free substitution fluid. Dialysate is used to create a concentration gradient for solute removal by diffusion, as in standard HD. The substitution fluid can be administered before (pre-dilution) or after (post-dilution) filtration. However, post-dilution HDF is preferable when maximal clearances of small and larger solutes are targeted [9]. At the introduction of HDF in the late 1970s, the pharmaceutically prepared substitution fluid was supplied in bags. Due to the high costs and the laborious procedure, the infusion volumes were limited (limiting the efficiency of HDF); in fact, volumes were similar to those achieved by internal filtration in high-flux HD. In recent years, online preparation of infusate has become available for clinical practice, enabling the replacement of larger volumes of sterile substitution fluid, up to 20.60 L per treatment. As a result, convective transport increased considerably at reasonable costs [10]. Obviously the quality of the substitution fluid has to meet strict microbiologic and biochemical criteria. In clinical practice, the infusate for online HDF is prepared by a two-step (Fresenius ONLINEplus system) or threestep (Gambro ULTRA system) ultrafiltration process of ultrapure water and dialysate concentrates [10,11]. Bacterial contaminants and endotoxins are cleared by restricted passage through the filters, based on cumulative risk reduction. Furthermore, endotoxin fragments that are smaller than the cutoff point of the membrane are adsorbed on the inner surface of the dialyzer. In this way, the quality criteria for ultrapure water for dialysis are met so that long-term use of online HDF is safe and reliable [12,13].

Uremic Toxins

Many studies have addressed the effects of convective therapies on the removal of various MMs, such as β_2 microglobulin (β_2M) and leptin. Moreover, the removal of phosphate, which is a small molecule but behaves like a MM, has been the subject of research in several studies on convective transport. Finally, some small and intermediate MW substances, such as advanced glycation end products (AGEs), asymmetric dimethylarginine (ADMA), and homocysteine (Hcy) are of special interest because it has been suggested that various unspecified MMs, accumulating in patients with ESRD, play an important role in the metabolism of these molecules. As these substances have been implicated more and more in the pathophysiology of cardiovascular disease, lowering them by online HDF may contribute to a reduction in cardiovascular events in these patients. In this section we will discuss the above-mentioned uremic toxins more extensively. β_2M, having a molecular weight of 11.8 kDa, has been studied extensively because of its suspected role in the pathophysiology of dialysis-related amyloidosis and the relatively simple and cheap

method of determination. Low-flux membranes show virtually no removal, whereas β_2M is cleared to a variable extent during both high-flux HD and HDF [14]. In HDF, the reduction rate of β_2M is positively correlated with the total volume of infusate per session [15]. Therefore, high-volume HDF might be an appropriate modality to reduce predialysis β_2M levels in the long term. Indeed, after 8 months of treatment, predialysis β_2M levels were approximately 35% lower as compared to high-flux HD [15]. Leptin is another typical MM, and the concentrations of this substance are elevated to a variable extent in the majority of chronic HD patients [16]. As leptin plays a role in the regulation of food intake and energy expenditure, it has been speculated that retention of leptin is involved in the malnutrition that is frequently observed in these patients [17]. Considering the MW of leptin (16 kDa), it is to be expected that a long-lasting reduction can only be obtained by the use of highly permeable membranes. Indeed, in a recent prospective comparative study, predialysis leptin levels were lower after HD with polysulfone superflux membranes (ultrafiltration coefficient greater than 60 ml/hr/mmHg) than after HD with both low- and high-flux polysulfone membranes [18]. As mentioned above, phosphate is a small molecule, but phosphate removal follows kinetics resembling a middle rather than a small molecule. During online HDF, phosphate elimination was markedly increased compared to high-flux HD [19]. These findings were confirmed in a study that showed lower predialytic values of phosphate in patients after 3 months of treatment with HDF [20]. In non-renal patients, high levels of AGEs have been implicated in the pathophysiology of cardiovascular disease. However, although AGE levels are elevated in most patients with ESRD, their role as a risk factor for cardiovascular disease and mortality in this patient group has been challenged recently [21,22]. Nevertheless, several studies have shown that both high-flux membranes (reviewed in ref. [23]) and HDF treatment [24] lower AGEs more effectively than HD with conventional lowflux dialyzers. Predialysis AGE levels after 6 months of treatment were reduced only in the case of HDF, suggesting a significant influence of convective transport on these molecules. A large proportion of AGE peptides have a MW of less 6 kDa [53], which can, theoretically, be readily removed by high-flux HD. The observed difference between HDF and high-flux HD might be due to the removal of some larger MW uremic toxins promoting AGE formation. Another substance that has been implicated in the pathophysiology of cardiovascular disease in nonrenal patients is ADMA, an endogenous inhibitor of nitric oxide. In fact, in chronic HD patients, plasma ADMA levels were associated with left ventricular hypertrophy and left ventricular dysfunction and appeared to be a strong predictor of cardiovascular events and total mortality [25,26]. Interestingly, plasma ADMA levels, which are generally increased in these patients [27], declined intradialytically during both low-flux and high-flux HD, whereas an even greater reduction

was observed during online HDF [28]. However, currently it is unknown whether convective therapies, such as HDF and high-flux HD, lower ADMA levels more effectively than standard HD in the long term. Finally, plasma Hcy levels are generally higher in chronic HD patients than in healthy controls. Although Hcy levels can be lowered by vitamin supplementation, plasma concentrations usually remain elevated. As Hcy is a small molecule (MW 268 Da), it is not surprising that after a single HD treatment with both low- and highflux dialyzers, plasma Hcy levels were considerably reduced. However, whereas predialysis values remained stable after 3 months of treatment with both low- and high-flux dialyzers [29], Hcy levels were normalized in a substantial number of patients after treatment with superflux dialyzers [30]. This was explained by the accumulation of middle MW uremic toxins of unknown specificity involved in Hcy metabolism, which are cleared by convective transport [31]. Taken together, both theoretical considerations and clinical data indicate that several MMs implicated in the pathogenesis of cardiovascular disease are lower in patients who are treated with online HDF rather than with standard HD.

Inflammatory State

In chronic HD patients, a microinflammatory state is frequently observed. Similar to the nonrenal population, the presence of chronic (micro)inflammation has been associated with a poor cardiovascular outcome [32]. In patients with ESRD, microinflammation is probably multifactorial in origin. Besides the bioincompatibility of dialyzer membranes and bacterial contamination of the dialysate [33,34], the presence of an arteriovenous graft and intercurrent clinical events might play an important role [35]. During online HDF, only biocompatible high-flux membranes are used, whereas the dialysate complies with the most stringent criteria for bacteriological purity. Nevertheless, the administration of large quantities of online-produced substitution fluid may pose an extra risk for the patient by inducing monocyte activation and subsequent cytokine production. On the other hand, it has been suggested that enhanced MM removal by convective transport contributes to an improvement of the (micro)inflammatory state [36]. Since many proteins that are involved in inflammation have a MW of 10.50 kDa, dialysis modalities with a high convective potential might offer a benefit by removing these substances. For example, the serum level of tumor necrosis factor (TNF)-α (MW 17 kDa) increases during low-flux HD and decreases during high-flux HD and online HDF [36]. However, anti-inflammatory proteins will also be removed by convective therapies. The net effect of online HDF on the balance of pro- and anti-inflammatory factors remains to be established.

Hemodynamic Stability

Symptomatic hypotension is the most frequent intradialytic complication of HD. Several studies suggest that convective dialysis techniques provide better hemodynamic stability than diffusive techniques [37,38]. This effect does not seem to be mediated by differences in dialyzer material, type of dialysate, sodium balance, or rate of solute removal[39]. Furthermore, since both HDF and hemofiltration are associated with better hemodynamic stability, neither the presence nor the absence of dialysate seem to play a substantial role. It has been suggested that heat loss in the extracorporeal circuit during treatment with convective techniques results in peripheral vasoconstriction and better hemodynamic stability [40]. When the temperature of the dialysate in HD is adjusted to result in energy transfer rates identical to online HDF, the number of hypotensive episodes was similar during HD and HDF [41]. Better interdialytic blood pressure control has also been observed during long-term treatment with convective therapies. This suggests the multifactorial nature of hemodynamic control in the dialysis population [38]. Whether the observation can be explained by qualitative differences in solute removal is currently unclear.

Target Organ Damage

As mentioned before, target organ damage and atherosclerosis is present in the majority of chronic HD patients. A number of reports indicate that a more intensive dialysis strategy can ameliorate these derangements. Several observational studies have shown that left ventricular dimensions greatly improve after dialysis frequency increases, particularly with nocturnal HD [42,43]. Furthermore, aortic stiffness, as measured by pulse wave velocity, and carotid intima media thickness appeared to be partly reversible after renal transplantation [44,45]. Currently it is largely unknown whether these alterations depend mainly on an improved fluid status, better blood pressure control, or correction of the uremic environment. Whether convective therapies can reduce target organ damage in patients with ESRD is uncertain and can only be resolved in a large prospective study.

Clinical Outcome

Observational studies have associated high-flux HD with a lower morbidity and mortality as compared to low-flux HD, independent of biocompatibility [46,47]. Recently the results of the Hemodialysis (HEMO) study, the first prospective randomized study on flux and survival, were published [48]. This study, comprising 1846 chronic HD patients with a mean follow-up time of approximately 3 years, showed no difference in mortality between

patients treated with high-flux or low-flux membranes. However, a trend toward an improved cardiovascular morbidity in favor of high-flux HD was found. While the HEMO study findings suggest that a high convective transport does not result in a superior clinical outcome, it is conceivable that the difference in flux between the two dialysis strategies was not large enough to result in a difference in morbidity and mortality. Remarkably, in an in-depth analysis, the mortality risk among patients with more than 3.7 years of dialysis prior to randomization was lower in the high-flux group than in the low-flux group. However, a clear explanation for the improved outcome in this subgroup could not be established [49]. In the Dialysis Outcomes and Practice Patterns Study (DOPPS), an observational investigation on practice patterns, 4504 European patients were analyzed. Patients receiving HDF had a 23% lower risk of mortality than those receiving standard HD. However, when adjusted for Kt/V, the difference between the groups disappeared, which suggests that the mechanism for the improved mortality is the larger dialysis dose in HDF patients [50]. In another large observational study comparing convective with diffusive treatments, a nonsignificant trend toward better survival was observed [51]. Finally, relatively high survival rates were reported in patients who were treated with online HDF when compared with European Dialysis and Transplant Association (EDTA) registry data for standard HD [13]. However, both retrospective and prospective observational studies are subject to bias through a confounding (contra)indication mechanism: that is, the assignment to HDF is related to having either a high risk (indication) or having a low risk of future events (contraindication). As a consequence, HDF patients may not have a similar risk profile at the start of the study when compared to non-HDF patients. The number of randomized prospective trials comparing HDF with standard HD is limited. None of these trials was sufficiently powered for clinical endpoints such as cardiovascular morbidity and mortality or survival. One small prospective randomized study compared lowflux HD with online HDF in 44 patients and failed to show any effect of treatment on clinical parameters and survival [11]. Another prospective trial compared different membrane permeabilities and different ultrafiltration rates in 380 patients. The outcomes of 50 patients who were randomized to HDF were comparable to those for patients on synthetic low- or high-flux membranes in terms of morbidity and mortality [52]. Taken together, thus far no conclusive data are available on the effect of HDF on survival and morbidity in patients with ESRD.

Conclusion

Online HDF is obviously gaining in popularity and is increasingly being used as a first-choice mode of dialysis therapy. Recent technical advances have made it possible to replace

considerable amounts of fluid safely at a reasonable cost. Furthermore, both experimental and clinical studies have shown that correction of the uremic environment by online HDF is better than by standard HD. Whether the increased removal of uremic toxins results in an improved clinical outcome is presently unknown. As a result of these uncertainties, the Dutch study CONTRAST was initiated. In this study, approximately 800 incident and prevalent HD patients will be randomized to either low-flux HD or online HDF and followed for 3 years. The main hypothesis of the study is that online HDF reduces cardiovascular morbidity and mortality and improves survival compared to standard low-flux HD. Apart from the assessment of clinical endpoints, parameters of target organ damage (carotid intima media thickness, aortic pulse wave velocity, and left ventricular mass) and markers of inflammation, endothelial dysfunction, and oxidative stress will be investigated. The study will be conducted in more than 20 centers in the Netherlands, and the first patients were enrolled in the second quarter of 2004.

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Chapter 1.2

Hemodiafiltration, promise for the future?

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Introduction

During haemodiafiltration (HDF), diffusive and convective transport are combined for the removal of waste solutes. Fluid removal exceeds the desired weight loss, and fluid balance is maintained by infusion of a sterile pyrogen-free solution. This dialysis modality may offer advantages as compared to haemodialysis (HD) or haemofiltration (HF) used separately. This brief Editorial Comment summarizes presently available knowledge on technical and (pre-)clinical aspects of HDF, as well as currently ongoing trials.

Theoretical background

In HDF, not only small molecules (< 5 kDa) are removed more effectively as compared to low flux HD, but in addition, a considerable clearance of so called "middle molecular weight (MMW) substances" (5-50 kDa) is obtained [1]. β_2 microglobulin (β_2M, MW 11.8 kD) is a typical example of this category and strongly associated with the presence of the carpal tunnel syndrome and dialysis related amyloidosis in chronic HD patients. In the HEMO study (see for details below), predialysis β_2M levels were associated with all cause mortality, even when adjusted for residual renal clearance [2]. These data suggest that β_2M can be used as a marker for MMW toxins that contribute to the extremely high mortality in chronic HD patients, although a direct relationship between β_2M levels and mortality is lacking.
Other examples of the MMW molecules include markers of inflammation, such as IL-6, TNF-α and complement factor D, and other molecules that might be relevant in the pathogenesis of cardiovascular morbidity and mortality, such as advanced glycation end products (AGEs) and mediators of oxidative stress [3].
HD using high flux membranes can be considered as a form of HDF, because the pressure drop along the fibers induces filtration, that can be considerable (8-10 liters per treatment). The total amount of ultrafiltration exceeds the required weight loss and is compensated by backfiltration. However, the exact volume of filtration in high flux HD is unpredictable, unmeasurable and fluctuates per treatment.
In HDF, the volume of ultrafiltration can be larger (10-30 L per treatment in the post-dilution mode) and can be controlled. The substitution volume infused into the patient compensates for the total ultrafiltration volume (synonym: convection volume) minus the desired weight loss. It can be added downstream (post-dilution) or upstream (pre-dilution) from the dialyser. In the latter mode, less small molecular clearance is obtained for the same filtration rate, as diffusion is less effective as compared to the post-dilution mode. In pre-dilution HDF, the concentration gradient driving diffusion is reduced because of dilution, as substitution fluid is added ahead of the filter.

High flux HD and low efficiency HDF

As mentioned before, high flux HD can be considered as a form of "low efficiency HDF", because internal filtration induces convective clearance. Although a considerable amount of convective transport can be obtained by this modality, the HEMO study showed no difference in survival between low and high flux HD. However, significant risk reductions in death from cardiac causes and in the combined outcome of first hospitalization for cardiac causes or death from cardiac causes were observed [4]. In this prospective clinical trial, 1800 prevalent HD patients were randomized to either low flux or high flux membranes with a mean follow up of almost three years. One post-hoc sub-analysis of this study suggests a survival benefit of high flux membranes for patients who are on hemodialysis for more than 3.7 years [5]. In addition, another post-hoc subanalysis suggested a decreased risk of death from cerebrovascular disease (CBVD) for patients on high flux HD without baseline evidence of CBVD or with a duration of HD therapy longer than 3.7 years [6].

Very recently, the results of two other randomized clinical trials were presented. First, the European Membrane Permeability and ESRD Patient Outcome (MPO)-study, originally designed to study outcome in hypoalbuminaemic HD patients [7], showed a survival advantage in this group if they were treated with high flux membranes. However, the study was amended underway due to slow enrollment so that the study protocol was opened to normoalbuminaemic subjects as well. In the overall group (containing hypoalbuminaemic and normoalbuminaemic patients) no survival advantage for high flux could be observed. Diabetic patients showed a survival advantage for high flux HD, both for the overall and the hypoalbuminaemic group. Second, a post-hoc analysis of the 4D study, which was originally designed to analyze the effect of atorvastatin in diabetic chronic HD patients on the composite end point of cardiovascular mortality and morbidity, showed a superior survival in patients treated with high flux as compared to low flux membranes [8].

Thus, until now, high flux HD has not shown to reduce mortality in the general HD population, although selected subgroups, such as diabetic or hypoalbuminaemic patients, or patients who are on HD therapy for a long time, may benefit from high flux HD.

HDF used to be performed with commercially produced substitution fluid in 5 liter bags. The applicability of this treatment was limited due to its logistic complexity and high costs. As a consequence, only small convective volumes could be obtained (less than 15 L per session), so-called "low efficiency HDF" or "low volume HDF". These volumes are in the same range as those obtained with high flux HD. Furthermore, in the DOPPS cohort, for patients in the low efficiency HDF group, the overall mortality was 12.6 deaths per 100 patient years whereas in the high flux HD group, the overall mortality was 12.7 deaths per

100 patient years [9]. Therefore, no additional benefit was achieved from low efficiency HDF as compared to high flux HD both in terms of convective clearance and survival.
The development of on-line systems that prepare substitution fluid continuously, has made HDF easier and much less expensive [10-12]. The question whether on-line HDF (olHDF), in which much more convective volume can be obtained, will result in clinical and survival benefit will be addressed below.

Technical considerations

As a substantial amount of online produced substitution fluid is infused directly into the patient, assurance of its chemical quality and microbiological purity is mandatory. Ultrapure water is mixed with high-quality concentrate components for the production of ultrapure dialysis fluid, from which substitution fluid for olHDF is continuously obtained by an extra step of ultrafiltration. The water distribution system must be maintained in hygienic conditions, by guaranteeing a continuous water flow and periodical thermal or chemical disinfection to prevent the formation of a biofilm. Hence, the production process of substitution fluid includes strict periodic evaluation of its quality. These aspects are discussed elsewhere in more detail [10-12].
Apart from a more complex purification system for water and dialysis fluid, some specific requirements are needed to perform olHDF on a routine basis. High flux membranes are used and equipment able to deliver olHDF is needed. In order to obtain a high convection volume, the patient needs to have an adequate vascular access for achieving relatively high blood flows. Generally, the total ultrafiltration flow in the post-dilution mode can be maximally 25-30% of the blood flow. Thus, in order to obtain a convection volume of for instance 6 L/h (24 L during an average session), blood flow needs to be 300 to 400 mL/min.

Evaluation of olHDF as renal replacement therapy: effects on (pre-)clinical variables and survival

Preclinical variables

Kt/V_{urea} is a well established marker of dialysis adequacy and the removal of small molecular weight substances. It is used as a variable to compare different dialysis techniques, although the importance of a high Kt/V_{urea} has been challenged by the HEMO study [4]. Enhanced removal of those small molecules by post-dilution olHDF has been documented. However, as the removal of those substances is far more dependent on

diffusive than convective transport, the advantage of (post-dilution) olHDF over HD is only modest [13,14].

The removal of larger molecules accumulating in chronic HD patients depends almost exclusively on the permeability characteristics of the dialyser membrane and the convection volume. Therefore, these substances are removed by olHDF, in which high flux membranes are used, and not by conventional low flux HD. In pre-dilution olHDF, a higher ultrafiltration rate is needed as compared with post-dilution olHDF to obtain equal MMW clearance because of dilution ahead of the filter in pre-dilution olHDF.

Several observational and randomized studies have shown that predialysis levels of β_2M are reduced when patients are switched to olHDF [14-17]. As could be expected, clearance of larger molecules is related to the amount of convection volume: the "dosage". A clear relation was found between the convection volume and the β_2M clearance [15,16]. Therefore, different studies on HDF need to be compared with caution as convection volumes can vary enormously between clinical studies (Table 1).

Phosphate is a small molecule, however, because it is surrounded by water molecules, it has a clearance profile similar to that of MMW substances. Superior clearance of phosphate by HDF has been demonstrated in some studies [18,19], but not in all [17].

Given the importance of clearance of MMW substances, quantified for instance by β_2M clearance, the effects of the various treatment modalities may be compared in that respect and then appear to show substantial differences. Standard low flux HD and peritoneal dialysis do (virtually) not provide any clearance of β_2M. Measurable reductions can be obtained by HF and high flux HD, especially when long treatment times are applied, such as with nocturnal HD [2,20-23]. It is important to realize that as compared to high flux HD, online HDF gives the opportunity to improve clearance of MMW molecules within treatment times and frequencies that are presently considered as "conventional". When HDF is applied daily without inreasing total treatment time per week, even more removal of MMW solutes can be obtained [24].

Table 1. Selection of studies evaluating effect of HDF on various biochemical and clinical variables and on survival (NB some studies did not have the mentioned variable as a primary outcome).

Reference	Design	Number of patients	HDF: Target volume per session and method	Outcome
Phosphate				
Zehnder 1999 [19]	Cross-over	16	24 L post-dilution	HDF: increased phosphate clearance
Schiffl 2007 [41]	Cross-over	76	18-22.5 L, method NA	HDF: lower phosphate with same dose phosphate binders compared to hfHD
Wizemann 2000 [17]	Randomized	44	60 L mid-dilution	HDF vs. lfHD: no difference
Minutolo 2002 [18]	Randomized	12	6-12 L, post-dilution	HDF vs. lfHD: lower phosphate
β_2 microglobulin				
Lin 2001 [15]	Observational	58	20-22 L post-dilution	HDF: 35% decrease in predialysis β_2M
Lornoy 2000 [16]	Cross-over	8	9.6-24L post-dilution, 19.2 L pre-dilution	HDF vs. lfHD: higher reduction rate and clearance of β_2M
Ward 2000 [14]	Randomized	44	15.6-20.4 L post-dilution	HDF vs. hfHD: greater removal and clearance of β_2M; 15% decrease in predialysis β_2M, no difference
Wizemann 2000 [17]	Randomized	44	60 L mid-dilution	HDF vs. lfHD: 40% decrease in predialysis β_2M, significant difference
Hemodynamic stability				
Mion 1992 [30]	Cross over	8	18-20 L, method NA	HDF: improved cardiovascular stability
Movilli 1996 [31]	Cross over	6	15.8 L post-dilution	HDF: less hypotensive episodes
Donauer 2003 [29]	Cross over	11	12 L post-dilution	HDF: less hypotensive episodes
Schiffl 2007 [41]	Cross-over	76	18-22.5 L, method NA	HDF: less hypotensive episodes
Locatelli 1996 [42]	Randomized	380 (50 HDF)	8-12 L post-dilution	HDF vs. HD: no difference
Wizemann 2000 [17]	Randomized	44	60 L mid-dilution	HDF vs. lfHD: no difference
Anemia / erythropoietin resistance				
Bonforte 2002 [25]	Observational	32	19.5 L post-dilution	HDF: decrease erythropoietin resistance
Maduell 1999 [27]	Cross-over	37	4.1 and 22.5 L post-dilution	High efficiency HDF: decrease erythropoietin resistance
Lin 2002 [26]	Cross-over	92	20-22 L post-dilution	HDF: decrease erythropoietin resistance
Vaslaki 2006 [28]	Cross-over	129	20.2 L, method NA	HDF: decrease erythropoietin resistance
Schiffl 2007 [41]	Cross-over	76	18-22.5 L, method NA	HDF and hfHD: decrease erythropoietin resistance as compared to lfHD
Ward 2000 [14]	Randomized	44	15.6-20.4 L post-dilution	HDF vs. hfHD: no difference

Reference	Design	Number of patients	HDF: Target volume per session and method	Outcome
Wizemann 2000 [17]	Randomized	44	60 L mid-dilution	HDF vs. lfHD: no difference
Nutritional parameters				
Schiffl 2007 [41]	Cross-over	76	18-22.5 L, method NA	HDF and hfHD: improvement as compared to lfHD
Wizemann 2000 [17]	Randomized	44	60 L mid-dilution	HDF vs. lfHD: no difference
Locatelli 1996 [42]	Randomized	380 (50 HDF)	8-12 L post-dilution	HDF vs. HD: no difference
Quality of life				
Schiffl 2007 [41]	Cross-over	76	18-22.5 L, method NA	No difference (KDQ)
Ward 2000 [14]	Randomized	44	15.6-20.4 L post-dilution	No difference (KDQ)
Dialysis related amyloidosis				
Locatelli 1999 [43]	Observational	6444	NA	HDF: decreased need for carpal tunnel syndrome surgery
Nakai 2001 [44]	Observational/ retrospective	1196	Online and offline HDF, not specified	HDF: decreased incidence of dialysis related amyloidosis
Mortality				
Locatelli 1999 [7]	Observational	6444	NA	HDF and HF: decreased mortality (10%), not significant
Jirka 2006 [32]	Observational	2564	NA	HDF: Decreased mortality (37%)
Canaud 2006 [9]	Observational	2165	Low efficiency 5-14.9 L; high efficiency 15-24.9L	High efficiency HDF: Decreased mortality (35%)
Bosch 2006 [33]	Observational	183	NA	HDF: Decreased mortality (60%)
Locatelli 1996 [42]	Randomized	380 (50 HDF)	8-12 L post-dilution	HDF vs. HD: no difference
Wizemann 2000 [17]	Randomized	44	60 L mid-dilution	HDF vs. lfHD: no difference

HD=haemodialysis, HDF=haemodiafiltration, lfHD=low flux HD, hfHD=high flux HD, NA= not available, KDQ=Kidney Disease Questionnaire

Clinical variables

Because olHDF removes substances in a broader range of molecule sizes as compared to conventional low flux HD, it provides a therapy somewhat better mimicking the human kidney. Therefore, it might provide a real improvement on clinically meaningful variables. For example, several reports of non-randomized trials have come up with the interesting finding of decreased erythropoietin resistance in patients treated with HDF [25-28]. In this respect, the removal of poorly defined erythropoiesis inhibiting factors and the creation of a

more biocompatible environment with less inflammation, possibly also by the use of ultrapure dialysate, might play a role. However, two randomized trials were not able to confirm these data, although both studies were not specifically designed to answer this question [14,17].

A number of studies suggests that olHDF is associated with an improvement of hemodynamic stability and blood pressure control [29-31]. However, no difference in hemodynamic stability was demonstrated between olHDF and temperature-controlled HD. Thus, rather than from increasing MMW clearance, this beneficial effect seems to be mainly caused by cooling of the blood via enhanced thermal energy losses within the extracorporeal system in olHDF [29].

Beneficial effects of HDF on other clinical parameters, e.g. markers of nutritional status, quality of life and prevalence of dialysis related amyloidosis, have been reported in a number of studies (Table 1). Most of these studies were performed in a non-randomized, observational or cross-over design and/or in small patient groups. Adequately powered randomized trials on the effect of HDF on clinical parameters are currently lacking.

Survival

Several observational studies suggest a benefit of olHDF on survival (Table 1). The use of high efficiency HDF in the DOPPS cohort (convection volume of more than 15 L per session, which means olHDF in most cases for logistic reasons) was associated with a 35% reduction in mortality risk, even after correction for various confounding factors [9]. In contrast, low efficiency HDF (convection volume less than 15 L per session) was not associated with any significant reduction of risk. Another large observational study from Eastern Europe reported 37% mortality risk reduction in patients on olHDF [32]. In a smaller observational study from the USA, olHDF was associated with an almost 60% reduction of risk of mortality [33]. In a systematic review on the effect of HDF (low and high volume) on survival, including data from 336 patients in four randomized controlled trials, a significantly greater mortality risk in patients treated with HDF was found, as compared to HD. However, even the sum of the available trials was not adequately powered to detect superior survival, most trials had suboptimal methodological quality and were difficult to compare because of different study protocols [34-36].

Ongoing trials

Despite the observational data as mentioned above, the merits of olHDF still have to be determined in properly designed and adequately powered randomized controlled trials.

The Dutch Convective Transport Study (CONTRAST) is a prospective randomized trial on the effect of online HDF on all cause mortality and CV morbidity and mortality. 700 patients are enrolled in more than 23 centers and followed until the end of 2010. According to the main hypothesis, better clearance of MMW substances results in a better correction of the uremic environment, ultimately leading to a reduction in all cause mortality and CV morbidity and mortality. Patients on standard low flux HD with an adequate $spKt/V_{urea}$ are either switched to olHDF or standard HD is continued. For HDF, target substitution volume is 6 L/h in the post-dilution mode. Rationale and design of the study are presented elsewhere [37, 38].

In a French prospective randomized trial, HDF is compared to high flux HD in 600 patients older than 65 years who are followed for two years. Primary endpoint is intradialytic morbidity (hypotension and symptoms), whereas secondary endpoints are all cause and cardiovascular mortality and laboratory markers of lipid metabolism, oxidative stress and inflammation [39].

In an Italian study, almost 250 chronic HD patients will be randomized for either a convective therapy [pre-dilution HDF (25%) or HF (25%)], or lowflux HD (50%), with a follow up period of two years. Hemodynamic stability and blood pressure control are studied as primary outcomes, whereas secondary outcomes are morbidity and overall and cardiovascular mortality [40]. As follow up has already ended, results of this study are soon to be expected.

Conclusion

Presently, HDF with online production of substitution fluid is very well possible and can be performed safely on considerable scale in every day clinical practice. Some specific technical requirements are needed, including standard use of high flux membranes. Reverse osmosis and dialysis machines have to be able to produce sterile and non-pyrogenic fluids, of which the microbiological and chemical quality are validated and controlled periodically. The patient needs to have a vascular access able to deliver a sufficient blood flow through the extracorporeal circuit.

OlHDF provides a measurable reduction in various substances suspected to be clinically relevant "uremic" toxins, which are not cleared by standard low flux HD. Observational studies suggest a dosage related substantial improvement in clinical parameters and reduction of mortality. However, observational studies are sensitive to bias. Therefore, properly designed randomized controlled trials need to establish whether olHDF reduces

cardiovascular morbidity and all cause mortality. Only after completing such studies, the question raised in the title might be answered.

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Chapter 1.3

Effect of increased convective clearance by on-line hemodiafiltration on all-cause and cardiovascular mortality in chronic hemodialysis patients - the Dutch CONvective TRAnsport STudy (CONTRAST): rationale and design of a randomised controlled trial

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Abstract

Background

The high incidence of cardiovascular disease in patients with end stage renal disease (ESRD) is related to the accumulation of uremic toxins in the middle and large-middle molecular weight range. As online hemodiafiltration (HDF) removes these molecules more effectively than standard hemodialysis (HD), it has been suggested that online HDF improves survival and cardiovascular outcome. Thus far, no conclusive data of HDF on target organ damage and cardiovascular morbidity and mortality are available. Therefore, the CONvective TRAnsport STudy (CONTRAST) has been initiated.

Methods

CONTRAST is a Dutch multi-center randomised controlled trial. In this trial, approximately 800 chronic hemodialysis patients will be randomised between online HDF and low-flux HD, and followed for three years. The primary endpoint is all cause mortality. The main secondary outcome variables are fatal and non-fatal cardiovascular events.

Conclusion

The study is designed to provide conclusive evidence whether online HDF leads to a lower mortality and less cardiovascular events as compared to standard HD.

Background and rationale

Atherosclerotic cardiovascular disease (CVD) is common among hemodialysis (HD) patients. In fact, approximately 50% of the deaths is attributed to cardiovascular causes, which is much higher than in the general population [1]. In addition, chronic HD patients suffer from atherosclerotic complications at a relatively younger age and die younger from ischemic heart disease [2]. The origin of CVD in chronic HD patients is most probably multi-factorial, as the extremely high prevalence in this patient group is not easily explained by traditional risk factors, either alone or in combination [3]. In recent years, other contributing factors have emerged, including the accumulation of uremic toxins, disturbances in the immuno-inflammatory system, as reflected by a chronic micro-inflammatory state, increased oxidative stress, and endothelial dysfunction [4-6]. In particular the retention of larger uremic toxins, the so-called middle molecules (MM, molecular weight [MW] 0.5 - 50 kDa), may play an important role in the pathogenesis of CVD [7,8]. Therefore, it is conceivable that dialysis modalities with superior MM removal reduce CVD and improve survival.

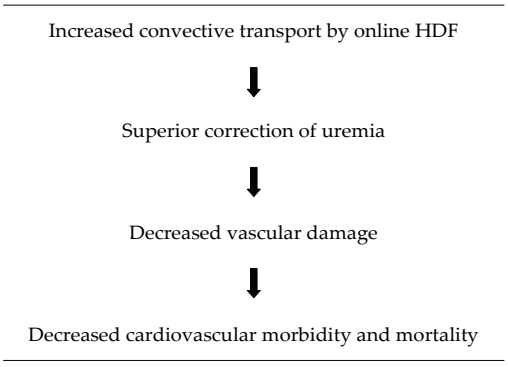
In contrast to diffusive dialysis strategies, which mainly remove small MW solutes, such as urea and creatinine, convective dialysis strategies are particularly effective in the removal of larger molecules. In hemodiafiltration (HDF), diffusive and convective transport are combined, providing an optimal removal of both small and larger MW substances up to the range of 30 - 40 kDa. Clinical studies have shown that β_2 microglobulin (β_2M), which is a typical MM with a MW of 11.8 kDa and therefore incapable of passage through the membrane of low flux devices, is effectively removed during HDF leading to lower pre-dialysis levels in the long term [9,10]. Similarly, the removal of other MM such as advanced glycation end-products (AGEs), leptin, and complement factor D is enhanced by convective transport [11-13]. Apart from the increased MM clearance, it has been suggested that HDF improves the removal of smaller molecules that are highly protein bound, due to a better elimination of the unbound fraction [14]. With respect to homocysteine, which is >90% protein bound, the observed decrease may also be explained by an improved removal of uremic substances with inhibitory effects on its metabolism [15]. Finally, it has been shown that treatment with online HDF leads to lower plasma phosphate concentrations, as compared to standard HD [16,17].

At present, it is unclear whether HDF has a favourable effect on the micro-inflammatory state in dialysis patients. Although a reduction of pro-inflammatory proteins has been shown during HDF [18], anti-inflammatory cytokines may also be removed. Of note, besides solute removal, other factors may influence the inflammatory-state as well, such as the bio-incompatibility of the dialyser membrane and the microbiological quality of the

dialysate [19,20]. Considering oxidative stress and endothelial dysfunction, data on the effects of HDF on these parameters are limited.

In summary, compared to standard HD, HDF improves the uremic state by an increased clearance of MM and other, mainly protein bound, uremic toxins. Circumstantial evidence implies that these effects result in less vascular damage and ultimately in decreased cardiovascular morbidity and mortality (Figure 1). Although observational studies suggest that online HDF improves cardiovascular outcome in chronic HD patients [21,22], two small randomised studies failed to show any differences between online HDF and standard HD [23,24]. However, the latter analysis lacked adequate power to detect differences in clinical endpoints.

Figure 1. Hypothesis.



Based on the above-mentioned theoretical considerations, the scarcity of reliable clinical data, and the growing interest in convective techniques under nephrologists, the CONvective TRAnsport STudy (CONTRAST) has been initiated. CONTRAST is a randomised controlled trial investigating the effects of online HDF on clinical endpoints, compared to low-flux HD. If online HDF indeed leads to an improvement in CV morbidity and mortality, this finding will imply a breakthrough in the treatment of chronic HD patients.

Methods

Objectives

The primary objective of CONTRAST is to assess the effect of on-line HDF on all cause mortality, when compared to standard low-flux HD. The main secondary outcomes are fatal and non-fatal cardiovascular events. Other secondary outcome measures include differences

between treatment regimens on the progression of left ventricular mass index (LVMi), as assessed by echocardiography, the progression of atherosclerosis as assessed by measurement of carotid intima-media thickness (CIMT) and the progression of arterial stiffness, as assessed by measurement of aortic pulse wave velocity (PWV). Furthermore, several laboratory markers of endothelial function, inflammatory state, and oxidative stress will be assessed over time and compared between the two treatment groups. In addition, subjective global assessment (SGA) is performed in the study patients as a measure of nutritional state, and a questionnaire is used to investigate the effects of on-line HDF on quality of life.

Study design

In this randomised controlled trial, participants are randomised centrally into a 1:1 ratio for treatment with online HDF or treatment with low-flux HD. Randomization is stratified by the participating centres and occurs in blocks. The follow-up period is three years. At present, 24 dialysis centres have agreed to recruit the required number of patients. The study is conducted according to good clinical practice (GCP) guidelines.

Patients

The in- and exclusion criteria are given in Table 1. Since the study results may be of importance for chronic HD patients of all ages, no upper age limit has been set. Severe incompliance is defined as non-adherence to the dialysis prescription, especially the frequency and duration of dialysis treatment. Permission for participation in other (e.g. observational) studies will be discussed with and decided by the executive committee.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria
patients treated by HD 2 or 3 times a week, for at least 2 months
patients able to understand the study procedures
patients willing to provide written informed consent
Exclusion criteria
current age < 18 years
treatment by HDF or high flux HD in the preceding 6 months
severe incompliance
life expectancy < 3 months due to non renal disease
participation to other clinical intervention trials evaluating cardiovascular outcome

Stabilisation period

Before randomization, patients will be dialysed 3 times (or 2 times) per week with low-flux synthetic dialysers (UF-coefficient < 20 ml/mmHg/h) for at least 6 months in case of a prevalent dialysis patient and at least 2 months in case of a new dialysis patient.

Blood flow will be maintained at 250-400 ml/min. Anticoagulation is performed with low molecular weight heparin (LMWH) before HD. Patients on coumarins receive 50% of the LMWH dose. Treatment times will be adapted to a target dialysis spKt/V urea of ≥ 1.2 per treatment. Ultra pure water is used for preparation of dialysis fluid. Bicarbonate is provided from powder cartridges to avoid the risk of a bacterial load from bicarbonate concentrates. For instance, the biBAGR system (Fresenius) and BiCartR system (Gambro) will be used. The dialysate flow is 500 ml/min and the temperature of the dialysate is 36oC.

Routine patient care

Metabolic control will be performed according to the guidelines of the Quality of Care Committee of the Dutch Federation of Nephrology. Anti-hypertensive medication, lipid lowering therapy, platelet aggregation inhibitors and medication to treat renal anemia and renal osteodystrophy will also be prescribed according to these guidelines, and, if not available, according to usual care.

Randomization

The patients will be randomised as soon as they are considered to be stable. When a patient has been randomised for low-flux HD, the treatment as performed in the stabilisation period will be continued. Treatment times will be adjusted only if dialysis spKt/V urea < 1.2 per treatment or if ultrafiltration goals can not be achieved, according to the attending nephrologist. When randomised for online HDF, patients will be treated with a target post-dilution dose of 6 l/h (~100 ml/min) and a high-flux synthetic dialyser (UF-coefficient > 20 ml/mmHg/h). Blood flow will be set at >300 ml/min, if possible, in order to achieve a substitution volume of 100 ml/min. If the blood flow is less than 300 ml/min, the post-dilution volume will be decreased accordingly (filtration and post-dilution <25-33% of blood flow). If necessary, the dose of LMWH will be increased and given in two separate doses. Treatment times will be fixed according to the prescription in the stabilisation period and adjusted only when spKt/V urea is < 1.2 / treatment. Metabolic control and medication is similar to the low-flux group, as described above.

Dialyser membranes

Dialysers with comparable biocompatible membrane material and surface area will be used in both treatment groups, to ascertain that differences in clearance result from differences in convective transport, rather than differences in dialyser characteristics. Only if the target dose of 6 l/h post-dilution is not achieved in the online HDF patients, it is allowed to prescribe a membrane with a larger surface area. The membranes advised by the study group are summarised in table 2. As many low-flux membranes with a membrane surface > 1.5 m^2 have a UF coefficient 10-20 ml/mmHg/h, in this study low-flux is defined as a UF coefficient of < 20 ml/mmHg/h.

Table 2. Dialyser characteristics for both treatment arms.

	Low-flux HD		**Online HDF**	
Company	Gambro	Fresenius	Gambro	Fresenius
Dialyser	Polyflux 17L	F8HPS	Polyflux 170H	FX80
Membrane material	polyamide	polysulfone	polyamide	polysulfone (helixone)
Sterilisation method	heat	heat	heat	heat
Surface area (m^2)	1.7	1.8	1.7	1.8
Membrane thickness (μm)	50	40	50	35
UF coefficient (ml/mmHg/h)	13	18	65	59
In vitro clearance:#				
Urea	260	251	268	276
Phosphate	198	193	229	239
Vit B12	111	118	158	175

(Qb=300 ml/min, Qd=500 ml/min)

Online HDF technique

During hemodiafiltration, the removal of larger solutes is increased by excess ultrafiltration, leading to solute removal by convection. As fluid removal exceeds the desired weight loss of the patient, fluid balance is maintained by the infusion of a pyrogen-free substitution solution. In addition, dialysate is used to create a concentration gradient for solute removal by diffusion, as in standard HD. At the introduction of HDF more than 20 years ago, the substitution fluid was supplied in bags. The infusion volumes were limited due to the high costs and laborious procedure, limiting the efficiency of HDF.

In recent years, however, technical advances have made it possible to prepare the substitution solution online from ultra pure water and dialysate concentrates. As a result, the volume of the substitution fluid could be increased considerably, without the disadvantages of inconvenient bags. Hence, the UF rate can be increased up to 50L per treatment in the pre-dilution mode and 25L in the post-dilution mode [25].

Online dialysate and substitution fluid preparation

Ultra-pure water is used for the preparation of bicarbonate-containing dialysis fluid, which undergoes one step of ultrafiltration converting it into ultra pure dialysis fluid. Dialysis fluid is produced at a rate of 600-800 ml/min of which approximately 100 ml/min is diverted for further processing into substitution fluid. The electrolyte composition of the dialysis fluid is: Na+ 138-140 mmol/l; K+ 1.0-3.0 mmol/l; HCO3- 30-35 mmol/l; Ca++ 1.0-1.7 mmol/l; Mg++ 0.5 mmol/l; Cl- 108-109.5 mmol/l; glucose 0-5.6 mmol/l; acetate 3 mmol/l.

The substitution fluid is prepared from the dialysis fluid by one additional step of controlled ultrafiltration, before it is infused post-filter into the blood. The electrolyte composition of the substitution fluid is the same as the composition of the dialysis fluid. Ultrafiltration procedures will be performed according to the manufacturers' instructions, as described below.

- The on-line system, ONLINEplus™ (Fresenius Medical Care, Bad Homburg, Germany) is integrated into the dialysis machine (4008 series; Fresenius Medical Care) and consists of two ultrafilters (DIASAFE® plus), an infusate pump module, and disposable infusate lines. Infusate is prepared continuously by double-stage ultrafiltration. Both filters are subjected to automated membrane integrity tests before dialysis, and are replaced after 100 treatments or 12 weeks of use, whichever comes first. Dialysis fluid downstream from the first filter stage enters the dialyser; part of the stream is subjected to cross-flow filtration in the second filter in order to produce infusate. The infusate stream is connected with the venous bubble catcher for post-dilution HDF [25,26].

- The AK 100/200 ULTRA dialysis machine (Gambro AB, Lund, Sweden) prepares ultra pure water and ultra pure dialysis fluid by stepwise ultrafiltration of water and bicarbonate – containing dialysis fluid (BiCart) using two polyamide ultrafilters (U8000 S). When used for HDF, sterile non-pyrogenic solution is prepared on-line from the ultra pure dialysis fluid by an additional step of ultrafiltration using a sterile polyamide ultrafilter (U2000) integrated in a sterile line set (Steriset). The hygiene of the fluid pathway, including the U8000S ultrafilters, will be assured by heat disinfections after each treatment. The U8000S filters are changed bimonthly. The final ultrafilter (U2000) is employed on a single-use basis [25,26].

Data collection

Baseline and follow-up data registration

At baseline, all relevant information will be documented: i.e. demographical data, information on cardiovascular risk factors, time on dialysis, cause of renal insufficiency, and

medication. A follow-up visit will be scheduled every three months. During this visit, the occurrence of CV events, death, and hospitalisation will be documented. In addition, blood pressure, body weight and the achieved filtration and substitution dose per treatment will be registered. Case record forms are provided using the TeleForm system (version 8.1.1, Cardiff Software Inc, Vista, CA, USA). As all completed forms are scanned, no data entry by typing is needed. Registration of all data will be performed in each centre by the attending nephrologists and research nurses.

Recording outcome events

CV events include fatal or non-fatal myocardial infarction, stroke, therapeutic coronary procedure (PTCA or stenting), therapeutic carotid procedure (endarterectomy or stenting), and PTA and vascular intervention (revascularisation, PTA or stenting). Congestive heart failure is excluded as a CV event, since the discrimination with fluid overload is often hard to make in chronic HD patients. Furthermore, hospitalisations, duration of the hospitalisations and main diagnosis (including the occurrence of infections) will be recorded during the study period.

An independent event committee will evaluate all causes of death, cardiovascular events, and infections. The primary investigators will collect sufficient information of the events for the event committee. The event committee is blinded for information on the received treatment and consists of physicians with different specialisations: neurologists, vascular surgeons, nephrologists, internists, and cardiologists. Events will be coded as fatal and non-fatal, definite, probable and possible and not codeable (i.e. insufficient information). Only definite and probable events will be used in the final analysis. This procedure is successfully applied in a number of studies coordinated by the Julius Center, e.g. in the SMART study [27].

Left ventricular hypertrophy

Using transthoracic M-mode echocardiography from the parasternal long axis position, left ventricular end-diastolic diameter (LVEDD), end-systolic diameter (LVESD) as well as posterior and septal wall thickness will be determined at baseline, after 6 months, after 12 months and annually afterwards, on a midweek non-dialysis day according to a central uniform protocol. From these parameters left ventricular ejection fraction (LVEF) will be determined as LVEDD – LVESD/LVEDD, while the left ventricular mass index (LVMi) will be calculated using the formula of Devereux and Reichek [28], modified in accordance with the recommendations of the American Society of Echocardiography [29]. The ultrasound

investigations will be recorded on a compact disc and analysed off-line by experienced cardiologists in a core laboratory.

Vessel wall measurements

With respect to carotid intima-media thickness (CIMT), the outcome is the change in mean common CIMT, defined as the average of the intima-media thickness measurements performed circumferentially at pre-defined angles for the near and far wall of 10 mm segments of the right and left distal common carotid arteries [30]. A limited number of centres will be involved in the CIMT measurements in this study. Centres will be trained according to a central uniform carotid ultrasound protocol. Before actually starting the study, sonographers need to be certified as outlined in the CIMT ultrasound protocol. Measurements will be performed at baseline and then annually on a midweek non-dialysis day. The ultrasound scan is being recorded on videotape and analysed off line by a core laboratory. Quality Assurance and Quality Control procedures as existing and applied in several (inter)national trials will be implemented [31,32].

Pulse wave velocity (PWV) is determined to provide additional information on functional changes of the arterial wall [33]. The outcome measurement is the change in aortic PWV. A limited number of centres will be involved in the PWV measurements in this study. Centres will be trained according to a central uniform PWV protocol. Data are checked regularly on quality control aspects as defined in the PWV protocol as described earlier [34]. Measurements will be performed at baseline and then annually on a midweek non-dialysis day.

Nutritional state

At base-line, after 1, 2 years and at the end of the study, nutritional state is assessed by subjective global assessment (SGA), pre-albumin and dry weight [35].

Quality of life

Patient well-being will be estimated at baseline, and once a year by the Kidney Disease Quality of Life Short Form (KDQOL-SF). This version is validated in American and Dutch dialysis patients [36,37].

Laboratory assessments

Three monthly, blood samples will be drawn for routine laboratory assessments. In addition, blood samples will be taken at baseline, and after 6, 12, 18, 24, and 36 months for determinations of oxidative stress, inflammatory and endothelial function markers. Finally,

a whole blood sample will be stored for future research on the effect of genetics on the response to HDF, after specific permission of the patients in the informed consent form.

Statistical methods

The results of the study will be analysed according to the 'intention to treat' principle.

Primary outcome

The primary outcome variable is the time until the occurrence of an event defined as 'all cause mortality'. Results will be presented as Kaplan-Meier curves for the two treatment arms and the difference between the two treatments will be analysed using a log-rank test. The log-rank test will be adjusted for the effect of cumulative data analyses (see below).

Secondary outcomes

CV events are considered as secondary outcome variables. They will be analysed and presented as described for the primary outcome variable.

The primary analysis of CIMT progression will employ a linear random coefficient (Laird-Ware) model using real visit days, treatment and clinical center as independent variables. for each participant, the intercept and slope of CIMT change over time is assumed to be a normally distributed random variable with different means for the two treatment groups. The mean slope for the HDF treatment group will be compared to that for the low flux group using linear contrasts and a 5% significance level. Additional exploratory analyses will evaluate the impact of including baseline CIMT, lumen diameter, and ultrasound reader as additional co-variates.

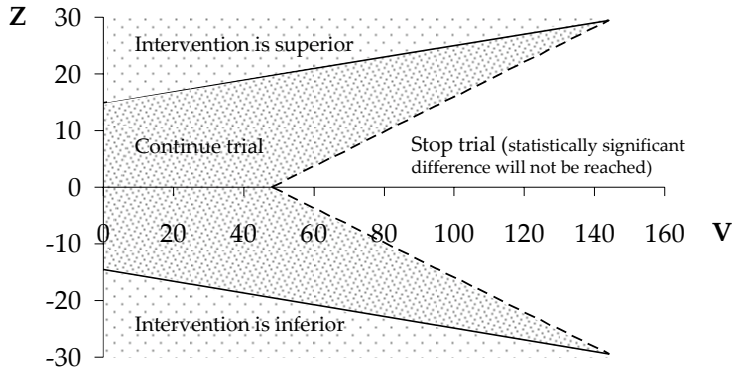
The data analytic approach to arrive at the PWV outcome variable and the LVMi outcome variable is similar to that of the CIMT outcome. Adjustments that will be taken into account in the estimates are changes in MAP and changes in heart rate, since both are closely related to PWV.

Sample size considerations

The sample size of the present study is based on the following event rates: the 3-year all cause mortality rate among subjects with ESRD is 44% based on data from the Dutch renal replacement registry (RENINE) [38]. CV mortality constitutes 40-60% of the total group of deaths, leading to a 3 year CV mortality rate of 22% in HD patients. Assuming that the incidence of non-fatal CVD is equal to the CV mortality rate (22%), the three-year incidence of fatal and non-fatal CVD is 44%. In addition, based on experience ± 8% of the ESRD patients will undergo renal transplantation yearly and as such is being censored in the trial.

Assuming that HDF will reduce all cause mortality with 20%, it has been estimated that with a two-sided alpha of 0.05 and a power of 80%, about 772 patients need to be enrolled and followed for three years. In these patients about 250 events are expected to come to a decision. Note that the total number of patients to be included cannot be specified in advance because of the planned sequential interim analyses, as described below.

Figure 2. Sequential analysis. Boundaries for a double sequential triangular test with α=0.05, power 0.80 and hazard ratio 0.75. Z is the observed number of events in the control group minus the expected number of events given treatment equivalence. V is approximately equal to a quarter of the number of events observed.



Interim analysis

In this study, group sequential interim analyses will be performed to evaluate the primary outcome variable. The reason for this approach is that, on average, fewer patients are needed in the study when the expected difference in the primary outcome variable is real or when no difference of the hypothesised magnitude can be expected anymore.

Sequential analysis is a statistical approach where one conducts significance tests over time as the data are collected. Sequential analysis and its application in clinical trials have been described extensively by Whitehead [39]. Sequential design and analysis is implemented in the computer program PEST version 4 [40].

The general approach is as follows. A null hypothesis H0 and an alternative hypothesis H1 are formulated for a suitable measure θ of treatment difference. For this study with a survival type outcome variable, θ is equal to the negative of the logarithm of the hazard ratio (HR). The HR is defined as the ratio of the logarithm of the (expected) cumulative survival under HDF (=0.648) and the logarithm of the (expected) cumulative survival under HD (=0.56). H0 is formulated as "no difference in the occurrence of the primary endpoint between the two trial arms" or $\theta = 0$ (i.e. HR = 1). The alternative hypothesis H1 is formulated as $|\theta| \geq -\log(0.75) = 0.29$. Two test statistics, Z and V, can be derived depending

on the type of response variable. Z is a measure of the treatment difference; for survival data Z is the observed number of events in the control group minus the expected number of events given treatment equivalence. V reflects the amount of information about θ contained in Z; for survival data V is approximately equal to a quarter of the number of events observed. The sequential analysis requires critical boundaries to be specified in advance. These boundaries depend on θ, the type I error α and the power 1-β. For each new group of patients, values of Z and V are calculated and presented graphically by plotting Z against V (see Figure 2 for an illustration of a double-sided sequential test). Based on the path of cumulative (Z,V)-points, one of the following three decisions is made:
the upper or the lower (continuous) boundary is crossed: stop the data collection and reject the null hypothesis;
one of the inner wedge-shaped (dashed) boundaries is crossed: stop the data collection and accept the null hypothesis;
continue the data collection: the cumulative data are inadequate to draw a conclusion yet.
An independent Data Safety and Monitoring Board (DSMB) will evaluate the results of the sequential interim analyses. The DSMB consists of a biostatistician (chair), a nephrologist, an internist, and a clinical epidemiologist. The biostatistician will perform the sequential analyses. The executive committee will provide the DSMB every 2 months with the relevant database to perform the unblinded analyses. The main task of the DSMB is to decide whether the analyses provide enough evidence of either efficacy or no efficacy with respect to the primary outcome and formulates recommendations for the executive committee on the continuation of the trial. The DSMB may also offer unsolicited recommendations on the continuation of the trial, for example after publication of results of similar trials.

Conclusion

Online HDF is gaining popularity, as recent technical advances have made it possible to safely replace considerable amounts of fluid at reasonable cost. In addition, accumulating evidence indicates that the correction of the uremic state is improved by online HDF, if compared to standard HD. However, at present it is unclear whether long-term treatment with HDF ultimately results in an improved clinical outcome. Therefore, CONTRAST is initiated, a randomised controlled trial of sufficient sample size to detect differences in survival and cardiovascular events. Patients will be randomised between low-flux HD and online HDF and followed for 3 years. Over 20 Dutch dialysis centers participate in this study and approximately 800 incident and prevalent HD patients will be recruited. By April 2005, more than 150 patients were included.

Acknowledgements

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Chapter 2

Treatment optimization

Chapter 2.1

Patient and treatment related determinants of convective volume in post-dilution hemodiafiltration in clinical practice

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Abstract

Background

Large convective volumes are recommended for online HDF to maximize solute removal. There has been little systematic evaluation of factors that determine convective volumes in routine clinical practice.

Methods

In the present study, potential patient and treatment related determinants of convective volume were analyzed in 235 consecutive patients on post-dilution HDF using multivariable linear regression models. All patients (age 64 ± 14 years; 61% male) participated in the ongoing CONvective TRAnsport STudy (CONTRAST). Additionally, differences in convective volumes between dialyzers were evaluated.

Results

The mean convective volume was 19.4 ± 4.0 L (± SD) per treatment, with a large variation between the participating centers (center means ranging from 13.4 ± 0.9 L to 24.5 ± 0.12 L, ± SE). The mean filtration fraction of the blood flow was 25.9 ◎ 3.6. In the multivariable analysis, factors that were significantly related to convective volume were hematocrit (inversely, regression coefficient (B)=-1.4 ± 0.4 L per 10%), serum albumin (positively, B=1.0 ± 0.4 L per 10 g/L), blood flow rate (positively, B=0.4 ± 0.04 L per 10 mL/min) and treatment time (positively, B=5.1 ± 0.4 L per hour). In addition, significant differences between dialyzers were observed, likely explained by different operational conditions.

Conclusions

Apart from increasing the treatment time and blood flow rate, convective volumes could be optimized by increasing the filtration fraction in each individual, provided that transmembrane pressures are well within safe limits. The precise role of dialyzer characteristics on maximal achievable convective volumes in clinical practice is a topic for further research.

Introduction

During hemodiafiltration (HDF) diffusive and convective transport are combined to maximize the removal of uremic toxins. The addition of convective transport leads to enhanced clearance of middle molecular weight (MMW) uremic toxins. This has been shown most extensively for β_2 microglobulin (β_2M), an established MMW marker molecule with a molecular weight of 11.8 kDa [1-4]. Since β_2M and other MMW uremic substances have been related to the extremely high cardiovascular mortality and morbidity in patients with end-stage renal disease [5,6], it has been proposed that increased convective transport improves clinical outcome. In fact, HDF has been associated with improved survival in some observational studies [7-9].

High convective volumes have been recommended to maximize MMW solute removal during HDF [10]. Indeed, a positive relation between the convective volume and the β_2M reduction ratio during post dilution HDF has been shown [1,2]. Moreover, in the DOPPS study high efficiency HDF (arbitrarily defined as infusion volumes 15-24 L per treatment) appeared to be related to an improved survival, whereas low-efficiency HDF (infusion volume < 15 L per treatment) did not [7].

There is currently limited information available which factors determine maximal convective volumes in post-dilution HDF in routine clinical practice. Obvious factors include treatment time and dialyzer blood flow rate [11]. Apart from that, hemoconcentration within the dialyzer leads to high hydraulic and transmembrane pressures and has been recognized as a limiting factor for total convective volumes [12]. Accordingly, a recent study showed a clear inverse relationship between hematocrit levels and the ratio of total convective volume and treated blood volume [13]. However, a systematic evaluation of these and other patient and treatment related factors on achieved convective volumes in routine clinical practice have not yet been performed.

The CONvective TRAnsport STudy (CONTRAST) is an ongoing randomized controlled clinical trial comparing online (post-dilution) HDF with low-flux (HD) on all-cause mortality and cardiovascular events [14]. As patient and dialysis characteristics are prospectively collected in CONTRAST, this study offers the unique opportunity to identify the determinants of convective volume in clinical practice. We especially aimed to recognize modifiable factors in an effort to optimize HDF treatment.

Patients and Methods

Patients

In the present study we included 256 consecutive HDF patients from 26 dialysis centers (24 Dutch centers, 1 Norwegian and 1 Canadian center), who were enrolled in the CONvective TRAnsport STudy (CONTRAST, ISRCTN38365125) and had completed six months of follow-up. Patient and dialysis characteristics at the six month visit were used for the present analyses. Twenty-one patients were not treated with HDF at the six month study visit because of vascular access problems (5 patients), unavailability of substitution fluids (3 patients) or other reasons (13 patients). Analyses were therefore restricted to 235 patients.

Before randomization in the CONTRAST trial, all patients were treated with low-flux HD (two or three times per week, for at least two months) with a minimum dialysis urea Kt/V ≥ 1.15 and age ≥ 18 year. Main exclusion criteria were: 1) treatment with high-flux HD or hemo(dia)filtration in the preceding six months, 2) severe incompliance and 3) life expectancy less than three months due to non-renal disease. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines and was approved by the institutional review board of each participating center. Written informed consent was obtained from all patients prior to randomization.

HDF prescription

Online HDF was performed in the post-dilution mode, using one of the dialyzers as summarized in Table 1. The target convective volume was arbitrarily set at 6 L/hr (or 100 mL/min). According to the CONTRAST protocol the filtration fraction should not exceed 33%. The operator's user manual of the FX-class dialyzers (Fresenius Medical Care, Bad Homburg, Germany) recommends that 25% of the blood flow should be the maximum substitution flow. The manual of the Polyflux dialyzers (Gambro Corporation, Lund, Sweden) does not give specific advice on maximum filtration fraction.

Treatment times were fixed at baseline and could only be increased if the dialysis urea spKt/V was below 1.2. Sterile and non-pyrogenic substitution fluids were produced by ultrafiltration of the ultrapure dialysate. Ultrapure quality was defined as bacterial counts < 0.1 colony forming unit per mL (CFU/mL) and endotoxin levels < 0.025 endotoxin units per mL (EU/mL). The microbiological water quality of the dialysis fluid was regularly monitored. Anticoagulation was performed with low molecular weight heparin before treatment. All routine patient care, e.g. metabolic control was performed according to the guidelines of the Quality of Care Committee of the Dutch Federation of Nephrology. The following dialysis systems were used for online HDF: 4008/5008 ONLINE (Fresenius), AK

100/200 ULTRA (Gambro), and DBB05 (Nikkiso, Tokyo, Japan) dialysis systems. At the time of analysis, the dialysis frequency of the patients was two times per week in 6.4% (n=15), three times per week in 93% (n=218), four times per week in 0.4% (n=1) and six times per week in 0.4% (n=1).

Table 1. Characteristics of dialyzers for HDF

Company	Dialyzer	Membrane material	Effective surface area (m^2)	Fiber inner diameter (μm)	UF coef.# ($mL \cdot hr^{-1} \cdot mmHg^{-1}$)	Effective fiber length (mm)
Fresenius Medical Care	FX80	Polysulfone	1.8	185	59	225
Fresenius Medical Care	FX100	Polysulfone	2.2	185	73	225
Fresenius Medical Care	Optiflux F 200NR	Polysulfone	2.0	200	56	280
Gambro Corporation	Polyflux 170H	Polyarylethersulfone/ polyamide	1.7	215	70	270
Gambro Corporation	Polyflux 210H	Polyarylethersulfone/ polyamide	2.1	215	85	270

#UF coefficients as given by the manufacturer, measured *in vitro* at 37ºC with bovine blood (Ht=32% and protein=60 g/L).

Data collection

Demographical data and data on medical history, including diabetic state, previous cardiovascular disease (CVD), vascular access and time on renal replacement therapy (RRT) were collected at the CONTRAST baseline study-visit. All other data were collected at the six months study-visit, including blood pressure level, body mass index (BMI) and various parameters of dialysis adequacy and prescription (treatment time, blood flow rate, intradialytic weight loss and infusion volume). Blood samples were collected before the start of a dialysis session for determination of hemoglobin, hematocrit, thrombocytes and serum albumin. All laboratory assessments were analyzed in the local hospitals by standard laboratory techniques.

The infusion volume (L per treatment) represents the amount of substitution fluid infused directly downstream of the dialyzer (post-dilution). The convective volume (L per treatment) was calculated by the sum of the intradialytic weight loss and the infusion volume per treatment and reported as the mean value of three consecutive dialysis treatments (i.e. three different days). The convective flow rate represents the convective volume per minute (mL per min). The filtration fraction (FF in %) is defined as the ratio between the convective flow rate and the dialyzer blood flow rate. High-efficiency HDF was

defined as a total infusion volume of ≥15 L per treatment [7]. Blood pressure (BP, in mmHg) was registered both as pre-dialysis systolic BP (SBP) and diastolic BP (DBP) and as the mean of pre- and post-dialysis values of three consecutive dialysis days. The reported blood flow rates represent the flow rates as displayed by the dialysis machines. Body mass index (BMI) was calculated by weight (kg) divided by the square of height (m^2).

Data analysis

Data were reported as proportions or as means with standard deviation (SD) or standard errors (SE) when appropriate. To study the independent relation of each variable with convective volume, we used multivariable linear regression analysis. We entered all patient and dialysis related variables in a multivariable model that showed a univariable relation with the convective volume using a cut-off p-value < 0.15. In addition, hematocrit, blood flow rate, treatment time were entered in the multivariable model up front. The multivariable model was adjusted for dialyzer type and dialysis machine, to correct for possible differences between dialyzers, dialysis operating procedures and center effects.

In a separate analysis, differences between dialyzers were evaluated. Mean values of all characteristics that were significant in the multivariate regression model were reported for each dialyzer category. Convective volumes were reported both unadjusted and after multivariable adjustment. Results were considered statistically significant when $p<0.05$ (two-tailed). We used SPSS software for all analyses (version 16.0.1; SPSS Inc. Headquarters, Chicago, Illinois, US).

Results

The mean age of the patients was 64 ± 14 years and 61% were male (Table 2). The mean convective volume was 19.4 ± 4.0 L (±SD) per treatment (range 6.7 to 28.2 L) and the mean filtration fraction was 25.9 ⊚ 3.6 % (range 11.2 to 36.8 %). The blood flow rate was 333 ± 44 mL/min. Seventy-eight percent of the patients were treated with high-efficiency HDF defined as infusion volumes ≥ 15 L (i.e. convective volume ≥ 17 L). The pre-defined target convective volume of ≥ 6 L per hour was reached in 43 of the 235 patients (18%). These 43 patients had lower hematocrit levels (37 ± 0.3 versus 34 ± 0.6 L/L [±SE, $p<0.001$] in the remaining patients), higher albumin levels (38 ± 0.5 versus 36 ± 0.3 g/L, $p=0.002$) and were treated with higher blood flow rates (384 ± 5 mL/min versus 322 ± 3, $p<0.001$). In 10 of the 26 centers the target convective volume of ≥ 6 L per hour was reached in none of the patients. In 2 centers the target was reached in all patients. Center means of convective volume ranged from 13.4 ± 0.9 L to 24.5 ± 0.12 L (± SE) per treatment (Figure 1).

Table 2. Patient characteristics of 235 chronic HDF patients

	mean ± SD or %
Male	61
Age (years)	64 ± 14
Body Mass Index (kg/m²)	25 ± 4.5
History of cardiovascular disease	44
Diabetes Mellitus	26
Time on renal replacement therapy (years)	3.6 ± 4.1
Systolic Blood Pressure (mmHg)	141 ± 20
Diastolic Blood Pressure (mmHg)	72 ± 12
Vascular access:	
- Fistula	78
- Graft	18
- Central venous catheter	4
Hemoglobin (mmol/L)	7.4 ± 0.8
Hematocrit (%)	36 ± 4.3
Thrombocytes (x 10^9/L)	227 ± 73
Serum albumin (g/L)	36 ± 4.5
Treatment time (min)	225 ± 24
Blood flow rate (ml/min)	333 ± 44
Intradialytic weight loss (L per treatment)	1.9 ± 0.9
Infusion volume (L per treatment)	17.5 ± 3.8
Convection volume (L per treatment)	19.4 ± 4.0
Convective flow rate (ml/min)	86.1 ± 16
Filtration fraction (%)	25.9 ± 3.6

To convert hemoglobin from mmol/L to g/dL multiply by 1.61. To convert albumin from g/L to d/dL divide by 10.

Patient related factors

In the univariable analysis, male sex, body mass index, serum albumin, hemoglobin and hematocrit were related to the convective volume. Age, a history of cardiovascular disease, diabetic state, dialysis vintage, blood pressure (either measured as predialysis DBP and SBP, or as the average of pre- and postdialysis values), vascular access and thrombocyte levels did not show an association with the convective volume (Table 3). In the multivariable analysis, serum albumin was positively and hematocrit was inversely related to the convective volume. Male sex and BMI were no longer significant in the multivariable model. In Figure 2 the relations between albumin and hematocrit with the convective volume are depicted after multivariable adjustments.

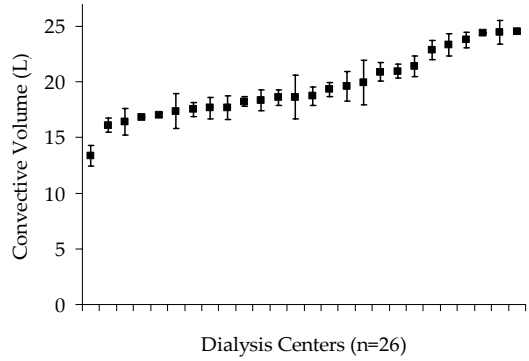

Figure 1. Mean convective volumes per dialysis center. Bars represent SE of the means.

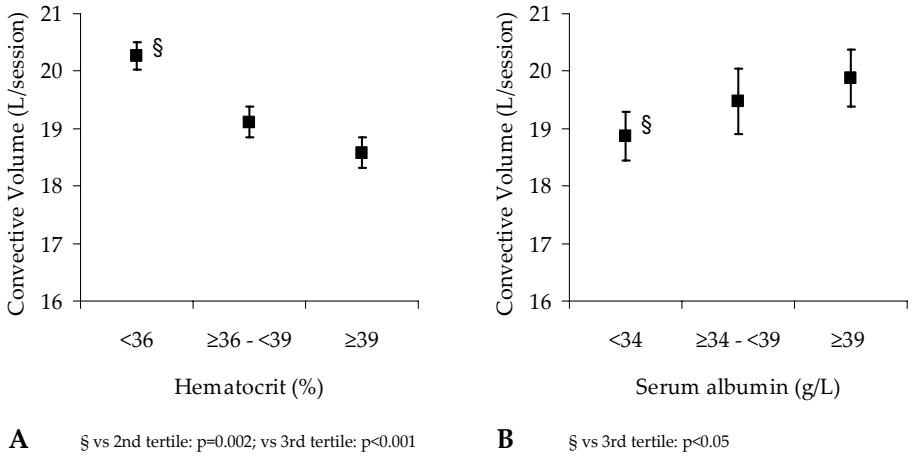

Figure 2. Relation between hematocrit and convective volume (panel A) and relation between serum albumin and convective volume (panel B). Convective volumes are adjusted for dialysis system, type of dialyzer, serum albumin, blood flow rate and treatment time. Hematocrit and albumin levels in tertiles, bars represent SE.

Table 3. Determinants of convective volume in post-dilution HDF: univariable and multivariable linear regression analysis

	Univariable model		Multivariable model	
Determinant	**B**	**95% CI**	**B**	**95% CI**
Sex (Male)	1.8	0.7 to 2.8†	0.47	-0.16 to 1.1
Age (years)	0.0	-0.04 to 0.03		
BMI (kg/m²)	0.14	0.03 to 0.25†	0.028	-0.04 to 0.09
History of CVD	-0.14	-1.2 to 0.90		
DM	0.67	-0.50 to 1.8		
Time on RRT (years)	0.01	-0.1 to 0.1		
SBP (mmHg)	0.0	-0.03 to 0.02		
DBP (mmHg)	0.01	-0.03 to 0.06		
Vascular access (Fistula)	-0.3	-1.5 to 1.0		
Hemoglobin (mmol/L)	-0.89	-1.5 to -0.28†		
Hematocrit (%)	-0.18	-0.30 to -0.06†	-0.14	-0.22 to -0.07†
Thrombocytes (x 109/L)	-0.003	-0.01 to 0.004		
Serum albumin (g/L)	0.19	0.07 to 0.3†	0.10	0.02 to 0.18†
Treatment time (min)	0.09	0.07 to 0.10†	0.09	0.07 to 0.10†
Blood flow rate (mL/min)	0.05	0.04 to 0.06†	0.04	0.03 to 0.05†

The multivariable model was adjusted for dialyzer and for dialysis system.
† $p<0.05$
R^2 of the multivariable model=0.71.
The B reflects the change of the total convective volume (in L per treatment) related with one unit increment of the determinant.

Treatment related factors

Blood flow rate (B=0.04 L per mL/min [95% CI 0.03 to 0.05, p<0.001) and treatment time (B=0.09 L per min [95% CI 0.07 to 0.10, p<0.001) were positively related to the convective volume in the multivariable model (Table 3).

In Table 4, the different prescribed dialyzers are compared. FX80 dialyzers were prescribed in 24% of the patients, FX100 dialyzers in 20%, Polyflux 170H and 210H dialyzers in 20% and 33%, respectively. Optiflux F 200NR dialyzers were prescribed in 7% of the patients, all from one single center. Three patients (1%) used alternative dialyzers and were excluded from the multivariable analyses. Hematocrit and serum albumin levels, treatment times, blood flow rates and convective volumes were all different between the dialyzers (Table 4).

Table 4. Comparison of dialyzers.

	European centers§				**Canadian center**
	FX80	**FX100**	**Polyflux 170H**	**Polyflux 210H**	**Optiflux F 200NR**
N (patients)	**57**	**34**	**46**	**78**	**17**
N (centers)#	**8**	**4**	**7**	**14**	**1**
Dialysis system					
4008/5008	53 (93%)	17 (50%)	6 (13%)	11 (14%)	17 (0%)
AK 100/200	4 (7%)	1 (3%)	37 (78%)	59 (76%)	0 (0%)
DBB05	0 (0%)	16 (47%)	3 (7%)	8 (10%)	0 (0%)
Hematocrit (%)	38 ± 0.6	36 ± 0.8	37 ± 0.7	36 ± 0.4	33 ± 1.0 [a,b,c,d]
Serum albumin (g/L)	37 ± 0.5	38 ± 0.8	33 ± 0.6 [a,b,d,e]	36 ± 0.5 [b]	37 ± 0.6
Blood flow (mL/min)	301 ± 4.5	325 ± 6.5 [a]	322 ± 5.1 [a]	356 ± 4.0 [a,b,c]	372 ± 10.3 [a,b,c]
Treatment time (min)	231 ± 3.2	208 ± 4.7 [a,c,d]	227 ± 3.0	229 ± 2.3	215 ± 5.2[d]
Unadjusted convective volume (L per treatment)	17.9 ± 0.4	16.6 ± 0.8	18.8 ± 0.5 [b]	20.9 ± 0.4 [a,b,c]	23.8 ± 0.7 [a,b,c,d]
Filtration fraction (%)	25.8 ± 0.4	24.6 ± 0.9	25.7 ± 0.4	25.6 ± 0.3	29.9 ± 0.6 [a,b,c,d]
Adjusted convective volume¶ (L per treatment)	18.2 ± 0.4	18.7 ± 0.5	19.5 ± 0.4 [a]	19.8 ± 0.3 [a]	22.2 ± 0.6 [a,b,c,d]

Mean ± SE or n (%).

§ 24 Dutch and 1 Norwegian dialysis centers.

More than 1 dialyzer can be used per center.

¶ Adjusted for: dialysis system, hematocrit, albumin, blood flow rate and treatment time.

[a] p<0.05 vs FX80; [b] p<0.05 vs FX100; [c] p<0.05 vs Polyflux 170H; [d] p<0.05 vs Polyflux 210H; [e] p<0.05 vs Optiflux F 200NR.

When dialyzers of similar materials were compared (FX100 versus FX80 and Polyflux 210H versus Polyflux 170H), blood flow rates were significantly higher in the patients treated with the largest dialyzers (i.e. FX100 and Polyflux 210H). However, after multivariable adjustment (including adjustment for blood flow rate), convective volumes were not significantly different between FX100 and FX80 dialyzers and between Polyflux 210H and Polyflux 170H dialyzers (Table 4). The highest convective volumes were achieved in the patients using Optiflux F 200NR dialyzers (all from one center). In these patients, the hematocrit level was significantly lower (33 ± 1.0% versus 37 ± 4.2%, $p<0.002$) and the filtration fraction was higher (29.9 ± 0.6% versus 25.5 ± 0.2%, $p<0.001$) in comparison with patients using other dialyzers.

Discussion

To the best of our knowledge, the present study is the first in which patient and treatment related determinants of convective volume were systemically evaluated in a large group of patients on post-dilution HDF in clinical practice. We observed higher convective volumes in patients with low hematocrit and/or high serum albumin levels. Dialysis treatment time and blood flow rate were identified as treatment related determinants of the convective volume. Apart from that, we found considerable differences in convective volume between centers, which could only partly be explained by patient or treatment characteristics.

The mean convective volume of 19.4 ± 4.0 L per treatment in the present study is comparable to the 18 to 23 L achieved in most other studies in patients on post-dilution HDF [1,3,9,13]. In few small studies, post-dilution infusion volumes up to 30 L per treatment have been reported [4,15]. In these studies, especially mean blood flow rates were much higher (400 mL/min) as compared to 333 mL/min in the present study. At the time of the conception of the study it was unclear which amount of convective volume could be considered adequate or sufficient. Target volume was arbitrarily set at 6 L per hour. More recently, the DOPPS data became available, indicating that volumes of approximately 17 L per session (15 L infusion + 2 L net weight loss) and higher were associated with survival benefit [7]. To date, these are still the only data relating volume to clinical outcome. The pre-defined treatment target of 6 L per hour was achieved in only 18% of the patients. However 78% of the patients were treated with volumes of 17 L per session and higher.

Patient related factors

Blood viscosity has been recognized as an important limiting factor of convective transport [12]. Post-dialyzer hematocrit values rise up to 54% during treatment [16], which together

with a comparable rise in plasma protein levels may reduce the hydraulic permeability of the dialyzer. As a consequence, the resistance to the flow in the dialyzer will increase, and eventually, clotting of dialyzer fibers may occur [17]. In line with this notion, we and others [13] found an inverse relation between the pre-dialysis hematocrit level and convective volume. The optimal hematocrit level for dialysis patients remains to be established, but for post-dilution HDF values should not exceed treatment targets. In the present study, 52% of the patients had hemoglobin levels above the current KDOQI target of 7.4 mmol/L (12 g/dL), which may have contributed to suboptimal convective volumes.

Serum albumin was positively related to the convective volume, although its contribution was limited (1 L per treatment increase of convective volume per 10 g/L albumin, Table 3). It is likely that higher colloid osmotic pressures in patients with high serum albumin levels enhance plasma refill rates, thus allowing more convective transport [18]. Alternatively, increased platelet aggregation [19] or reduced red cell deformability [20] in patients with hypoalbuminemia may limit the convective volume. On the other hand, high protein levels may decrease membrane permeability by increasing the thickness of the protein layer on the membrane [17]. However, total protein levels were not measured in this study. Likely, the beneficial effects of high albumin on the convective volume is the result of opposite factors. It is therefore unclear whether albumin levels higher than the range in this study would still be beneficial. Nevertheless, paying attention to the nutritional and inflammatory state in patients with low albumin levels may contribute to high convective volumes and may, more importantly, improve clinical outcome [21].

Treatment related factors

Apart from treatment time and blood flow rate, the dialyzer type was related to the convective volume, also after multivariable adjustments. However, the present study was not designed to investigate differences between dialyzers. A reliable comparison between dialyzers can only be made in relation to the applied transmembrane pressure. Since transmembrane pressures were not assessed, it is unknown whether maximal tolerable and safe transmembrane pressures were applied in all patients. On the contrary, the large differences in convective volumes between centers suggested that HDF was not performed at maximal transmembrane pressures in several centers. Nevertheless, it can be hypothesized that the design of the dialyzer plays a role on maximal achievable convective volumes. Dialyzer characteristics such as fiber length or fiber diameter have substantial effects on the pressure profile [22-24], which could influence maximal convective transport. In addition, the membrane type may have an effect on the water permeability. Recently, it has been shown that almost similar synthetic high-flux dialyzers may be different in terms

of middle molecule removal and solute trapping [25]. Finally, a large surface area may allow higher blood flow rates and could therefore be beneficial to optimize exchange volumes. The precise role of the abovementioned dialyzer characteristics in clinical practice needs further evaluation.

The user manual of some manufacturers recommends that the filtration fraction should not exceed 25%. However, the present study demonstrates that higher filtration fractions are tolerated in many patients. For example, the mean filtration fraction in the Canadian center was 30%. It should be noted that an increase of the filtration fraction from 25% to 30% has substantial effects on the total convective volume. During a regular HDF session (treatment time 4 hours, blood flow rate 350mL/min) a 5% increase of the filtration fraction leads to an increase of the convective volume of 4.2 L.

To improve convective volumes in post-dilution HDF in clinical practice, the treatment time and blood flow rate should be increased first if possible. Then, the filtration fraction should be increased within the limits of safe transmembrane pressures, taking into account hematocrit and protein levels, and possibly also dialyzer characteristics.

Limitations and strengths

All patients participated in a randomized trial and satisfied predefined inclusion criteria, which may have limited the generalizability of the results. On the other hand, the inclusion criteria were meant to include the average chronic HD patient and all patients were randomly allocated to HDF treatment. Furthermore, the data for this study were not specifically collected to investigate determinants of convective volume. For example, the indication to prescribe a certain dialyzer to a patient was unknown. It is possible that larger dialyzers were more often prescribed for patients with lower convective volumes in an effort to improve treatment. Moreover, transmembrane pressures were not assessed, limiting the possibilities to compare different dialyzers. Effects of dialyzer characteristics on convective volumes should be evaluated in specifically designed prospective studies. Finally, it should be noted that the definition of filtration fraction (i.e. filtered fraction of the blood flow) as frequently used in clinical practice is theoretically incorrect, since it does not take into account differences in hematocrit level. Ideally, the fraction of the plasma flow should be used instead. However, this is not very practical in everyday clinical practice. The strengths of this study were the large number of patients, allowing for multivariable comparisons, and the prospective and standardized data collection.

Optimizing convective volumes may be of clinical relevance and has been recommended by the European Best Practice Guidelines [10]. Moreover, data from DOPPS suggest that infusion volumes should be at least 15 L per treatment in order to improve clinical outcomes

with online HDF [7]. Whether the differences within the observed range of convective volumes in the present study are of any clinical relevance is currently unknown. The CONTRAST study may provide data to answer that question.

Conclusion

The present study suggests that sufficient treatment time and/or blood flow rate are a prerequisite to achieve convective volumes which are presently considered adequate [7]. In addition, attention should be paid to current hemoglobin treatment targets and the nutritional and inflammatory state, since convective volumes may be attenuated by high hematocrit or low albumin levels. The precise role of dialyzer characteristics on maximal achievable convective volumes is a topic for further research. The data suggest that within the hematocrit range of this study, which represents everyday clinical practice, filtration fraction could be higher than recommended by some of the manufacturers. As minor changes of the filtration fraction have substantial effects on the total convective volume, the maximal tolerable filtration fraction for each individual patient should be assessed for treatment optimalization.

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Chapter 2.2

Microbiological quality and quality control of purified water and ultrapure dialysis fluids for online hemodiafiltration in routine clinical practice

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Abstract

During online hemodiafiltration patients are directly infused with sterile substitution solutions to maintain fluid balance. Adequate water treatment and a well organized quality control process are essential to provide non-pyrogenic fluids with consistent optimal quality. We sought to assess water quality, the water treatment system and the methods for surveillance of microbiological water quality in ten Dutch dialysis centers that routinely treat patients with hemodiafiltration. Microbiological monitoring results (micro-organisms and endotoxins) were collected over a one year period representing 11258 hemodiafiltration sessions covering 97 patients. In all centers, water purification was based on a reverse osmosis module in combination with a second reverse osmosis and/or an electrodeionizer. All centers regularly and routinely monitored the microbiological purity of the dialysis water with adequate analytical methods but with variable monitoring frequency. Microbiological assessments were compliant with reference quality levels in 3923 out of 3961 samples. Our study suggests that non-pyrogenic substitution fluids can be produced online for a prolonged period of time. It is likely that the current Dutch Quality of Care Guideline has contributed to high quality water treatment and a well organized control process.

Introduction

Convective dialysis strategies such as online hemodiafiltration (HDF) are increasingly used as the dialysis modality of choice because of their suggested clinical benefits [1]. During online HDF, convective solute removal is increased by filtering considerable amounts of plasma water through the dialyzer. At the same time, sterile substitution fluids are infused directly into the bloodstream to maintain fluid balance. These substitution fluids are manufactured online from the municipal water supply after multiple steps of water purification and ultrafiltration, as depicted in Figure 1 [2,3].

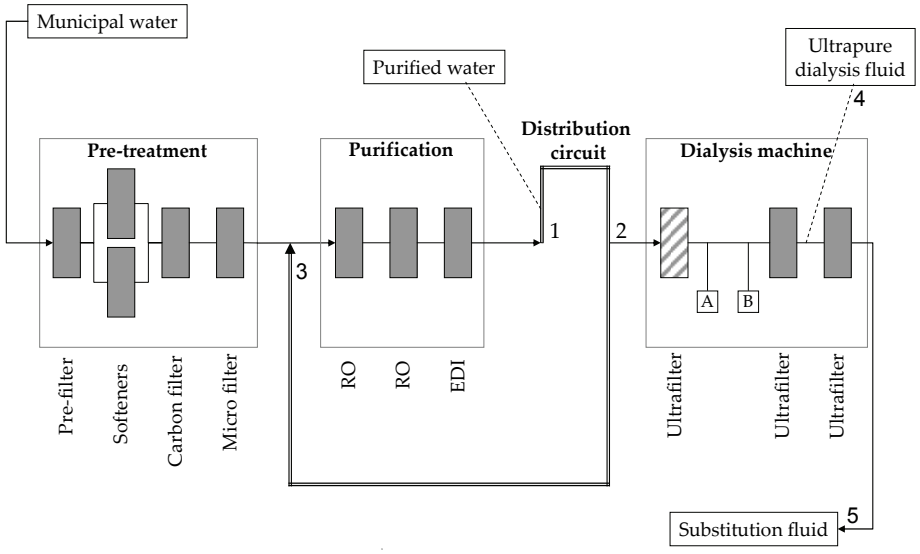

Figure 1. Online production of substitution fluid. Sampling sites of dialysis solutions: 1) entry distribution circuit (purified water), 2) machine feeding water (purified water), 3) exit distribution circuit (purified water), 4) ultrapure dialysis fluid and 5) substitution fluid. Online production of substitution fluids consists of water pre-treatment, water purification, a well designed distribution circuit for the delivery of purified water to the dialysis machines and several ultrafiltration steps on the dialysis machine [2,3]. Water pre-treatment involves down sizing microfilters, water softener(s) and in some cases an activated carbon filter. Softeners mainly remove calcium and magnesium from the water. Activated carbon filters remove chlorine and chloramines. The purification system is based on one or two reverse osmosis (RO) modules and/or an electrodeionizer (EDI). The RO modules remove most ions and virtually all organic compounds, including bacteria, viruses and pyogens; an EDI removes mostly inorganic ions. The water distribution circuit should be designed as a loop system, constructed from stainless steal or appropriate synthetic materials and should be disinfected regularly by ozone, heat or chemicals. Storage tanks or dead ends within the circuit should be avoided. Continuous high-speed flow of water minimizes the risk of biofilm formation. All approved dialysis systems incorporate at least two ultrafilters, which are regularly replaced. Some dialysis systems contain a 3rd ultrafilter (▨). After the addition of acid and bicarbonate concentrates (A and B), the purified machine feeding water is ultrafiltered to produce the ultrapure dialysis fluid. A final ultrafiltration step provides the substitution fluid. All manufacturers guarantee sterility of substitution fluid, provided that the machine feeding water (sampling site 2) contains < 100 CFU/mL and < 0.25 EU/mL.

Since large volumes are administered each treatment, delivery of sterile and non-pyrogenic substitution fluids should be guaranteed for prolonged periods of time with persistent optimal quality. Monitoring of the microbiological purity of the water is therefore an essential part of the quality control process. Hence, samples should be drawn routinely and regularly from strategic points in the fluid path of the water treatment system for determination of micro-organism and endotoxin levels. For standard hemodialysis (HD) in Europe, the maximum contamination level for dialysis fluid has been defined by the European Pharmacopoeia as < 100 colony forming units (CFU)/mL and < 0.25 endotoxin units (EU)/mL [4]. In contrast, dialysis fluid for HDF should be ultrapure, defined as < 0.1 CFU/mL and < 0.03 EU/ml [5-8]. Substitution fluids are produced by ultrafiltration of ultrapure dialysis fluids, using a filter with a logarithmic reduction value ≥ 6 for micro-organisms [9]. In Table 1 the reference quality levels for dialysis solutions are summarized.

Table 1. Reference quality levels for dialysis solutions for HD and HDF.

		Micro-organisms (CFU/mL)	**Endotoxins (EU/mL)**
Standard HD			
Purified water		< 100	< 0.25
Dialysis fluid		< 100	< 0.25
Online Hemodiafiltration			
Purified water	Sampling site †: 1,2,3	< 100	< 0.25
Ultrapure dialysis fluid	Sampling site †: 4	< 0.1	< 0.03
Substitution fluid	Sampling site †: 5	< 10-6	< 0.03

Adapted from references: [4-8]
† See Figure 1 for the location of the sampling sites.

No specific international guidelines are currently available on how to maintain persistent high quality of dialysis solutions for online HDF. Therefore, the Dutch Quality of Care Guideline on "Water treatment for hemodialysis and online hemodiafiltration" has been developed [8]. This guideline defines basic requirements for the configuration of a water treatment system when used for online convective therapies and describes analytical methods and frequency of microbiological monitoring of the water purity.

The aim of the present study was to assess the water quality for online HDF in a sample of Dutch dialysis centers that routinely treat patients with online HDF. In addition, we assessed the configuration of the water treatment systems in these centers and we evaluated methods for surveillance of microbiological water quality, in comparison with the current Dutch guideline.

Methods

Data collection

Ten dialysis centers (four of which university medical centers) volunteered to participate in the present study, all taking part in the ongoing CONvective TRAnsport STudy (CONTRAST) [10]. These dialysis centers were asked to fill out a questionnaire about the design of the water treatment system and analytical methods and frequency of microbiological monitoring. The topics of this questionnaire were based on the current Dutch guideline and are summarized in Table 2. Furthermore, we collected all microbiological monitoring test results of these centers from January 2007 to January 2008. Microbiological monitoring results included bacterial contamination, expressed as colony forming units per milliliter (CFU/mL) and endotoxin levels, expressed as limulus amoebocyte lysate (LAL) reactivity in endotoxin units per milliliter (EU/mL) [11]. Results from tests were based on center specific laboratory results rather than from a central laboratory since our approach reflected usual practice. Finally, we reviewed the policy in case of sample contamination and we assessed the occurrence of pyrogenic reactions in patients on online HDF related to contamination, i.e. temperature > 38.5°C or chills, otherwise unexplained, during the study period.

Data analysis

Results of microbiological tests of all sampling sites were reported as percentages of the samples below the reference quality levels as defined in Table 1. Data from all cultures and endotoxin tests of the purified water (Figure 1, sampling sites 1, 2 and 3) were pooled.

To evaluate the impact of components of the water treatment systems on the microbiological water quality, we defined the microbiological quality as the proportion of the samples of purified water with less than 0.1 CFU/mL. These samples were categorized according to the configuration of the water treatment system: 1) number of RO modules (one or two), 2) EDI (yes/no), 3) UV disinfection (yes/no), 4) material of distribution loop (stainless steel/polyethene), 5) disinfection method of distribution loop (ozone/heat) and 6) disinfection frequency of the distribution loop (< 4 times per week or ≥ 4 times per week). For each category, the proportion of samples < 0.1 CFU/mL was assessed.

The differences in proportions between groups were statistically evaluated using Chi-square tests. A two-sided p-value < 0.05 was considered as statistically significant. We used SPSS software (version 16.0.1; SPSS Inc. Headquarters, Chicago, Illinois, US).

Table 2. Overview of assessment of quality control methods.

Questionnaire topics	Dutch Quality of Care Guideline [8]
1. Water pre-treatment	
- pre-filter	
- softener	Double, parallel, to regenerate resins without discontinuation of water production
- activated carbon filter	Potential contamination source, installation preferably only when municipal water is chlorinated.
- micro-filter	
2. Design of water purification	
- reverse osmosis (RO), single or double	Preferably double, placed in series
- electrodeionizer (EDI)	Prerequisite when only 1 RO
- ultraviolet (UV) disinfection	Prerequisite when using ozone disinfection
3. Distribution circuit	
- Type of material	Preferably stainless steel, polyvinyldifluoride or crosslinked polyethene
- Disinfection method	Chemical, thermal or ozone disinfection
- Connection water distribution circuit with dialysis machines	A double lumen connecting tube (secondary loop) may be used to prevent water stagnation when the dialysis machine is turned off.
4. Dialysis machines	
- Type and number of dialysis machines	All approved systems, with 2 or 3 ultrafilters
- Disinfection method	
5. Microbiological monitoring	
- Culture method	Nutrient poor media are recommended such as Reasonar's 2 agar (R2A) or tryptone glucose extract agar (TGEA), with prolonged incubation (5-7 days) at ambient temperature (17-23°C) [12].
- Type of endotoxin tests	Use limulus amebocyte lysate (LAL) assay according to the chromogenic method, gel-clot method or turbidimetric technique, as discribed by the European Pharmacopoeia [11].
- Monitoring frequency	Micro-organisms: monthly for distribution loop entry and ultrapure dialysis fluid (sampling sites 1 and 4, Fig 1), weekly for distribution loop exit (sampling site 3). Endotoxins: monthly for distribution loop entry and exit and for ultrapure dialysis fluid (sampling sites 1,3 and 4). Monitoring of substitution fluid is not required. For validation of a water treatment system, a higher sample frequency is recommended.

Results

Water treatment systems

The water treatment systems were constructed between 1999 and 2006. Pre-treatment of the municipal water was comparable in all centers and included down sizing microfilters and two parallel water softeners. In two centers an activated carbon filter was installed. Nine centers were equipped with a double reverse osmosis (RO) module. One center combined a single RO module with an Electrodeionizer (EDI) (Table 3). In addition, five centers used ultraviolet (UV) disinfection. The distribution loop was disinfected with heat in seven and with ozone in three centers, with a median frequency of 4 (range 2 to 7) times per week (Table 3). In 5 centers, the machines were connected to the distribution circuit by a double lumen connecting tube.

Table 3. Characteristics of water purification system and distribution circuit.

Center	Water purification				Distribution circuit		
	RO (1st)	*RO (2nd)*	*EDI*	*UV*	*Type*	*Disinfection method*	*Disinfection frequency*
1	+	+	-	-	Polyethene	Heat	7x per week
2	+	+	+	+	Polyethene	Ozone	3x per week
3	+	+	-	-	Stainless steel	Heat	3x per week
4	+	-	+	+	Polyethene	Heat	7x per week
5	+	+	-	-	Stainless steel	Heat	4x per week
6	+	+	-	+	Polyethene	Ozone	3x per week
7	+	+	-	-	Polyethene	Heat	2x per week
8	+	+	+	+	Polyethene	Ozone	2x per week
9	+	+	+	+	Stainless steel	Heat	7x per week
10	+	+	-	-	Polyethene	Heat	7x per week

Abbreviations: RO=reverse osmosis module; EDI=electrodeionizer; UV=ultraviolet desinfection; UF=ultrafiltration. The components of the water purification system are placed in series. All centers with ozone disinfection use UV treatment for ozone breakdown. UV disinfection was located at the entry (in centers 2 and 4) or exit (in centers 8 and 9) of the distribution loop, or both (in center 6).

A total of 174 dialysis machines were used for online HDF (61 4008/5008 ONLINE machines (Fresenius Medical Care, Bad Homburg, Germany), 83 AK100/200 ULTRA (Gambro AB, Lund, Sweden, and 30 DBB05 (Nikkiso, Tokyo, Japan)). All dialysis machines were heat disinfected after each treatment. In addition, machines were chemically disinfected with citric or peracetic acid at least once on each treatment day. Only bicarbonate powder cartridges were used. Ultrafilters on the dialysis machines were replaced after two months (U8000S, Gambro, for AK100/200 ULTRA systems), three months (DIASAFE plus, Fresenius, for 4008/5008 ONLINE systems) or after 750 operational hours (i.e. approximately two months, EF 02, Nikkiso, for DBB05 systems), according to manufacturers instructions.

All centers complied with the Dutch Guideline regarding the technical design of the water treatment system, distribution circuit and use of appropriate dialysis machines.

Microbiological monitoring

Dedicated persons (i.e. specifically trained dialysis nurse, medical analist, or dialysis technician) were responsible for sampling under aseptic conditions. Median sample volumes were 100 mL (range 10 to 1000 mL) for the purified water and 500 mL (300 to 1000) for the ultrapure dialysis fluid. All centers applied a membrane filtration technique for the ultrapure dialysis fluid cultures, using a microfilter with pore size 45 μm (22 to 45).

Tryptone Glucose Extract Agar (TGEA) media was used in six centers and Reasonar's 2 agar (R2A) media in four centers, with a median culture time of 7 days (5 – 7) incubated at room temperature. In one center cultures were incubated at 37°C in stead, which has been shown to underestimate bacterial contamination levels of dialysis solutions [12]. All centers used LAL assays for determination of endotoxins (chromogenic method in seven centers and gel-clot method in three centers). These analytical methods complied with the guideline. In one center, the detection limit of the LAL assay (0.125 EU/mL) was higher than the quality level of ultrapure dialysis fluid (0.03 EU/mL), which may have resulted in an underestimation of endotoxin levels. Cultures and endotoxin tests were performed in the local hospital by the departments of Pharmacy and/or Medical Microbiology.

Sampling sites and corresponding monitoring frequencies are listed in Table 4. Purified water was monitored in all centers and ultrapure dialysis fluid was monitored in eight centers (centers 1-7 and 10). Although not required by the guideline, machine feeding water was monitored in four centers (centers 3, 4, 6 and 9) and substitution fluid was monitored in three centers (centers 7, 8 and 9). Monitoring frequency varied between centers. Four centers had a more intensive monitoring schedule (centers 3, 4, 5 and 7) and two centers clearly had a less intensive monitoring schedule (centers 8 and 10) as compared to the guideline.

Table 4. Microbiological monitoring frequency of dialysis solutions for online HDF, according to the Dutch guideline and as performed by the centers.

Type of water	**Purified water**	**Purified water**	**Purified water**	**Ultrapure dialysis fluid**	**Substitution Fluid**
Sampling site location	**Distribution loop entry**	**Machine feeding water**	**Distribution loop exit**	**Dialysis machine**	**Dialysis machine**
Sampling site number (Fig. 1)	**1**	**2**	**3**	**4**	**5**
Dutch Guideline [8]	Monthly	-	Weekly/ Monthly#	Monthly	-
Center					
1	Monthly		Weekly/ Monthly#	Monthly	-
2	Monthly		2 x per Month	Monthly	-
3	Monthly	Monthly†	Weekly/ Monthly#	Monthly	-
4	Monthly	Monthly*	Weekly	Monthly	-
5	Weekly		Weekly	Monthly	-
6	Monthly	Monthly	Monthly	Monthly	-
7	Monthly¶	-	Weekly	Monthly	Monthly
8	Monthly	-	Monthly	-	Yearly*
9	-	Monthly	Weekly	-	Monthly
10	1x per 3 months	-	1x per 3 months	1x per 3 months	-

Monitoring frequency is similar for micro-organisms and endotoxins, except for:
weekly: micro-organisms and monthly: endotoxins.
* Only micro-organisms, no endotoxins
† Random selection of 3 different machines
¶ Fixed point halfway distribution loop, in stead of distribution loop entry

Water quality

In total, microbiological assessments during the one-year study period were compliant with reference quality levels in 3923 out of 3961 samples (99.0%). Bacterial counts were too high in 6 of the 685 purified water samples (0.9%), in 8 of 1185 ultrapure dialysis fluid samples (0.7%) and in 4 of 193 substitution fluid samples (2.1%). Endotoxin levels were too high in 2 of 663 purified water samples (0.3%), in 14 of 1058 ultrapure dialysis fluid samples (1.3%) and in 4 of 177 substitution fluid samples (2.3%) (Figure 2). Contamination of purified water and ultrapure dialysis fluid samples did not occur simultaneously and was equally distributed over the centers and study period. In all incompliant cases, new samples were collected to repeat microbiological cultures or endotoxin tests. Contamination was not confirmed in any of these repeated samples. When purified water samples were incompliant (n=8), HDF was temporarily discontinued in the unit (n=5), or no action was taken (n=3), in addition to repeated microbiological tests. When dialysis fluid samples were incompliant (n=22), the machine was removed temporarily (n=16, i.e. 9% of all dialysis machines per year), only HDF was temporarily stopped (n=3), or no action was taken (n=3), in addition to repeated microbiological tests. No pyrogenic reactions during 11258 HDF sessions in 97 patients were reported.

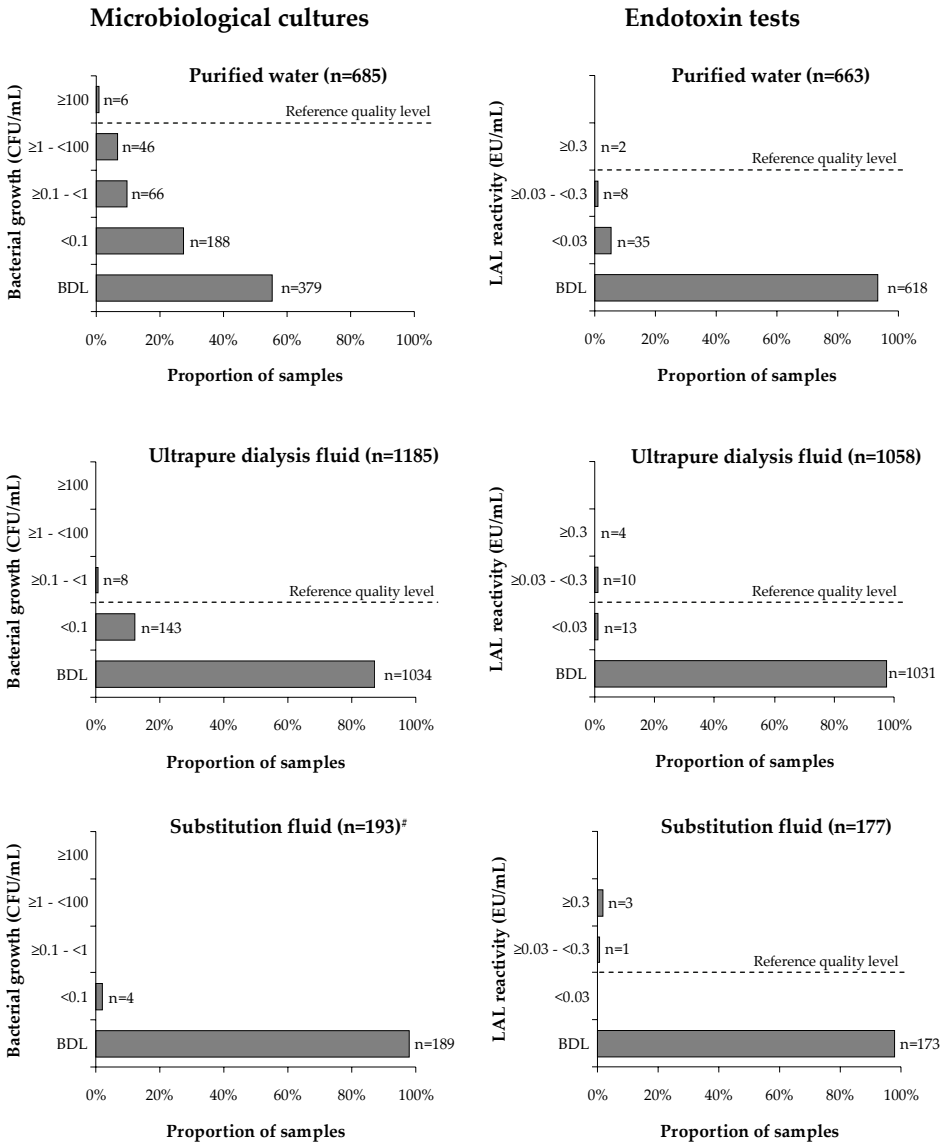

Figure 2. Proportions of samples meeting reference quality levels in the participating centers during the one year follow-up period

BDL: Below detection limit; CFU: colony forming unit; LAL: limulus amoebocyte lysate; EU: endotoxin units.

The detection limits varied according to sampling volume.

Detection limits ranged from 5 x 10-4 CFU/mL to 5 x 10-5 CFU/mL (samples volumes 2 to 20 L), which was higher than the reference quality level (i.e. 10-6 CFU/ml). To comply with the reference quality level, sample volumes up to 1000 L would be required. Therefore, the proportion of cultures complying with the reference quality could not be calculated.

Impact of water treatment system on quality of purified water

The bacterial contamination level of the purified water was below 0.1 CFU/mL in 82.8% of the samples. Water quality in the center with a single RO (in combination with an EDI and UV disinfection) was not worse (90.6% < 0.1 CFU/mL) than in centers with a double RO (80.2% <0.1 CFU/mL). We did not analyze this statistically, since only one center was equipped with a single RO module (Table 3, center 4). In centers with an EDI, 85.9% of the cultures were below 0.1 CFU/mL as compared to 77.4% in centers without an EDI (Figure 3, p=0.004). UV disinfection, type of material of the distribution circuit and disinfection methods did not have impact on the microbiological water quality (Figure 3).

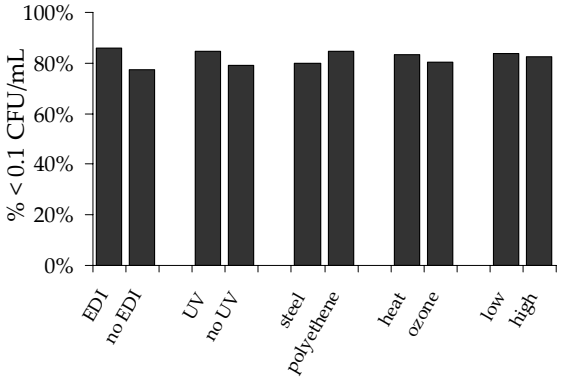

Figure 3. Effect of components of water treatment systems on microbiological quality of purified water.
† low disinfection frequency of the distribution circuit: < 4 times per week.
‡ high disinfection frequency of the distribution circuit: ≥ 4 times per week.
The amount of RO modules was not statistically analyzed, since only one center had a single RO and all other centers had a double RO module.

Discussion

The present study shows that ultrapure dialysis fluids can be produced online for a prolonged period of time and with persistent adequate quality. For online HDF, these ultrapure dialysis fluids are subject to an additional ultrafiltration step to provide the substitution fluids that are used for infusion. Although the microbiological quality of substitution fluids is usually not measured in routine clinical practice, the absence of any adverse reaction in HDF patients during the study period strengthens the idea that the substitution fluids were non-pyrogenic. Probably, the current Dutch Quality of Care Guideline contributed to high quality water treatment and a well organized control process in all ten participating centers.

The quality of the purified water in the present study was much better than in several other studies in which water quality for online HDF was evaluated [13-15]. In two studies, low-grade contamination of the machine feeding water (purified water) was reported in a large proportion of the samples, with mean contamination levels of 33 and 68 CFU/mL [13,14]. Another study observed contamination levels above 100 CFU/mL in 15 to 20% of the samples [15]. Water purification methods were not described in these studies. In comparison, in the present study more than 80% of the purified water samples were below 0.1 CFU/mL and only 0.9% of the samples contained more than 100 CFU/mL. Notably, despite the much lower quality levels of purified water in the studies mentioned above, sterile substitution fluids were obtained after two ultrafiltration steps [15-17]. In a single center evaluation of a new water treatment system, composed of a double RO module with weekly thermal disinfection of the distribution loop, the quality of the purified water was slightly better than the present study. In that study only 0.48% of the samples contained more than 100 CFU/mL and 93.3% of the samples were below detection limit [18].

In the present study no pyrogenic reactions were encountered in more than 11 000 HDF sessions, indicating that online HDF executed as described in this paper is a safe dialysis technique. Within the last 10 years, three studies have specifically addressed patient safety of online HDF, analyzing in total more than 30 000 HDF sessions [15-17]. Two of these studies did also not report any pyrogenic reaction [15,16]. The third study reported six pyrogenic reactions in 19 200 sessions [17], which was lower than reported for standard low and high flux dialysis [19].

All participating centers complied with the Dutch Guideline with regard to the technical design of the water treatment system, distribution circuit and use of approved dialysis machines. The guideline recommends water purification by a double RO module, since there is some indication that microbiological water quality after purification with a single RO does often not comply with the reference quality level for purified water (i.e. < 100 CFU/mL and < 0.25 EU/mL, Table 1) [20,21]. Moreover, considerable biofilm formation in the piping system has been observed with water treatment systems based on a single RO module [22]. In our study, nine out of ten centers were equipped with a double RO system, resulting in adequate water quality. However, the water quality in the center with a single RO (center 4) was not inferior to the water quality in the centers with a double RO. Hence, based on these results, the added value of a second RO module in the presence of an otherwise well designed water treatment system is questionable. Our data further suggest that the addition of an EDI to a water treatment system improves water quality, which is in agreement with a previous observation [20], but conflicting with another study [21]. Although an EDI is mainly used for removal of ions from the water supply, its bactericidal

effects may be explained by extreme pH changes. Furthermore, we found no effects of type (thermal or ozone) and frequency (2 to 7 times per week) of disinfection on the water quality. However, these results should be interpreted with caution, since a limited number of centers was evaluated in our study, which did not allow for multivariable analysis to assess the impact of components of water treatment systems on water quality.

In addition to the impact of the water treatment and distribution system on water quality, the dialysis machine itself is a potential source of contamination [15,21]. Water stagnation in the machine and elevated temperatures (water heated to 36ºC) favor microbiological growth and biofilm formation in the tubing of the dialysis system [21,23]. Moreover, liquid bicarbonate concentrate is recognized as potential source of contamination, which can be avoided by using bicarbonate powder cartridges. Apart from that, biofilm formation in the connection tube between distributing circuit and dialysis machine may contaminate the machine feeding water. A double lumen tube, which was used in five of the centers, provides continuous flow and may reduce this risk. Regular disinfection of the complete flow path to minimize biofilm in the tubing system is recommended, although the impact of disinfection methods on water quality has not been studied.

The monitoring frequency and sampling sites were highly variable between the centers. The Dutch guideline recommends weekly bacterial monitoring of the distribution circuit exit (sampling site 3, Figure 1), but only six of the ten centers complied with this recommendation. On the other hand, monitoring of machine feeding water is not recommended, but was performed in four of the centers. In agreement with a previous report [21], we found that contamination of purified water (sampling sites 1, 2 and 3, Figure 1) and ultrapure dialysis fluid (sampling site 4, Figure 1) did not coincide. This suggests that sampling of purified water may be of limited value. Substitution fluids were monitored in three centers. However, to comply with the reference quality level for substitution fluids (i.e. < 1 x 10-6 CFU/mL) sample volumes up to 1000 L should be required, which is not practical in clinical practice. Sample volumes in the three centers ranged from 2 to 20 L, thus resulting in insufficient detecting limits and inconclusive interpretation of results.

The present study was not designed to develop new or validate existing guidelines. However, the data allow making several recommendations on the design of water treatment systems and the optimal frequency of microbiological surveillance. In the first place, the present study suggests that adequate water quality can be produced with each of the water treatment systems as described in the study. However, it is possible that a more basic water treatment system might provide identical water quality. The added value of a second RO module remians unclear, but it is unlikely that a properly designed trial comparing one and two RO modules on water quality or on biofilm formation will ever be performed. Most

likely, the best available data will be obtained by large observational studies. Nevertheless, such studies are needed to assess which type of water treatment and disinfection is most cost-effective and has the lowest environmental burden. Secondly, the present data support the idea that microbiological surveillance should be focused on ultrapure dialysis fluids (sampling site 4, Figure 1). Upstream sampling (sampling sites 1, 2 and 3, Figure 1) should only be mandatory in case of abnormal results, and may be valuable to locate a source of contamination. Further downstream sampling, i.e. sampling of substitution fluids (sampling site 5, Figure 1) is not practical in clinical practice and is therefore not recommended. Notably, a high monitoring frequency may give a false feeling of security [9] and is expensive. For example, the estimated sampling costs of € 1000,- per dialysis machine per year in center 1, would increase by 50% to € 1500,- per machine when the monitoring strategy of center 4 is followed (Table 4, assuming € 35,- per LAL test and € 30,- per culture). A monthly sampling frequency of ultrapure dialysis fluids, as recommended by the Dutch guideline, is arbitrary and should be a topic for debate.

Limitations and strengths

The Dutch guideline has been developed to guarantee a sustained and adequate quality of dialysis solutions for HDF in all Dutch dialysis centers. The guideline promotes uniformity of water treatment and monitoring methods. However, within the scope of the guideline, there are differences in for instance water disinfection strategies or analytical methods. As the centers participated on a voluntary basis, this might have introduced a minor bias in the results of this study.

All microbiological samples were obtained in aseptic conditions to prevent contamination. Nevertheless, it is conceivable that in the present study a proportion of the incompliant samples were false positive due to contamination during the sampling procedure or processing. Although we did not specifically evaluate sampling procedures, it is possible that variation in procedures among centers has contributed to apparent differences in water quality. Furthermore, all centers used LAL assays for determination of endotoxins, which are only sensitive to intact lipopolysaccharides (LPS). Hence, endotoxins produced by waterborne bacteria, such as Pseudomonas species, or bacterial DNA fragments remain undetected. Recently it was shown that with a novel bio-assay, an inflammatory response could be detected in approximately 10% of dialysis fluid samples that complied to the definition of ultrapure when using a standard LAL assay [24]. The role of such bio-assays for the quality control process of substitution fluids is not yet clear, especially since in the present study no pyrogenic reactions were observed.

We are among the first to describe the quality control process for online production of substitution fluids in clinical practice. Although the importance of high water quality for HD and HDF has been acknowledged [25], only a limited number of studies have been performed on this topic. Our data may be of interest for health care specialists involved in convective dialysis modalities, to further improve the quality control process and quality of substitution fluids.

Conclusion

The present study demonstrated sustained and adequate quality of ultrapure dialysis fluids. Moreover, no pyrogenic reactions were reported in HDF patients. This suggests that sterile and non-pyrogenic substitution fluids for online HDF can be ensured for a prolonged period of time, provided that quality standards for water treatment systems and microbiological monitoring are satisfied. The current Dutch Quality of Care Guideline on water treatment for HDF may have contributed to the fact that all dialysis centers under study had installed a high quality water treatment system and had implemented a well organized quality control process. The frequency of microbiological monitoring and the policy in case of contaminated samples was variable among centers, which should be the subject of future guideline development.

Acknowledgements

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Chapter 3

Effects on biochemical parameters

Chapter 3.1

Benefits of residual renal function on ß2 microglobulin, phosphate control and anemia management in hemodialysis patients

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Abstract

Background

In contrast to peritoneal dialysis, the benefits of residual renal function (RRF) in hemodialysis (HD) patients have not been well studied. The aim of the present study was to investigate the role of RRF on β_2 microglobulin (β_2M), phosphate control and anemia management in a large cohort of HD patients.

Methods

For the present analysis baseline data of 569 consecutive patients (274 anuric patients and 295 patient with residual renal function [RRF]) from the Convective Transport Study (CONTRAST) was used. Patients with RRF were subdivided in tertiles, according to degree of glomerular filtration rate (GFR). Several parameters, including β_2M, phosphate, hemoglobin, use of phosphate binding agents and erythropoietin stimulating agents (ESA), and achievement of phosphate and hemoglobin treatment targets (i.e. phosphate between 3.5 and 5.5 mg/dL and hemoglobin larger than 11g/dL, respectively) were evaluated. The independent relations of GFR with β_2M, phosphate and ESA index (ESA dose divided by hematocrit) were analyzed with multivariable linear regression models.

Results

Pre-dialysis β_2M levels were much lower in patients with GFR > 4.13 mL/min/1.73m^2 (i.e. upper tertile) as compared to anuric patients (17.6 ± 0.62 g/L versus 39.0 ± 0.84 g/L, ± SE, $p<0.001$). Phosphate treatment targets were achieved in 68% of patients within the upper tertile, as compared to 45% in anuric patients (p for trend=0.007), despite a decrease in prescription of phosphate binding agents (p for trend=0.001). Moreover, ESA requirements were 30% lower in patients within the upper tertile as compared to anuric patients (p-value for trend = 0.003), while hemoglobin levels were comparable (overall mean 11.9 ± 1.2 g/dL). In multivariable regression models, GFR showed an independent relation with β_2M level (B = -2.3, 95% CI -2.6 to -1.9, $p<0.001$), phosphate level (B= -0.10, 95% CI -0.15 to -0.05, $p<0.001$) and ESA index (B=-0.12, 95% CI -0.24 to -0.01, p=0.02).

Conclusion: This study demonstrated beneficial effects of RRF on β_2M, phosphate control and anemia management in HD patients, which may contribute to improved clinical outcome. Efforts to preserve RRF in HD patients should be encouraged.

Introduction

The benefits of residual renal function (RRF) have been widely recognized in peritoneal dialysis (PD) patients [1]. In these patients, the presence of RRF not only contributes to improved dialysis adequacy, it also contributes to increased clearance of middle molecules and protein-bound solutes [2]. It has been clearly shown that concentrations of uremic substances such as phosphate, uric acid and β_2 microglobulin are lower in patients with RRF as compared to anuric patients [3-5]. In addition, the need for dietary and fluid restriction is reduced, which may partly explain the better nutritional state [6] and quality of life [7]. Moreover, there is some indication that requirements of epoeitin stimulating agents (ESA) are lower in PD patients with RRF [8]. Besides, the degree of RRF has been associated with left ventricular hypertrophy, independent of blood pressure or anemia level [8]. In view of these multiple beneficial effects, it is not surprising that RRF has been associated with improved survival in these patients [9,10].

In contrast to PD patients, the role of RRF in hemodialysis (HD) patients is often ignored. This may be explained by the previous notion that renal function rapidly declines after initiation of HD. Yet, several studies have demonstrated that RRF can be preserved for several years after start of HD in many patients [11,12]. Moreover, it has been suggested that the rate of decline in RRF in HD may be similar to PD when biocompatible high flux membranes are used [12]. Since the benefits of RRF may also apply for HD patients, the limited attention for RRF in HD patients is not justified.

It has been clearly shown that pre-dialysis β_2M levels are lower in HD patients with RRF, similar to PD, indicating the importance of RRF for middle molecule removal [13-15]. Moreover, it has been shown that HD patients with RRF have a survival benefit [16,17]. However, there is only limited evidence available on the effects of RRF on for instance phosphate control or anemia management. Recently, a relation between phosphate levels and RRF was observed in a cohort consisting of both HD and PD patients, but the use of phosphate binding agents was not accounted for in this study [11]. Another recent study reported lower ESA dose in HD patients with GFR ≥ 1 mL/min/1.73m^2 [18]. However, in this retrospective study ESA dose was not related to the degree of RRF and was not adjusted for potential confounders.

The aim of the present study was to investigate the role of RRF on phosphate control and anemia management in chronic HD patients. In addition, β_2M levels were evaluated to confirm previous observed relations between β_2M and RRF. For these analyses, baseline data of the CONvective TRAnsport STudy (CONTRAST) were used [19].

Materials and methods

Patients and study design

For the current analysis, baseline data of 569 consecutive patients from the CONTRAST study (NCT00205556) were used [19]. The patients were recruited from 26 dialysis centers in Canada (n=1), Norway (n=1) and The Netherlands (n=24). All patients were treated two or three times per week with chronic HD for at least two months and were considered stable with a minimum dialysis single pool Kt/V for urea of 1.2 or higher. Exclusion criteria were: age below 18 years, treatment with hemo(dia)filtration or high-flux HD in the six months preceding randomization, a life expectancy less than three months due to non-renal disease, participation in another clinical intervention trial evaluating cardiovascular outcomes and severe incompliance regarding frequency and duration of dialysis treatment. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines and was approved by all local medical ethics review boards. Written informed consent was obtained from all patients prior to randomization. Routine patient care was performed according to Quality of Care Guidelines of the Dutch Federation of Nephrology. Patients were treated with ultrapure dialysis fluids, defined as less than 0.1 colony forming units (CFU) per mL and less than 0.03 endotoxin units (EU) per mL. In most centers, water purification was based two reverse osmosis modules placed in series. The quality of the dialysis solutions were regularly monitored as part of the Dutch Quality of Care Guideline on water quality. All patients were treated with low-flux synthetic dialyzers (F6: 11%, F7: 2%, F8: 40%, Optiflux 18NR: 4% [Fresenius Medical Care, Bad Homburg, Germany], Polyflux 14L: 10%, 17L: 21%, or 21L: 3% [Gambro Corporation, Lund, Sweden], or other dialyzers: 9%). Mean dialyzer blood flow was 301 ± 40 mL/min.

Data collection

Data on demographics and medical history (i.e. history of cardiovascular disease (CVD), diabetic state and duration of dialysis) were prospectively collected, in addition to several clinical parameters (i.e. pre-dialysis blood pressure, dry weight and body mass index [BMI]) and dialysis treatment parameters (i.e. dialysis frequency, session length, intradialytic weight loss and type of vascular access). Prior to dialysis, samples were drawn for routine laboratory assessments including: hemoglobin (g/dL), β_2M (g/L), urea (mg/dL), creatinine (mg/dL), albumin (g/dL), phosphate (mg/dL), calcium (mg/dL) and intact parathyroid hormone (iPTH, pg/mL). An additional blood sample was drawn after this dialysis session for determination of post-dialysis urea and creatinine concentration. All laboratory samples were analyzed in the local hospitals by standard laboratory techniques. Calcium

concentrations were corrected for albumin using the formula: corrected calcium = calcium + 0.8 × (4 – albumin) [20]. Interdialytic urinary samples were collected in patients with a urinary production of 100 mL per day or more. Residual renal function (RRF) was expressed as glomerular filtration rate (GFR), calculated as the mean of creatinine and urea clearance and adjusted for body surface area (mL/min/1.73m^2) [21]. The plasma concentrations used for this calculation were the mean of the values before and after dialysis. GFR was considered zero in patients with a urinary production below 100 mL per day. The second generation Daugirdas formula was used to calculate single pool Kt/V for urea [22]. The normalized protein equivalent of total nitrogen appearance (nPNA), also known as protein catabolic rate (nPCR), was assessed for evaluation of nutritional state. nPRA (g/kg/day) was calculated from two blood urea nitrogen measurements and adjusted for residual renal urea clearance as described by Depner [23]. Prescription of phosphate binding agents and erythropoietin stimulating agents (ESA) was collected in 22 of 26 centers (in 490 of the 569 patients). Prescribed dosages were converted to daily defined doses (DDD), using conversion factors as provided by the World Health Organization (WHO) Drug Classification (http://www.whocc.no/atcddd/). Phosphate lowering agents comprised: calcium carbonate (ATC code A12AA04, DDD 3 g), calcium acetate (ATC code A12AA12, DDD 2 g), calcium carbonate/calcium lactogluconate (ATC A12AA20, DDD 0.5 g), sevelamer (ATC code V03AE02, DDD 6.4 g) and lanthanum carbonate (ATC code V03AE03, DDD 2.25 g). ESA comprised: darbepoetin (ATC code B03XA02, DDD 4.5 μg) and epoetin α and β (ATC code B03XA01, DDD 1000 IU). ESA index was expressed as DDD devided by hematocrit level.

Statistical analysis

All variables were reported as mean ± standard deviation (SD) or standard error (SE), median with inter quartile ranges or as proportion, when appropriate. Patients were subdivided into four groups. The first group comprised anuric patients. The second, third and fourth groups comprised patients with RRF subdivided in tertiles according to GFR. Comparisons between these groups were statistically analyzed with ANOVA for normally distributed variables, Kruskal-Wallace tests for not normally distributed variables and chi-square test for binominal variables.

A multivariable linear regression model was developed to investigate the independent relation between GFR and β_2M level. Variables were selected for the multivariable model if they were related with β_2M in a univariable regression model, using a cut-off value of $p<0.15$. Similar models were developed to investigate the relation between GFR and phosphate and between GFR and ESA index. Sex and age were entered up front in all

multivariable regression models. Additionally, body weight was entered in the model evaluating ESA index. All regressions were adjusted for clinical center.
Two-sized $p<0.05$ were considered statistically significant. All statistical analyses were performed with SPSS software (version 15.0.0; SPSS Inc. Headquarters, Chicago, Illinois, US).

Results

Patient characteristics

The primary renal diagnosis of the 569 patients is shown in Table 1. Median age of the patients was 63.9 ± 13.9 (±SD) years and 61% was male (Table 2). Patients with RRF were slightly older as compared to anuric patients (65.3 ± 0.80 [± SE] versus 62.3 ± 0.85 year, p=0.01). Ninety-five percent of the patients were treated three times per week with an arteriovenous (AV) fistula being the predominant type of vascular access (79%). Treatment times and dialysis Kt/V were higher in anuric patients as compared to patients with RRF (Table 2). The total weekly Kt/V was higher in patients with RRF (4.7 ± 0.05 [± SE]) as compared to 4.2 ± 0.04 in anuric patients ($p<0.001$).

Table 1. Primary renal diagnosis

Category	%
Renal vascular disease	28.7
Diabetes Mellitus	17.6
Primary glomerulopathy	13.4
Interstitial nephropathy	9.6
Cystic kidney disease	7.8
Multisystem disease	4.6
Other	10.5
Unknown	7.7

β_2 microglobulin

Pre-dialysis serum β_2M levels were more than two times higher in anuric patients as compared to patients with GFR > 4.13 mL/min/1.73m^2 (i.e. third tertile, Table 2). GFR was strongly related to pre-dialysis β_2M level in univariable and multivariable linear regression models (data not shown). After multivariable adjustments (for sex, age, history of CVD, diabetic state, BMI, duration of dialysis and serum albumin level), the regression coefficient (B) was -2.3 mg/dL for each 1 mL/min/1.73m^2 increment of GFR (95% CI -2.6 to -1.9, $p<0.001$).

Table 2. Patient characteristics

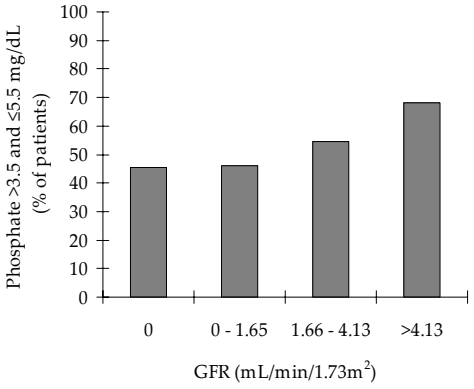
	Patients without RRF	**GFR >0 and ≤ 1.66 mL/min1.73m²**	**GFR > 1.66 and ≤ 4.13 mL/min/1.73m²**	**GFR > 4.13 mL/min/1.73m²**	**All patients**	**P-value#**
N	**274**	**98**	**99**	**98**	**569**	
Sex (% male)	61	63	67	55	61	0.40
Age (year)	62.3 ± 14	64.2 ± 16	65.1 ± 13	66.6 ± 12	63.9 ± 14	0.045
History of cardiovascular disease (%)	41	40	44	52	43	0.08
Diabetes mellitus (%)	19	28	26	26	23	0.10
Body mass index (kg/m²)	24.8 ± 4.2	25.7 ± 5.3	24.7 ± 4.3	26.0 ± 5.0	25.1 ± 4.6	0.09
Duration of dialysis (yr)	3.0 (1.5 – 5.4)	1.8 (0.8 – 3.1)	1.5 (0.83 – 2.5)	1.2 (0.7 – 2.2)	2.0 (1.0 – 4.0)	<0.001
Treatment time (hours per week)	12 (10.5 – 12)	12 (10.5 – 12)	11.3 (10.5 – 12)	10.5 (9 – 12)	12 (10.5 – 12)	<0.001
Kt/V – dialysis (per week)	4.23 ± 0.66	4.09 ± 0.54	4.04 ± 0.80	3.60 ± 0.64	4.07 ± 0.70	<0.001
Kt/V – renal (per week)	-	0.21 ± 0.10	0.66 ± 0.23	1.65 ± 0.76	0.42 ± 0.68	<0.001
Kt/V – total (per week)	4.23 ± 0.66	4.30 ± 0.54	4.69 ± 0.81	5.27 ± 0.87	4.50 ± 0.80	<0.001
Intradialytic weight loss (L)	2.13 ± 0.78	2.17 ± 0.89	1.67 ± 0.88	0.95 ± 0.91	1.86 ± 0.95	<0.001
GFRa (mL/min/1.73m²)	-	0.87 (0.56 – 1.17)	2.7 (2.2 – 3.6)	6.3 (5.1 – 8.1)	0.31 (0.0 – 2.9)	<0.001
Diuresis (L per day)	-	246 (160 – 400)	739 (500 – 1000)	1293 (990 – 1813)	700 (350 – 1150)	<0.001
Hemoglobin (g/dL)	11.8 ± 1.3	11.9 ± 1.2	11.9 ± 1.1	12.0 ± 1.3	11.9 ± 1.2	0.48
β_2 microglobulin (g/L)	39.0 ± 13.3	30.4 ± 10.3	28.3 ± 9.9	17.6 ± 5.9	32.0 ± 13.7	<0.001
Albumin (g/dL)	3.65 ± 0.44	3.60 ± 0.45	3.63 ± 0.47	3.79 ± 0.44	3.66 ± 0.45	0.12
Phosphate (mg/dL)	5.17 ± 1.63	5.54 ± 1.57	5.0 ± 1.42	4.8 ± 1.17	5.1 ± 1.53	0.003
Calcium (mg/dL)	9.30 ± 0.71	9.18 ± 0.76	9.23 ± 0.66	9.38 ± 0.65	9.28 ± 0.70	0.21
iPTH (pg/mL)	21.2 (10.0 – 38.3)	28.0 (14.4 – 38.8)	17.6 (8.6 – 32.4)	15.4 (7.7 – 26.4)	20.5 (10 – 35)	0.003

Values expressed as mean ± SD or median (interquartile range). # between groups. To convert hemoglobin in g/dL to mmol/L multiply by 0.62; albumin in g/dL to g/L multiply by 10; phosphate in mg/dl to mmol/l to multiply by 0.323; calcium in mg/dL to mmol/L multiply by 0.25.

Phosphate control

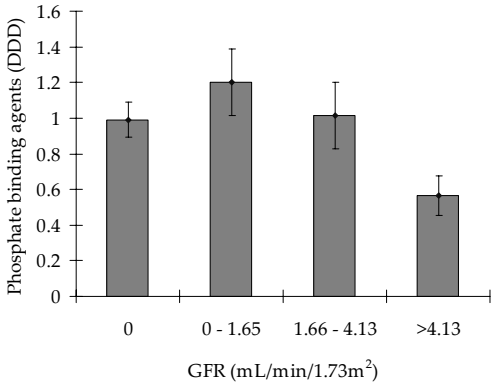
The proportion of patients with a phosphate level between 3.5 and 5.5 mg/dL increased from 45% in anuric patients to 68% in patients with GFR > 4.13 mL/min/1.73m^2 (Figure 1, p-value for trend=0.007). In addition, the patients with the highest GFR levels used less phosphate binding agents (Figure 2, p-value for trend=0.001). In a multivariable regression model (Table 3), GFR was inversely related to pre-dialysis phosphate concentration (B= -0.10, 95% CI -0.15 to -0.05, p<0.001). Apart from GFR, age and duration of dialysis were also inversely related to phosphate levels. nPNA and dose of phosphate binding agents were positively related with phosphate levels (Table 3).

Figure 1. Proportion of patients achieving phosphate treatment targets.



p=0.007 (for trend)

Figure 2. Dose of phosphate binding agents used per day subdivided per GFR category.



DDD: daily defined dose, bars represent 95% CI
p=0.001 (for trend)

Table 3. Univariable and multivariable regressions# of pre-dialysis phosphate level

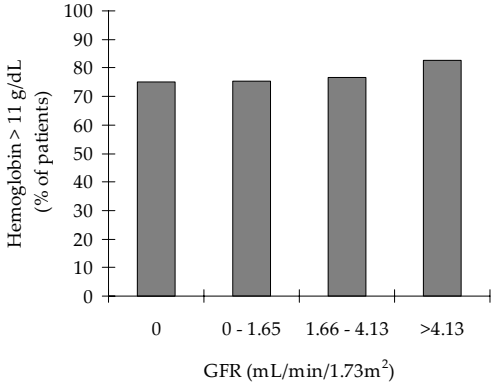
	Univariable model		Multivariable model	
Determinant	**B**	**95% CI**	**B**	**95% CI**
Sex (Male)	-0.04	-0.31 to 0.22	-0.003	-0.28 to 0.28
Age (per 10 year)	-0.25	-0.34 to -0.16†	-0.22	-0.32 to -0.12†
History of CVD	-0.04	-0.29 to 0.23		
DM	-0.06	-0.38 to 0.26		
Body mass index (per kg/m²)	0.02	-0.01 to 0.05		
Duration of dialysis (per year)	-0.05	-0.09 to 0.00†	-0.09	-0.14 to -0.04†
Weekly Kt/V (per 1 increment)	0.05	-0.15 to 0.24		
GFR (per mL/min/1.73m²)	-0.06	-0.11 to -0.02†	-0.10	-0.15 to -0.05†
Serum albumin (per g/dL)	0.70	0.34 to 1.07†	0.34	-0.05 to 0.74
nPNA (per g/kg/day)	1.20	0.70 to 1.69†	1.36	0.82 to 1.91†
Phosphate binders (per DDD)	0.35	0.17 to 0.54†	0.23	0.05 to 0.42†

adjusted for clinical center
DDD: daily defined dose.
† p<0.05

Anemia management

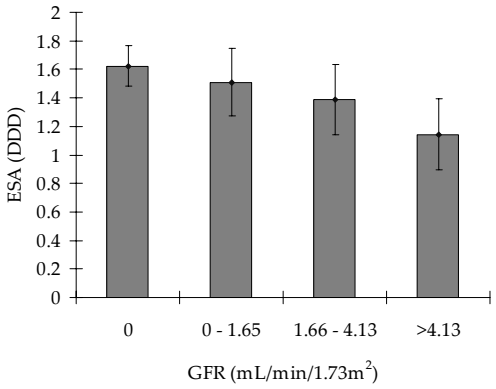
Mean hemoglobin levels were 11.9 ± 1.2 g/dL (± SD) and were not different for patients with and without RRF (Table 2). Seventy-seven percent of the patient had hemoglobin levels > 11 g/dL, which was also not different for patient with or without RRF (Figure 3, p-value for trend=0.23). ESA dose was inversely related to GFR (Figure 4, p-value for trend=0.003). In patients with GFR > 4.13 mL/min/1.73m² the mean ESA dose was 30% lower as compared to anuric patients (DDD 1.14 ± 0.13 versus 1.62 ± 0.07, p<0.001). ESA index was inversely related to male sex, GFR and albumin level (i.e. lower ESA doses were needed in males and in patients with high GFR and high albumin levels, Table 4). ESA index was inversely related with body weight (i.e. more ESA are needed in patients with large body weight, Table 4).

Figure 3. Proportion of patients achieving hemoglobin treatment targets



p=0.23 (for trend)

Figure 4. Role of residual renal function on ESA dose. #



ESA dose adjusted for body weight.
DDD: daily defined dose.
p=0.003 (for trend)

Table 4. Univariable and multivariable regressions# of ESA index

	Univariable model		Multivariable model	
Determinant	**B**	**95% CI**	**B**	**95% CI**
Sex (Male)	-0.64	-1.2 to -0.04†	-0.84	-1.5 to -0.18†
Age (per 10 year)	-0.11	-0.32 to 0.10	-0.16	-0.37 to 0.05
History of CVD	-0.25	-0.84 to 0.33		
DM	0.12	-0.60 to 0.85		
Body weight (per kg)	0.01	-0.01 to 0.03	0.03	0.003 to 0.05†
Body mass index (per kg/m²)	0.05	-0.02 to 0.12		
Duration of dialysis (per year)	-0.05	-0.15 to 0.05		
Weekly Kt/V (per 1 increment)	0.41	-0.03 to 0.85	0.19	-0.29 to 0.68
GFR (per mL/min/1.73m²)	-0.15	-0.26 to -0.04†	-0.12	-0.24 to -0.01†
Serum albumin (per g/dL)	-2.0	-2.8 to -1.2†	-2.2	-3.0 to -1.4†
nPNA (per g/kg/day)	-0.73	-1.9 to 0.41		

adjusted for clinical center
† p<0.05

Discussion

Only few studies have thus far evaluated the effects of RRF in HD patients. The present study showed substantial effects of RRF on β_2M, phosphate control and anemia management in a large cohort of adequately dialyzed HD patients. Phosphate treatment targets were reached much more often in patients with RRF, while these patients used less phosphate binding agents. Moreover, lower ESA dosages were required to achieve target hemoglobin levels. Importantly, dialysis dose and treatment time tended to be lower in the patients with RRF.

In confirmation with several other reports [13-15,24], we found a strong relation between GFR and β_2M level. β_2M is a middle molecule with a molecular weight (MMW) of 11.8 kDa and has been implicated in the development of dialysis related amyloidosis [25]. Moreover, pre-dialysis β_2M levels have been widely studied as a marker for middle molecule removal in chronic kidney disease. β_2M levels already start to rise when kidney function is only mildly impaired [26]. When patients reach stage 5 kidney disease, β_2M levels can be increased by 20-fold or more as compared to healthy controls. The present data adds evidence to the notion that even very low levels of RRF in HD patients contribute substantially to clearance of middle molecules. Recently, β_2M levels have been identified as an independent risk factor for mortality in these patients [13]. This may imply that improved middle molecule removal is one of the mechanisms explaining the lower mortality in HD patients with RRF.

Hyperphosphatemia is a well known risk factor for all-cause and cardiovascular mortality in HD patients [27-29]. It contributes to secondary hyperparathyroidism and renal osteodystrophy and has been implicated in the development of vascular calcifications [30]. Conversely, low phosphate levels have also been associated with increased mortality, partly reflecting low nutritional state in these patients [29]. Despite dietary counseling and treatment with phosphate binding agents, adequate phosphate control is not established in many HD patients. In the present study almost 50% of the patients did not comply to current treatment targets, which is in agreement with previous reports [29,31]. However, phosphate treatment targets were reached much more often in HD patients with GFR > 4.13 mL/min/1.73m2 as compared to anuric patients, while less phosphate binding agents were required. Since phosphate levels are associated with outcome, this finding may be of clinical importance. Moreover, lower medication intake may contribute to improved quality of life and reduces treatment costs.

The present data identifies RRF as an additional important determinant of ESA index (i.e. ESA dose divided by hematocrit level). ESA dosages in patients with high RRF were 30% lower than in anuric patients, which imply relevant reductions in treatment costs of these patients. The inverse relation with albumin, reflecting inflammatory state, has been well recognized [32]. Underdialysis is another determinant of ESA sensitivity, however, by design all patients in the present study had a dialysis Kt/V of 1.2 or higher. Notably, a high ESA index has been associated with increased mortality in some [33,34], but not all studies [35]. The present data suggest that it is important to control such studies for RRF. It is appealing to hypothesize that the relation between ESA sensitivity and mortality may partly be explained by RRF.

In view of the numerous beneficial effects of RRF in HD patients, efforts to preserve RRF should be encouraged. Several modifiable factors have been implicated in the preservation of renal function in HD patients [36]. In the first place, it has been recognized that both hyper- and hypotension should be avoided. Hypertension should be treated preferably with angiotensin converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB), as there is some evidence that these drugs may decrease the decline of RRF, independent of blood pressure level [37]. Diuretic use is associated with lower intradialytic weight gain [17]. Hence, it may help to prevent intradialytic hypotensive episodes and may therefore preserve residual renal function. Secondly, there is some evidence that biocompatibility of HD plays a role. The use of ultrapure dialysis fluids [38] and biocompatible membranes [12] have been associated with better preservation of renal function. Finally, nephrotoxic medication or contrast agents should be avoided as possible. Whether dialysis modalities such as daily dialysis or online hemodiafiltration (HDF), which are associated with improved hemodynamic stability, contribute to decrease in decline of RRF remains to be established.

Due to the cross-sectional design of this study, causality of the relationships could not be established. For instance, the beneficial effects of RRF may be partly explained by inflammatory state. Data from pre-dialysis chronic kidney disease patients [39] and from PD patients [40] have shown a relation between GFR and inflammatory state. Although we did adjust for serum albumin in the multivariable analysis, the value of albumin as a marker for inflammation has been questioned. The observation that phosphate was lower in anuric patients as compared to patients with GFR up to 1.66 mL/min1.73m^2 may partly be explained by misclassification. As GFR was only measured in patients with a urinary production of 100mL or more per 24 hours, it is possible that patients classified as anuric may have been producing some urine from which they received clinical benefit. Furthermore, the observation that anuric patients were somewhat younger, less often diabetic and had experienced less CVD, may be explained by the fact that the healthiest patients have the biggest chance to survive on long-term HD. The strengths of this study are the large sample size and the prospective data collection. Thus far, only few studies have evaluated medication use in large groups of HD patients.

In conclusion, the present study clearly demonstrates beneficial effects of RRF on middle molecule removal, phosphate control and anemia management in HD patients. Since the large and wide range effects of RRF in HD patients, RRF should be controlled for in clinical studies. Moreover, efforts to preserve RRF in HD patients should be encouraged.

Acknowledgements

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Chapter 3.2

Role of residual renal function on decrease of serum ß2 microglobulin levels in patients on online hemodiafiltration

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Abstract

Background

Removal of β_2 microglobulin (β_2M) may be increased by adding convective transport to hemodialysis (HD). The aim of this study was to investigate the change in β_2M levels after 6 months treatment with hemodiafiltration (HDF) and to evaluate the role of residual kidney function (RKF) and the amount of convective volume on this change.

Methods

Predialysis serum β_2M levels were evaluated in 230 patients with and 176 patients without RKF from the CONvective TRAnsport STudy (CONTRAST) at baseline and 6 months after randomization for online HDF or low-flux HD. In HDF patients, potential determinants of change in β_2M were analyzed using multivariable linear regression models.

Results

Mean serum β_2M levels decreased from 29.5 ± 0.8 (±SE) at baseline to 24.3 ± 0.6 mg/L after 6 months in HDF patients and increased from 31.9 ± 0.9 to 34.4 ± 1.0 mg/L in HD patients, the difference of change in β_2M levels between treatment groups being statistically significant (regression coefficient -7.7 mg/L, 95% CI -9.5 to -5.6, $p<0.001$). This difference was more pronounced in patients without RKF as compared to patients with RKF. In HDF patients, β_2M levels remained unchanged during follow-up in patients with GFR > 3.5 ml/min/1.73m^2. The β_2M decrease was not related to convective volume.

Conclusions

This study demonstrated effective lowering of β_2M levels by HDF, especially in patients without RKF. The role of convective volume on β_2M decrease appears limited, likely due to resistance to β_2M transfer between body compartments.

Introduction

β2 Microglobulin (β2M, 11.8 kDa) accumulates in kidney failure and has been implicated in the development of dialysis associated amyloidosis [1]. In addition, β2M levels have been widely studied as a marker for uremic toxins within the middle molecular weight (MMW) range. β2M is eliminated from the extracellular volume almost exclusively by the kidneys. Consequently, serum β2M levels already rise when kidney function is only mildly impaired [2]. In hemodialysis (HD) patients, serum β2M levels may be increased by 20-fold or more as compared to the general population, the highest levels being observed in patients without residual kidney function (RKF) [3-5]. Moreover, it has been shown that pre-dialysis β2M levels predict all-cause and infectious related mortality in these patients [3,6,7].

Because of its size, removal of β2M is negligible during low-flux HD. In contrast, significant removal of β2M can be established with high-flux HD, due to convective transport by internal filtration within the dialyzer. Both the Hemodialysis (HEMO) and the Membrane Permeability Outcome (MPO) studiy showed lower serum β2M levels in high-flux HD as compared to low-flux HD patients[3,8]. In addition, it has been shown that removal of β2M is further increased with online hemodiafiltration (HDF), by using excess ultrafiltration to provide increased convective transport. Actually, lower pre-dialysis β2M have been reported after three to twelve months treatment with HDF, as compared to low-flux or high-flux HD [9-14]. It has been proposed that the improved survival of HDF patients, as reported in few observational studies [15-17], can be partly attributed to increased removal of β2M and other MMW uremic toxins by convective transport.

For optimal efficiency of HDF treatment, the use of large convective volumes has been recommended [18]. Indeed, a relation between the delivered convective volume and β2M reduction ratio has been reported during a dialysis session [13,14]. Besides, in the Dialysis Outcomes and Practice Patterns Study (DOPPS) a survival benefit was observed only in HDF patients who were treated with high convective volumes (replacement of ≥ 15 L per treatment) [15]. However, as of yet, a direct relationship between the amount of convective volume and decrease in β2M levels on the short or long term has not been investigated.

The ongoing CONvective TRAnsport STudy (CONTRAST) has been designed to investigate the effects of increased convective transport by online HDF as compared to low-flux HD on all-cause mortality and cardiovascular morbidity and mortality [19]. As part of CONTRAST, pre-dialysis serum β2M levels were measured to evaluate short term treatment effects. The aim of the present study was to investigate the change in β2M levels from baseline to six month in patients randomized to HDF and HD. Since β2M strongly relates to RKF, the change in β2M during the study period was analyzed separately for patients with and

without RKF. In addition, the relations of the extent of RKF and the amount of convective volume with the change in β_2M levels were evaluated in HDF patients.

Materials and methods

Patients and study design

For the current analysis, the first 406 consecutive patients participating in the CONTRAST study (NCT00205556) were included who all had completed six months of follow-up by January 2009 and had serum β_2M assessments at baseline and after six months. Patients were recruited from twenty-six dialysis centers in The Netherlands (n=24), Canada (n=1) and Norway (n=1). Primary diagnoses of kidney disease were: vascular disease (30%), diabetes mellitus (17%), primary glomerulopathy (12%), interstitial nephropathy (8%), cystic kidney disease (8%), multisystem disease (5%), other (13%) or unknown (7%). The study design of CONTRAST has been described previously [19]. In short, all patients were randomized centrally into a 1:1 ratio for treatment with online HDF or continuation of low-flux HD, stratified per participating center. Upon randomization, patients were stable with a minimum dialysis single pool Kt/V for urea of 1.2 or higher. Patients were eligible for inclusion if they were treated two or three times per week with chronic HD for at least two months. Exclusion criteria were: age below 18 years, treatment with hemo(dia)filtration or high-flux HD in the six months preceding randomization, a life expectancy less than three months due to another cause than kidney disease, participation in another clinical intervention trial evaluating cardiovascular outcomes and severe incompliance regarding frequency and duration of dialysis treatment. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines and was approved by a central and local medical ethics review boards. Written informed consent was obtained from all patients prior to randomization.

Dialysis procedures

Routine patient care was performed according to Quality of Care Guidelines of the Dutch Federation of Nephrology. Treatment times were fixed during follow-up in both treatment arms, unless dialysis single pool Kt/V for urea was below 1.2. Online HDF was performed in the post-dilution mode. Blood flow rates could be increased in HDF patients to improve convective volumes. HDF patients were treated with synthetic high-flux dialyzers (FX80: 27%, FX100: 11% and Optiflux F200NR: 8% [Fresenius Medical Care, Bad Homburg, Germany], Polyflux 170H: 25% and Polyflux 210H: 27% [Gambro Corporation AB, Lund, Sweden] or other dialyzers: 2%). HD patients were treated with synthetic low-flux dialyzers

(F6HPS: 5%, F8HPS: 45% and Optiflux 18NR: 9% [Fresenius], Polyflux 14L: 2% and Polyflux 17L: 30% [Gambro], or other dialyzers: 9%). Both HD and HDF patients were treated with ultrapure dialysis fluids, defined as less than 0.1 colony forming units (CFU) per mL and less than 0.03 endotoxin units (EU) per mL.

Data collection

At baseline, data on demography, history of cardiovascular disease (CVD), diabetes mellitus (DM), type of vascular access and the duration of dialysis (dialysis vintage) were collected. In addition, treatment time, blood flow rate, intradialytic weight loss, infusion volume and blood pressure were assessed at baseline and each visit thereafter (i.e. three monthly). Systolic and diastolic blood pressures were reported as the values measured prior to dialysis. In HDF patients, infusion volumes (liter per treatment) were reported as the mean value of three consecutive treatment sessions at three and six months. Convective volumes (liter per treatment) were defined as the sum of the intradialytic weight loss and the infusion volume.

At each visit, samples were drawn prior to dialysis for assessment of urea (mmol/L), creatinine (μmol/L), phosphate (mmol/L), albumin (g/L) and hemoglobin (mmol/L). Serum β_2M (mg/L) was assessed at baseline and after six months. In addition, samples for determination of urea and creatinine were also drawn after this dialysis session. All laboratory samples were analyzed in the local hospitals by standard laboratory techniques.

Interdialytic urinary samples were collected each visit in patients with a urinary production of 100 mL per day or more. In these patients, residual kidney function (RKF) was expressed as glomerular filtration rate (GFR), calculated by the mean of creatinine and urea clearance and adjusted for body surface area (mL/min/1.73m^2). The plasma concentrations used for this calculation were the mean of the values before and after the dialysis session. GFR was considered zero in patients with a urinary production below 100 mL per day. The second generation Daugirdas formula was used to calculate single pool Kt/V for urea [20].

Statistical methods

All variables were reported as proportions or as means with standard deviation (SD) or standard errors (SE) when appropriate. Paired t-tests were used to evaluate changes from baseline to six months in the HDF and HD groups. Moreover, differences in rate of change during follow-up between the two treatment modalities were evaluated with linear regression models. To explore whether RKF modified the relation between change in β_2M and treatment modality, a multiplicative interaction term (RKF x treatment modality) was added to the regression model. This interaction term was statistically significant (p=0.006),

indicating modification (i.e. interaction). Hence, the effect of treatment modality on β_2M was reported separately for patients with and without RKF.

In the HDF patients, determinants of change in β_2M were studied using multivariable linear regression. Sex, age, history of CVD, DM, dialysis vintage, BMI, type of vascular access, dialysis frequency and serum albumin level were selected for the multivariable model if they showed a univariable relation ($p<0.15$) with the change in β_2M. In addition, GFR and convective volume were added to the multivariable model beforehand. All models were adjusted for participating center, to adjust for possible differences in β_2M assays. A p-value below 0.05 was considered statistically significant. SPSS software was used for all statistical analyses (version 15.0.0; SPSS Inc. Headquarters, Chicago, Illinois, US).

Results

Patient and dialysis characteristics

The median age of the patients (n=406) was 66 years (interquartile range 54 – 74) and 64% was male. Fifty-seven percent (n=230) had RKF with median GFR 3.2 (1.1 – 5.5) mL/min/1.73m^2. Patient characteristics were balanced between the treatment groups (Table 1). HDF patients were slightly more often diabetic (25%) than HD patients (20%) and had lower dialysis vintage (1.7 [0.8 – 3.4] versus 2.2 [1.1 – 4.1] years). Patients were predominantly dialyzing three times per week (93%). In the HDF patients, the mean convective volume was 19.1 ± 5.0 L (±SD) per treatment (interquartile range 16.4 – 22.0 L).

During follow up, Kt/V increased from 1.39 ± 0.02 (±SE) at baseline to 1.61 ± 0.03 after six months in HDF patients and from 1.36 ± 0.01 to 1.39 ± 0.02 in HD patients. The rate of change in Kt/V between the two treatment modalities reached statistical significance ($p<0.001$). The dialyzer blood flow rate increased from 302 ± 3.4 to 325 ± 4.5 mL/min in the HDF patients and remained stable in the HD patients (299 ± 3.8 and 300 ± 4.0 mL/min, respectively), with a statistically significant difference between the two treatment groups ($p<0.001$). Treatment time was stable during follow-up in both groups.

Table 1. Patient characteristics at baseline

	HDF (n=199)	HD (n=207)
Sex (% male)	62	65
Age (year)	66 (53 – 74)	66 (55 – 75)
History of cardiovascular disease (%)	45	44
Diabetes mellitus (%)	25	20
Dialysis vintage (yr)	1.7 (0.8 – 3.4)	2.2 (1.1 – 4.1)
Body mass index (kg/m²)	24.9 ± 4.7	25.0 ± 4.2
Systolic blood pressure (mmHg)	147 ± 21	150 ± 21
Diastolic blood pressure (mmHg)	76 ± 13	77 ± 11
Vascular access (% arteriovenous fistula)	76	80
Treatment time (min)	240 (210 – 240)	240 (210 – 240)
Blood flow (mL/min)	302 ± 37	299 ± 43
Dialysis Kt/V_{urea}	1.39 ± 0.22	1.36 ± 0.17
Residual kidney function (%) [a]	58	55
Urinary volume (mL/24 hours) [b]	700 (288 – 1150)	750 (350 – 1210)
Glomerular filtration rate (mL/min/1.73m²) [b,c]	3.1 (1.3 – 5.6)	3.3 (1.1 – 5.4)
Hemoglobin (mmol/L)	7.4 ± 0.8	7.4 ± 0.7
Phosphorus (mmol/L)	1.69 ± 0.52	1.63 ± 0.49
Albumin (g/L)	36.5 ± 4.6	36.7 ± 4.5
β2 microglobulin (mg/L)	29.5 ± 12	31.9 ± 13

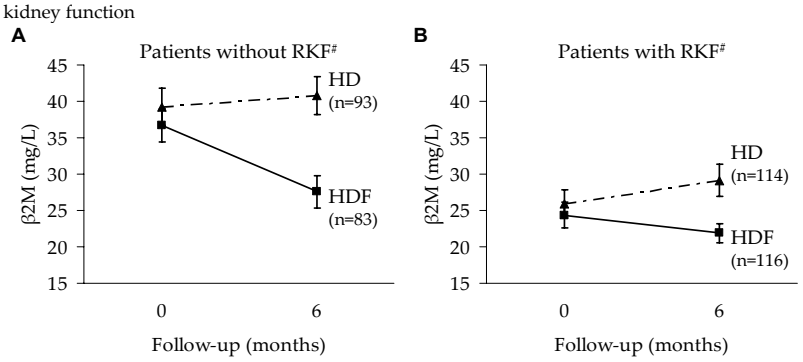
Values represent mean ± SD, median (interquartile range) or proportion (%).
a Defined as the proportion of patients with diuresis > 100 mL / 24 hours.
b In selection of patients with residual kidney function,
c Mean of urea and creatinine clearance.
To convert hemoglobin in mmol/L to g/dL, divide by 0.62; phosphate in mmol/L to mg/dL, divide by 0.323; albumin in g/L to g/dL, divide by 10.

Change in serum β2M levels from baseline to six months in HDF and HD patients

Mean serum β2M levels decreased from 29.5 ± 0.8 mg/L (±SE) at baseline to 24.3 ± 0.6 mg/L (i.e. 18% decrease, p<0.001) after six months in HDF patients and increased from 31.9 ± 0.9 mg/L to 34.4 ± 1.0 mg/L (i.e. 8% increase, p<0.001) in HD patients. The regression coefficient (B), indicating the difference of change in β2M from baseline to six months between the treatment groups was -7.7 mg/L (95% confidence interval [CI] -9.5 to -5.6, p<0.001).

Baseline β2M levels were higher in 176 patients without RKF (38.0 ± 0.9 mg/L) as compared to 230 patients with RKF (25.1 ± 0.7 mg/L, p<0.001). The difference in change of β2M from baseline to six months between the treatment groups was more pronounced in patients without RKF than in patients with RKF (B = -10.7 mg/L, 95% CI -13.9 to -7.5, p<0.001 and B = -5.6 mg/L, 95% CI -7.7 to -3.6, p<0.001, respectively, Figure 1).

Figure 1. Changes in pre-dialysis serum β2M levels by dialysis modality, in patients without (A) and with (B) residual kidney function

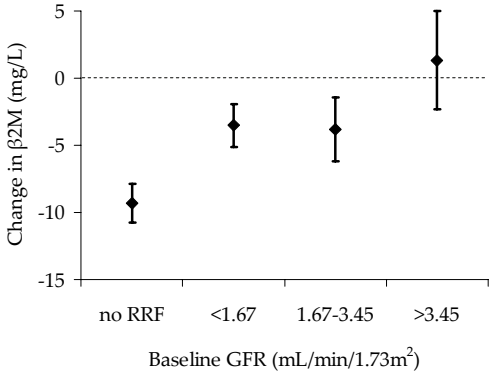


Bars represent 95% CI
RKF: residual kidney function; β2M: β2 microglobulin
# p<0.001 (for difference between HDF and HD)

Determinants of change in β2M levels in HDF patients

In HDF patients, β2M levels decreased from 36.7 ± 1.2 mg/L to 27.6 ± 1.1mg/L in absence of RKF (25% decrease, p<0.001, Figure 1A) and from 24.3 ± 0.9 mg/L to 21.9 ± 0.7 mg/L in the presence of RKF (10% decrease, p=0.001, Figure 1B). Baseline GFR in HDF patients was related to the decrease in β2M (p for trend<0.001, Figure 2). In HDF patients with a GFR > 3.45 mL/min/1.73m^2, β2M levels did not statistically change from baseline.

Figure 2. Relationship between baseline GFR and change in β2M level from baseline to six months in HDF patients§



§ adjusted for participating center
RKF: residual kidney function. Baseline GFR is subdivided in tertiles.
Bars represent 95% CI.
p-value for trend < 0.001

In a multivariable model, GFR was the only significant determinant of the change in β2M (Table 2). The regression coefficient was 0.9 mg/L per mL/min/1.73m² (95% CI 0.4 to 1.5, p<0.001), indicating that the decrease in β2M levels after six months of treatment with HDF was smaller in patients with higher GFR. Change in β2M was not related to the delivered convective volume during HDF, or any other of the evaluated variables (Table 2). In a subgroup analysis of patients without RKF, change in β2M was also not related to the delivered convective volume (data not shown).

Table 2. Results from univariable and multivariable linear regression analyses[§] of 6 month change in β2M in HDF patients

Determinant	Univariable regression		Multivariable regression	
	B	95% CI	B	95% CI
Sex (Male)	1.3	-2.0 to 4.6		
Age (per 10 year)	0.2	-0.9 to 1.3		
History of cardiovascular disease	2.5	-0.7 to 5.7	1.9	-1.3 to 5.2
Diabetes mellitus	2.7	-1.0 to 6.3	1.7	-2.1 to 5.5
Dialysis vintage (per year)	-0.4	-0.9 to 0.2		
Body mass index (per kg/m²)	0.0	-0.3 to 0.3		
Arteriovenous fistula	0.2	-3.5 to 3.9		
Convective volume (per L / treatment)	0.1	-0.3 to 0.6	0.3	-0.2 to 0.7
Dialysis frequency (per session / week)	-6.3	-12.7 to 0.1	-3.7	-10 to 3.0
GFR (per mL/min/1.73m²)	0.9	0.5 to 1.4[♯]	0.9	0.4 to 1.5[♯]
Serum albumin (per g/dL)	-0.2	-0.7 to 0.2		

The regression coefficient (B) reflects the change in β2M levels between baseline and 6 month. Positive values of B indicate smaller decreases in β2M during the study period.

[§] adjusted for participating center

[♯] p<0.001

Discussion

This study demonstrated that serum β2M levels decreased after six months of treatment with online HDF, whereas β2M levels slightly increased in HD patients. To our knowledge, the present study is the first showing that the effect of HDF on β2M levels is larger in patients without RKF as compared to patients with RKF. Moreover, in HDF patients the degree of RKF was related to the decrease in β2M, whereas the amount of delivered convective volume was not.

Previous studies in HDF patients have shown a decrease in pre-dialysis β2M levels varying from 10 to 40%, after switch from low-flux or high-flux HD. However, most studies were small (number of HDF patients ranging from 16 to 76) [9-12] or uncontrolled [13,14]. Besides, none of these studies specifically addressed the impact of RKF or convective volume on β2M decrease. Relatively large effects of HDF were reported in a randomized study in 42 patients (of which 23 HDF patients), comparing mid-dilution HDF with exchange volumes of 60 L per treatment to low-flux HD. In that study, pre-dialysis β2M

levels decreased up to 40% during the first nine months of follow-up and remained stable thereafter in HDF patients, whereas β_2M levels did not change in low-flux HD. All patients in that study were treated with ultrapure dialysis fluids. However, the presence of absence of RKF was not reported [9]. In a randomized cross-over study in 76 patients, pre-dialysis β_2M levels were 22% lower in HDF (using substitution volumes ranging from 17 to 21 L) as compared to high-flux HD patients after twelve months [10]. Sixty-eight percent of these patients were anuric at the end of the two year study period and ultrapure dialysis fluids were used in all patients. Finally, in two randomized studies in 50 and 20 mostly anuric HDF patients (post-dilution, convective volumes 8 to 12 L and 16 to 20 L, respectively), β_2M levels decreased by 10 to 15% as compared to baseline treatment with either low-flux or high-flux HD in 12 months. However, this decrease was not significantly different from high-flux HD [11,12], perhaps due to the small number of subjects. In the present study, β_2M levels decreased up to 25% in anuric patients, which is well within the range as previously reported.

An inverse relation between β_2M levels and RKF in dialysis patients has been shown previously [4,21]. In agreement, we found much higher β_2M levels in anuric patients as compared to patients with RKF. Yet, we are the first to report that the decrease in β_2M in HDF patients after conversion from conventional HD strongly relates to the degree of RKF. Whereas β_2M levels significantly decreased after six months of treatment with HDF in patients without RKF or with a GFR up to 3.5 ml/min/1.73m^2, β_2M levels did not change in HDF patients with a GFR larger than 3.5 ml/min/1.73m^2. This finding underscores the importance of RKF and suggests that a GFR above 3.5 ml/min/1.73m^2 may outweigh the effects of convective clearance by HDF for clearance of β_2M and possibly also for other MMW uremic toxins. From this perspective, more attention to preservation of RKF may be justified. At the same time, it may be proposed that especially anuric patients may benefit from HDF treatment.

The decline in β_2M levels during follow-up in the HDF patients was not related to the amount of delivered convective volume in this study, which represented everyday clinical practice. Also in the subgroup of patients without RKF no relation between convective volume and decrease in β_2M could be established. In contrast, two previous studies have observed a positive relation between the convective volume and the reduction of β_2M during HDF, although the magnitude of this relation was modest. In those studies, an increase of the convective volume from 15 L to 25 L was associated with an increase in the β_2M reduction ratio by approximately 10% (i.e. increase in β_2M reduction ratio from approximately 70 to 80%). The present data suggest that such small increases in β_2M removal during HDF do apparently not result in lower pre-dialysis β_2M levels after six

months of treatment. The absence of a relation between the amount of convection and the change in pre-dialysis β2M levels is counterintuitive but may be explained by the multicompartmental distribution of β2M. Since the removal rate of β2M by HDF is almost similar to the transfer rate from the extravascular to the vascular compartment, efforts to increase β2M removal by increasing convective transport will be disappointing [22]. Hence, alternative dialysis strategies, such as increased dialysis frequency or treatment time, are needed to further reduce β2M concentrations [22]. In fact, this has been suggested for short daily HDF [23] and daily nocturnal high-flux HD [24]. Notably, it is possible that a relation between convective volume and change in β2M could be found at lower volumes than applied in this study (i.e. below 10-15 L).

For completeness, it should be noted that other factors may contribute to the level of β2M in dialysis patients, such as bio-compatibility of treatment and inflammatory state of patients. Patients treated with cellulose membranes were shown to have higher β2M levels, especially if RKF was low [21]. Notably, adsorption to the dialyzer membrane partly contributes to β2M removal [25], which may also differ between various types of dialyzers [26]. Besides, several studies have observed a decrease in β2M concentrations after a switch from conventional to ultrapure dialysis fluid, possibly due to a decreased inflammatory response and subsequent decreased β2M production [27-29]. However, it is unlikely that these factors have played a major role in the present study, as both HD and HDF patients were treated with similar water quality and only synthetic biocompatible dialyzers were used. Interestingly, some evidence exists that RKF can be preserved with the use of biocompatible membranes [30,31] or with ultrapure dialysis fluids [32]. Whether HDF plays a role in preservation of kidney function, in addition to biocompatibility aspects of dialysis, is currently unclear.

It can not be concluded from the present data whether maximal effects of HDF on β2M levels were already reached after six months of treatment. However, in a preliminary analysis of CONTRAST data, β2M levels at twelve months seemed to be similar to those at six months [33]. Other studies indicate that a steady state is reached after three to twelve months [9,11,12]. The strength of the present study is that the data comprise a large cohort of patients from a randomized controlled trial. Importantly, HDF and HD patients were treated according to similar protocols and with similar treatment times.

In conclusion, the present study demonstrated that a considerable decrease in pre-dialysis β2M levels can be obtained after six-months of treatment with HDF in comparison with low-flux HD. This decrease was much more pronounced in patients without RKF, suggesting that especially these patients are most likely to benefit from HDF. In addition, kidney clearance of β2M (and possibly also other MMW solutes) seems to be much more important

than convective clearance by HDF in patients with a GFR >3.5 mL/min/1.73m^2. Furthermore, this study showed that the amount of convective volume, within the range used during HDF in clinical practice, is not related to the decrease in pre-dialysis β_2M levels, likely due to resistance to β_2M transfer between extracellular and intracellular body compartments. More intensified treatment regimes in terms of duration and frequency could possibly further decrease β_2M levels. Whether this leads to improved outcome remains to be established.

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Chapter 3.3

Short term effects of online hemodiafiltration on phosphate control: results from the randomized controlled CONvective TRAnsport STudy (CONTRAST)

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Submitted

Abstract

Background

Hyperphosphatemia is an independent risk factor for all-cause and cardiovascular mortality in hemodialysis (HD) patients. Phosphate control is often unsuccessful with conventional dialysis therapies.

Study design

Randomized controlled trial.

Setting and participants

493 consecutive patients from 26 centers in 3 countries, participating in the CONvective TRAansport STudy (CONTRAST).

Intervention

Online hemodiafiltration (HDF) versus continuation of low-flux HD.

Outcomes

Difference of change from baseline to six months in phosphate levels and in proportion of patients reaching phosphate treatment targets (Phosphate ≤ 5.5 mg/dL).

Measurements

Phosphate levels, use of phosphate binding agents and the proportion of patients achieving treatment targets at baseline, three months and six months.

Results

Phosphate levels decreased from 5.18 ± 0.10 mg/dL (±SE) at baseline to 4.87 ± 0.10 mg/dL at six months in the HDF patients ($p<0.001$) and remained stable in the HD patients (5.10 ± 0.10 mg/dL at baseline and 5.03 ± 0.10 mg/dL after six months, $p=0.49$). The difference of change in phosphate levels between HD and HDF patients (B=-0.24; 95% CI -0.52 to 0.03, $p=0.08$) increased after adjustment for phosphate binder usage (B = -0.36; 95% CI -0.65 to -0.06, $p=0.02$). The proportion of patients reaching phosphate treatment targets increased from 64% to 74% in the HDF patients and remained stable in the HD patients (66% and 66%), the difference between groups reaching statistical significance ($p=0.04$). Nutritional parameters and residual renal function were similar in both treatment groups.

Limitations

Patients participated in a randomized controlled trial with pre-defined inclusion criteria, which may limit the generalizibility of the results.

Conclusion

HDF may help to improve phosphate control. Whether this contributes to improved clinical outcome remains to be established.

Introduction

Treatment of hyperphosphatemia in dialysis patients remains a therapeutic challenge. It has been shown that almost 50% of hemodialysis (HD) patients have phosphate levels above recommended treatment targets, despite the use of phosphate lowering agents and dietary recommendations [1,2]. Hyperphosphatemia contributes to secondary hyperparathyroidism and renal osteodystrophy. Furthermore, it has been well recognized as an independent predictor of mortality in dialysis patients [1-6], possibly by facilitating the development of vascular calcifications [7]. It has been estimated that each mg/dL (or 0.323 mmol/L) increment of phosphate increases the relative mortality risk by 5% and increases the risk of cardiovascular mortality by 10% [2,3]. Especially when phosphate levels exceed 5.5 to 6.0 mg/dL (1.78 to 1.94 mmol/L) mortality risks start to rise considerably [1,3,8]. Hence, lowering of phosphate levels in these patients may improve clinical outcome.

There is increasing awareness that bone mineral parameters can be improved with intensified dialysis therapies. Several studies have demonstrated decreased phosphate concentrations or reductions in the use of phosphate binding agents after increasing the dialysis frequency with short daily HD [9,10], and/or increasing treatment time with nocturnal dialysis [11]. Yet, most of these studies were small and observational, whereas the magnitude of the effect was highly variable. In one of the few randomized studies, pre-dialysis phosphate concentrations decreased from 5.5 to 4.4 mg/dL (20%) and oral phosphate binders were reduced or discontinued in 19 of the 26 patients after six months of treatment with nocturnal HD [12]. Comparable results have been reported for short daily HD [13].

During hemodiafiltration (HDF), the removal of small and large uremic toxins is increased by the addition of convective solute removal to diffusion, but the dialysis frequency and session length are mostly similar to conventional HD. It has been shown that phosphate removal is increased during HDF [14-17]. However, it is currently unclear whether this increased intradialytic removal leads to lower pre-dialysis phosphate levels on the short or long term. Whereas some small trials reported decreased phosphate levels after three to twelve months of HDF [14,18,19], other trials did not find such effects [20,21]. As of yet, conclusive evidence from large trials is lacking.

The aim of the present study was to investigate whether phosphate control improves after six months of treatment with HDF, as compared to conventional HD with similar dialysis frequency and session length. We therefore analyzed bone mineral parameters of patients participating in the CONvective TRAnsport STudy (CONTRAST), a large ongoing randomized controlled trial investigating the effects of HDF on all-cause and cardiovascular

mortality as compared to HD [22]. In addition, we evaluated the use of phosphate binding agents, nutritional state and residual renal function on phosphate levels in these patients.

Methods

Patients and study design

For the current analysis, the first 493 consecutive patients from the CONvective TRAnsport STudy (CONTRAST, NCT00205556) that completed six months of follow-up were included. These patients were recruited from 26 dialysis centers; i.e. in Canada (n=1), Norway (n=1) and The Netherlands (n=24). All patients were randomized centrally into a 1:1 ratio for treatment with online HDF or continuation of low-flux HD, stratified per participating center [22]. Patients were eligible for inclusion if they were treated two or three times per week with chronic HD for at least two months. Exclusion criteria were: age below 18 years, treatment with hemo(dia)filtration or high-flux HD in the six months preceding randomization, a life expectancy less than three months due to non-renal disease, participation in another clinical intervention trial evaluating cardiovascular outcomes and severe incompliance regarding frequency and duration of dialysis treatment. The study was conducted in accordance with the Declaration of Helsinki and was approved by a central and by all local medical ethics review boards. Written informed consent was obtained from all patients prior to randomization.

Treatment protocol

Upon randomization, all patients were stable with a minimum dialysis single pool Kt/V for urea of 1.2 per treatment or higher. Treatment times were fixed at baseline and could only be increased during follow-up when the Kt/V dropped below 1.2. Online HDF was performed in the post-dilution mode. Routine patient care was performed according to Quality of Care Guidelines of the Dutch Federation of Nephrology. Calcium level of the dialysis fluid was established according to current practice in the participating center, and was similar for HD and HDF. All medication, e.g. phosphate binders, was prescribed as needed, based on clinical judgment by the attending nephrologist. Both HDF and HD patients were treated with ultrapure dialysis fluids, containing less than 0.1 colony forming units (CFU) per mL and less than 0.03 endotoxin units (EU) per mL [23]. In most centers, water purification was based on two reverse osmosis modules placed in series. The quality of the dialysis solutions were regularly monitored according to the Dutch Quality of Care Guideline on water quality. Patients in the HDF group were treated with high-flux synthetic dialyzers (FX80: 25%, FX100:14%, Optiflux F 200NR: 8% [Fresenius Medical Care, Bad

Homburg, Germany], Polyflux 170H: 24% and Polyflux 210H: 27% [Gambro Corporation AB, Lund, Sweden], or with other dialyzers: 2%). Patients in the HD group were treated with low-flux synthetic dialyzers (F6: 5%, F8: 48%, FX8: 2% and Optiflux 18NR: 8% [Fresenius], Polyflux 14L: 3%, 17L: 28% and 21L: 2% [Gambro], or other dialyzers: 4%).

Data collection

At baseline, data on demography, history of cardiovascular disease (CVD), diabetes mellitus (DM), type of vascular access and the duration of dialysis (dialysis vintage) were collected. At baseline and at the three and six months study visits, clinical parameters (blood pressure level, body mass index (BMI) and dry weight) and treatment parameters (dialysis frequency, session length, dialyzer blood flow, intradialytic weight loss and infusion volume) were assessed. The systolic and diastolic blood pressures reported are values prior to dialysis (mmHg). Convective volumes were defined as the sum of the infusion volume and the intradialytic weight loss.

At each study visit, samples were drawn prior to dialysis for routine laboratory assessments. In addition, samples for the determination of urea and creatinine were also drawn after this dialysis session. All laboratory samples, including hemoglobin (g/dL), hematocrit (%), urea (mg/dL), creatinine (mg/dL), albumin (g/dL) and the bone mineral parameters phosphate (mg/dL), calcium (mg/dL) and intact parathyroid hormone (iPTH, pg/mL) were analyzed in the local hospitals by standard laboratory techniques. Calcium concentrations were corrected for albumin using the formula: corrected calcium = calcium + 0.8 × (4 – albumin) [24]. Interdialytic urinary samples were collected in patients with a urinary production of 100 mL per day or more. In these patients, residual renal function was expressed as glomerular filtration rate (GFR), calculated as the mean of creatinine and urea clearance and adjusted for body surface area (mL/min/1.73m^2) [25]. GFR was considered zero in patients with a urinary production below 100 mL per day. The second generation Daugirdas formula was used to calculate single pool Kt/V for urea [26]. To evaluate nutritional state at baseline and follow-up the normalized protein equivalent of total nitrogen appearance (nPNA), also known as protein catabolic rate (nPCR), was evaluated in addition to dry weight, BMI and serum albumin. The nPRA (g/kg/day) was calculated from two blood urea nitrogen measurements and adjusted for residual renal urea clearance as described by Depner [27].

Information on the prescription of phosphate binders was collected each visit in 24 of the 26 centers (in 438 of 493 patients). The proportion of patients using phosphate binders during the study was reported separately for calcium containing salts (calcium carbonate, calcium acetate and calcium lactogluconate) and for non-calcium phosphate binding agents (sevelamer and lanthanum carbonate). We defined the phosphate treatment goal as a

phosphate concentration ≤ 5.5 mg/dL (i.e. ≤ 1.78 mmol/L), which is the upper limit of the range defined by KDOQI [24].

Data analysis

All variables were reported as means ± standard deviation (SD) or standard errors (SE) medians with interquartile range or as proportions, when appropriate. Comparisons of bone mineral and nutritional parameters within HDF and HD patients over time were statistically evaluated with paired t-tests. Proportions of patients using phosphate binders and achieving the phosphate treatment goal within the groups over time were compared with McNemar tests. Between groups comparisons were analyzed with t-tests for continuous or chi-square tests for dichotomous variables.

The difference of change in phosphate concentration and in the proportion of patients reaching phosphate treatment targets from baseline to six months between the two treatment modalities was evaluated with a linear regression model. To evaluate the effects of residual renal function, nutritional state and medication on the change in phosphate concentrations during follow-up, GFR, nPNA and phosphate binder usage were added to a similar linear regression model. Two-sided $p<0.05$ were considered statistically significant. All statistical analyses were performed with SPSS software (version 15.0.0; SPSS Inc. Headquarters, Chicago, Illinois, US).

Results

Patient characteristics

Baseline patient characteristics of the 251 HDF and 242 HD patients are summarized in Table 1. The HDF patients were more often diabetic (26%) as compared to the HD patients (20%). Median dialysis vintage was 2.2 (inter quartile range 1.1 – 4.0) in the HD patients and 1.8 (0.9 – 3.3) in the HDF patients. Overall, baseline characteristics were well balanced between the groups. The majority of the patients was treated three times per week with a mean session length of 226 ± 23 min (± SD). Dialysis frequency and treatment time did not change during follow-up in both groups. Mean blood flow values were 323 ± 39 mL/min for the HDF patients and 307 ± 37 mL/min for the HD patients. The HDF patients were treated with mean convective volumes of 19.5 ± 4.3 L per treatment. Single pool Kt/V urea increased from 1.38 ± 0.01 (± SE) at baseline to 1.59 ± 0.02 after six months in the HDF patients ($p<0.001$) and from 1.36 ± 0.01 to 1.40 ± 0.01 in the HD patients ($p<0.001$). The rate of change in Kt/V was significantly different across the treatment groups ($p<0.001$).

Table 1. Patient characteristics in HDF and HD patients at baseline

	HDF (n=251)	HD (n=242)
Patient characteristics		
Sex (% male)	60	64
Age (year)	66 (54 – 74)	66 (54 – 75)
Body mass index (kg/m^2)	25.0 ± 4.6	25.0 ± 4.0
Systolic blood pressure (mmHg)	148 ± 21	150 ± 21
Diastolic blood pressure (mmHg)	76 ± 12	77 ± 12
History of cardiovascular disease (%)	44	43
Diabetes mellitus (%)	26	20
Dialysis vintage (yr)	1.8 (0.9 – 3.3)	2.2 (1.1 – 4.0)
Vascular access (% Fistula)	77	82
Biochemical characteristics		
Hemoglobin (g/dL)	11.9 ± 1.3	11.9 ± 1.1
Hematocrit (%)	36.2 ± 4.1	36.0 ± 3.5
Phosphate (mg/dL)	5.18 ± 1.59	5.10 ± 1.49
Calcium (mg/dL)	9.59 ± 0.74	9.53 ± 0.72
Calcium x Phosphate (mg^2/dL^2)	49.7 ± 15.7	48.4 ± 13.6
PTH (pg/mL)	20.5 (9.1 – 35.0)	19.9 (10.4 – 26.2)
Albumin (g/dL)	3.66 ± 0.44	3.68 ± 0.45
nPRA (g/kg/day)	1.31 ± 0.31	1.30 ± 0.32
GFR# (mL/min/1.73m2)	2.64 (1.57 – 4.94)	2.53 (1.09 – 5.03)
Treatment parameters		
Session length (min)	226 ± 23	226 ± 23
Dialysis frequency 2x per week (%)	8.4	5.8
Dialysis frequency 3x per week (%)	91.6	94.2
spKt/V urea	1.38 ± 0.21	1.36 ± 0.19

Mean ± SD or median (interquartile range)

Glomerular filtration rate (GFR) in 141 HDF patients (56%) and 133 HD patients (55%) with baseline diuresis ≥ 100 mL/24hr.

To convert hemoglobin in g/dL to mmol/L multiply by 0.62; phosphate in mg/dl to mmol/l to multiply by 0.323; calcium in mg/dL to mmol/L multiply by 0.25; albumin in g/dL to g/L multiply by 10.

Bone mineral parameters

Pre-dialysis phosphate concentrations decreased from 5.18 ± 0.10 mg/dL (±SE) at baseline to 4.87 ± 0.10 mg/dL after six months in the HDF patients (p=0.001) and remained unchanged in the HD patients (5.10 ± 0.10 mg/dL at baseline and 5.03 ± 0.10 mg/dL after six months, p=0.49) (Table 2). The difference in rate of change of phosphate levels from baseline to six months between the treatment modalities was not statistically significant. The regression coefficient indicating mean difference in rate of change in phosphate levels (B) was -0.24 (95% CI -0.52 to 0.03, p=0.08). After adjustment for phosphate binder usage the effect of HDF on phosphate levels increased (B = -0.36 with 95% CI -0.65 to -0.06, p=0.02). Additional adjustments for nPNA and GFR did not further change this relationship (B = - 0.35 with 95% CI -0.63 to -0.07, p=0.02).

Pre-dialysis calcium and iPTH concentrations were similar in both groups and did not change during the study period, except for a slightly lower calcium concentration in the HDF patients at three months as compared to baseline (Table 2). The calcium-phosphate

product decreased significantly in the HDF patients during follow-up, mainly due to the lower phosphate levels in these patients (Table 2).

Table 2. Effects of HDF and HD on bone mineral parameters and nutritional parameters in time

		Baseline	3 months	6 months
Bone mineral parameters				
Phosphate (mg/dL)	HDF	5.18 ± 0.10	4.90 ± 0.10a	4.87 ± 0.10a
	HD	5.10 ± 0.10	5.08 ± 0.10	5.03 ± 0.10
Calcium (mg/dL)	HDF	9.59 ± 0.05	9.51 ± 0.05a	9.54 ± 0.04
	HD	9.53 ± 0.05	9.51 ± 0.05	9.50 ± 0.05
PTH (pg/mL)	HDF	20.5 (9.1 – 35.0)	-	18.6 (11.0 – 33.3)
	HD	19.9 (10.4 – 26.2)	-	21.6 (10.4 – 36.0)
Calcium x Phosphate (mg2/dL2)	HDF	49.7 ± 1.0	46.4 ± 1.0a,b	46.6 ± 0.9a
	HD	48.5 ± 0.9	48.1 ± 1.0b	47.9 ± 1.0
Nutritional parameters				
Dry Weight (kg)	HDF	71.2 ± 0.9	71.3 ± 0.9	71.2 ± 0.9
	HD	72.1 ± 0.8	72.0 ± 0.8	71.7 ± 0.8
Serum albumin (g/dL)	HDF	3.66 ± 0.03	3.63 ± 0.03	3.61 ± 0.03a
	HD	3.68 ± 0.03	3.65 ± 0.03	3.66 ± 0.03
nPNA (g/kg/day)	HDF	1.14 ± 0.02	1.06 ± 0.02a	1.07 ± 0.02a
	HD	1.14 ± 0.02	1.06 ± 0.02a	1.07 ± 0.02a
Residual renal function				
GFR# (mL/min/1.73m2)	HDF	2.64 (1.57 – 4.94)	2.08 (1.24 – 4.14)a	1.72 (1.02 – 3.74)a
	HD	2.53 (1.09 – 5.03)	1.94 (0.89 – 4.39)a	1.65 (0.37 – 3.44)a

Mean ± SE or median (interquartile range).
In 141 HDF patients (56%) and 133 HD patients (55%) with baseline diuresis ≥ 100 mL/24hr
a $p<0.05$ (versus baseline)
b $p < 0.05$ (difference in rate of change between groups)

Nutritional parameters

All nutritional parameters were similar in HDF and HD patients at baseline and during follow-up (Table 2). In both groups, nPNA decreased from 1.14 ± 0.02 g/kg/day at baseline to 1.07 ± 0.02 g/kg/day at six months ($p<0.001$). Serum albumin concentrations also tended to decrease. In the HDF patients, serum albumin was slightly lower at six months (3.61 ± 0.03 g/dL) as compared to baseline (3.66 ± 0.03 g/dL, $p=0.02$). Dry weight and BMI did not change during the study period.

Residual renal function

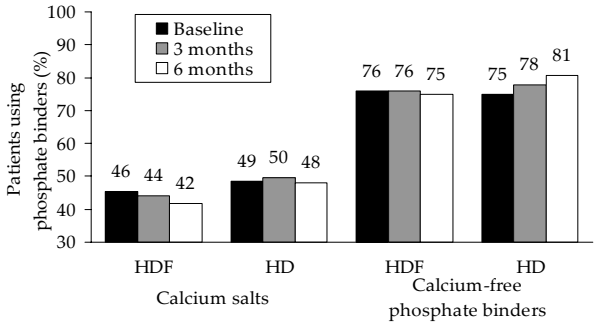
Fifty-five percent of the patients had a residual diuresis at baseline. This percentage decreased to 50% after six months. From baseline to six months, GFR decreased from 2.64 (inter quartile range 1.57 – 4.94) to 1.72 (1.02 – 3.74) mL/min/1.73m^2 in the HDF patients and from 2.53 (1.09 – 5.03) to 1.66 (0.37 – 3.44) mL/min/1.73 m^2 in the HD patients (both $p<0.001$) (Table 2). The rate of change in GFR over time did not significantly differ across treatment modalities.

Use of phosphate binders

The proportion of patients using phosphate binding agents at baseline and during follow-up is shown in Figure 1. At baseline, 87% of the patients used phosphate binding agents (calcium salt, calcium-free phosphate binding agent, or a combination of these drugs). During follow-up, this proportion slightly decreased in the HDF group to 83% and increased in the HD group to 90%. The difference in proportions between the groups at six months was statistically significant (p=0.04).

The number of tablets prescribed per day was 6.8 ± 0.35 (± SE) in HDF patients and 7.3 ± 0.43 in HD patients. After six months follow-up, the number of tablets changed to 6.5 ± 0.35 in HDF patients and remained stable in HD patients (7.3 ± 0.40).

Figure 1. Patients using calcium salts and calcium-free phosphate binders at baseline and after three or six months follow-up

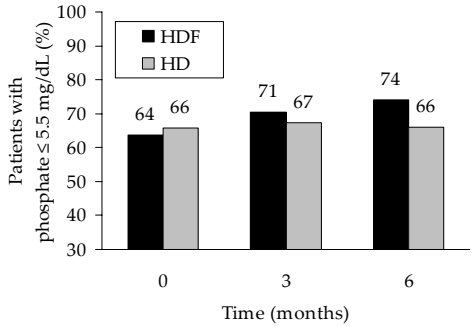


Numbers above bars represent percentages.

Phosphate treatment targets

The proportion of HDF patients with phosphate concentrations ≤ 5.5 mg/dL increased from 64% to 74% during follow-up and remained stable in the HD patients (p=0.04, difference between groups) (Figure 2).

Figure 2. Patients achieving phosphate treatment targets at baseline and after three or six months follow-up.



Numbers above bars represent percentages.
[a] p<0.05 (versus baseline)
[b] p<0.05 (difference in rate of change between groups)

Discussion

The present study demonstrated decreased pre-dialysis phosphate levels after six months of treatment with online HDF. In addition, phosphate treatment targets were satisfied more often, while the use of phosphate binding agents was reduced. These effects could not be explained by nutritional status or residual renal function, indicating a role for increased phosphate removal during HDF. Notably, the dialysis frequency and session length did not change during follow-up.

Increased phosphate removal by HDF may potentially improve clinical outcome. For example, it has been suggested that increased phosphate removal by short daily HD contributes to a decrease of left ventricular hypertrophy, independent of improvements in extracellular fluid volume control, blood pressure level or anemia management [28]. In the present study, phosphate concentrations decreased by 6% (i.e. 0.31 mg/dL or 0.10 mmol/L) after six months of treatment with HDF, despite a decrease in the prescription of phosphate binding agents. However, it is currently unclear whether such a modest decrease of phosphate concentrations will substantially contribute to an overall improved clinical outcome. Nevertheless, HDF might be an efficient dialytic strategy to improve phosphate control in specific patient populations, without the need to increase treatment time, session length, dose of phosphate binders, or to modify dietary recommendations.

Our finding that compliance with phosphate treatment targets increased by 10% with HDF, suggests that HDF may be especially beneficial for high-risk patients with difficult to control phosphate levels. Notably, the mean phosphate concentration in the present study was 5.1 mg/dL, which is lower than reported in several large databases [1,4,8], indicating that the

patients were relatively well treated. The largest effects of HDF on phosphate levels were found in a small randomized controlled study in which mean baseline phosphate levels were considerably higher (5.8 mg/dL) than in the present study. In that study in 12 highly-selected patients, pre-dialysis phosphate concentrations decreased by 24% after three months of HDF and remained stable during HD, while dietary intake and medication were kept constant [14]. On the contrary, two other randomized trials in 44 and 45 patients did not show any benefits of HDF on phosphate control [20,21]. In these trials baseline phosphate levels were 4.9 mg/dL and 5.0 mg/dL, respectively, which was even lower than in the present study.

The effects of HDF on phosphate concentrations should be considered in view of its multi-compartmental distribution and kinetic behavior during treatment. It has been shown that most phosphate is removed during the first two hours of treatment, when extracellular concentrations are high. Thereafter, phosphate removal is mainly dependent on the shift of phosphate from the intracellular compartment [29]. The increased phosphate removal during HDF has been explained by both increased removal during the first part of the treatment and increased mobilization of phosphate during the second part of treatment [14]. However, due to complex phosphate rebound mechanisms, total (weekly) phosphate removal will be higher during daily dialysis treatment.

The contribution of increased dialysis treatment time and/or session length on phosphate levels has been quantified by computer simulations [30], using a previously developed multi-compartment kinetic model [31]. With this model it was calculated that an increase in treatment time from 4 to 8 hours would result in a 6% decrease of pre-dialysis phosphate concentrations. Phosphate concentrations would be lowered by 20% after increasing dialysis frequency from 3 to 6 times per week. When session length and frequency were both increased, phosphate levels decreased by 27% [30]. Thus, whereas increased session length and dialysis frequency were both capable to lower phosphate, an increase in the dialysis frequency appeared most effective. Interestingly, these data further suggest that an increase in treatment time from 4 to 8 hours might be equally effective as a change from conventional HD to HDF. In this respect, short daily HDF may be an interesting treatment option [32].

In agreement with previous studies, nutritional parameters did not differ between HDF and HD patients, neither at baseline nor after six months [18,21,33]. Notably, improved nutritional state after conversion to HDF has been shown when conventional dialysis fluids, i.e. non-ultrapure, were used in HD patients [18]. However, in the present study patients were treated with ultrapure dialysis fluids. The importance of dialysate quality on nutritional status has been recognized previously [34] and should be considered when different dialysis techniques are compared. It is not readily apparent from the present data

why nPNA decreased during follow-up in both treatment arms. Possible explanations include the decline in GFR during follow-up [35] or regression to the mean due to the enrolment of relatively well nourished patients at the start of the study. The slight reduction of albumin levels in the HDF patients is in agreement with other reports [20,21]. Increased albumin loss during HDF as compared to HD has been described previously [36], but its clinical significance is currently unclear.

Finally, fibroblast growth factor-23 (FGF-23), which is an important regulator of phosphate and vitamin D metabolism, has recently been identified as an independent risk factor for mortality in hemodialysis patients [37]. FGF-23 (molecular weight 32 kDa) is not removed during conventional hemodialysis [38]. However, the role of HDF on FGF-23 removal remains to be elucidated.

The present study was limited by the fact that all patients participated in a randomized trial with predefined inclusion criteria, which may have compromised the generalizability of the results. However, these criteria were meant to include the average chronic HD patient and all patients were randomly allocated to HDF treatment. The strength of this study is its large sample size and the fact that all patients were treated according to routine clinical practice. Up till now, the role of HDF in improving bone mineral parameters had been conflicting. The present study is by far the largest cohort reported thus far and provides conclusive evidence on the short term effects of HDF on phosphate control.

In conclusion, the present study demonstrates that pre-dialysis phosphate levels were lowered by HDF and that treatment targets were achieved more often, while the use of phosphate binders decreased. Nutritional parameters and residual renal function were similar in both treatment modalities, suggesting that the lower phosphate levels were caused by an increased intradialytic removal. Yet, the magnitude of the effects of HDF on phosphate control was modest, especially when compared to dialysis strategies with increased frequency. Whether these effects contribute to an improved clinical outcome remains to be established.

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Chapter 4

Conclusions and perspectives

Chapter 4.1

Optimizing hemodiafiltration: tools, strategy and remaining questions

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Hemodiafiltration (HDF) is considered the best dialysis therapy currently available in terms of optimal removal of both small and middle molecules. This is obtained by combining diffusive and convective clearance. The efficacy of diffusive transport is usually quantified and monitored by the assessment of small molecule clearance. For this purpose, urea clearance expressed as Kt/V_{urea} has been widely accepted and used as parameter for dialysis adequacy. In contrast, it is currently unclear how the efficacy of convective transport should be quantified and monitored.

Pre-dialysis ß2 microglobulin (β_2M, 12.8 kD) levels have been accepted as a marker for middle molecules [1]. In two large studies β_2M levels have been associated with mortality risk, at least within the range as usually found in hemodialysis patients [2,3]. However, the clinical use of β_2M levels to monitor the effects of increased convective clearance by HDF is severely limited by its strong dependence on residual kidney function [4]. To overcome this limitation, the β_2M reduction ratio or $Kt/V_{ß2M}$ could be assessed to measure clearance during HDF, similar to urea. However, in the Hemodialysis (HEMO) study β_2M clearance did not relate to clinical outcome [5].

Alternatively, convective transport could be quantified by monitoring convective volumes. It has been shown that the convective volume is related to the β_2M reduction ratio during a single HDF session [6,7]. Importantly, the Dialysis Outcomes and Practice Patterns Study (DOPPS) showed that in order to benefit from HDF in terms of survival, the infusion volume should be at least 15 L/session (i.e. effective convective volume of 17 L/session, assuming an average net ultrafiltration of 2 L/session) [8]. These data suggest a dose and effect relation between the convective volume and clinical outcome. Current European Guidelines recommend to apply convective volumes as high as possible, with consideration of safety [9]. In practice, these large amounts of infusion fluids should be produced online. Furthermore, post-dilution HDF (infusion occurs post-filter) results in a more effective removal of uremic toxins than pre-dilution HDF (infusion occurs pre-filter) and is therefore recommended [10,11].

Recently, we have shown that achieved convective volumes were highly variable among patients and centers in a large cohort of HDF patients in a multi-center setting (Penne et al, submitted). Factors determining the convective volume in post-dilution HDF are summarized in Table 1. Modifiable treatment related factors include obvious ones as treatment time and dialyzer blood flow rate. In addition, dialyzer characteristics such as surface area, fiber inner diameter, fiber length, pore size and membrane material might be of relevance. As of yet, the role of these characteristics has not been systematically evaluated. In general, it seems wise to choose for a dialyzer with a relatively large surface area, for instance the Polyflux 210H (Gambro, Lund, Sweden) or the FX1000 (Fresenius Medical Care,

Bad Homburg, Germany). Variations in intradialytic weight loss may influence the convective volume in either direction. A large weight loss per se contributes to the convective volume. Moreover, in fluid-overloaded patients the blood may be diluted at the beginning of treatment, facilitating high filtration rates. Conversely, hemoconcentration by decreased blood volumes at the end of the session may diminish the convective volume.

Table 1. Factors determining convective volume in post-dilution HDF

Determining factors	Effect on convective volume
Patient related:	
Hematocrit ↑	↓
Total Protein ↑	↓
Serum Albumin[a] ↑	↑
Vascular access	↑↓
Treatment related:	
Blood flow rate ↑	↑
Session length ↑	↑
Filtration fraction[b] ↑	↑
Intradialytic weight loss	↑↓
Dialyzer characteristics	↑↓

[a] The association between albumin and convective volume is the result of opposite factors. High albumin levels may facilitate large convective volumes by enhancing plasma refill rates, thus diminishing blood volume changes during treatment. Alternatively, high albumin levels may compromise convective volumes by increasing the thickness of the protein layer on the dialyzer membrane, thereby decreasing membrane permeability.
[b] Maximal tolerable filtration fraction is dependent on transmembrane pressure.

The relation between convective volume and dialyzer blood flow rate is often referred to as the filtration fraction (i.e. the ratio of convective flow and blood flow). Theoretically, the ratio of convective flow and plasma flow should be used instead to account for individual differences in hematocrit level, but this is not very practical for clinical use. The relations between on the one hand treatment time, blood flow rate and filtration fraction an on the other hand the convective volume that can be achieved in post-dilution HDF are illustrated in Table 2. It is apparent from this table that the convective volume can be increased with 7.1 L per session (i.e. approximately 50%) by increasing the filtration fraction from 20 to 30% in a patient treated for 4 hours with a blood flow rate of 300 mL/min. Blood flow rates ranging from 300 to 400 mL/min as indicated in Table 2 assume the presence of an adequate vascular access. In our experience, filtration fractions between 25% and 30% are generally feasible when hematocrit levels are kept within the range as advised by current guidelines. When one of the above mentioned dialyzers is used, transmembrane pressures (TMP) are usually acceptable.

Table 2. Convective volumes in post-dilution HDF in relation to treatment time, blood flow rate, filtration fraction

Treatment time = 3h00min														
FF (%):	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Q_B = 300 mL/min	10.8	11.3	11.9	12.4	13.0	13.5	14.0	14.6	15.1	15.7	16.2	16.7	17.3	17.8
Q_B = 350 mL/min	12.6	13.2	13.9	14.5	15.1	15.8	16.4	17.0	17.6	18.3	18.9	19.5	20.2	20.8
Q_B = 400 mL/min	14.4	15.1	15.8	16.6	17.3	18.0	18.7	19.4	20.2	20.9	21.6	22.3	23.0	23.8

Treatment time = 3h30min														
FF (%):	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Q_B = 300 mL/min	12.6	13.2	13.9	14.5	15.1	15.8	16.4	17.0	17.6	18.3	18.9	19.5	20.2	20.8
Q_B = 350 mL/min	14.7	15.4	16.2	16.9	17.6	18.4	19.1	19.9	20.6	21.3	22.1	22.8	23.5	24.3
Q_B = 400 mL/min	16.8	17.6	18.5	19.3	20.2	21.0	21.8	22.7	23.5	24.4	25.2	26.0	26.9	27.7

Treatment time = 4h00min														
FF (%):	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Q_B = 300 mL/min	14.5	15.1	15.8	16.6	17.3	18.0	18.7	19.4	20.2	20.9	21.6	22.3	23.0	23.8
Q_B = 350 mL/min	16.8	17.6	18.5	19.3	20.2	21.0	21.8	22.7	23.5	24.4	25.2	26.0	26.9	27.7
Q_B = 400 mL/min	19.2	20.2	21.1	22.1	23.0	24.0	25.0	25.9	26.9	27.8	28.8	29.8	30.7	31.7

Percentages represent filtration fractions (i.e. convective flow rate divided by blood flow);
Convective volumes > 17 L per treatment are indicated in grey.
Values represent convective volumes in L per treatment.
Abbreviations: Q_B=dialyzer blood flow, FF=filtration fraction.

Based on the abovementioned considerations and our clinical experience, we suggest the following steps when initiating HDF treatment in the post-dilution mode: 1) ascertain the presence of a vascular access allowing blood flows of at least 300 mL/min, 2) choose for a relatively large dialyzer, 3) set treatment time at four hours or more, 4) keep hematocrit levels within the currently recommended range, 5) start with a filtration fraction of 25% and adjust upwards as long as TMP levels are within safe limits. With this strategy, convective volumes of at least 20 liters per session will be achieved in most cases.

At present, several uncertainties remain. Firstly, it is questionable whether β_2M is a reliable marker of middle molecules, since levels may be influenced by factors other than kidney and dialyzer clearance, for instance by inflammation. Moreover, it remains to be investigated whether lowering of β_2M by HDF leads to improved survival. Secondly, the role of β_2M for monitoring middle molecule clearance and therefore convective transport is unclear. In the HEMO study, only pre-dialysis β_2M levels were predictive for mortality in the total cohort, but β_2M clearance was not [12]. Thirdly, modeling suggests that β_2M levels may only slightly decrease when convective volumes are increased in the range as used for post-dilution HDF, which has been explained by resistance to β_2M transfer between body compartments [13]. This may indicate that favorable effects on pre-dialysis β_2M levels by increasing the convective volume from for instance 15 to 25 L per treatment may be limited.

It is therefore very well possible that increasing frequency of treatment sessions is much more effective than improving individual sessions per se.

In conclusion, results from DOPPS provide the only data relating convective transport to a meaningful clinical endpoint. This study suggested a survival benefit when infusion fluids were between 15 and 25 L per session (i.e. convective volumes approximately between 17 and 27 L per session). As explained above, the assessment of β_2M as surrogate for convective transport has important drawbacks. Given the increasing interest in online HDF, we considered it appropriate to present a practical set of recommendations which might be helpful for dialysis staff who wishes to optimize treatment with online HDF. With these recommendations, effective convective volumes of 20 L or more will often be achieved. In order to confirm and detail the observational data provided by DOPPS, properly designed clinical studies relating various levels of convective volume to clinical endpoints seem the only appropriate way to define the minimal and/or optimal convective dose.

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Chapter 4.2

Benefits and potential drawbacks of increased convective transport by online hemodiafiltration

Several studies have associated online hemodiafiltration (HDF) with improved biochemical and clinical outcomes when compared to conventional hemodialysis (HD). Since these studies were mostly small and/or observational, results should be interpreted with caution. Final results from large clinical trials such as the ongoing CONvective TRAnsport STudy (CONTRAST) are warranted to provide conclusive evidence on the clinical effects of online HDF.

The short term data from the CONTRAST study as presented in this thesis have extended the knowledge on the biochemical effects of HDF. In more than 400 CONTRAST participants it was shown that β_2 microglobulin (β_2M) levels and phosphate control significantly improved after six month of treatment with online HDF as compared to HD (Chapters 3.2 and 3.3). In addition, it was suggested that not all patients equally benefit from short-term treatment with online HDF. For instance, the favorable effects of HDF on β_2M levels were more pronounced in anuric patients as compared to patients with residual kidney function (RKF). Apart from that, it was postulated that for a valid comparison between HDF and HD, the microbiological quality of dialysis fluids should be similar for both HDF and HD patients, since suggested benefits of HDF could otherwise be confounded by differences in water quality.

In this Chapter, current understanding how increased convective transport by HDF could lead to improved clinical outcome is briefly reviewed. For a complete overview of all suggested benefits of HDF is referred to Chapter 1.1 and 1.2. Furthermore, the role of RKF on the effects of HDF in the CONTRAST study is discussed and mechanisms by which HDF could preserve RKF are proposed. In addition, the important topic of microbiological quality of dialysis solutions for online HDF is discussed separately, providing recommendations for future research. Finally, potential drawbacks and limitations of online HDF are summarized, based on CONTRAST data and current literature.

Benefits of increased convective transport

High-flux membranes have been developed to resemble more closely the clearance properties of the normal kidney. These membranes have a larger pore size as compared to low-flux membranes, resulting in clearance of solutes with a molecular weight up to 50 kD. In comparison, low-flux membranes do not remove solutes larger than 10 kD. Besides, the membrane characteristics of high-flux devices allow considerable solute removal by convective transport through a complex interplay of filtration and backfiltration within the dialyzer [1].

Many studies have demonstrated a wide range of benefits of high-flux HD. For instance, it has been shown that lipid profiles improve and clearance of several uremic toxins increase as compared to low-flux HD [2-4]. Moreover, high-flux HD has been associated with improved survival in large observational studies [5,6]. At first glance, it was therefore surprising that in the Hemodialysis (HEMO) study and in the Membrane Permeability and Outcome (MPO) study, both large randomized controlled trials, no significant effects of membrane flux on all-cause mortality were demonstrated [7,8]. Nevertheless, in post hoc analyses high-flux HD was associated with improved survival in patients who had been on dialysis for more than 3.7 years (HEMO) or in patients with diabetes, with albumin levels below 4 g/dL (MPO). The latter findings supported the notion that increased solute removal by dialysis was beneficial, especially in certain high risk patient groups, but suggested that the differences between low-flux and high-flux HD were too small to improve mortality in the overall dialysis population.

During online HDF, solute removal can be further improved by using excess ultrafiltration to increase convective transport. In this way, convective transport is two to three times higher than during high-flux HD. To date, many studies have demonstrated benefits of HDF on clearance of uremic toxins, hemodynamic stability, erythropoietin resistance and nutritional parameters in comparison with low-flux or high-flux HD (reviewed in Chapters 1.1 and 1.2). Additionally, several observational studies have reported 20 to 60% lower mortality rates in HDF patients [9-12]. At present, there are no results from clinical trials confirming these observational data. It is anticipated that by December 2010 over 700 patients will be enrolled in the CONTRAST study with one to seven years of follow-up. Hopefully, this study will provide conclusive evidence on the effects of online HDF on clinical outcomes.

Role of residual kidney function (RKF)

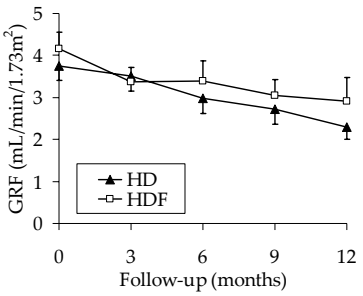
Whereas the importance of RKF has been well recognized in peritoneal dialysis (PD) patients, the attention for RKF in HD patients has thus far been limited [13]. In view of the multiple benefits of RKF this limited attention is not justified. Data from the CONTRAST study have clearly shown that HD patients with RKF have lower levels of uremic toxins, such as β_2M and phosphate, do more often reach treatment targets and use less phosphate binding agents and epoetin stimulating agents (ESA) as compared to anuric patients (Chapter 3.1). Moreover, it has been shown previously that both HD and PD patients with RKF have a better survival than patients without RKF [14-17].

Apart from these favorable effects of RKF on several metabolic and clinical parameters, the data from the CONTRAST study suggested for the first time a relation between RKF and the effect size of HDF treatment (Chapter 3.2). Whereas pre-dialysis β_2M levels decreased substantially after six month of treatment with HDF in anuric patients, the effect of HDF was less pronounced in patients with RKF. Actually, β_2M levels did not decrease at all in patients with a glomerular filtration rate (GFR) of 3.5 mL/min or higher. These data suggested that the presence of a GFR > 3.5 mL/min is more important for β_2M removal than increased convective clearance by HDF. It is conceivable, but not yet investigated, that this also applies for other uremic toxins in the middle molecular weight range. Hence, it can be postulated that HDF may be especially beneficial for anuric patients.

Since RKF is associated with improved prognosis, efforts to preserve RKF should be encouraged. Several factors implicated in decline of RKF in HD patients, of which some are modifiable, have been summarized in Chapter 3.1. Among these, hypotension during dialysis and biocompatibility of dialysis are probably the most important [18-20]. Of interest, online HDF is associated with improved hemodynamic stability and the reduced hypotensive episodes [21-23] and is exclusively performed with ultrapure dialysis fluids and highly biocompatible synthetic membranes. Therefore, it can be postulated that online HDF might contribute to preservation of RKF as compared to conventional HD.

In a preliminary analysis of the CONTRAST study, the effect of treatment modality on decline of RKF was evaluated in the first 178 consecutive patients with twelve months of follow-up (Figure 1). Although at this stage no statistically significant differences between HDF and HD were observed, further analyses in more patients and with longer follow-up are warranted.

Figure 1. Decline of RKF in 92 HDF and 85 HD patients with twelve months of follow-up.



Bars represent the standard error of the mean.
P=0.70 (p-value of the difference in decline between HDF and HD patients)

Role of microbiological water quality

It is increasingly recognized that ultrapure dialysis fluids should be used routinely for all types of HD. Ultrapure dialysis fluids have been implicated in decreased inflammatory state, preservation of RKF, lower levels of β_2M and lower requirements of ESA [24-27]. As mentioned above, for online HDF the use of ultrapure dialysis fluid (i.e. <0.1 colony forming units [CFU] per mL and < 0.03 endotoxin units [EU] per mL) is a prerequisite for safe production of non-pyrogenic substitution fluids. The production process and quality control of the ultrapure dialysis solutions used for online HDF are described extensively in Chapter 2.2. In contrast, for standard HD the quality of dialysis fluids is frequently not ultrapure (contamination levels up to 100 CFU/mL and 0.25 EU/mL are allowed for conventional HD).
In clinical studies comparing online HDF with conventional HD, it is therefore of utmost importance to treat both HD and HDF patients with ultrapure dialysis fluids. Differences in water quality may significantly confound the association between HDF and mortality as reported in observational studies [28,29]. Therefore, reporting of water quality in clinical studies should be promoted. In the Contrast study, water quality has been evaluated in 10 participating centers (Chapter 2.2), but this evaluation should be extended to the remaining centers. Further studies are needed to investigate the precise role of water quality on clinical outcomes. Large multi-center observational studies would be most appropriate for this purpose, since well powered randomized studies appear not feasible.
Although the use of ultrapure dialysis solutions is advocated, it is unclear how pure these fluids should be and how these should ideally be produced [30]. The Dutch Quality of Care Guidelines on water treatment [31] have provided recommendations for water treatment, disinfection and monitoring methods to produce sustained high quality of dialysis fluids (Chapter 2.2). However, it is currently unclear whether a water treatment system more basic than recommended by this guideline would provide similar water quality. In addition, the ideal sampling sites and frequency for microbiological monitoring of dialysis fluids should be a matter of debate, since on the one hand monitoring is expensive and on the other hand a too high monitoring frequency may give a false feeling of safety. Finally, it could be of interest to investigate the added value of an additional ultrafilter on the dialysis machine to purify the machine feeding water in the presence of an otherwise well designed and frequently disinfected water treatment system.

Potential hazards of HDF

As of yet, many different uremic toxins have been identified [32]. For several of these, increased removal by HDF has been demonstrated. However, it should be noted that

dialyzer membranes do not discriminate between harmful uremic toxins on the one hand and beneficial substances on the other hand. Accordingly, removal of beneficial substances such as water soluble vitamins, amino acids and albumin may also be increased during HDF, which could result in deficiencies or malnutrition [33].
For vitamin C (molecular size of 176 Da) it has indeed been shown that convective transport is responsible for a substantial amount of removal during HDF [34]. Nevertheless, vitamin C concentrations were not lower in HDF as compared to HD patients [35]. Moreover, folate and cobalamin levels were also similar for both HDF and HD patients [35]. Amino acid losses have been shown during high-flux HD, especially after dialyzer reuse [36], but have not been well studied in HDF patients. Finally, it has been shown that albumin loss in the dialysate is significantly greater during HDF as compared to HD [37]. Additionally, serum albumin levels in CONTRAST decreased from 3.7 g/dL to 3.6 g/dL after six months of treatment with HDF (Chapter 3.3). The clinical significance of increased albumin loss during HDF is unclear, but needs further attention. Of note, there is at present no indication that HDF is associated with malnutrition (summarized in Chapter 1.2).

Limitations of HDF

With state of the art HDF treatment, levels of uremic toxins decrease, but do not normalize. For example, pre-dialysis β_2M levels are still more than ten times higher in HDF patients as compared to healthy controls (Chapter 3.2). Moreover, despite HDF treatment, adequate phosphate control was not established in 25% of the patients (Chapter 3.3). In part, this may be explained by the multi-compartmental distribution of many uremic toxins.
During dialysis, uremic toxins are exclusively removed from the plasma. However, the plasma volume comprises only a small part of the total distribution volume. Total solute removal may therefore be limited, despite high plasma reduction rates during treatment. For β_2M is has been shown that the transfer rate from the extravascular compartment to the plasma is slower than the dialytic removal [38], suggesting that efforts to further increase the efficiency of dialysis will not result in significantly increased β_2M removal. In line with this notion, decline of β_2M levels in CONTRAST did not depend on the amount of convective volume (Chapter 3.3). For future improvements of uremic state by HD or HDF it will be required to substantially increase the frequency and/or duration of treatment. With this respect, daily HDF may be an interesting treatment option. Of note, the absence of a relation between convective volume (within the range 15 to 25 L per treatment) and β_2M decline in HDF patients does not automatically imply that there is no relationship between convective transport and clinical outcome.

Protein bound substances represent a third group of uremic toxins apart from the small water-soluble compounds and those within the middle molecular weight range [39]. Removal of these substances is limited during conventional dialysis. However, current evidence suggests that removal is not much better during convective dialysis strategies. For instance, dialytic removal of folate and cobalamin was negligible during HDF and HD, due to high protein binding. Removal of asymmetric dymethylarginine (ADMA), another highly protein bound uremic toxin, was also not different during HDF as compared to HD [40]. In contrast, removal of p-cresol was improved by HDF as compared to HD [41]. It was suggested that better elimination of the unbound fraction was the most plausible underlying mechanism. Whether short or long term treatment with HDF will result in lower pre-dialysis p-cresol levels will be a topic of investigation in the CONTRAST study.
Finally, is should be noted that all currently available dialysis therapies are inferior to the beautiful complexity of the human kidney with its interplay of glomerular filtration, tubular absorption and excretion, and endocrine functions.

Conclusion

The data presented in this thesis add evidence to the notion that removal of uremic toxins within the small and middle molecular weight range can be improved significantly with increased convective transport by HDF. Furthermore, long term HDF is safe, provided that all requirements for online production of substitution fluids are satisfied. Importantly, there is currently no indication that potentially increased removal of beneficial compounds during HDF is harmful, although increased albumin loss may be of concern. Removal of uremic toxins by HDF may be limited due to the multi-compartmental distribution of many compounds, the slow transfer rate between body compartments and the relatively short treatment times. Moreover, removal of small and middle molecular sized uremic toxins may be less apparent in patients with substantial RKF. Results from randomized controlled trials such as CONTRAST are warranted to conclude whether increased convective transport by HDF is a promise for the future.

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Summary & Samenvatting

Summary

Chapter 1. Introduction

Cardiovascular morbidity and mortality is extremely high among chronic hemodialysis (HD) patients. Already at a relatively young age, atherosclerotic complications may become manifest and approximately 50% of chronic HD patients die from cardiovascular diseases. This can only partly be explained by traditional risk factors, such as hypertension, diabetes and dyslipidemia. In recent years, novel cardiovascular risk factors have emerged in the dialysis population, such as anemia, disorders of calcium/phosphate metabolism, bio-incompatibility of HD treatment, and incomplete removal of uremic toxins. However, despite major advances in anemia management, phosphate control and the use of bio-compatible dialyzers, yearly mortality rates remain unacceptably high. Therefore, it has been suggested that retention of uremic toxins in patients treated with conventional HD is the culprit of the accelerated atherosclerosis that is observed in these patients. In particular, the retention of uremic toxins within the middle molecular weight (MMW) range (10-40kD) may play a key role in the development of cardiovascular disease. MMW uremic toxins have been associated with endothelial dysfunction and inflammation, ultimately leading to target organ damage and cardiovascular events. Hence, it has been postulated that dialysis therapies with increased removal of MMW uremic toxins, such as online hemodiafiltration (HDF) may improve the prognosis of dialysis patients.

Online hemodiafiltration.

During standard HD, small solutes, such as urea and creatinine, are almost exclusively removed by diffusion, whereas MMW solutes are largely retained. During online HDF, the removal of uremic toxins is increased by combining diffusive and convective transport. The addition of convective transport to dialysis is especially beneficial for the removal of MMW uremic toxins, such as β_2 microglobulin (β_2M, 12.8 kD). To obtain significant convective transport, filtration volumes (i.e. on average 20 L) by far exceed the desired weight loss (on average 1.5-2.5 L). Therefore, a non pyrogenic substitution fluid, which is produced 'online' by the dialysis machine, is continuously infused into the bloodstream of the patient in order to maintain fluid balance during treatment.

CONvective TRAnsport STudy (CONTRAST).

Several small and mostly non-randomized studies have shown that treatment with online HDF results in superior removal of MMW uremic toxins in comparison with conventional HD. However, it is not yet clear whether treatment with online HDF improves survival in

these patients. The CONvective TRAnsport STudy (CONTRAST) has been initiated to provide conclusive evidence whether online HDF leads to a lower mortality and less cardiovascular events as compared to conventional HD. Secondary outcomes include differences in specific vessel wall characteristics, nutritional state, quality of life and selected laboratory parameters. In this ongoing trial, approximately 700 chronic HD patients will be randomized between online HDF and low-flux HD and followed-up for one to seven years. The main results are expected in 2011 and may substantially contribute to our understanding of online HDF and its role in the treatment of chronic HD patients. The studies presented in this thesis are part of the first data of the CONTRAST study.

Chapter 2. Treatment optimization

Chapter 2.1. Determinants of convective volume

High convective volumes have been recommended to maximize solute removal during HDF. However, currently there is limited data available on factors determining maximal tolerable convective volumes in post-dilution HDF. Therefore, the aim of study presented in Chapter 2.1 was to assess the relation between patient and dialysis characteristics and the achieved convective volume in clinical practice. This study clearly suggested that sufficient treatment time (≥ 4 hours) and blood flow rate (> 300 ml/L) are a prerequisite to achieve convective volumes which are presently considered adequate (> 17 L per treatment). In addition, the data suggested that attention should be paid to current hemoglobin targets and the nutritional and inflammatory state, since convective volumes may be attenuated by high hematocrit and/or low albumin levels. Because of the observational design of the study, the exact role of dialyzer characteristics on the convective volume could not be established and should be topic for further research. Finally, it was noticed that the fraction of the blood that is filtrated during each treatment could be considerable higher in many patients, even higher than recommended by manufacturers. It was postulated that assessment of the maximal tolerable filtration fraction for each individual patient would be an effective approach to optimize HDF treatment.

Chapter 2.2. Microbiological water quality for online HDF

During online hemodiafiltration, patients are exposed to large volumes of online produced substitution fluids. Adequate water treatment and a well organized quality control process are prerequisites to provide non-pyrogenic fluids with consistent optimal quality. In Chapter 2.2 water quality, the water treatment system and the methods for surveillance of microbiological water quality in ten Dutch dialysis centers that routinely treat patients with hemodiafiltration were assessed. Microbiological monitoring results (micro-organisms and

endotoxins) were collected over a one year period, representing 11258 hemodiafiltration sessions covering 97 patients. In all centers, the equipment for water purification consisted of a reverse osmosis module in combination with a second reverse osmosis and/or an electrodeionizer. All centers regularly and routinely monitored the microbiological purity of the dialysis water with adequate analytical methods but with variable monitoring frequency. Microbiological assessments were compliant with the reference quality levels in 3923 out of 3961 samples (99%). This study suggested that non-pyrogenic substitution fluids can be produced online for a prolonged period of time. It is likely that the current Dutch Quality of Care Guideline has contributed to high quality water treatment and a well organized control process.

Chapter 3. Effects on biochemical parameters

Chapter 3.1. Benefits of residual renal function in HD patients

In contrast to peritoneal dialysis (PD) patients, the role of residual renal function (RRF) is often ignored in HD patients. However, in view of the numerous potential benefits of RRF this limited attention may not be justified. The aim of the study in Chapter 3.1 was to investigate the role of RRF on phosphate control and anemia management in chronic HD patients. In addition, serum β_2M levels were evaluated to confirm previous observed relations between β_2M and RRF. It was shown that HD patients with RRF had lower pre-dialysis β_2M and phosphate levels, and used lower dosages of erythropoietin stimulating agents to achieve target hemoglobulin levels. These data clearly showed clinical benefits of RRF in chronic HD patients. Therefore, efforts to preserve RRF in these patients should be encouraged.

Chapter 3.2. Change in β_2M levels by hemodiafiltration

Previously, some small and/or non-randomized studies have shown that the removal of β_2M can be increased by adding convective transport to HD. In Chapter 3.2 the change in serum β_2M levels after six months of HDF treatment was evaluated. In addition, the role of RRF on the change in β_2M and the relation between the amount of delivered convective volume and the change in β_2M were analyzed. This study demonstrated that pre-dialysis β_2M levels considerably decreased by HDF treatment as compared to low-flux HD. Interestingly, this decrease was much more pronounced in patients without RRF, suggesting that anuric patients may benefit the most from HDF. Moreover, it appeared that residual kidney clearance of β_2M (and possibly also other MMW toxins) was more important than convective clearance by HDF in patients with a glomerular filtration rate [GFR] >3.5 mL/min/1.73m^2. Interestingly, the amount of convective volume was not related to the

decrease in pre-dialysis β_2M levels, which was explained by resistance to β_2M transfer between extracellular and intracellular body compartments. These data suggested that more intensified treatment regimes in terms of duration and frequency could possibly further decrease β_2M levels. Whether this would lead to improved outcome needs further evaluation in large clinical trials.

Chapter 3.3. Effects of hemodiafiltration on phosphate control

Hyperphosphatemia is an independent risk factor for all-cause and cardiovascular mortality in HD patients. However, phosphate control is often unsuccessful with conventional HD and optimal medical treatment. Small studies have suggested that phosphate control may be improved by online HDF. The aim of the study presented in Chapter 3.3 was to investigate the effects of six months HDF treatment on serum phosphate levels, as compared to conventional HD with similar dialysis frequency and session length. It was shown that pre-dialysis serum phosphate levels were lowered by HDF and that treatment targets were achieved more often, while the use of phosphate binders decreased. Nutritional parameters and RRF were similar in both treatment modalities, suggesting that the lower phosphate levels were caused by increased intradialytic phosphate removal. Yet, the magnitude of the effects of HDF on phosphate control was modest, especially when compared to dialysis strategies with increased frequency. Whether the effects of HDF on phosphate control contribute to improved clinical outcome remains to be established.

Chapter 4. Conclusions and perspectives

Chapter 4.1. Optimizing hemodiafiltration

Current treatment guidelines have recommended to apply the highest possible convective volumes during online (post-dilution) HDF, providing that safety is guaranteed. In Chapter 4.1, a practical set of recommendations is presented, which might be helpful for dialysis staff who wishes to optimize treatment with online HDF. These recommendations include: increasing treatment time and blood flow rate, and assess the maximal tolerable filtration fraction, taking into account hematocrit and protein levels. With these recommendations, effective convective volumes of 20 L or more likely to be achieved.

Chapter 4.2. Benefits and drawbacks of online HDF

In this Chapter, current understanding how increased convective transport by HDF could lead to improved clinical outcome is briefly reviewed. Furthermore, the role of RRF on the effects of HDF in the CONTRAST study is discussed and mechanisms by which HDF could preserve RRF are proposed. Although preliminary data do not show any differences in the

decline of RRF between HDF and HD after one year of follow-up, further analyses with more subjects and longer follow-up are needed. In addition, the important topic of microbiological quality of dialysis solutions for online HDF is discussed, providing recommendations for future research. Finally, potential drawbacks and limitations of online HDF are summarized, based on CONTRAST data and current literature. For example, it could be postulated that not only uremic toxins, but also beneficial solutes, such as vitamins or amino acids, are increasingly removed by HDF. Thus far, however, no clinical significant loss of any beneficial substance has thus far been reported.

Results from randomized controlled trials such as CONTRAST are warranted to provide conclusive evidence on the role of online HDF to improve the prognosis of chronic HD patients.

Samenvatting

Hoofdstuk 1. Introductie

Patiënten die chronisch worden behandeld met hemodialyse hebben een sterk verhoogd risico op het krijgen van hart- en vaatziekten. Vaak blijkt slagaderverkalking al op jonge leeftijd voor te komen en uiteindelijk overlijdt ongeveer de helft van alle dialysepatiënten aan hart- en vaatziekten. Dit kan slechts gedeeltelijk worden verklaard door traditionele risicofactoren zoals hoge bloeddruk, suikerziekte en afwijkingen van de vetstofwisseling. Daarom neemt men tegenwoordig aan dat bij chronische hemodialysepatiënten ook nog andere risicofactoren een rol spelen, zoals de bij nierziekte optredende bloedarmoede en verstoorde calcium- en fosfaathuishouding. Daarnaast zou ook de hemodialysebehandeling zelf kunnen bijdragen aan het verhoogde risico op hart- en vaatziekten, doordat afvalstoffen in het bloed tijdens de behandeling onvolledig verwijderd worden en doordat het bloed in contact komt met een lichaamsvreemde spoelvloeistof en met het dialysemembraan.

Ondanks verbeteringen in de behandeling van bloedarmoede met behulp van erytropoëtine (EPO) en door de introductie van zogenaamde bio-compatibele dialysemembranen, blijft de sterfte onder dialysepatiënten met ongeveer 20% per jaar onverminderd hoog. Waarschijnlijk is dit voor een aanzienlijk deel toe te schrijven aan hoge concentraties van middelgroot- en grootmoleculaire afvalstoffen in het bloed, die tijdens standaard hemodialyse onvoldoende worden verwijderd. Daarom zijn pogingen ondernomen om de hemodialysebehandeling te intensiveren door het gebruik van extra doorlaatbare dialysemembranen en door de dialysedosis (dialyseduur en stroomsnelheid van het bloed door de kunstnier) te verhogen, maar tot op heden hebben deze aanpassingen niet geresulteerd in een afname van hart- en vaatziekten. Er wordt voortdurend gezocht naar alternatieve dialysemethoden die de prognose van hemodialysepatiënten wel zullen verbeteren.

Online hemodiafiltratie

Een van deze alternatieve methoden is de zogenaamde online hemodiafiltratie (HDF). Bij deze techniek worden afvalstoffen niet alleen verwijderd door diffusie zoals bij standaard hemodialyse (afvalstoffen verplaatsen zich door de dialysemembraan vanuit het bloed naar de spoelvloeistof doordat de concentratie van de opgeloste deeltjes lager is in de spoelvloeistof), maar ook door convectie (afvalstoffen worden uit het bloed verwijderd door filtratie van plasmawater door de dialysemembraan op basis van een drukverschil tussen bloed en spoelvloeistof). Hoe groter het convectievolume, des te meer afvalstoffen er verwijderd worden. Om de vochtbalans bij de patiënt te waarborgen wordt tijdens de

behandeling een hoeveelheid steriele en uitgebalanceerde substitutievloeistof aan de patiënt teruggegeven dat precies gelijk is aan het gefiltreerde volume. Hoewel hemodiafiltratie al sinds eind jaren zeventig bestaat, is het pas door de huidige generatie dialysemachines die de substitutievloeistof direct ter plekke (online) kunnen produceren en reguleren, mogelijk geworden om hemodiafiltratie met grote volumes (dat wil zeggen circa 20 L per behandeling) toe te passen. Hierdoor is de effectiviteit van deze behandeling sterk toegenomen. De middelgroot- en grootmoleculaire afvalstoffen , zoals β_2 microglobuline (β_2M), die tijdens standaard hemodialyse niet of nauwelijks worden verwijderd, kunnen wel adequaat verwijderd worden met online hemodiafiltratie. Er zijn aanwijzingen dat juist deze afvalstoffen schadelijk zijn voor de vaatwand en het ontstaan van slagaderverkalking bevorderen. Daarom zou behandeling met hemodiafiltratie kunnen leiden tot minder vaatschade, met uiteindelijk minder hart- en vaatziekten en een lagere sterfte.

CONvective TRAnsport STudy (CONTRAST)

Uit klinische studies blijkt inderdaad dat online hemodiafiltratie de concentratie van afvalstoffen in het bloed kan verminderen, maar het is nog onduidelijk of dit ook effect heeft op klinische uitkomsten. Enkele studies suggereren weliswaar een verminderd sterfterisico, maar door beperkingen in de onderzoeksopzet konden er geen betrouwbare conclusies aan worden verbonden. Toch wordt online hemodiafiltratie steeds vaker toegepast.

In 2004 is in Nederland de CONvective TRAnsport STudy (CONTRAST) van start gegaan. In dit onderzoek worden sterfte en hart- en vaatziekten bij online hemodiafiltratie vergeleken met die bij standaard hemodialyse gedurende 1 tot 7 jaar follow-up. Ongeveer 700 chronische hemodialysepatiënten afkomstig uit meer dan 20 dialysecentra in Nederland, Noorwegen en Canada doen mee aan dit onderzoek. De helft van deze patiënten wordt behandeld met online hemodiafiltratie, de andere helft met standaard hemodialyse. Een loting bepaalt welke behandeling een patiënt krijgt toegewezen. De eindresultaten worden in 2011 verwacht en zullen hopelijk een belangrijke bijdrage leveren aan onze kennis over online hemodiafiltratie en de rol die deze techniek kan spelen bij de behandeling van chronische hemodialysepatiënten. In dit proefschrift worden de eerste resultaten van dit onderzoek beschreven.

Hoofdstuk 2. Optimalisatie van de behandeling

Hoofdstuk 2.1 Determinanten van het convectievolume

Om de effectiviteit van online hemodiafiltratie te vergroten wordt geadviseerd om tijdens de behandeling te streven naar zo groot mogelijke convectievolumes. Echter, het is vooralsnog onduidelijk welke patiënt- en welke dialyse-gerelateerde factoren van belang

zijn om een zo groot mogelijk convectievolume te bereiken. Het onderzoek zoals beschreven in dit hoofdstuk had als doel om deze factoren te onderzoeken. Het bleek dat een lange behandelduur (minstens 4 uur) en een hoge stroomsnelheid van het bloed door de kunstnier (minstens 300 mL/min) beide gerelateerd waren aan een groot convectievolume. Daarnaast was het convectievolume groter bij patiënten met een lage hematocrietwaarde of een hoge albumineconcentratie in het bloed. Tenslotte bleek dat de fractie van het bloed dat tijdens de hemodiafiltratiebehandeling gefiltreerd wordt in veel gevallen hoger ingesteld zou kunnen worden, zelfs hoger dan door sommige fabrikanten van kunstnieren en dialysemachines wordt geadviseerd. Aldus heeft deze studie heeft meer inzicht gegeven in de factoren die het convectievolume bepalen, wat mogelijkheden biedt om de hemodiafiltratiebehandeling in de toekomst verder te verbeteren.

Hoofdstuk 2.2. Microbiologische kwaliteit van de dialysevloeistoffen voor hemodiafiltratie

Tijdens online hemodiafiltratie krijgen patiënten grote hoeveelheden substitutievloeistof toegediend. De substitutievloeistof wordt door de dialysemachine ter plekke 'online' geproduceerd. Online hemodiafiltratie is alleen toegestaan als de kwaliteit van de substitutievloeistof is gegarandeerd. In Hoofdstuk 2.2 wordt de microbiologische waterkwaliteit in 10 Nederlandse dialysecentra beschreven. Daarnaast zijn de verschillen in het productieproces van de substitutievloeistof geëvalueerd en is bekeken hoe de kwaliteit van de substitutievloeistof in deze centra gewaarborgd is. In totaal zijn er 11258 hemodiafiltratie behandelingen verspreid over 1 jaar onderzocht. De microbiologische waterkwaliteit was hoog gedurende deze gehele periode. In 3923 van de 3961 (99%) watermonsters werden geen of minder dan de toegestane concentraties micro-organismen aangetroffen. Het productieproces was vergelijkbaar tussen de centra en alle centra hadden een uitgebreid kwaliteitscontrole systeem. Wel waren er grote verschillen tussen de centra met betrekking tot de frequentie van controle. De huidige kwaliteitsrichtlijn 'Waterbehandeling voor hemodialyse en hemo(dia)filtratie' van de Nederlandse Federatie voor Nefrologie heeft waarschijnlijk bijgedragen aan de hoge waterkwaliteit en goede kwaliteitscontrole.

Hoofdstuk 3. Effecten op biochemische parameters

Hoofdstuk 3.1. Voordelen van restnierfunctie bij hemodialyse patiënten

Er wordt relatief weinig aandacht besteed aan het belang van restnierfunctie bij hemodialysepatiënten. Gezien de vele voordelen van enige restnierfunctie, zoals aangetoond bij patiënten met peritoneaal dialyse (buikspoeling, is dit gebrek aan aandacht waarschijnlijk onterecht. In dit hoofdstuk is de rol van restnierfunctie op de behandeling

van stoornissen in de calcium-fosfaat huishouding en op de behandeling van bloedarmoede onderzocht. Daarnaast is de relatie tussen de mate van restnierfunctie en β_2M geanalyseerd. Deze studie liet zien dat het fosfaatgehalte in het bloed beter gereguleerd is bij hemodialysepatiënten met restnierfunctie in vergelijking met patiënten zonder restnierfunctie, terwijl er minder fosfaatverlagende medicatie voorgeschreven werd. Daarnaast gebruikten hemodialysepatiënten met restnierfunctie minder EPO. Zoals verwacht bleek er een sterke relatie te zijn tussen de restnierfunctie en de β_2M-concentratie in het bloed. Deze studie heeft het belang van restnierfunctie bij hemodialysepatiënten laten zien. Aanvullend onderzoek naar methoden om zoveel mogelijk van de restnierfunctie bij hemodialysepatiënten te behouden zou daarom klinisch waardevol kunnen zijn.

Hoofdstuk 3.2. Verandering van β2M-concentratie door online hemodiafiltratie

De β_2M-concentratie in het bloed kan effectief verlaagd worden door het toepassen van convectief transport, zoals tijdens hemodiafiltratie. In dit hoofdstuk is onderzocht in welke mate de concentratie van β_2M verlaagd was na 6 maanden behandeling met online hemodiafiltratie. Daarnaast is gekeken wat de invloed van een zekere restnierfunctie en het convectievolume zijn op de verandering van β_2M. De restnierfunctie bleek een belangrijke factor in de afname van β_2M concentratie na 6 maanden hemodiafiltratiebehandeling. Verder bleek dat als de restnierfunctie hoger was dan een bepaald niveau, deze zelfs van groter belang was voor de verwijdering van β_2M dan de toepassing van convectie door hemodiafiltratie. Een tweede bevinding van deze studie was dat de grootte van het convectievolume geen invloed had op de verandering van de β_2M-concentratie, in tegenstelling tot wat van tevoren was verondersteld. Het blijft dus de vraag of de β_2M-concentratie nog verder verlaagd kan worden door de frequentie of de duur van de dialysebehandeling te verhogen. Om deze vraag te kunnen beantwoorden is vervolgonderzoek nodig.

Hoofdstuk 3.3. Effecten van online-hemodiafiltratie op de fosfaathuishouding

hemodialysepatiënten hebben vaak een hoog fosfaatgehalte in het bloed, hetgeen een risicofactor is voor hart- en vaatziekten. Helaas lukt het vaak niet om het fosfaatgehalte onder controle te krijgen met standaard hemodialyse en medicamenteuze therapie. Enkele kleine studies hebben laten zien dat de fosfaathuishouding met online hemodiafiltratie mogelijk verbetert. Om dit verder uit te zoeken is in Hoofdstuk 3.3 de fosfaathuishouding in een groot aantal patiënten onderzocht na 6 maanden online hemodiafiltratie respectievelijk standaard hemodialyse. Er bleek een klein verschil te bestaan in het voordeel van online hemodiafiltratie. Daarnaast hadden de hemodiafiltratiepatiënten iets minder

fosfaatverlagende medicatie nodig om de streefwaarden te bereiken. Deze resultaten werden niet beïnvloed door verschillen in voedingstoestand of restnierfunctie. Toekomstige studies moeten uitwijzen of deze kleine verschillen zich vertalen in een langere overleving van patiënten die behandeld worden met hemodiafiltratie.

Hoofdstuk 4. Conclusies en perspectieven

Hoofdstuk 4.1. Optimalisatie van online hemodiafiltratie

Huidige richtlijnen adviseren om te streven naar zo groot mogelijke convectievolumes, zolang de behandeling maar veilig kan worden uitgevoerd. In Hoofdstuk 4.1 wordt een aantal praktische aanbevelingen gedaan, die voor het dialysepersoneel bruikbaar kunnen zijn om het convectievolume te maximaliseren. Als deze aanbevelingen worden gevolgd kan er bij de meeste patiënten een convectievolume van 20 L per behandeling worden gehaald.

Hoofdstuk 4.2. Voor- en nadelen van online-hemodiafiltratie

In Hoofdstuk 4.2 worden de huidige inzichten beschreven hoe het verwijderen van afvalstoffen uit het bloed door middel van convectie zou kunnen bijdragen tot een verbeterde prognose van dialysepatiënten. Om de resultaten van eerdere studies goed te interpreteren zal onder andere rekening gehouden moeten worden met de aan- of afwezigheid van restnierfunctie en met de microbiologische kwaliteit van de substitutievloeistof. Er wordt ingegaan op de vraag of hemodiafiltratie zelf kan bijdragen tot het behoud van de restnierfunctie, hoewel voorlopige data dit vooralsnog niet laten zien. Behandeling met hemodiafiltratie kan potentieel nadelig zijn voor de patiënt als niet alleen afvalstoffen uit het bloed worden verwijderd, maar ook nuttige stoffen zoals vitamines. Gelukkig zijn er tot dusver weinig aanwijzingen dat dit inderdaad het geval is. De eindconclusie is dat grote klinische onderzoeken zoals CONTRAST onontbeerlijk zijn om vast te stellen of online hemodiafiltratie een rol kan spelen bij het verbeteren van de prognose van chronische dialysepatiënten.

Appendix

Appendix: CONTRAST Investigators

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Dankwoord

Curriculum Vitae

List of Publications

Dankwoord

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Lars Penne
Amsterdam, juli 2009

Curriculum Vitae

Lars Penne was born on August 2, 1977 in Tiel, the Netherlands. After graduating secondary school (gymnasium) at the Lingecollege in Tiel in 1995, he started his medical studies at Utrecht University. During his studies, he completed a research traineeship in the field of Nephrology at the Kolling Institute, University of Sydney, Australia (Prof. Dr C.A. Pollock). He obtained his medical degree in 2003. In the same year, he started a PhD program as a research physician for the CONvective TRAnsport Study (CONTRAST), which resulted in this thesis. He followed a Master of Sience program in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES) in Rotterdam and graduated in 2007. In 2006, he started his specialist training in Internal Medicine at the VU medical center in Amsterdam (Prof. Dr S.A. Danner and Prof. Dr M.H. Kramer). He received a Kolff Fellowship from the Dutch Kidney Foundation for a research fellowship at the Renal Research Institute in New York.

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