PART I: INTRODUCTION
MIDDLE MOLECULAR WEIGHT CLEARANCE AND HEMODIAFILTRATION: TECHNICAL ASPECTS AND CLINICAL IMPLICATIONS.

Based in part on:

Haemodialfiltration: promise for the future?


Nephrol Dial Transplant. 2008; 23(2): 438-43

Is there clear evidence that middle molecule removal is important in renal replacement therapies?


Semin Dial. 2011; 24(4): 411-3
INTRODUCTION

The first step towards the development of hemodialysis (HD) was made in 1854 by Thomas Graham, who described the principle of solute transport across a semipermeable membrane. Almost a century later, in 1944, this pre-clinical physiologic research formed the basis for the first artificial kidney for clinical use in humans developed by doctor Willem Kolff in Kampen. In 1945, he successfully treated a patient with HD for the time: a 67-year-old woman with uremic encephalopathy who regained consciousness after 11 hours of hemodialysis with Kolff’s dialyzer. Since then, the evolution of the vascular access and the progression towards more manageable dialysis equipment and more biocompatible dialyzers and dialysis fluids have highly contributed to the extracorporeal renal replacement therapy as it is applied nowadays.

Hemodiafiltration (HDF) can be regarded as conventional HD with an extra waste solute removal technique added. Actually, during HDF, diffusive and convective solute transport are combined. 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 HD or hemofiltration (HF) used separately.

HDF was first introduced in the early 1970s and initially performed with substitution fluid in plastic bags. The production of “online” sterile pyrogen-free substitution fluid, which enabled the administration of much higher volumes, was already possible as early as 1978. However, the implementation of online HDF in Europe was delayed for some 20 years because of regulatory objections, but was finally approved in 1995.

Below, theoretical and technical aspects of HDF and its implications on pre-clinical and clinical parameters, are discussed.

THEORETICAL BACKGROUND

In 2003, the European Uremic Toxin (EUTox) Work Group has composed a list of uremic retention solutes that accumulate in patients with chronic kidney disease (CKD). These substances are divided into three groups, namely (1) small free water-soluble compounds (molecular weight [MW] <500 D); (2) protein bound solutes and (3) middle molecular weight (MMW) substances (MW 500-60.000 D). As retention of toxic MMW substances may play a key role in the pathogenesis of the uremic syndrome, it is plausible that removal of these toxins by convective therapies alleviates the burden of disease in CKD patients.

In HDF, not only small molecules (<500 D) are removed more effectively as compared to low-flux HD, but in addition, a considerable clearance of MMW substances is obtained. Beta-2-microglobulin (ß2M, MW 11.8 kD) is a typical example of this category and is strongly associated with the presence of carpal tunnel syndrome and dialysis-related amyloidosis in chronic HD patients. In the HEMO study (see details below), predialysis ß2M levels were associated with all-cause mortality, even when adjusted for residual renal clearance. These data suggest that ß2M can be used as a marker for MMW toxins which contribute to the extremely high mortality in chronic HD patients, although a direct relationship between ß2M levels and mortality is lacking.
MMW CLEARANCE AND HDF

Other examples of MMW molecules include markers of inflammation such as interleukin-6 (IL-6; 24.5 kD), tumor-necrosis-factor α (TNF-α; 26.0 kD) and complement factor D (23.8 kD) 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.8 HD using high-flux membranes can be considered as a form of HDF, because the pressure drop along the fibres induces filtration that can be considerable (8–10 L per treatment). The total amount of ultrafiltration exceeds the required weight loss and is compensated by backfiltration.4 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 (i.e. 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 a given filtration volume, as diffusion is less effective when compared to the post-dilution mode.11 Therefore, post-dilution online HDF is regarded the most optimal extracorporeal convective RRT.

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, albeit 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.12 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 3 years. One post hoc sub-analysis of this study suggests a survival benefit of high-flux membranes for patients on HD for more than 3.7 years.13 In addition, another post hoc sub-analysis suggested a decreased risk of death from cerebrovascular disease for patients on high-flux HD who had no baseline evidence of cerebrovascular disease, or with a duration of HD therapy longer than 3.7 years.14

The randomized controlled European Membrane Permeability Outcome (MPO) study, originally designed to study the outcome inhypoalbuminemic HD patients,15 showed a survival advantage in patients with low albumin levels (≤ 4 g/dL) if they were treated with high-flux membranes. However, the study was amended underway due to slow enrolment so that the study protocol was opened to normoalbuminemic subjects as well. In the overall group (containing hypoalbuminemic and normoalbuminemic patients) no survival advantage for high-flux was observed.16 Moreover, diabetic patients showed a survival advantage for high-flux HD, both for the overall and the hypoalbuminemic group.

Furthermore, a post hoc analysis of the 4D study, a randomized controlled trial which was originally designed to analyze the effect of atorvastatin in diabetic chronic HD patients on the composite endpoint of cardiovascular mortality and morbidity, showed a superior
survival in patients treated with high-flux as compared to low-flux membranes.\(^{17}\) In addition, in a prospective cohort study in prevalent HD patients, those treated with high-flux dialysis membranes had a better survival, after adjustment for other risk factors.\(^{18}\)

To summarize, high-flux HD has not been shown to reduce mortality in the general HD population, although subgroups composing a substantial fraction of the dialysis population, such as diabetic or hypoalbuminemic patients, or patients with long dialysis vintage, may benefit from high-flux HD.

As mentioned in the introduction, HDF used to be performed with commercially produced substitution fluid in 5 L 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 (<15 L per session), the 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 Dialysis Outcomes and Practice Patterns Study (DOPPS) cohort, for patients in the low-efficiency HDF group, the (unadjusted) 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.\(^{19}\) 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 online systems that prepare substitution fluid continuously has made HDF easier and much less expensive.\(^{4, 6, 20}\) The question whether online HDF, in which much more convective volume can be obtained, will result in clinical and survival benefit, will be addressed below (in the rest of this chapter, the term “HDF” is mentioned for “online HDF”, unless mentioned otherwise).

### 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 online HDF 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.\(^{4, 6, 20}\)

Apart from a more complex purification system for water and dialysis fluid, some specific requirements are needed to perform online HDF on a routine basis. High-flux membranes (ultrafiltration coefficient >20 mL/mmHg/h) are used and equipment able to deliver online HDF 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.\(^{21}\)
EFFECTS OF HDF ON (PRE-)CLINICAL VARIABLES AND SURVIVAL

Effects of HDF on preclinical variables

Kt/V<sub>urea</sub> is a well-established marker of dialysis adequacy and 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<sub>urea</sub> has been challenged by the HEMO study. Enhanced removal of those small molecules by post-dilution HDF has been documented. However, as the removal of those substances is far more dependent on diffusive than convective transport, the advantage of (post-dilution) HDF over HD is only modest.<sup>11,22</sup>

The removal of larger molecules accumulating in chronic HD patients depends almost exclusively on the permeability characteristics of the dialyzer membrane and the convection volume. Therefore, these substances are removed by HDF, in which high-flux membranes are used, and not by conventional low-flux HD. In pre-dilution HDF, a higher ultrafiltration rate is needed as compared with post-dilution HDF, to obtain equal MMW clearance because of dilution ahead of the filter in pre-dilution HDF.<sup>11</sup>

Several observational and randomized studies have shown that predialysis levels of β<sub>2</sub>M are reduced when patients are switched to HDF.<sup>23-28</sup> The beneficial effect of HDF in terms of β<sub>2</sub>M removal seemed to be most pronounced in patients without residual kidney function (RKF),<sup>29</sup> suggesting that the presence of RKF might outweigh the effect of convective transport on MMW removal. Furthermore, as may 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<sub>2</sub>M clearance in some studies,<sup>23,24</sup> but not in all.<sup>29</sup> As a consequence, it may be important to compare different studies on HDF with caution as convection volumes can vary enormously between clinical studies (table 1).

Improved clearance with HDF of other MMW substances than β<sub>2</sub>M have been described in various studies, e.g. complement factor D,<sup>27</sup> osteocalcin,<sup>22</sup> myoglobin,<sup>22,30</sup> retinol binding protein<sup>30</sup> and cystatin C.<sup>30</sup> 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 various studies,<sup>25,26,31-35</sup> but not in all.<sup>28</sup> Obviously, the effect of convective therapies on phosphate removal was most explicit in patients with highest phosphate levels at baseline.<sup>11</sup>

Concerning protein-bound uremic toxins, e.g. indoxylsulphate (251 D) and p-cresol (108 D) and its metabolite p-cresylsulphate, it has been shown that improved clearance with HDF, either with pre- or post-dilution, can be obtained.<sup>11,30,36</sup> When comparing pre- and post-dilution, no differences were observed when similar convection volumes were applied.<sup>11,36</sup>

Micro-inflammation and an impaired immunological response are important considerations in CKD patients. In this respect, retention of inflammatory cytokines in the MMW range, such as IL-6 and TNFα, might play an important role. Other potential sources of inflammation in HD patients are the bio-incompatibility of the extracorporeal system and contact with contaminated dialysate. Several studies have shown decreased levels of C-reactive protein, IL-6, TNFα and pro-inflammatory CD14+/CD16+ mononuclear cells in patients treated with high-flux HD with ultrapure dialysate and HDF.<sup>34,52-54</sup> However, whether
**Table 1:** Studies on the effect of HDF on various (pre-)clinical variables.

<table>
<thead>
<tr>
<th>Phosphate</th>
<th>Design</th>
<th>Number of patients</th>
<th>HDF: Method and target convection volume per session</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zehnder 1999</td>
<td>Cross-over</td>
<td>16</td>
<td>Post 24 L</td>
<td>HDF vs HD: 33% ↑ phosphate clearance</td>
</tr>
<tr>
<td>Wizemann 2000</td>
<td>RCT</td>
<td>44</td>
<td>Mid 60 L</td>
<td>HDF vs HD: no difference</td>
</tr>
<tr>
<td>Minutolo 2002</td>
<td>RCT</td>
<td>12</td>
<td>Post 6-12 L</td>
<td>HDF vs HD: 23% ↓ predialysis phosphate</td>
</tr>
<tr>
<td>Schiff 2007</td>
<td>Randomized cross-over</td>
<td>76</td>
<td>Method NA 18-22.5 L</td>
<td>HDF vs HD: 13% ↓ predialysis phosphate</td>
</tr>
<tr>
<td>Davenport 2010</td>
<td>Observational</td>
<td>5366 (HDF: 851)</td>
<td>Post 15-20 L</td>
<td>HDF 6% ↓ predialysis phosphate and HD =, HDF ↓ phosphate binders</td>
</tr>
<tr>
<td>Penne (CONTRAST) 2010</td>
<td>RCT</td>
<td>493 (242 HDF)</td>
<td>Post 19.5 L</td>
<td>HDF 8% ↓ predialysis phosphate, = phosphate binders</td>
</tr>
<tr>
<td>Oates 2011</td>
<td>Observational</td>
<td>34 HDF, 44 HD</td>
<td>Post &gt;16 L</td>
<td>HDF vs HD: 8% ↓ predialysis phosphate, = phosphate binders</td>
</tr>
<tr>
<td>Pedrini 2011</td>
<td>Randomized cross-over</td>
<td>62</td>
<td>Post 19.7 L; Mixed 37.7 L; Pre 46.3 L</td>
<td>HDF vs HD: 8% ↓ predialysis phosphate, = phosphate binders</td>
</tr>
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<td>* continued on the next page</td>
</tr>
</tbody>
</table>

**β2-microglobulin**

| Lornoy 2000                | Cross-over         | 8                  | Post 9.6-24 L; pre 19.2 L                          | HDF vs HD: ↑ reduction rate and clearance of β2m                                                  |
| Ward 2000                 | RCT                | 44                 | Post 15.6-20.4 L                                  | HDF vs HD: ↑ removal and clearance of β2m; = decrease in predialysisβ2m over time               |
| Wizemann 2000             | RCT                | 44                 | Mid 60 L                                          | HDF: 40% ↓ in predialysisβ2m, if HD =                                                              |
| Lin 2001                  | Observational      | 58 HDF             | Post 20-22 L                                      | HDF vs HD: 36% ↓ in predialysisβ2m                                                                  |
| Penne (CONTRAST) 2010     | RCT                | 406                | Post 19.1 L                                       | HDF: 18% ↓ in predialysisβ2m, most pronounced in those without residual kidney function (28% ↓) |
| Oates 2011                | Observational      | 34 HDF, 44 HD      | Post >16 L                                        | HDF vs HD: 14% ↓ in predialysisβ2m                                                                 |
| Pedrini 2011              | Randomized cross-over | 62                | Post 19.7 L; Mixed 37.7 L; Pre 46.3 L             | Predialysisβ2m 34% ↓ in HDF vs HD                                                                  |
### Hemodynamic stability

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Number of patients</th>
<th>HDF: Method and target convection volume per session</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mion 1992&lt;sup&gt;37&lt;/sup&gt;</td>
<td>Cross-over</td>
<td>8</td>
<td>Method NA 18-20 L</td>
<td>HDF: ↑ cardiovascular stability</td>
</tr>
<tr>
<td>Movilli 1996&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Randomized cross-over</td>
<td>6</td>
<td>Post 15.8 L</td>
<td>HDF vs HD: ↓ hypotensive episodes</td>
</tr>
<tr>
<td>Locatelli 1996&lt;sup&gt;39&lt;/sup&gt;</td>
<td>RCT</td>
<td>380 (50 HDF)</td>
<td>Post 8-12 L</td>
<td>HDF vs HD: no difference</td>
</tr>
<tr>
<td>Wizemann 2000&lt;sup&gt;28&lt;/sup&gt;</td>
<td>RCT</td>
<td>44</td>
<td>Mid 60 L</td>
<td>HDF vslfHD: no difference</td>
</tr>
<tr>
<td>Donauer 2003&lt;sup&gt;40&lt;/sup&gt;</td>
<td>Cross-over</td>
<td>11</td>
<td>Post 12 L</td>
<td>HDF vshfHD: ↓ hypotensive episodes; HDF vs temp. controlledhfHD: = hypotensive episodes</td>
</tr>
<tr>
<td>Schiff 2007&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Randomized cross-over</td>
<td>76</td>
<td>Method NA 18-22.5 L</td>
<td>HDF vshfHD: ↓ hypotensive episodes</td>
</tr>
<tr>
<td>Vilar 2009&lt;sup&gt;41&lt;/sup&gt;</td>
<td>Observational</td>
<td>858 (232 HDF)</td>
<td>NA</td>
<td>HDF vshfHD: ↓ hypotensive episodes</td>
</tr>
<tr>
<td>Locatelli 2010&lt;sup&gt;42&lt;/sup&gt;</td>
<td>RCT</td>
<td>146 (40 HDF)</td>
<td>Pre 39.9 L</td>
<td>HDE: ↓ hypotensive episodes</td>
</tr>
</tbody>
</table>

### Anemia/ ESA resistance

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Number of patients</th>
<th>HDF: Method and target convection volume per session</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maduell 1999&lt;sup&gt;43&lt;/sup&gt;</td>
<td>Cross-over</td>
<td>37</td>
<td>Post, low-efficiency 4.1 L; high-efficiency 2.2 L</td>
<td>High-efficiency HDF: ↓ ESA resistance</td>
</tr>
<tr>
<td>Ward 2000&lt;sup&gt;27&lt;/sup&gt;</td>
<td>RCT</td>
<td>44</td>
<td>Post 15.6-20.4 L</td>
<td>HDF vshfHD: no difference</td>
</tr>
<tr>
<td>Wizemann 2000&lt;sup&gt;28&lt;/sup&gt;</td>
<td>RCT</td>
<td>44</td>
<td>Mid 60 L</td>
<td>HDF vslfHD: no difference</td>
</tr>
<tr>
<td>Bonforte 2002&lt;sup&gt;44&lt;/sup&gt;</td>
<td>Observational</td>
<td>32 HDF</td>
<td>Post 19.5 L</td>
<td>↓ ESA resistance</td>
</tr>
<tr>
<td>Lin 2002&lt;sup&gt;45&lt;/sup&gt;</td>
<td>Cross-over</td>
<td>92</td>
<td>Post 20-22 L</td>
<td>HDF: ↓ ESA resistance</td>
</tr>
<tr>
<td>Vaslaki 2006&lt;sup&gt;46&lt;/sup&gt;</td>
<td>Randomized cross-over</td>
<td>129</td>
<td>Method NA 20.2 L</td>
<td>HDF: ↓ ESA resistance</td>
</tr>
<tr>
<td>Schiff 2007&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Randomized cross-over</td>
<td>76</td>
<td>Method NA 18-22.5 L</td>
<td>HDF and hfHdvsfHd: ↓ ESA resistance</td>
</tr>
<tr>
<td>Vilar 2009&lt;sup&gt;41&lt;/sup&gt;</td>
<td>Observational</td>
<td>858 (232 HDF)</td>
<td>NA</td>
<td>HDF vshfHD: no difference</td>
</tr>
<tr>
<td>Pedrini 2011&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Randomized cross-over</td>
<td>62</td>
<td>Post 19.7 L; Mixed 37.7 L; Pre 46.3 L</td>
<td>HDF 13% ↓ ESA dose than lfHDF, ESA index =</td>
</tr>
<tr>
<td>Oates 2011&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Observational</td>
<td>34 HDF, 44 HD</td>
<td>Post &gt;16 L</td>
<td>No difference</td>
</tr>
</tbody>
</table>

* continued on the next page
### Nutritional parameters

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Number of patients</th>
<th>HDF: Method and target convection volume per session</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locatelli 1996</td>
<td>RCT</td>
<td>380 (50 HDF)</td>
<td>Post 8-12 L</td>
<td>HDF vs HD: no difference</td>
</tr>
<tr>
<td>Wizemann 2000</td>
<td>RCT</td>
<td>44</td>
<td>Mid 60 L</td>
<td>HDF vs lHDF: no difference</td>
</tr>
<tr>
<td>Schiff 2007</td>
<td>Randomized cross-over</td>
<td>76</td>
<td>Method NA 18-22.5 L</td>
<td>HDF and hfHD vs lHDF: improvement (albumin, predialysis weight, arm muscle circumference)</td>
</tr>
<tr>
<td><strong>Nutritional parameters</strong></td>
<td><strong>RCT</strong></td>
<td><strong>44</strong></td>
<td><strong>Post 15.6-20.4 L</strong></td>
<td><strong>HDF vs lHDF: no difference (KDQ)</strong></td>
</tr>
<tr>
<td><strong>Quality of life</strong></td>
<td><strong>Observational</strong></td>
<td><strong>2165 (156 low-efficiency HDF, 97 high-efficiency HDF)</strong></td>
<td><strong>Method NA; low-efficiency 5-14.9 L, high-efficiency 15-24.9 L</strong></td>
<td><strong>HDF vs lHDF: no difference (KDQ)</strong></td>
</tr>
<tr>
<td>Schiff 2007</td>
<td>Randomized cross-over</td>
<td>76</td>
<td>Method NA 18-22.5 L</td>
<td>No difference (KDQ)</td>
</tr>
<tr>
<td>Mazairac (CONTRAST) 2012</td>
<td>RCT</td>
<td>714</td>
<td>Post 19 L</td>
<td>No difference (KDQ)</td>
</tr>
</tbody>
</table>

### Mortality

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Number of patients</th>
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<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locatelli 1996</td>
<td>RCT</td>
<td>380 (50 HDF)</td>
<td>Post 8-12 L</td>
<td>HDF vs HD: no difference</td>
</tr>
<tr>
<td>Locatelli 1999</td>
<td>Observational</td>
<td>6444 (1082 HDF/ HF)</td>
<td>NA</td>
<td>HDF/HF vs HD: 10% ↓ mortality, not significant</td>
</tr>
<tr>
<td>Jirka 2006</td>
<td>Observational</td>
<td>2564 (394 HDF)</td>
<td>NA</td>
<td>HDF vs HD: 35% ↓ mortality</td>
</tr>
<tr>
<td>Canaud (DOPPS) 2006</td>
<td>Observational</td>
<td>2165 (156 low-efficiency HDF, 97 high-efficiency HDF)</td>
<td>Method NA; low-efficiency 5-14.9 L, high-efficiency 15-24.9 L</td>
<td>High-efficiency HDF vs lHDF: 35% ↓ mortality; Low-efficiency HDF vs lHDF: no difference</td>
</tr>
<tr>
<td>Bosch 2006</td>
<td>Observational</td>
<td>183</td>
<td>NA</td>
<td>HDF vs general US dialysis population: 59% ↓ mortality</td>
</tr>
<tr>
<td>Vilar 2009</td>
<td>Observational</td>
<td>858 (232 HDF)</td>
<td>NA</td>
<td>HDF vs lHDF: 55% ↓ mortality</td>
</tr>
</tbody>
</table>
the reduction of the inflammatory state is mainly caused by the removal of pro-inflammatory MMW toxins or by use of ultrapure dialysate, is not readily apparent from these studies. Given the importance of clearance of MMW substances, quantified for instance by ß2M 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 provide no or limited clearance of ß2M. Measurable reductions can be obtained by HF and high-flux HD, especially when long treatment times are applied, such as with nocturnal HD.\textsuperscript{10, 55-58} 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 increasing total treatment time per week, even more removal of MMW solutes can be obtained.\textsuperscript{59}

**Effects of HDF on clinical variables**

Because HDF 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, a number of reports of non-randomized trials have come up with the interesting finding of decreased resistance to erythropoiesis stimulating agents (ESA) in patients treated with HDF\textsuperscript{26, 34, 43-46} that has been associated with several factors among which accumulation of uremic toxins that inhibit erythropoiesis\textsuperscript{60} and reduce red blood cell lifespan.\textsuperscript{61} However, two randomized trials were not able to confirm these data, although neither of these studies were specifically designed to answer this question.\textsuperscript{27, 28}

Intradialytic hypotension (IDH) is of major concern in HD treatment and independently associated with mortality.\textsuperscript{62} Several studies suggested that HDF is associated with an improvement of IDH and blood pressure control, among which one randomized controlled trial with 146 Italian patients.\textsuperscript{38, 40, 42} However, no difference in IDH was demonstrated between HDF and temperature-controlled HD.\textsuperscript{40} Hence, rather than from increasing MMW clearance, this beneficial effect seems to be mainly caused by a cooling of the blood via enhanced thermal energy losses within the extracorporeal system in HDF.

Quality of life (QoL) remains an important area of interest, especially for chronic dialysis patients. Several studies have shown beneficial effects of convective therapies on various domains of QoL.\textsuperscript{53} In a preliminary analysis of the CONTRAST study, patients randomized to HDF showed an improved QoL, although the change was not significantly different compared to the HD group.\textsuperscript{47}

In table 1, the effect of HDF on various clinical variables are summarized. It can be seen that most of these studies were performed in a non-randomized, observational or crossover design and/or in small patient groups.

**Effects of HDF on survival**

Several observational studies suggest a benefit of HDF on survival (Table 1). The use of high-efficiency HDF in the DOPPS cohort (convection volume of \(>15\) L per session, which will mean online HDF in most cases for obvious logistical reasons) was associated with a
35% reduction in mortality risk, even after correction for various confounding factors. In contrast, low-efficiency HDF (convection volume <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 HDF. In a smaller observational study from the USA, HDF was associated with an almost 60% reduction of risk of mortality. Two other observational studies from the UK and Italy showed 55 and 22% decreased mortality, respectively, although the latter study exhibited some serious methodological drawbacks. In a systematic review from 2005 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.

Until recently, data from properly designed and adequately powered randomized controlled trials on the effect of HDF on mortality were limited. One small randomized trial showed a survival benefit for online pre-dilution hemofiltration as compared to low-flux HD, although statistical significance was only reached after exclusion of drop-outs and adjustment for age.

The Dutch CONvectiveTRAnsportStudy (CONTRAST; NCT00205556) is multicenter randomized clinical trial (RCT) in which the hypothesis is tested whether increased MMW clearance by hemodiafiltration (HDF) is beneficial on clinical endpoints. 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. In this study, 714 chronic hemodialysis (HD) patients were randomized to either post-dilution HDF or continuation of low-flux HD with a variable follow-up. All-cause mortality was the primary endpoint. The CONTRAST study ended in January 2011 and the results are presented in this thesis (Chapter 6).

A Turkish study (NCT00411177) in which patients were randomized to either HDF or high-flux HD was recently presented and will be discussed in the general discussion (Chapter 11).

In a French prospective randomized trial, HDF is compared to high-flux HD in 600 patients older than 65 years, who are followed for 2 years. The 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. The results of this study are not published yet.

POTENTIAL DRAWBACKS AND SIDE EFFECTS OF HDF

As studies on convective therapies have shown indefinite and conflicting results, the question arises whether the technique itself has undesirable side effects, such as an augmented bio-incompatibility and loss of essential proteins and nutrients. Indeed, during HDF, substantial leakage of albumin and vitamin C has been reported. In addition, both
considerable platelet activation\textsuperscript{73} and substantial procoagulatory activity,\textsuperscript{74} possibly due to an increased transmembrane pressure and/or hemoconcentration within the dialyzer, have been described. Furthermore, safety concerns on water and infusate quality have been raised as with online H(D)F, considerable amounts of substitution fluid are directly infused into the patient. However, it has been shown by our group that with strict guidelines and a well-organized quality control process, HDF is a safe treatment.\textsuperscript{75}

\textbf{CONCLUSION}

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

HDF provides a measurable reduction in various substances suspected to be clinically relevant “uremic” toxins, which are not cleared by standard low-flux HD, although studies of convective therapies on clinical parameters have shown mixed results. Observational studies suggest a dosage-related substantial improvement in clinical parameters and reduction of mortality. However, observational studies are sensitive to bias, and data from randomized controlled trials have been scarce so far.

Therefore, to answer the question whether MMW clearance is an important consideration for patients with end stage renal disease and whether convective therapies should be applied in these patients, several issues ought to be taken into consideration: (1) Besides MMW clearance, the beneficial effects of convective therapies might be caused by other factors, such as the use of ultrapure dialysate and blood temperature reduction; (2) In addition to potential benefits of convective therapies, undesirable side-effects should be taken into consideration as well; (3) randomized clinical trials (RCTs) on convective therapies mainly showed beneficial effects in specific patient categories, although most of these specific groups compose a substantial fraction of the general dialysis population, e.g. those with no RKF, high phosphate levels, high ESA resistance, low albumin levels and long dialysis vintage. (4) As many RCTs with survival as primary endpoint revealed to be negative in dialysis patients, other endpoints such as the burden of cardiovascular disease and QoL may turn out to be clinically important.

With current knowledge, there is not sufficient evidence available to suggest convective strategies as a standard treatment option. For the future, pooling data from ongoing RCTs might be of eminent importance to obtain definite evidence on the clinical effects of convective therapies, especially in selected subgroups.
CHAPTER 1

REFERENCES


HEPCIDIN IN PATIENTS WITH END STAGE RENAL DISEASE
RENAL ANEMIA EN RESISTANCE TO ERYTHROPOIESIS STIMULATING AGENTS.

Renal anemia is a common feature of patients with chronic kidney disease (CKD) and is mainly caused by a reduced renal capacity to synthesize erythropoietin and a diminished bone marrow response to its biological action. In this respect, accumulation of uremic toxins that inhibit erythropoiesis and reduce red blood cell lifespan are supposed to be important contributing factors.

Routine administration of erythropoiesis stimulating agents (ESA) for the control of anemia improves left ventricular mass and quality of life of patients with CKD not on dialysis and in those with end stage renal disease (ESRD) treated with renal replacement therapy. Actually, a wide variation in the individual response to ESA is observed, and target hemoglobin levels as defined by current guidelines are often not reached. Furthermore, the scientific evidence of these guidelines for dialysis patients is rather weak. A recent meta-analysis of studies evaluating higher hemoglobin targets by administering ESA, mainly from studies in patients with CKD not yet on dialysis suggests that there is a greater risk for stroke, cardiovascular events and mortality when aiming at higher hemoglobin levels. It is not clear whether the adverse outcomes of targeting for higher hemoglobin levels are related to high ESA dosing or simply to a higher hemoglobin mass. Alternatively, the negative results may be explained by the unfavorable and high-risk patient category in which a high ESA dose is required when aiming for high hemoglobin levels (so-called ESA resistant patients). In fact, a high hemoglobin mass without administration of ESA does not seem to be harmful, as illustrated by a study in which a subgroup of hemodialysis (HD) patients with high hemoglobin levels who were not treated with ESA, did not have an increased mortality risk.

ESA resistance in CKD patients has been associated with various conditions, including inflammation, oxidative stress, malnutrition, hyperparathyroidism, vitamin D deficiency, use of angiotensin modulating agents and deficiency of various nutrients such as folium acid and vitamin C. Another important underlying factor is (functional) iron deficiency. Iron deficiency in ESRD patients can be caused by poor nutrition, decreased intestinal uptake and depletion of available iron stores due to the supra-physiological proliferation of erythroid cells resulting from ESA therapy. Furthermore, there is an estimated loss of 2 g of elemental iron per year in adult patients on maintenance HD. Sources of blood loss are frequent blood sampling, blood loss in the extracorporeal system (i.e. in blood lines and dialyzers) and gastrointestinal bleeding, for example because of uremic gastritis or angiodysplasias. It has been well established that intravenous iron supplementation therapy is beneficial in HD patients as hemoglobin levels increase, even when ferritin levels are well above 500 ng/ml, which used to be the upper limit target level as suggested in the guidelines. However, the effect of intravenous iron seems to be dependent on several factors, for example inflammation, which is associated with a decreased response.

Hepcidin is a key regulator of the systemic iron homeostasis and has been identified as a very relevant peptide in ESRD patients, since it may play a critical role in the balance between iron availability and ESA responsiveness.
Outline of this chapter
In this chapter, clinically relevant characteristics of hepcidin and the available quantification methods are reviewed. Subsequently, the relevance of hepcidin in CKD and dialysis patients will be discussed, with a focus on the role of hepcidin in ESA resistance and cardiovascular disease, and the possibilities and potential benefits of hepcidin lowering therapies, including dialysis.

HEPCIDIN: STRUCTURE, SYNTHESIS, AND KINETICS
Hepcidin was identified in 2001 as a peptide with antimicrobial properties playing a role in iron overload. It is mainly produced in the liver, but expression by other cells than hepatocytes has been described, namely in kidney tubuli, heart, retina, monocytes, neutrophils, fat cells, alveolar cells and pancreatic cells. Hepcidin is mainly present in its bioactive form hepcidin-25, a 25 amino acid peptide of 2.8 kD. Hepcidin-20 and hepcidin-22 are isoforms with unknown biological function. Under physiological conditions, these isoforms are present in the urine, but absent, or present in very low concentrations, in the blood. In patients with CKD, and especially in those on dialysis, however, serum levels of hepcidin-25 as well as hepcidin-20 and -22 are elevated.

Circulating hepcidin-25 has a high affinity to α-microglobulin and to a lesser extent to albumin, whereas only 11% was estimated to be freely circulating. Its clearance is assumed to occur via cellular degradation at its sites of action, and via excretion with the urine. Under physiological conditions, the fractional excretion of hepcidin is only 0-3%, suggesting that hepcidin is either almost completely reabsorbed by the tubuli or, due to its protein-bound character, not freely filtered by the glomerulus.

HEPCIDIN: FUNCTION AND REGULATION
Hepcidin is able to express its regulatory function in iron homeostasis by impairing the expression of ferroportin. Ferroportin is a cellular iron exporter in hepatocytes, macrophages and on the basolateral side of enterocytes. Hepcidin-25 induces the internalization and degradation of ferroportin, resulting in enhanced intracellular iron stores, diminished iron adsorption and circulating iron concentrations, and hence decreased iron availability.

Hepcidin is produced in response to iron loading, erythropoietic demand, hypoxia and inflammatory signals. Iron administration enhances hepcidin expression, resulting in a feedback mechanism that limits further iron adsorption, e.g. from the gastrointestinal tract. Anemia and hypoxia inhibit hepcidin expression, hence increasing iron availability for erythropoiesis. Inflammation increases hepcidin expression, which is mainly mediated via IL-6. Iron sequestration in the reticuloendothelial system, impaired intestinal iron absorption and decreased transferrin and serum iron levels are well-known characteristics of anemia of chronic disease, which is present in many (chronic) inflammatory conditions.

Originally, it was postulated that hepcidin expression is part of a defence mechanism against infection and malignancy by reducing serum iron levels, as iron availability is
necessary for bacterial growth and results in oxidative stress.\textsuperscript{34} Although the antimicrobial effects of hepcidin require much higher serum concentrations than have been observed in the human circulation, local concentrations in macrophages may be sufficient for this purpose.\textsuperscript{32}

**MEASUREMENT OF HEPcidIN**

Measurement of hepcidin in human sera has been challenged by the fact that its amphipathic structure results in adsorption to surfaces and sticking to the plastic of the blood-tubes.\textsuperscript{32} Furthermore, under physiological circumstances, hepcidin concentrations increase during the day.\textsuperscript{45} This circadian rhythm can be explained by diurnal transcription of genes involved in hepcidin expression, and by the influence of iron intake during the day. In dialysis patients, these circadian rhythms are virtually absent.\textsuperscript{46}

Currently, two types of hepcidin quantification methods have been studied in patients with CKD and those on dialysis, namely (1) immunochemical assays, including radioimmunoassays and ELISA, of which most are relatively easy to apply and some are commercially available, and (2) mass spectrometry, which is more expensive and technically demanding, but able to detect each isoform separately.\textsuperscript{32, 47} Thus far, there has not been one general worldwide accepted assay yet, and several questions are remaining. First, there are considerable differences in absolute hepcidin concentrations using the different methods.\textsuperscript{37, 47} In healthy controls, measured values can differ by a factor 10, and even more in dialysis patients. This precludes the definition of universal reference intervals. Nevertheless, between-sample variation and the analytical variation of the methods are fairly similar.\textsuperscript{48} Second, with several methods, it is unclear whether total hepcidin is measured, or whether its isoforms are quantified separately. Especially with most immunoassays, total hepcidin concentrations are measured, unless specific antibodies are used. Third, it is not completely established yet whether free, bound, or total hepcidin is measured, as hepcidin is highly protein-bound (see before).

Currently, initiatives are undertaken to standardize techniques and measurements worldwide and to be able to define reference values in healthy subjects and in different patient categories such as dialysis and CKD patients.\textsuperscript{32, 48}

**HEPCIDIN IN CKD AND ESRD PATIENTS**

It is well known that in CKD and ESRD patients, anemia, iron deficiency and inflammation are highly prevalent. Therefore, many studies on hepcidin in these patient categories have been performed.

It has been well established that hepcidin levels are increased in CKD patients and even more in those on dialysis.\textsuperscript{38, 49} Research on the relation between hepcidin and residual kidney function (expressed by the estimated glomerular filtration rate [eGFR]) has shown conflicting results. It has been suggested that the relation between eGFR and hepcidin was due to the measurement of inactive isoforms, as in CKD patients hepcidin measured with an ELISA was associated with eGFR,\textsuperscript{49} whereas hepcidin-25 specifically
measured with mass spectrometry was not.\textsuperscript{38, 50} The bioactive form of hepcidin measured with a specific radioimmunoassay, however, was associated with eGFR in CKD patients.\textsuperscript{46} In dialysis patients with residual diuresis of more than 500 mL per day, lower hepcidin levels (measured with a non-specific radioimmunoassay) were reported as compared to those with a residual diuresis of less than 500 mL per day, but the residual kidney function was not quantified with an eGFR.\textsuperscript{51}

Virtually all studies on hepcidin in HD patients groups have observed a strong association between ferritin and hepcidin.\textsuperscript{38, 52-55} Concerning markers of inflammation, several studies have demonstrated a relation between CRP\textsuperscript{38, 55-57} or IL-6\textsuperscript{53, 56} in small groups of chronic HD patients, whereas others did not.\textsuperscript{54, 58}

### Hepcidin as a clinical tool in CKD and ESRD patients

It has been speculated that hepcidin measurements could be applied in the clinical assessment of anemia in CKD and dialysis patients, to identify patients who might benefit from increasing either ESA or iron dosing.\textsuperscript{59} Furthermore, it has been suggested that hepcidin might contribute to ESA resistance. Animal data, for example, have shown that overexpression of hepcidin impairs the response to even very high ESA doses.\textsuperscript{60} However, in humans, the association between hepcidin and ESA resistance has not been definitely demonstrated. In a study of almost 100 HD patients on maintenance therapy with ESA, hepcidin levels were inversely related to ESA dose, which was consistent across each tertile of hemoglobin.\textsuperscript{46} In the same study, hepcidin levels decreased after ESA administration in seven ESA-naive patients.\textsuperscript{46} These data indicate that hepcidin levels are (directly or indirectly) reduced by ESA, which was confirmed in a study showing that hepcidin levels decreased after administration of ESA in patients who had been withheld from ESA for two weeks.\textsuperscript{54} Interestingly, another study revealed that hepcidin-25 levels in ESA responsive HD patients did not differ from ESA resistant patients, suggesting that measurement of hepcidin is not helpful when assessing ESA resistance.\textsuperscript{52} Contrarily, in HD patients and in patients with the cardio-renal syndrome, ESA responders showed higher hepcidin-25 levels than non-responders, suggesting that hepcidin might rather be a marker of ESA responsiveness than associated with ESA resistance.\textsuperscript{56, 61}

Concerning the utility of hepcidin in managing iron supplementation therapy, overall, negative results have been reported. In a study in 56 chronic HD patients, neither hepcidin-20 nor hepcidin-25 (measured with mass spectrometry) could predict an increase in hemoglobin levels after administration of intravenous iron.\textsuperscript{58} Instead, the percentage of hypochromic red blood cells was the only biomarker independently associated with iron responsiveness. Furthermore, in a small group of HD patients, hepcidin-25 levels were similar before and after the administration of intravenous iron.\textsuperscript{54}

Importantly, in stable HD patients, intra-individual hepcidin levels varied widely in a relative short period of time, likely dependent on fluctuations in the inflammatory state.\textsuperscript{57} This finding implicates that short-term measurements of serum hepcidin might not be appropriate to guide clinical decisions regarding ESA or iron management in individual patients.

In conclusion, thus far, conflicting or negative data are available to answer the question whether measurement of hepcidin may be a suitable tool for making decisions concerning
HEPCIDIN IN ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

In an effort to explain the sex difference in the incidence of cardiovascular disease, the so-called "iron hypothesis" was formulated, in which it was suggested that a mild iron deficiency or sustained iron depletion generated a protective effect against ischemic heart disease. However, older epidemiological studies on the relation between iron content and atherosclerotic disease in the general population showed conflicting results. Yet, newly induced atherosclerotic plaques in animal experiments contained high iron concentrations, and either an iron deficient diet or iron chelation therapy resulted in reduced plaque formation and diminished atherosclerotic iron content in these plaques (reviewed in ). In patients with stable symptomatic peripheral arterial disease, iron reduction by phlebotomy did not result in improved survival and reduction of cardiovascular events, although phlebotomy was beneficial in the youngest patient group (age 43-61 years) included in this randomized controlled trial. In one study, it was shown that atherosclerotic vascular lesions contained more iron than arterial walls of healthy controls, and that the iron content in these lesions was correlated with cholesterol levels. Moreover, another study revealed that in patients with extensive atherosclerotic plaques, iron content was associated with pro-oxidant activity within the plaque. Iron can be sequestered in macrophages by phagocytosis, for example of senescent erythrocytes. In an experimental model, it has been shown that macrophages with increased iron levels within the vascular wall exhibit pro-atherogenic activity by oxidation of low density lipoprotein and increased cellular cholesterol accumulation. All these data indicate that atherogenic characteristics of macrophages in vessel walls may be associated with iron accumulation.

As mentioned before, hepcidin is a key regulator of iron homeostasis, and high hepcidin levels result in increased iron content in macrophages. Therefore, it can be hypothesized that elevated hepcidin levels are associated with atherosclerotic disease by increasing iron content in macrophages in the vascular wall and hence inducing oxidative stress. Several recent (pre-)clinical studies support this hypothesis. In an experimental mouse model, suppression of hepcidin resulted in reduced intracellular iron content in macrophages. This resulted in an augmented efflux capacity of cholesterol, which was associated with diminished foam cell formation and atherosclerosis. Moreover, in human monocytes derived from atherosclerotic plaques, intracellular iron content was increased in the presence of hepcidin, resulting in enhanced reactive oxygen substances, which prevented cholesterol efflux from these cells. In patients with non-alcoholic fatty liver disease and a normal hemochromatosis (HFE) genotype, high ferritin levels were independently
associated with the prevalence of carotid plaques (measured with cIMT). Moreover, these patients had the highest levels of hepcidin, whereas in patients with a HFE mutation (and as a consequence a decreased hepcidin release), the prevalence of plaques was significantly lower. In a similar group of patients with non-alcoholic fatty liver disease, monocyte chemo-attractant protein-1, a chemokine that plays a crucial role in both the initiation and progression of atherosclerosis, was correlated with hepcidin-25 and an independent predictor of the presence of atherosclerotic plaques.

In HD patients, atherosclerosis, as measured by carotid artery intima-media thickness, has been associated with high iron stores and high doses of iron supplementation therapy. Although recently, much attention has been paid to the role of hepcidin in CKD and dialysis patients, studies on the effects of hepcidin on atherosclerosis and the high burden of cardiovascular disease in this patient group, are virtually absent. Only one recent study in chronic HD patients showed an association between arterial stiffness (measured with brachial-ankle pulse wave velocity) and hepcidin-25. As the cardiovascular risk profile of CKD patients seems to differ from the general population, the question arises whether the atherogenic effect of iron accumulation and oxidative stress in the vascular wall, is relevant in this specific high risk patient group as well.

HEPCIDIN-LOWERING THERAPIES AND THEIR POTENTIAL BENEFITS

It is tempting to speculate on the possible beneficial effects of lowering hepcidin levels, as this might improve the response to iron therapy and ESA resistance in CKD and ESRD patients, and possibly results in decreased cardiovascular disease in this patient group. In experimental studies, hepcidin “knock-in” mice with overexpression of hepcidin were treated with hepcidin antibodies, which resulted in decreased hepcidin levels, enhanced erythropoiesis and increased iron levels. Besides hepcidin-antibodies, several substances targeting various pathways in hepcidin expression have been developed (reviewed in 32, 84). An example of an anti-hepcidin target is dorsomorphin, a small molecule that inhibits the bone morphogenic protein (BMP) receptor, which is important for hepcidin transcription. Recently, it has been established that heparin and heparin derivates inhibit hepcidin expression via the BMP signalling pathway, which may be interesting information as (low molecular weight) heparin is applied for anti-coagulation during HD treatment. Furthermore, early human studies of stabilizers of the hypoxia-induced factor (HIF) have shown to improve anemia in CKD patients and lower hepcidin levels. Another anti-hepcidin pathway is the interruption of the IL-6 activation of hepcidin. In this respect, tocilizumab, which is a neutralizing antibody to IL-6, might be promising. However, all these investigational treatments are not without risks: reducing hepcidin levels might promote infections and HIF stabilizers may enhance tumor growth.
Effect of dialysis strategies on hepcidin levels

Hepcidin is a small molecule, and even though it is highly protein-bound, various studies have shown that its levels are reduced by HD, with a reduction ratio in different studies of 14-51%\(^{38, 51, 53-55, 89}\). Hepcidin was present in the ultrafiltrate of patients treated with HD and in the dialysate of patients treated with peritoneal dialysis (PD), but levels were too low for quantification.\(^{38, 55}\) Prohepcidine was present in both the ultrafiltrate in HD patients and peritoneal effluent in PD patients, although concentrations were much lower than in the urine.\(^{51}\) Furthermore, hepcidin has been detected after elution of a biocompatible polysulfone dialyzer, which suggests that hepcidin binds to the HD filter.\(^{38}\) This observation is in line with the amphipathic characteristics of hepcidin (see “Measurement of hepcidin” before). One study showed that the inter-patient variability of the hepcidin reduction ratio was substantial, suggesting that postdialysis levels may be influenced by factors other than removal by dialysis, such as increased production of hepcidin during the dialysis treatment.\(^{89}\)

The latter might be caused by an inflammatory response due to bio-incompatibility of the dialyzer and blood tubes, and use of non-ultrapure dialysis fluids. Moreover, although hepcidin levels initially decreased during a dialysis session, post-dialysis levels were back to pre-dialysis levels already one hour after the end of a dialysis session.\(^{53}\) This suggests that sustained lowering of hepcidin levels with extracorporeal renal replacement therapy might not be possible.

Considering the permeability of the dialyzer, one study showed a superior reduction of hepcidin in patients treated with high-flux as compared to low-flux HD,\(^{54}\) whereas no difference was observed in another study.\(^{89}\) Hemodiafiltration (HDF) resulted in superior reduction of hepcidin, especially when a double filter was used.\(^{51, 89}\) In one study, it was suggested that HDF decreased the inflammatory status, which resulted in less production of hepcidin during a dialysis session.\(^{89}\)

Finally, it should be mentioned that hepcidin levels were similar in patients treated with different dialyzer membranes, namely cellulose triacetate, polysulfone, ethylene-vinyl and polyester-polymer dialyzers.\(^{52}\) Yet, the studied groups were very small.

CONCLUSION AND REMAINING QUESTIONS

ESA resistance is common in chronic dialysis patients, and (functional) iron deficiency is an important contributing factor. Hepcidin is a key regulator in iron homeostasis, as the active isoform hepcidin-25 induces the internalization and degradation of the iron exporter ferroportin, resulting in decreased iron absorption and circulating iron concentrations, and hence decreased iron availability, and increased intracellular iron stores. Hepcidin can be measured with several techniques, although measurements are not standardized yet and there are considerable differences between various techniques used in the different studies.

In CKD and dialysis patients, hepcidin levels are increased, possibly due to the diminished clearance of hepcidin and/or enhanced production. The latter may be associated with an inflammatory state. In the general population as well as in CKD and ESRD patients, hepcidin is associated with ferritin. Studies in limited numbers of CKD patients on the
association of hepcidin with inflammation and residual kidney function have shown conflicting results. Furthermore, the role of hepcidin in predicting the response to ESA and iron administration, and its role as a marker of ESA resistance, is debatable.

Hepcidin might prove to be a novel determinant of cardiovascular disease as enhanced hepcidin levels may result in iron sequestration in macrophages in vessel walls, which is associated with oxidative stress in atherosclerotic plaques. Whether this hypothesis is relevant in CKD patients, remains to be established.

Substances that interact with various pathways in the regulation of hepcidin might be applicable as hepcidin antagonists. Currently, it is unknown if these therapies are beneficial in CKD patients and whether they are safe. Furthermore, various dialysis techniques can be used as hepcidin lowering strategies, although its short-term results are rather disappointing. Whether dialysis can result in a reduction of hepcidin levels over a long-term period, and what dialysis modality will be most effective for this purpose, is not known yet.

In conclusion, the discovery of hepcidin enhanced our knowledge with respect to the pathophysiology of renal anemia and functional iron deficiency in CKD and ESRD patients. Furthermore, the role of hepcidin in cardiovascular disease may be relevant. Whether hepcidin-lowering therapies will be beneficial in this high-risk patient group remains to be established.

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3

AIM AND OUTLINE OF THIS THESIS
In this thesis, the current evidence on the clinical benefits of middle molecular weight (MMW) clearance by hemodiafiltration (HDF) will be extended. Furthermore, the role of hepcidin in dialysis patients will be addressed.

The first aim of this thesis is to evaluate the benefits of increased MMW clearance as obtained by hemodiafiltration (HDF) with respect to renal anemia and cardiovascular disease. The second aim is to study the role of hepcidin in renal anemia in chronic hemodialysis (HD) patients, and to investigate whether this peptide can serve as a novel biomarker for cardiovascular disease in this patient group.

Resistance to erythropoiesis stimulating agents (ESA) in the treatment of renal anemia has been associated with various clinical factors, such as inflammation and malnutrition. However, thus far, the role of residual kidney function on anemia management and ESA resistance in HD patients has not been fully revealed. In chapter 4, we describe clinical and biochemical factors associated with ESA resistance in the baseline cohort of the CONvective TRAnsport STudy (CONTRAST), with emphasis on residual kidney function. In addition, the possible beneficial effect of residual kidney function on levels of β2-microglobulin, a MMW toxin, and phosphate control, are evaluated.

One of the secondary endpoints of the randomized controlled CONTRAST study was the effect of treatment with online HDF on ESA resistance, anemia management and iron parameters, as compared to low-flux HD. These results are presented in chapter 5.

The main hypothesis of the CONTRAST study was that enhanced MMW clearance obtained by online HDF would result in a decreased mortality and less cardiovascular events, as compared to low-flux HD. Furthermore, the question arose whether specific patient groups could be identified that would benefit most from treatment with HDF. The primary outcome and the main secondary outcome of the CONTRAST study are described in chapter 6.

During the last decade, hepcidin, a key regulator of the systemic iron homeostasis, appeared to be a relevant peptide regarding the pathophysiology of impaired erythropoiesis and (functional) iron deficiency in patients with CKD. In chapter 7, we evaluate which patient-, biochemical- and treatment related characteristics are related to levels of hepcidin-25 in chronic HD patients in the baseline cohort of the CONTRAST study, and whether hepcidin might be a potential biomarker for ESA resistance.

Apart from its role in anemia and iron metabolism, hepcidin-25 might be involved in iron accumulation in atherosclerotic plaques, and hence may play a role as a biomarker of cardiovascular disease. In chapter 8, the relation between the bioactive hepcidin-25 and both all-cause mortality and fatal and non-fatal cardiovascular disease is assessed in HD patients participating in CONTRAST.

Hepcidin-25 has a molecular weight of 2.8 kD and is a MMW toxin as such. Therefore, we hypothesized that HDF could result in enhanced clearance as compared to low-flux HD. The question whether the decrease in ESA resistance in a subgroup of patients in the CONTRAST study was mediated by a decrease in hepcidin-25 levels, is addressed in chapter 9.

Several guidelines, adopted by the Dutch Federation of Nephrology, have been developed to guide the clinical management of renal anemia in patients with chronic kidney disease and in those on dialysis. However, especially for the latter category, evidence from
randomized clinical trials is virtually absent. In **chapter 10**, the compliance with targets on hemoglobin levels and iron parameters as formulated in the abovementioned guidelines are described in the baseline cohort of CONTRAST. Furthermore, this chapter focuses on demographic and clinical characteristics that are related to compliance with the guidelines, and to what extent compliance differs between treatment facilities.

**Chapter 11** comprises the overall discussion and perspectives of this thesis. Furthermore, suggestions for further research are made.