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GENERAL DISCUSSION
INTRODUCTION

During the last decades, the conventional thrice-weekly low-flux hemodialysis (HD) treatment for 3-4 hours has developed into a variety of other extracorporeal renal replacement therapies, such as HD with high permeable (high-flux or super-flux) dialyzers, longer and/or more frequent dialysis (so-called “intensified dialysis modalities”, e.g. thrice-weekly HD with 8 hour sessions, short daily and nocturnal HD), performed either in-center or at home, and hemodiafiltration (HDF), mostly performed in-center within conventional treatment times and frequency.

Nevertheless, the prognosis of HD treated patients in general is still extremely poor. The nephrology community kept on searching for novel treatment options to improve this poor prognosis, for example by starting dialysis in an earlier phase of renal failure, improving solute clearance by increasing the dialysis dose, performing HD with high-flux dialyzers or hemo(dia)filtration, and by prescribing drugs such as statins and erythropoiesis stimulating agents (ESA), the latter aimed at obtaining high hemoglobin levels. Studies on these interventions, however, do not always show unequivocally positive results, and findings from observational studies and randomized controlled trials (RCTs) can be conflicting. Furthermore, specific treatments that appeared to be beneficial in observational studies turned out to be harmful when tested in RCTs, which was the case when aiming for high hemoglobin levels in patients with chronic kidney disease (CKD) resulting in increased risk for stroke and in HD patients resulting in an increased incidence of vascular access thrombosis. Therefore, performing well-designed RCTs in CKD and dialysis patients is of utmost importance, as will be highlighted below.

Outline of this chapter

In this chapter, the studies that are described in this thesis will be discussed and compared with studies on other chronic extracorporeal dialysis modalities focusing on clinical implications. In addition, topics for further research will be proposed.

First, the effect of HDF and various other HD modalities on renal anemia and resistance to erythropoiesis stimulating agents (ESA) will be discussed (in relation to part II: chapter 4 and 5). Second, the effects of various extracorporeal dialysis modalities on mortality and cardiovascular disease and implications of the results of the main outcome of the CONvectiveTRAnsportSTudy (CONTRAST study, part II: chapter 6) will be described. Third, the role of hepcidin for diagnostic and prognostic purposes will be reviewed (highlighted in part III: chapter 7, 8 and 9).

EFFECT OF HDF ON ANEMIA AND ESA RESISTANCE

In chapter 5, we presented a secondary analysis of the randomized controlled CONTRAST study on the effect of HDF on ESA resistance and iron parameters. We clearly demonstrated that there was no effect of online HDF on ESA resistance compared to low-flux HD. Furthermore, a trend towards an enhanced intravenous iron dose in patients treated with HDF was observed. The CONTRAST study is the largest RCT of online HDF versus
low-flux HD currently published and anemia management was a secondary endpoint. In chapter 1, we described that virtually all observational and small cross-over studies showed a beneficial effect of HDF on ESA resistance, whereas two small RCTs did not.\textsuperscript{10, 11} A recent review on the impact of different dialysis techniques on renal anemia shared our opinion that thus far, data from HDF studies on anemia are non-conclusive, possibly since different treatment protocols and dialyzers have been applied, and most studies exhibited a lack of control groups and/or enrolled a small number of patients.\textsuperscript{12} The recently published European randomized controlled MINOXIS trial (Modulation of Inflammation and Oxidative Stress by high-flux hemodialysis) did not show any difference in hemoglobin levels, ESA dose or ESA index (i.e. ESA dose normalized to body weight and hemoglobin level) between treatment with high-flux versus low-flux HD for 12 months.\textsuperscript{13} These data indicate that adding a relatively low convective volume to conventional dialysis appears not to be beneficial with respect to ESA resistance.

Simultaneously with the CONTRAST study, a Turkish study on HDF with a similar design was performed (NCT00411177).\textsuperscript{5} The results of this study were presented at the 48\textsuperscript{th} ERA-EDTA congress in June 2011 in Prague. A total of 782 patients were randomized to either high-flux HD or online HDF and followed for 22.7 months (median). In this study, equal Hb levels were observed in patients treated with online HDF and high-flux HD (11.5 ± 1.2 g/dL in both groups), albeit that in the HDF group, the mean ESA dose was significantly lower (2282 ± 2121 U/week versus 2852 ± 2706; p=0.001). The latter suggested improved ESA resistance with HDF. These results were different from our findings as described in chapter 5. However, since the study treatment protocol and statistical methods are not published yet, it is not possible to judge the results at this moment.

As described in chapter 4, residual kidney function seems to be of significant importance when aiming for low ESA resistance. The effect of HDF and time-intensified treatment modalities such as daily or nocturnal HD on residual kidney function is largely unknown. It has been shown that HDF is associated with less intradialytic hypotension,\textsuperscript{14, 15} which may be an important contributing factor to the preservation of residual kidney function.\textsuperscript{16} One small, non-randomized study showed that HDF resulted in better preservation of residual kidney function, indeed.\textsuperscript{17} In a short-term analysis of the first 180 consecutive patients included in the CONTRAST study, a protective effect of HDF on residual kidney function was not present (unpublished data),\textsuperscript{18} but a formal analysis in the total study population and with a total duration of follow-up will be performed in the future.

**Effect of time-intensified dialysis strategies on anemia and ESA resistance**

It can be hypothesized that intensified dialysis techniques result in improvement of renal anemia and ESA responsiveness, as compared to conventional dialysis. The possible underlying mechanisms could be a diminished inflammatory state and/or increased removal of uremic toxins,\textsuperscript{19} or increased red blood cell lifespan.\textsuperscript{20, 21} Increasing treatment time and/or frequency can be beneficial for additional removal of some specific toxins with multi-compartment kinetics, e.g. phosphate and β2-microglobulin, resulting in lower
pre-dialysis concentrations over time. However, more frequent and/or longer exposure to blood tubes, dialyzer and dialysis fluid may also result in an enhanced inflammatory state, as well as an increased risk for blood loss. These two factors may attenuate the assumed beneficial effect of longer and/or more frequent HD. With respect to HDF, the beneficial effects on inflammation have been controversial thus far.

Several studies on the effect of intensified different hemodialysis strategies on anemia treatment and ESA resistance are available. In a single-center observational study, a good anemia control in patients treated with low-flux HD performed three times per week for eight hours (so called “long dialysis”) was observed in Tassin, France. In only 56% of patients, ESA was prescribed. However, the results of this study are limited by the absence of a control group.

In 2008, a systematic review on the effects of short daily HD on various clinical parameters was published. During short daily HD, either in-center or at home, treatment frequency is increased whereas total weekly treatment time generally remains stable (i.e. 12 hours/week). The review consisted mainly of small studies (10-40 patients), with an observational switch or cross-over design. In six studies, ESA dose could be reduced during short daily HD, whereas in two studies, no difference was observed. In a randomized controlled study from the US Frequent Hemodialysis Network (FHN, see table 1 for study details), no difference in ESA dosing between short daily and conventional HD was shown either. Increasing the frequency of HDF treatment from three-weekly to short daily HDF in a very limited number of chronic HD patients, did not show an effect on hemoglobin levels with stable ESA dosis.

Studies on the effect of increasing both treatment frequency and time as in nocturnal HD, have shown mixed results. Two prospective observational studies (one switch [n=60] and one case-control [n=12]) showed an increase in hemoglobin concentrations in patients treated with daily nocturnal HD as compared to conventional thrice-weekly HD. In the case control study, the observed concomitant increase in ESA index was thought to be caused by more frequent blood loss. In the second randomized controlled FHN trial (see table 1 for study details) a non-significant higher ESA dose was observed in patients treated with nocturnal as compared to conventional HD.

As for long, frequent HDF, a cross-over study in 26 patients on the effect of a switch from 4-5 h thrice-weekly HDF to 7–8 h nocturnal every-other-day HDF with the same (20–30 L) or higher (35–50 L) convective volume was recently published. Whereas this resulted in a remarkable improvement in nutritional status, phosphate and hypertension control as well as a reduction in left ventricular mass (LVM) over 12 months' of follow-up, hemoglobin level, ESA index and iron parameters did not change.

In conclusion, in fact, no conclusive evidence exists on the effect of different dialysis strategies on ESA resistance. Hence, when aiming at an optimal anemia management and lowest ESA resistance, no single dialysis modality seems to be preferable.
EFFECT OF HDF AND INTENSIFIED DIALYSIS MODALITIES ON CLINICAL ENDPOINTS

As described in chapter 1, evidence from RCTs on the effect of HDF with respect to survival has been limited so far. In chapter 6, the results of the CONTRAST study on all-cause mortality and cardiovascular events are presented. In this RCT in 714 prevalent dialysis patients comparing treatment with online HDF and conventional low-flux HD, treatment with HDF was not associated with improved survival or with diminished composite of fatal and non-fatal cardiovascular events in the overall analysis. Furthermore, in none of the pre-specified subgroups, such as patients with long dialysis vintage, patients without residual kidney function or with low albumin levels, beneficial effects of HDF were shown. However, in an on-treatment analysis, patients treated with HDF had an improved survival when a convection volume (i.e. substitution volume plus net ultrafiltration volume) of more than 22.0 L (substitution volume ± 20.1 L) was applied.

In the Turkish HDF study (NCT00411177), results were similar to those of the CONTRAST study: the composite of mortality and non-fatal cardiovascular events (primary endpoint) was not different between the two groups(hazard ratio HDF vs. high-flux HD 0.82; 95% CI 0.59-1.16, p=0.28; table 1), neither was the secondary outcome of all-cause mortality. In the on-treatment analysis, however, HDF patients who were treated with a substitution volume (i.e. convection volume minus net ultrafiltration volume) of more than 17.4 L did have a survival benefit (hazard ratio high-efficiency HDF vs high-flux HD 0.54; 95% CI 0.31-0.93, p=0.02; table 1).

In 2012, three large observational studies on the effect of intensive HD (i.e. nocturnal and/or frequent HD) were published, all with matched control groups. The results of these studies are summarized in table 1: all three studies showed a better survival in patients treated with time-intensified HD modalities as compared to conventional HD (three times per week). Of course, these studies have an important limitation: as they have an observational design, they cannot prove causality. Furthermore, despite all efforts to adjust for differences between the comparison groups, residual confounding cannot be excluded. However, RCTs on more time intensive dialysis modalities may not be realistic, and thus, these studies may be the best available for "evidence based clinical practice".

Nevertheless, in 2010 and 2011, the FHN published two RCTs on frequent short HD and nocturnal HD respectively, which were already briefly addressed above. In these trials, however, it was not feasible to recruit a patient sample large enough to provide adequate statistical power to assess individual end points of death, cause-specific death, hospitalization, or other events. Therefore, both studies had two primary composite endpoints: (1) death or change in LVM and (2) death or change in physical-health score (measured with the validated RAND 36-item health survey). Of note, these endpoints may be difficult to translate into clinical practice. The study on frequent short HD showed a beneficial effect on both composite endpoints as compared to conventional thrice-weekly HD. Regarding frequent nocturnal HD, effects on both endpoints were similar as compared to conventional HD, although the number of patients included in this study was smaller due to difficulties with the recruitment of patients. The results of both trials are summarized in table 1.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Intervention</th>
<th>Outcome</th>
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<tr>
<td>Chertow 2010</td>
<td>RCT</td>
<td>Frequent HD</td>
<td>Composite death/non-fatal cardiovascular events: HR 0.70 (95% CI 0.53-0.92)</td>
</tr>
<tr>
<td>Rocco 2011</td>
<td>RCT</td>
<td>Nocturnal and frequent HD</td>
<td>Composite death/non-fatal cardiovascular events: HR 0.68 (95% CI 0.44-1.07)</td>
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<tr>
<td>Lacson 2012</td>
<td>Observational</td>
<td>Nocturnal HD</td>
<td>Death: HR 0.75 (95% CI 0.61-0.91)</td>
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<tr>
<td>Nesrallah 2012</td>
<td>Observational</td>
<td>Nocturnal and frequent HD</td>
<td>Death: HR 0.55 (95% CI 0.34-0.87)</td>
</tr>
<tr>
<td>Weinhandl 2012</td>
<td>Observational</td>
<td>Daily home HD</td>
<td>Death: HR 0.87 (95% CI 0.78-0.97)</td>
</tr>
<tr>
<td>Grooteman 2012</td>
<td>RCT</td>
<td>Online HDF</td>
<td>Death: HR 0.95 (95% CI 0.75-1.20)</td>
</tr>
<tr>
<td>Ok 2012 (Turkish HDF Study)</td>
<td>RCT</td>
<td>Online HDF</td>
<td>Composite death/non-fatal cardiovascular events: HR 0.82 (95% CI 0.59-1.16)</td>
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Table 1. Results from large trials on the effect of different dialysis strategies on all-cause mortality.
Performing randomized controlled trials in HD patients

The above-mentioned studies emphasize the difficulty when performing RCTs in chronic HD patients, especially regarding time-intensified treatments such as nocturnal and daily HD. In general, the number of RCTs in nephrology patients is very limited when compared to other internal medicine disciplines. Furthermore, performing (double-blinded) RCTs is a real challenge in a chronic HD population with a very high rate of comorbidity and many elderly patients. Especially RCTs on intensified dialysis regimes with hard clinical endpoints may be difficult to conduct, since the intervention (e.g. daily or nocturnal HD) requires major changes to patient lifestyle. In addition, the outcome of most (hemo-) dialysis trials will be influenced by the fact that the contribution of “relatively fit” patients may be limited since (pre-emptive) kidney transplantation is nowadays the renal replacement therapy of choice.

Although RCTs may be difficult to design and to implement and its results are often not as positive as expected, this type of study has traditionally been regarded as the highest level of evidence. Nevertheless, it has been argued that the comparison of observational versus randomized controlled evidence may not identify large differences in effect estimates. There are well known examples in the literature, however, in which this is clearly not the case. In HD patients, observational and randomized studies have shown conflicting results, e.g. regarding the use of statins.

As for implementing guidelines in dialysis patients, a novel classification system for the available evidence has been recently introduced by the Kidney Diseases Improving Global Outcome (KDIGO) initiative and by the European Renal Best Practice (ERBP) working groups. The reason for this change was the limited availability of RCTs in dialysis patients, and the fact that good clinical practice or widespread experience is unlikely to be tested by an RCT. In this new system, RCTs are not unequivocally considered as the highest level of evidence. Instead, the level of confidence is dependent on the judgment of a group of experts in the field as well, taking benefit versus risk stratification into account.

Nevertheless, for novel treatment options in which RCTs seem feasible, it is important they are performed in the high-risk dialysis population. The CONTRAST study and the Turkish HDF study demonstrated that RCTs on HDF are feasible and emphasized once more the different outcome between observational and randomized studies.

Lessons from HDF trials

Do the “negative” results of the two HDF landmark studies (the CONTRAST study and the Turkish HDF study) mean that HDF can be regarded as a failed treatment option, and that nephrologists should focus on alternative dialysis strategies? This question can be answered with “no” for several reasons.

High convection volume

Both CONTRAST and the Turkish HDF study have shown in an on-treatment analysis that HDF treatment with high convection volume did result in improved survival, even after adjustment for other determinants of convection volume, suggesting a dose-response
relationship. This is in accordance with the observational data from the DOPPS study in 2006, that showed a survival benefit for treatment with so-called “high-efficiency HDF” (substitution volume >15 L), whereas the outcome of “low-efficiency HDF” was similar to high- and low-flux HD.\(^46\) Previously, we have shown that convective volumes vary to a large extent between treatment facilities.\(^47\) We demonstrated that convection volume is associated with various potentially modifiable factors such as treatment time, hematocrit and blood flow rate.\(^47\) Furthermore, we presented some practical treatment recommendations to achieve convection volumes of more than 20 liters.\(^48\)

Of note, conclusions on the pre-specified on-treatment analysis in the CONTRAST study should be interpreted with caution. Patients in which a high convection volume could be reached might have specific characteristics that are associated with a favorable outcome. In this secondary analysis of the CONTRAST study, adjustments were made for factors that have previously been related to convection volume (i.e. age, gender, albumin, and hematocrit),\(^47\) and for potential determinants of mortality (i.e. age, gender, previous vascular access, diabetes, previous transplantation, spKt/V, baseline eGFR, albumin, creatinin and hematocrit, use of alpha- and betablockers, calciumantagonists and RAS inhibitors). One can argue that the results of this secondary on-treatment analysis are biased by the fact that a high blood-flow, and thus a proper vascular access (i.e. a native fistula), are essential for obtaining high convection volumes, and that a decent vascular access is associated with a better outcome in itself. However, in a recent analysis of the CONTRAST study, the percentage of patients in the group who obtained the highest convection volume (i.e. >22 L/treatment) with a fistula and a central catheter was 79 and 9%, respectively, whereas these numbers were 78 and 3 in the group who obtained the lowest (i.e. <18.3 L/treatment) convection volumes (unpublished data). These data suggest that the results of the on-treatment analysis in CONTRAST are not biased by the presence of a proper vascular access in the group with high convection volumes.

Furthermore, the favorable outcome in patients with high convection volumes might be considered as an example of a "dose-targeting bias" which was also described in HEMO study in patients who were targeted for a high or low Kt/V: patients that reached the target Kt/V level had a better survival than those who did not, regardless whether they were allocated to the high or low Kt/V group.\(^49\)

Still, future research in HDF should focus on interventions with high convection volumes and on the determination of the optimum amount of fluid replacement with highest benefits at the price of minimum complications (e.g. clotting in the extracorporeal system).

**Selection of the primary endpoint**
The CONTRAST study and the Turkish HDF study were powered for (cardiovascular) survival as a primary endpoint. However, in today’s chronic dialysis population, with very high comorbidity and an increasing age, endpoints such as quality of life, relief of symptoms such as pruritus, fatigue, neuropathy, loss of appetite, restless legs, sleeping disturbances and sleep apnea, and a decrease in the number of pills prescribed, may be as important as “hard” clinical endpoints such as survival, especially with regard to geriatric patients.\(^50\) Randomized studies on these quality-of-life related endpoints are limited. In the CONTRAST study, no
difference between HD and HDF was observed with respect to health related quality of life, although a trend towards a stable quality of life during treatment with HDF and a decrease in quality of life during HD was suggested.\textsuperscript{51} As comorbidity and disease burden are high, the above-mentioned endpoints should be important focuses for further research.

Subgroup analyses
It is important to identify subgroups that could benefit from HDF, especially since in the overall population, no effect of HDF on all-cause mortality was demonstrated. In the HEMO study, no difference in all-cause mortality was observed between high-flux as opposed to low-flux HD and high-dose as opposed to low-dose HD in the overall study population.\textsuperscript{3} However, the pre-specified subgroup of patients with a dialysis vintage $\geq$3.7 years benefited from treatment with high-flux HD, especially with respect to cardiac death and cerebrovascular disease.\textsuperscript{52, 53} Furthermore, women benefited from a higher eqKt/V of around 1.53.\textsuperscript{3} In the European MPO study, treatment with high-flux HD resulted in improved survival in the pre-specified subgroup of patients with an albumin level of $\leq$40 g/L as compared to low-flux HD.\textsuperscript{4} Moreover, patients with diabetes had a survival advantage when treated with high-flux HD in a post-hoc analysis. Of note, both hypoalbuminemic and diabetic patients represent a substantial amount of the total dialysis population.\textsuperscript{54, 55} In the overall MPO study population, however, no difference between high- and low-flux HD with respect to mortality, hospitalizations and infections were observed.\textsuperscript{4}

To perform valid subgroup analyses, a large patient population randomized to HDF or HD should be studied. For this purpose, data pooling from various (ongoing) studies on HDF is of eminent importance. Recently, the ERA-EDTA European Dialysis Working Group (EUDIAL) group has been established, of which the main goal is to improve renal outcomes by upgrading renal replacement therapies.\textsuperscript{56} Such an initiative could be very helpful in data pooling of individual patients from different HDF studies, which facilitates subgroup analyses. Furthermore, a meta-analysis (combining results from different studies) by the independent Cochrane Library group is currently being performed.

Treatment frequency and duration
Finally, another significant aspect of HDF is that the intervention modality can be performed within a treatment time and frequency that is traditionally regarded as “conventional”, whereas short daily and nocturnal HD are characterized by longer and/or more frequent treatments. For many patients, HDF, when performed with high convection volumes, could be a more acceptable and applicable treatment.

HEPCIDIN: A NOVEL CLINICAL TOOL FOR ANEMIA MANAGEMENT?

In chapter 2, several aspects of hepcidin in chronic kidney disease (CKD) and dialysis patients are reviewed. Measuring hepcidin was expected to be a useful clinical tool for distinguishing absolute from functional iron deficiency, as was illustrated in a paper titled “Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation”,\textsuperscript{57} suggesting that
hepcidin could be a marker of ESA resistance. However, in both a study in HD patients and one in patients with the cardio-renal syndrome, hepcidin levels were higher in ESA responders than in non-responders, suggesting that hepcidin is more a marker of ESA responsiveness rather than being associated with ESA resistance. Furthermore, neither hepcidin-20 nor hepcidin-25 was associated with iron responsiveness, whereas only the percentage of hypochromic red blood cells could predict a response to intravenous iron. In another study in HD patients, hepcidin (measured with a competitive enzyme-linked immunosorbent assay) showed a large intra-individual variability over 2- and 6-weeks periods, which was also correlated with inflammation markers. The same observations were made for ferritin, regarded as the classical biomarker for iron stores. These findings indicate that as of yet, hepcidin does not provide an advantage over ferritin as a clinical useful indicator of iron status in chronic stable HD patients as of yet.

In our study on the determinants of hepcidin in chronic HD patients as described in chapter 7, the (patho-) physiological pathways of hepcidin that have been suggested in smaller studies were confirmed in a large cohort of chronic HD patients, namely the association between hepcidin and iron stores, erythropoiesis and inflammation. The cross-sectional design of the study impeded conclusions on the role of hepcidin as a predictor of ESA- or iron-responsiveness. However, both with univariate and multivariate statistics, no relations between hepcidin and either ESA or iron doses were observed. Therefore, apart from the fact that thus far, specific hepcidin-25 assays are either expensive and/or time-consuming, the role of hepcidin to guide clinical anemia management in HD patients may be limited.

Mechanism of reduced ESA resistance by HDF: via hepcidin?

Although hepcidin may not be suitable as a clinical tool to predict the response to ESA administration, it has been suggested that hepcidin is associated with anemia of chronic disease (ACD). Moreover, ESA resistance in HD patients is associated with malnutrition and inflammation, which may accompany ACD. Hence, we hypothesized that extremely ESA resistant patients would have high hepcidin levels and that the beneficial effect of HDF on ESA responsiveness in this ESA resistant patient group, as was suggested in a post-hoc analysis of the CONTRAST study (chapter 5), might be accompanied and explained by a concomitant reduction in serum hepcidin levels. However, as was shown in chapter 9, we could not confirm this hypothesis: after one-year follow-up, pre-dialysis hepcidin levels were not reduced in patients treated with HDF. This negative finding might be explained by several factors. First, as highlighted above, the association between high hepcidin levels and ESA resistance in HD patients on maintenance ESA therapy is debatable. Second, the CONTRAST study may not have the optimal design to test the above-mentioned hypothesis. Only pre-dialysis hepcidin levels were measured at baseline and after 12 months, whereas ideally, pre-dialysis hepcidin levels on more time-points should have been measured, as well as post-dialysis levels. Third, it is not known whether treatment with dialysis is able to diminish hepcidin levels over a long-time period. It was shown that hepcidin levels initially decrease during a dialysis session, although post-dialysis levels were back to pre-dialysis levels already one hour after the end of a dialysis session.
Hopefully, the questions raised above can be answered by an ongoing Italian randomized cross-over study in which the effects of HDF and HD on ESA dose, hemoglobin, iron and inflammation markers and hepcidin are evaluated (EU Clinical Trials Register 2010-018718-57). Results are to be expected soon.

HEPCIDIN: A NOVEL BIOMARKER FOR CARDIOVASCULAR DISEASE?

The role of hepcidin as a biomarker of cardiovascular disease seems promising. As discussed in chapter 2, the “iron hypothesis” has gained renewed interest suggesting that hepcidin promotes iron trapping in the vascular wall, thereby enhancing oxidative stress, instability of atherosclerotic plaques and cardiovascular disease. HD patients suffer from a high burden of cardiovascular disease, are often iron loaded and exhibit high hepcidin levels. Therefore, it is remarkable that besides one study in HD patients on the relation between hepcidin and vascular stiffness, further studies in this patient category are virtually absent. In chapter 8, we demonstrated an association between hepcidin-25 levels and cardiovascular disease, persistently present after adjustment for various cardiovascular risk factors and determinants of hepcidin-25, including inflammation markers. The mechanism by which this association is established in these high-risk patients is not clarified yet. It may be similar as has been described in patients with non-alcoholic fatty liver disease or in non-CKD patients with cardiovascular disease, but this has not been investigated thus far. Furthermore, it is completely unknown whether iron sequestration in macrophages plays the same role in atherosclerotic plaques in the arterial intima as in calcified plaques in the arterial media, as the latter is a characteristic of cardiovascular disease in dialysis patients. Since the associations between hepcidin and both all-cause mortality and cardiovascular events were confounded to a large extent by inflammation, it remains to be established whether hepcidin plays a causal role or is an epiphenomenon of an inflammatory state.

If the observed association between hepcidin and cardiovascular events appears to be causal, this might challenge the safety of administering large amounts of intravenous iron in dialysis patients, especially in patients who already exhibit high hepcidin levels and/or an inflammatory state. This concern was also argued in a recent position statement of the Anemia Working Group of the European Renal Best Practice. The Dialysis Patients’ Response to IV Iron with Elevated Ferritin (DRIVE) trial showed that hemoglobin levels increased after administration of intravenous iron in patients on stable ESA therapy with high ferritin levels and low TSAT. This might result in the prescription of iron supplementation even in patients with high ferritin levels. Yet, the design of the DRIVE study was not appropriate to draw any conclusions on the safety of iron administration with respect to cardiovascular events, since the follow up (six weeks) was too short. In a cohort study of over 32,000 HD patients, iron administration of over 1000 mg per 6 months was associated with increased mortality. However, after adjustment for time-varying measures of iron administration as well as for other fixed and time-varying measures of morbidity, no significant association between iron supplementation and mortality was observed any more.
So far, no prospective trials on the safety of intravenous iron supplementation, especially with respect to cardiovascular events, have been published.\textsuperscript{76, 77} Nevertheless, such a study would provide important information to guide treatment of renal anemia in HD patients.

Finally, it would be interesting to evaluate the effect of kidney transplantation on hepcidin levels and the susceptibility to cardiovascular disease. In transplant patients with known coronary artery disease, hepcidin levels were significantly higher (in univariate analysis).\textsuperscript{79} Further studies in this patient group are currently lacking.

CONCLUSION

The data presented in this thesis provide evidence on the effects of increasing middle molecular weight (MMW) clearance by HDF on renal anemia and cardiovascular disease in chronic HD patients. Furthermore, the knowledge on the role of hepcidin in chronic HD patients is extended.

Measurement of hepcidin provides information on the pathways of functional iron deficiency regarding renal anemia in HD patients, although its use as a clinical tool to guide treatment seems to be limited. At the same time, its role as a biomarker of cardiovascular disease in this patient category may be promising and deserves further research.

Increasing MMW clearance by HDF does not result in improved ESA responsiveness, and may result in increased iron requirements. Furthermore, treatment with HDF does not improve survival or decrease cardiovascular morbidity and mortality. Nevertheless, treatment with HDF might have a beneficial effect on survival provided that sufficiently high convection volumes are obtained (i.e. more than 22.0 L/session).

The latter paragraph may implicate that HDF is not such a “promise for the future” as we had hoped before.\textsuperscript{79} However, within a treatment duration and frequency that is traditionally regarded as “conventional”, it still may have beneficial effects, provided that convection volumes are high enough. This message might especially be applicable for the growing dialysis population that is not in the condition for kidney transplantation or willing to be treated with more frequent and/or longer HD strategies such as nocturnal and short daily HD.

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