General discussion and future perspectives
Acute kidney injury (AKI) in the intensive care unit (ICU) still has a high incidence and is associated with increased morbidity and mortality. It is defined using criteria according to relative changes of serum creatinine and urine output and is a serious condition as even a slight deterioration of renal function implies a worse overall prognosis [1-5]. Once AKI is present in patients and supportive measures are insufficient to reverse it, renal replacement therapy (RRT) is often inevitable. In RRT, blood contacts the extracorporeal circuit and hemofilter, inducing an inflammatory response in the patients defined as bioincompatibility. Also, the risk of clotting necessitates continuous anticoagulation for maintaining patency and function of the extracorporeal circuit and filter. Heparin remains to be the most widely used anticoagulation. Current guidelines, however, suggest regional citrate anticoagulation for continuous RRT rather than heparin in patients without contraindications for citrate [6]. This recommendation is based, amongst other studies, on the study by Oudemans et al., who observed improved patient survival in continuous venovenous haemofiltration (CVVH) with citrate as opposed to CVVH with low molecular weight heparin [7]. In contrast, Hetzel et al. did not find a survival difference between predilution citrate-CVVH as compared to CVVH with unfractionated heparin, yet did observe improved filter survival times in the citrate group [8].

The type of anticoagulation appears to contribute to the magnitude of the inflammatory response during RRT, as has been demonstrated in intermittent hemodialysis [9-12]. In this thesis we aim to answer the question whether regional citrate is indeed superior to the classic choice heparin in CVVH in critically ill patients with AKI. We address several aspects including patient- and filter survival and biocompatibility.

In chapter 2 we present the results of the CASH trial (Citrate Anticoagulation versus Systemic Heparinisation). In this multi-center randomized controlled trial, patients admitted to the ICU requiring CVVH were randomly assigned to citrate or heparin. Of the 139 patients enrolled, 66 were randomized to citrate and 73 to heparin. Mortality rates at 28 and 90 days did not differ between groups: 22/66 (33%) and 27/65 (42%) of citrate-treated patients died versus 25/72 (35%) and 29/69 (42%) of heparin-treated patients at respectively 28 and 90 days (P = 1.00 for both). Renal outcome, i.e. independency of RRT 28 days after initiation of CVVH in surviving patients, did not differ between groups: 29/43 (67%) in the citrate-treated patients versus 33/47 (70%) in heparin-treated patients (P = 0.82). Heparin was discontinued in 24/73 (33%) of patients whereas citrate was discontinued in 5/66 (8%) of patients (P < 0.001). Filter survival times were superior for citrate (median 46 versus 32 hours, P = 0.02), similar to the findings by Hetzel et al. [8], as were the number of filters used (P = 0.002) and the off time within 72 hours (P = 0.002). The costs during the first 72 hours of prescribed CVVH were lower in citrate-based CVVH. We therefore conclude that renal outcome and patient
mortality were similar for citrate and heparin anticoagulation during CVVH in the critically ill patient with AKI, in contrast to the study by Oudemans et al. [7], where citrate reduced mortality (90-day mortality 45% for citrate, 62% for nadroparin). Mortality rates in our study were somewhat lower than in previous studies and this could, at least in part, be attributed to a lower frequency of sepsis in our patient group. Also, APACHE-II scores at baseline, and age, were higher as reported by Oudemans et al. [7], yet similar to those reported by Hetzel et al. [8]. We could not confirm the beneficial effect on mortality rates in cases of sepsis that was suggested by Oudemans et al. [7]. Survival rates were similar for septic and non-septic patients and the type of anticoagulation used did not affect this number. Moreover, the lack of benefit for citrate regarding mortality persisted in subgroup analyses. However, citrate was superior in terms of safety, efficacy and costs.

The pathophysiological basis of superiority of citrate to heparin in terms of improved filter survival and possible improved patient outcome observed by others [7], remains to be elucidated. One hypothesis is that citrate may induce less bioincompatibility and consequently a lower inflammatory response to CVVH in critically ill patients. Interestingly, Oudemans et al. found citrate to reduce mortality particularly in septic patients or patients with a high degree of organ failure, suggesting a role of citrate in inflammation [7]. Citrate creates an almost calcium-free environment in the extracorporeal circuit. Apart from being important for coagulation, calcium may be pivotal in cell activation and degranulation [9-13]. Calcium signaling may contribute to activation of NFκB and subsequent release of pro-inflammatory mediators like interleukin-6 (IL-6) and -8 (IL-8) from mononuclear cells, since calcium antagonists appear to inhibit such responses [14-16].

In chapter 3 we studied the influence of citrate, heparin and no anticoagulation on handling of IL-6 and IL-8 during CVVH. Both cytokines play a pivotal role in innate immunity and are markedly elevated in patients suffering from sepsis particularly when complicated by AKI and in case of non-survival [17,18]. We also focus on whether these harmful cytokines were removed by CVVH by convection or adsorption. This feature of CVVH has been described in the literature, however, the magnitude and significance of this potential removal is debated [19-24]. We observed that blood to membrane contact, adsorption/clearance and anticoagulation did not increase nor attenuate high circulating levels of IL-6 and IL-8 during CVVH for AKI, irrespectively of the anticoagulation administered. The inflammatory response to various components of CVVH, however, also includes complement activation and C5a release followed by polymorphonuclear cell degranulation with release of intra-cellular products such as elastase, complexed in the circulation to alpha1-antitrypsin, and myeloperoxidase (MPO). As demonstrated in intermittent hemodialysis and in blood in vitro, calcium-chelating
citrate anticoagulation may lower polymorphonuclear cell degranulation [9-12]. Moreover, the use of citrate may also prevent activation of complement by blood-membrane contact and endothelial release of MPO, as seen during heparin anticoagulation [25]. The single prior, albeit small, study suggesting prevention of neutrophil degranulation with citrate-CVVH as compared to heparin lacked a control group (no anticoagulation) and measurements of complement activation products [13].

In chapter 4 we focused on differences between citrate, heparin and no anticoagulation in C5a release and subsequent release of elastase and MPO from neutrophils during CVVH. We observed that in the heparin group, there was C5a production across the filter which decreased most over time as compared to other groups (P = 0.007). There was also net production of elastase and MPO across the filter during heparin anticoagulation (P = 0.049 or lower), while production was minimal and absent in the no anticoagulation and citrate groups, respectively. During heparin anticoagulation, plasma concentrations of MPO at the inlet increased in the first 10 minutes of CVVH (P = 0.024). We therefore conclude that citrate confers less filter-induced, potentially harmful complement activation and neutrophil degranulation and less endothelial activation than heparin when used for anticoagulation during CVVH. These findings argue in favour of citrate being superior to heparin as anticoagulation in CVVH in terms of biocompatibility.

The diagnosis of AKI heavily relies on serum creatinine and urinary output, but these markers are imperfect in non-steady-state conditions such as AKI. In patients with subclinical AKI, further damage may be prevented by appropriate measures. Therefore, biomarkers to predict AKI before the increase of serum creatinine have been studied intensively. Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein (25-kDa) and one of the neutrophil secondary granule proteins and it is also rapidly induced in distal tubular segments of injured nephrons with stress [26,27]. Therefore, urinary and plasma levels are helpful in early prediction of AKI, in prediction of severity of AKI and AKI-related outcomes, such as the need for renal-replacement therapy (RRT), as well as mortality [28-33]. There is only one case series (n=3) addressing the effect of CVVH on plasma NGAL in vivo [34]. We examined whether CVVH affects plasma levels by clearance or release of NGAL by activated neutrophils in the filter, dependent on the anticoagulation regimen applied (chapter 5). We observed no net removal or production of NGAL during CVVH. Concentrations of NGAL correlated with disease severity at initiation of CVVH and at the end of a CVVH run. The plasma level and biomarker value of NGAL in critically ill patients with AKI, therefore, are not affected by CVVH. These findings are in concordance with results from the small case series by de Geus et al. [34]. Furthermore, we did not find evidence for intrafilter release of
NGAL by neutrophils, irrespective of the anticoagulation method applied and can therefore not judge the inhibitory effect of citrate on neutrophil activation in this study. Dissimilar degranulation patterns of primary and secondary granules of neutrophils might account for the absence of NGAL release in the filter, because primary granular markers elastase and myeloperoxidase (MPO) are released during heparin-CVVH as described in chapter 4, whereas secondary granular NGAL apparently is not [13]. Others indeed observed that there was less release of lactoferrin, a secondary granular product similar to NGAL, than of MPO during citrate-based dialysis [10].

Although the pathogenesis of AKI is often multifactorial, novel mediators have been described that are implicated in the development of AKI, for instance in the course of sepsis. These mediators include TNF-associated weak inducer of apoptosis (TWEAK), a member of the super tumor necrosis factor (TNF) family, angiopoietin-2 (Ang-2), and pentraxin-3 (PTX3). Similar to chapter 5, in chapter 6 plasma levels of TWEAK, Ang-2 and PTX3 were studied over time during citrate- and heparin-CVVH focusing on whether release or removal of these markers occur and furthermore, whether the administered anticoagulation is of influence. We observed that concentrations of TWEAK, Ang-2 and PTX3 were hardly affected by CVVH since the mediators were not or hardly detectable in ultrafiltrate, indicating negligible clearance by the filter. Heparin use, however, was associated with an increase in in- and outlet plasma TWEAK. This finding may relate to proinflammatory actions of high concentrations of heparin [35]. Platelet activation by heparin may also play a role since TWEAK is also stored in platelets [36]. High concentrations of TWEAK are undesirable, especially in an inflammatory microenvironment. Regional citrate and CVVH without anticoagulation did not induce this undesirable phenomenon occurring in heparin-CVVH.

In chapter 7, we aimed to explore the mechanisms responsible for improved filter survival times in citrate-CVVH as compared to heparin, as observed in chapter 3, by analysing different markers of coagulation. In critically ill patients, in whom sepsis is common, the coagulation/fibrinolysis balance is often deranged at initiation of CVVH and this may affect circuit survival, even when anticoagulation is used. Simultaneous pro- and anticoagulant and pro- and antifibrinolytic processes may occur. We aimed to unravel the mechanisms of filter failure by measuring specific coagulation mediators, such as thrombin-antithrombin (TAT) complex, representing a sensitive marker for thrombin generation, activated protein C-protein C inhibitor (APC-PCI) complex, representing protein C activation and thus inhibition of coagulation, and type I plasminogen activator inhibitor (PAI-1), the principal inhibitor of fibrinolysis. In case of early filter failure (<24h), we observed that inlet concentrations of TAT and APC-PCI were higher over time, irrespective of anticoagulation administered. There was
more production of APC-PCI and platelet-derived PAI-1 in the filter after 10 minutes in the heparin than in the other groups. In clotted filters, production of APC-PCI and PAI-1 was also higher with heparin than with citrate. It is therefore suggested that coagulation activation in plasma and inhibited anticoagulation in plasma and filter may partly result in early CVVH filter failure due to clotting, particularly when heparin is used. Regional anticoagulation by citrate circumvents inhibition of anticoagulation and fibrinolysis by platelet activation following heparin.

Conclusions

In the search for the best anticoagulation regimen for critically ill patients with AKI requiring CVVH, we demonstrate in this thesis that patient mortality and renal outcome are similar for trisodium citrate and heparin anticoagulation in predilution CVVH. Citrate is superior than heparin in terms of efficacy demonstrated by improved filter survival and less off-time. In addition there is less need for discontinuation of the anticoagulant due to adverse events during CVVH. Also, the costs of the first 72 hours of prescribed CVVH are lower in citrate-based CVVH as compared to heparin-based CVVH. In terms of bioincompatibility occurring after blood to membrane contact, citrate did not attenuate high circulating levels of IL-6 and IL-8 during CVVH, nor was there adsorption or clearance of these interleukins. We did show, however, that citrate confers less potentially harmful complement activation and neutrophil degranulation, as well as less endothelial activation than heparin when used in CVVH. We showed that the plasma level and biomarker value of NGAL are not affected by CVVH, because of low clearance. Furthermore, intrafilter release of NGAL by neutrophils, irrespective of the anticoagulation method applied, could not be detected. The plasma levels of novel AKI mediators TWEAK, Ang-2 and PTX3 are also not affected by CVVH. Heparin anticoagulation, however, undesirably increased TWEAK levels in patient’s plasma whereas citrate did not. A deranged coagulation profile may partly determine early CVVH filter failure, particularly when heparin is used. Citrate partly circumvents this derangement. In conclusion, citrate has advantages over heparin in terms of safety, efficacy, costs and biocompatibility and should therefore be considered as first choice for anticoagulation in CVVH.

Future perspectives

While this thesis adds novel arguments to support the gaining popularity of citrate as anticoagulation of choice in CVVH in AKI in the ICU, there remain numerous issues to
address in the future. The heterogeneity of existing citrate protocols is impeding the necessary standardization of therapy, especially with regard to dosage and filtration modality. Its widespread use is hampered by fears of severe metabolic side effects, such as citrate accumulation leading to hypocalcaemia and acid-base disorders. Trials comparing hypertonic citrate solutions to isotonic citrate incorporated in the predilution replacement solution in terms of metabolic side effects and patient outcome have not been performed. The limited commercially available replacement solution containing trisodium citrate could partly contribute to the lack of availability of these data. The preferred citrate-based method, therefore, remains to be elucidated.

Even though we could not confirm the beneficial effect on mortality rates in cases of sepsis that were suggested by Oudemans et al. [7], clear suggestions of effects of citrate on inflammation were observed. In order to explore mechanisms underlying our observations, in vitro experiments with human umbilical vein endothelial cells (HUVECs) could add information. Different pro- and anti-inflammatory factors and pro- and anti-coagulation factors of interest could be studied when the endothelium is stimulated by citrated anticoagulated blood and exposed to stimuli that would normally evoke inflammatory responses. Indeed, citrate appears to reduce endothelial inflammation and dysfunction in hyperglycemic conditions in vitro in concentrations attained in patients during CRRT [37]. If this phenomenon occurs in vivo as well, even systemic citrate could have beneficial effects via anti-inflammatory and anti-oxidant pathways. A first step, however, would be confirmation of the in vitro results in animal models. Moreover, an experimental ex vivo extracorporeal circuit including hemofilter could be used in this setting to mimic in vivo conditions and thus unravel the influence of citrate on the endothelium and the inflammatory response in continuous RRT.

There is a substantial amount of interest in AKI research on biomarkers and mediators of AKI. Biomarkers to predict AKI before the increase of serum creatinine have been studied intensively. NGAL levels are helpful in early prediction of AKI. In order to be implemented in the everyday practice, fast and accurate biomarker assays are needed. These tests could potentially reduce morbidity and mortality by providing a diagnosis in hours rather than days. The readiness of such technology, and the benefit it confers versus its cost, must be considered. NGAL testing has the potential to facilitate rapid decision-making by producing results from small sample volumes in a matter of seconds. This approach was tested with point-of-care testing of NGAL in the emergency department and seems promising [38]. There is an urgent need to prove the viability of these techniques in order for them to be translated into clinical practice.
In terms of translating proposed mediators of AKI to therapeutical interventions, a phase I dose-ranging clinical trial demonstrated the safety of anti-TWEAK antibodies in humans [39]. A phase II randomized placebo-controlled clinical trial exploring the efficacy, safety and tolerability of neutralizing anti-TWEAK antibodies as a tissue protection strategy in lupus nephritis is ongoing. The eventual success of this trial may expand the range of kidney diseases in which TWEAK targeting should be explored, including AKI [40].
General discussion and future perspectives

References