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Citrate Kinetics during Regional Citrate Anticoagulation in Extracorporeal Organ Replacement Therapy

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1 Introduction

With its widespread availability, haemodialysis supports the lives of approximately 920,000 people around the world. In European countries as well as in the United States it constitutes approximately 70-80% of renal replacement treatments for patients with end-stage renal disease (ESRD). The population of dialysis patients is growing constantly by around 3-7% per year.

1.1 Principles of haemodialysis

Since the first performance of the dialysis treatment in a human being in 1926 by G.Haas in Giessen many improvements were introduced to the treatment with the artificial kidney. However, the primary concept of dialysis remains unchanged. It is based on the diffusion of the small solutes through a semipermeable membrane in order to normalize blood solute concentrations that have been disturbed due to the failure of the natural organ. The semipermeable membrane is a porous structure that is placed between the blood and the dialysis solution compartment to allow the bi-directional passage of all molecules that are smaller than the size of the pores of the membrane. Diffusion of the solutes across a semipermeable membrane is governed by the Fick's first law, which is expressed by the formula:

$$J = - Ko*A*\Delta C$$

According to the formula above, the flux of the solute through a semipermeable membrane, J, is proportional to its concentration gradient over this membrane, ΔC, as well as to the membrane surface area, A, and the mass transfer coefficient, Ko. The last parameter is a function of the membrane resistance and the size of the molecules. The size of the molecule determines its diffusive velocity and for this reason a smaller molecule such as urea is easier transferred through the membrane than a bigger molecule such as creatinine. Membrane resistance depends largely on the thickness and the porosity of the membrane.
An additional mechanism of solute transfer during haemodialysis is ultrafiltration. It occurs, when water is pushed through the membrane by hydrostatic pressure. The flow of the water is dragging dissolved solutes if they are smaller than the size of the pores of the membrane. This is called convective transport.

The mechanical structure of a dialysis circuit can be divided into three components: the dialyzer, the blood delivery system, and the dialysis solution delivery system. The dialyzer is the "heart" of the haemodialysis circuit since it is the module where blood comes into contact with the dialysis solution through the pores of a semipermeable membrane. The most common type of dialyzer used nowadays is the hollow fibre dialyzer, also called capillary dialyzer. In these dialyzers bundles of capillary fibres made from semipermeable membrane allow for blood flow inside the

capillaries while dialysis solution is flowing outside the capillaries. The blood delivery system is an extracorporeal circuit connected to a pump, which is pulling the blood from the patient's blood vessel (fistula or a catheter), pushing it through the capillaries of the dialyzer and back to the patient. At the same time the dialysis solution delivery system provides the flow of the dialysis solution around the fibres of the dialyzer.

1.2 Heparin

Since the dialysis treatment exposes the blood to an artificial surface the coagulation system is activated. Effective anticoagulation is necessary to prevent premature occlusion of the extracorporeal circuit and is an important feature of the successful dialysis. The adoption of heparin as an anticoagulant in the late 1920s solved effectively the problem of clotting which was the major impediment to the development of haemodialysis. Since the introduction of dialysis heparin remains the standard anticoagulant for this extracorporeal procedure. Heparin is a mixture of heterogeneous mucopolysaccharides that differ in regard to: molecular weight, anticoagulant activity and pharmacokinetic properties. Currently there are two types of heparin in use for anticoagulation in renal replacement therapy: unfractioned heparin (UF heparin) with molecular weight in the range from 5000 to 30000 (mean: 15000) and low-molecular-weight heparin (LMW heparin) with a molecular weight between 4000 and 5000 (Hirsh et al. 1995). The main anticoagulant effect of both types of heparin is mediated by binding to and enhancing the plasma activity of natural thrombin inhibitor antithrombin III resulting in a prolonged bleeding time (Breiterman-White 1995; Ehrlich and Stivala 1973; Eikelboom and Hankey 2002; Hirsh et al. 1995; Kleinschmidt and Charles 2001; Rosenberg 1997). Since the activity of heparin depends on the adequate level of ATIII, the administration of heparin can be ineffective in critically ill patients, patients with severe liver failure or others with low ATIII levels (Kramer et al. 2003).

Although the heparinization for renal replacement therapies is relatively safe and effective it can be associated with two severe side effects: bleeding and heparin induced thrombocytopenia (HIT).

1.2.1 Side-effects of heparin

1.2.1.1 Haemorrhage

Bleeding after heparin administration occurs as a result of systemic anticoagulation.

In ESRD patients the tendency to bleed is additionally exacerbated by the uraemia-induced dysfunction of platelets. Despite this, most stable dialysis patients tolerate the heparin-induced prolongation of clotting time well. However, patients at increased risk of bleeding e.g. due to gastric ulcers, pericarditis, recent surgery or trauma, diabetic retinopathy, coagulopathy, thrombocytopenia, vascular malformation, malignant hypertension or patients with active bleeding are particularly susceptible to develop severe hemorrhagic complications. The increased risk of

bleeding due to systemic anticoagulation in the above-mentioned patient groups amounts to 25-50% (Coon and Willis, III 1974; Landefeld and Beyth 1993; Levine et al. 2001; Swartz 1981).

Haemorrhagic complications are particularly important in the treatment of intensive care patients since comorbid conditions predispose to fatal bleeding. The continuous renal replacement therapy, which has been advocated as the treatment of choice in critically ill patients, involves the prolonged exposure to heparin further promoting the bleeding risk. The reported incidence of bleeding even on low-dose heparin is as high as 50% and may contribute to the mortality in the intensive care units by up to 15% (Abramson and Niles 1999; Bellomo et al. 1993; Landefeld and Beyth 1993; Levine et al. 2001; Martin et al. 1994; van de Wetering J. et al. 1996).

LMW heparin requires a lower dose in comparison to UF heparin to provide an equivalent antithrombic effect and has the potential of a lower bleeding risk than UF heparin. This has been demonstrated in animal studies and also in the treatment of thrombosis. However, the results of the studies investigating the superiority of LMW heparin over UF heparin in reducing bleeding risk during haemodialysis remain controversial (Andriuoli et al. 1985; Bergqvist et al. 1985; Dolovich et al. 2000; Holmer et al. 1982; Lensing et al. 1995; Levine et al. 1991; Saltissi et al. 1999; Schrader et al. 1986; Schrader et al. 1988; Schrader et al. 1990).

1.2.1.2 Thrombocytopenia

Thrombocytopenia is the second well-recognized complication of heparin therapy. There are two types of heparin-induced thrombocytopenia (HIT).

Type 1 is believed to have a nonimmune pathogenesis, and is characterized by an early onset, mild course and spontaneous resolution after 4 days despite continued use of heparin. This type usually does not pose any serious clinical problems.

In contrast, HIT-2 manifests itself in a marked thrombocytopenia associated with the substantial risk of life-threatening arterial or venous thromboses. Despite a decline in the platelet count severe bleeding complications are much less frequent than thromboses, which have been observed in up to 60% of patients with a confirmed diagnosis of HIT-2. The thrombotic nature of HIT-2 results from the IgG-mediated platelet activation. In HIT-2 IgG antibodies are directed against the complex of heparin and platelet factor 4 (PF4/H). This immune form of HIT begins within 5 to 15 days after commencing heparin therapy. The platelet count falls to a nadir of around 50×10^9/L and cannot be normalized without discontinuing heparin. It has also been reported that symptoms can develop within hours after the initiation of the heparin treatment. This rapid thrombocytopenia was observed in patients who had received UF heparin within the previous three months (Hirsh et al. 1995). Although PF4/H antibodies are detectable in up to 12-30% of haemodialysis patients, the symptomatic HIT-2 appears to be a rare event in chronic dialysis patients with the exception of one study that reported a prevalence of 3.9% (Yamamoto et al. 1996).

It has been found that the use of LMW heparin is associated with a lower risk of inducing HIT-2 (Baglin 2001; Warkentin et al. 1995). Nevertheless, the application of LMW to patients with evident symptoms of HIT-2 is dangerous due to the cross activity of antibodies PF4/H with LMW heparin with the in vitro rates of more than 80% (Vun et al. 1996). The recognition of HIT-2 is critical since early diagnosis and intervention can be lifesaving and reduce the mortality from 30% to 10% (Baglin 2001; Lubenow and Greinacher 2002; Warkentin et al. 1998).

The disadvantages of heparin have led to the interest in other anticoagulation strategies in haemodialysis. Regional heparinization has been suggested as an option for patients with imminent bleeding, whereas hirudin and heparinoids are used to treat patients with established HIT-2. Other strategies applied if heparin is contraindicated include anticoagulant free dialysis, prostacyclin and regional citrate anticoagulation. The use of the above methods encounters considerable limitations and has not gained widespread acceptance making an alternative anticoagulation to heparin a persisting challenge.

1.3 Alternative anticoagulation

1.3.1 Regional heparinization

Regional heparinization utilizes protamine, which forms stable complexes with heparin and thus neutralises its anticoagulant effect. The use of this method is complicated by anticoagulation rebound due to the shorter half time of protamine compared to heparin. Heparin is released from the protamine complexes and again binds to antithrombin III (Blaufox et al. 1966). In addition, anaphylactic reactions, hypotension, cardiac depression, leucocytopenia, and thrombocytopenia were described after the administration of protamine (Horrow 1985). However, some authors reported successful application of this method in continuous renal replacement therapy (Bellomo et al. 1993; Kaplan and Petrillo 1987). Nevertheless, in a high risk group of patients the bleeding complications using protamine were more frequent in comparison to the regimen of low dose of heparin (Swartz and Port 1979). For these reasons the use of protamine protocols in dialysis patients at high risk for bleeding complications has been discouraged by recent European guidelines.

1.3.2 Hirudin & Danaparoid

Anticoagulation with hirudin or danaparoid is currently recommended by the European Best Practice Guidelines for haemodialysis in patients with HIT-2.
Hirudin is a natural protein that exerts its anticoagulant activity by direct inhibition of thrombin. For the use in haemodialysis a recombinant hirudin, lepirudin, is available. Hirudin is completely eliminated by the kidneys, and for this reason the half time of hirudin in patients with renal failure is considerably longer than the average 90 minutes in patients with normal kidney function. In

ESRD patients hirudin can be detected in the blood for at least 48 hours up to one week (Fischer et al. 1999; Nowak 1991; Nowak et al. 1992; Nowak et al. 1997; Nowak 2002; O'Shea et al. 2003; Vanholder et al. 1994). Therefore, hirudin application in haemodialysis patients can easily result in prolonged and excessive anticoagulation and requires extreme caution as well as exact dosage adjustment. Additionally no antidote is available in case of overdose. Therefore monitoring of the degree of anticoagulation is of especial importance for this anticoagulation method. Typically hirudin dose is guided by the prolongation of the activated partial thromboplastin time (aPTT) with the target prolongation of 1.5-2.5 times of the normal range (O'Shea et al. 2003). However, it has lately been postulated that the Ecarin clotting time (ECT) is preferable to monitor the anticoagulant effect of hirudin. Currently ECT is not readily available at most hospitals adding to the complexity of hirudin use (Nowak 1992; Nowak 2001; O'Shea et al. 2003).

Danaparoid is a substance derived from heparin, a “heparinoid”, but in contrast to heparin, it does not require antithrombin III as a cofactor. Danaparoid elicits its anticoagulation effect by interacting directly with the factor Xa. Like hirudin danaparoid is predominantly cleared by the kidneys and accumulates in patients with renal failure. The application of danaparoid should be monitored by the anti-factor Xa level, which is not routinely available in many laboratories. Additionally, in vitro studies have shown a 20% cross-reactivity of PF4/H antibodies with danaparoid, which is usually irrelevant in clinical practice, however should be excluded in cases with recurrent HIT-2 induced thrombosis.

Overall, the use of direct thrombin inhibitors (hirudin and danaparoid) is limited by their long half time, difficult but essential monitoring of the treatment, and lack of specific antidotes as well as high costs.

1.3.3 Anticoagulant free dialysis

Anticoagulant free dialysis can be performed in all haemodialysis patients with recognized contraindications to heparin. However, the frequent clotting of the extracorporeal circuit is likely when no anticoagulant is used and leads in many cases to premature termination of the haemodialysis treatment. To decrease the risk of clotting, anticoagulant free procedures are usually combined with periodical saline flushes and in continuous settings also with prophylactic changes of the bloodlines and the dialyzer. The use of high blood flow can also reduce the incidence of clotting, but may not be tolerated by all patients. Even when all above labour-intensive procedures are employed the reported incidence of clotting associated with this method reaches 20% in intermittent haemodialysis (Agresti et al. 1985; Caruana et al. 1987; Preuschof et al. 1988). In addition, this method induces the platelet activation in the extracorporeal circuit, which is particularly undesirable in patients with active HIT-2, since it may increase the already existing risk of thrombosis. In continuous renal replacement therapy the difficulty to maintain the extracorporeal circuit patent is a substantial limiting factor for the application of this method.

Some centres find anticoagulant free dialysis in continuous renal therapy only applicable to patients with congenital or acquired coagulopathies (Mehta 1994; Ward and Mehta 1993).

1.3.4 Prostacycline

Prostacycline is a physiological inhibitor of platelet aggregation and a natural vasodilator. It is a product of arachidonic acid in the cyclo-oxygenase pathway. In the early trial prostacycline was applied concomitantly with heparin to anticoagulate haemodialysis systems (Turney et al. 1980). In this study administration of prostacyclin proved to lower the necessary dose of heparin and reduced heparin induced platelet dysfunction. Later experiences using prostacyclin as the sole anticoagulant, both for intermittent and continuous haemodialysis, are mixed. While some authors report an unacceptable incidence of hypotension, others either fail to observe this complication or note only a clinically negligible drop in blood pressure (Caruana et al. 1991; Davenport et al. 1991; Davenport et al. 1994; Ede et al. 1985; Smith et al. 1982; Turney et al. 1980b; Turney et al. 1981; Turney and Weston 1981; Vane 1985; Weston et al. 1980; Zusman et al. 1981; Zusman et al. 1981). Besides the debate about side effects, the high costs of prostacycline remain another important limitation to its widespread use in haemodialysis.

1.4 Regional citrate anticoagulation

Among the alternatives to heparin regional citrate anticoagulation appears to be a very promising approach.

1.4.1 Citrate: general information

Citrate is a normal metabolite in the human body that acts as a first intermediate substance in the Krebs cycle. Since Krebs cycle takes place in mitochondria, all cells that contain these cellular organelles have enzymes for citrate production and metabolism. Tissues rich in mitochondria such as liver, skeletal muscles, and kidney posses higher amount of Krebs cycle enzymes and therefore represent higher capacity for citrate generation and elimination. Although red blood cells are devoid of intracellular organelles they were found to contain trace amounts of citrate (Baruch et al. 1975; Marangella et al. 1991). Interestingly, externally infused citrate is not transported into erythrocytes (Whitfield and Levy 1981). Therefore, it has been concluded that the wall of the red blood cells is impermeable to citrate.

Under physiological conditions small concentrations of citrate are detectable both in the plasma and in the urine.
Normal concentration of citrate in plasma ranges from 0.05 to 0.1 mmol/l and can be physiologically higher in children and after muscular exercises.
In disease states elevated plasma citrate levels were observed in patients with liver failure and were also described in the anhepatic phase of liver transplantation (Diaz et al. 1994; Diaz et al.

1995; Kost et al. 1986). It has also been noted that the bone tissues contain very high amount of citrate explaining an increase in citrate plasma levels in bone diseases (NORDMANN and NORDMANN 1961) (Baruch et al. 1975) (Dzik and Kirkley 1988). Since citrate is excreted into urine it has the potential to also accumulate in patients with renal failure. However, the reported data about citrate concentrations in patients with functional kidney disorders are contradictory.
The normal urinary excretion rate of citrate ranges from 200 to 1000 mg/day. The lower concentration of citrate in the urine correlates with a higher frequency of calcium lithiasis in the urinary tract. This phenomenon has been demonstrated after the administration of substances, which reduce urinary citrate without affecting urinary calcium excretion (acetazolamide). Thus, urinary citrate plays an important role in preventing the precipitation of calcium salts in the urinary tract by virtue of chelating ionized calcium (NORDMANN and NORDMANN 1961). This unique feature of citrate to form stable complexes with calcium ions is also the main principle of regional anticoagulation with citrate.

Due to the quality of citrate to form stable complexes with ionized calcium, adding citrate to the blood leads to a significant decrease in the concentration of ionized calcium. Since the coagulation process is a calcium dependent cascade of enzymatic reactions the marked reduction in ionized calcium concentration results in anticoagulation. Typically the efficient inhibition of clotting is attributable to ionized calcium levels lower than 0.1-0.5 mmol/l (Chadha et al. 2002; Hofmann et al. 2002; Kutsogiannis et al. 2000).

Since the early 1900 this citrate-mediated hypocalcemia has been utilized in transfusion medicine to store blood products.(Abbott 1983) (Dzik and Kirkley 1988) (Mollison 2000; Perkins et al. 1971) A few decades later in the mid-1960s, when continuous blood flow separation devices were introduced, citrate anticoagulation has also been adopted as a standard procedure for blood cell apheresis and later on for lipid apheresis (Bolan et al. 2002b; Bosch et al. 1993; Bosch 2003; Bosch et al. 2003; Drager et al. 1998; Hester et al. 1983; Makar et al. 2002; Rock and Sutton 1997; Toffaletti 1983; Uhl et al. 1997). The first description of the application of citrate anions for anticoagulation in renal replacement therapy was published in 1961 (Morita et al. 1961). However, the wider use of citrate anticoagulation in haemodialysis was initiated by the publication of Pinnick et al. in 1983 (Pinnick et al. 1983). In 1990 regional anticoagulation with citrate was also introduced for use in continuous renal replacement therapies (Mehta et al. 1990).

1.4.2 Regional citrate anticoagulation in RRT

For anticoagulation in haemodialysis, trisodium citrate is typically infused into the arterial line of the extracorporeal circuit resulting in the rapid formation of citrate-calcium complexes and in the prolongation of the blood clotting time within the circuit. A scheme of citrate anticoagulation is depicted in figure 1.

In the original description by Pinnick et al. the recommended dose of trisodium citrate was between 2.5 and 7.5 mmol per litre of blood. The dialyzer removes a part of the infused dose of citrate in the form of citrate-calcium complexes and as trisodium citrate while the rest reaches the systemic circulation of the patient. However, the citrate circulating in the systemic blood is repeatedly returning to the filter and therefore an additional portion of citrate is cleared into the dialysis solution. The citrate not removed into the dialysate needs to be metabolised by the human tissues. The degradation of 1 mol of calcium-citrate yields 3 mols of bicarbonate and 1 mol of ionized calcium (Dzik and Kirkley 1988). Since citrate is a ubiquitous molecule in the human body and takes part in regular metabolic pathways it has a high body clearance mostly due to its rapid metabolism in the liver. In patients receiving citrate-anticoagulated apheresis the body clearance of citrate has been estimated to amount to 481.8 ml/min resulting in a half time of 32.9 minutes (Apsner et al. 1997). ESRD patients are also able to metabolise citrate which is illustrated by the rapid decline of the systemic citrate concentration after the termination of the citrate infusion (Evenepoel et al. 2002; Faber et al. 1990; Hocken and Hurst 1987; Janssen et al. 1993; Janssen et al. 1996; Lohr et al. 1988; Lohr et al. 1989b). Hepatic failure patients show an increase of the citrate half time up to approximately 70 minutes since the liver plays the main role in the metabolism of citrate (Apsner et al. 1997) (Kramer et al. 2003).

The majority of citrate anticoagulation protocols for haemodialysis are carried out with the use of calcium free dialysis solution, which avoids recalcification of the blood inside the dialyzer. The normal ionized calcium concentration is restored by the separate infusion of calcium. Due to the maintenance of physiological ionised calcium levels in the patients' circulation the anticoagulation is not systemic but is limited to the extracorporeal circuit.

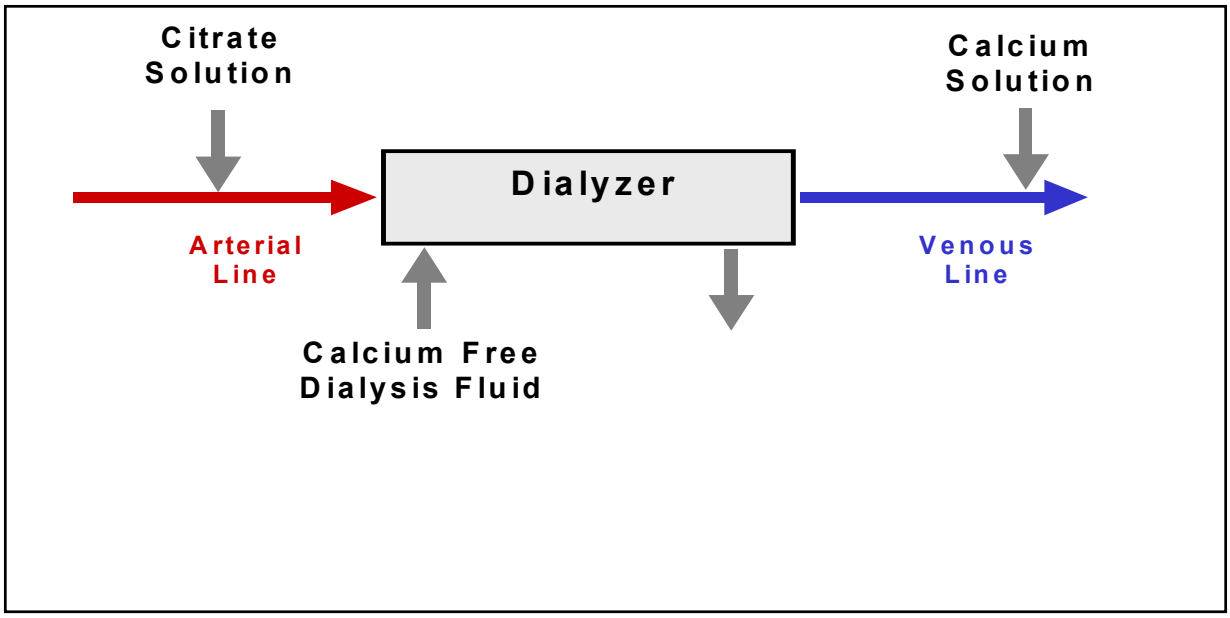

Figure 1 Schema of citrate anticoagulation during haemodialysis.

1.4.2.1 Prevention of bleeding

So far regional citrate anticoagulation has been successfully applied to haemodialysis patients at high risk of bleeding (Ashouri 1985; Collart et al. 1989; Flanigan et al. 1987; Monchi et al. 2003; Palsson and Niles 1999; Pinnick et al. 1983; Ward and Mehta 1993). One prospective, randomised trial studied haemodialysis patients that were judged to be at high or very high risk of bleeding. In this study regional citrate anticoagulation was associated with lower incidence of dialysis-induced bleeding in comparison to low-dose heparinization with no difference in regard to dialyzer clotting (Flanigan et al. 1987). In continuous renal replacement therapy regional citrate anticoagulation was also found to reduce the incidence of dialysis-related bleeding and the requirements for blood transfusions as demonstrated in a randomised, prospective trial (Monchi et al. 2003).

1.4.2.2 Other beneficial effects of citrate in RRT

It is worthwhile to note, that citrate not only prevents bleeding but may also provide a superior prevention of clotting of the dialyzer capillaries. It has convincingly been shown in the study by Hofbauer et al that citrate anticoagulation induces significantly less clotting of the dialyzer than both types of heparin (UF and LMW) (Hofbauer et al. 1999). Lately, the study by Monchi et al demonstrated longer filter life time with citrate anticoagulation in comparison to heparin anticoagulation in continuous therapies as well (Monchi et al. 2003). Since filter performance and prevention of bleeding are the most problematic issues in continuous renal therapies, citrate anticoagulation appears to be especially attractive in these treatment modalities. The better prevention of thrombus formation is also an important issue in intermittent HD. It contributes to an improved dialysis adequacy and is a precondition to achieve the desired dialysis dose.
In HIT-2, use of citrate anticoagulation is beneficial in the short term as well as in the long-term treatment (Dworschak et al. 2002; Unver et al. 2002). This suggests a great potential of citrate to avoid heparin-induced thrombocytopenia. This anticoagulation technique can be of special interest for long-term haemodialysis in patients with the diagnosis of HIT-2, because alternative anticoagulants (hirudin and danaparoid) are difficult to handle and expensive.

Furthermore the citrate-induced depletion of ionized calcium in the extracorporeal circuit has the potential to improve the biocompatibility profile of haemodialysis procedures. Böhler et al have demonstrated a significantly diminished release of proteolytic enzymes from neutrophils during citrate anticoagulation in comparison to standard UF heparin anticoagulation (Bohler et al. 1996). The biocompatibility of haemodialysis procedure is relevant both in acute and chronic haemodialysis patients. In patients with acute renal failure the poor biocompatibility of the dialysis membranes was associated with a reduced renal recovery and patient survival (Hakim et al. 1994). In chronic haemodialysis, activation of granulocytes with resulting release of proteolytic enzymes and generation of reactive oxygen species (ROS) have been linked to the progression of atherosclerosis in ESRD patients (Galli et al. 1999). The prolonged stimulation of the blood

cells is also presumed to promote cellular and humoral immunodeficiency (Angelini et al. 1993; Angelini et al. 1994; Lee et al. 1991), an important issue in uraemia, since infections are the second most common cause of death.

1.5 Objectives of the current study

Despite the many recognized advantages of regional citrate anticoagulation and numerous reports describing the successful application of this method, it has still not been established as a routine procedure in most haemodialysis centres. The major impediment to the wide spread use of this method is the potential of citrate to cause electrolyte disturbances, mainly hypocalcaemia, which in a severe form may lead to a fatal cardiac dysfunction. The lack of the well-defined pharmacokinetic features of this anticoagulation method in renal replacement therapy additionally contributes to the threat of inducing hypocalcaemia and limits its broad acceptance.

This study is a systematic investigation on citrate and calcium pharmacokinetics in haemodialysis patients. Pharmacokinetic data obtained from this evaluation may serve as a useful tool to understand the relationship between the clinical situation and citrate/calcium concentrations. This research also explores the body's compensatory mechansims that are affected by citrate anticoagulation and explain their contribution to the maintenance of ionized calcium level. Findings from this study suggest that the knowledge of the principles of citrate and calcium pharmacokinetics can help to apply citrate anticoagulation in a safe and effective way.

2 Methods

This study was conducted in three steps.
The first step was the development of a mathematical model to predict the systemic concentration of citrate and calcium and the measurements of the in vitro clearance of citrate.
The second part consisted of the patient study in which following kinetic parameters were measured:

- In vivo clearance of citrate
- Calcium loss into the dialysate
- Metabolic rate constant of citrate

At the third stage the data from the clinical study were implemented into the model in order to validate the mathematical model and also to estimate the distribution volume of citrate.

2.1 Description of the mathematical model

2.1.1 Model of citrate concentration

The model of the citrate concentration comprises of the development of the mathematical equations. In order to visualize the results the equations were applied to computer software VisSim 4.5.

2.1.1.1 Calculation of citrate concentration

Following citrate concentrations have to be considered:

- systemic citrate concentration $c_{Ci\ sys}$: concentration within the body and in the extracorporeal circuit before the citrate infusion site
- pre-dialyser citrate concentration $c_{Ci\text{-}art}$: concentration in the arterial line between the citrate infusion site and the inlet of the dialyser
- post-dialyser citrate concentration $c_{Ci\text{-}ven}$: concentration in the venous line between the dialyser outlet and the vascular access

For the calculation of the systemic citrate concentration a one-compartment distribution model of citrate is employed. This compartment is the sum of all body fluids containing citrate. By definition, all these fluids always contain the same concentration of citrate (no concentration gradient); there are no barriers between the fluids and no transport processes. With these assumptions all these fluids together can be considered for pharmacokinetic modelling as one homogenous fluid compartment. The volume of this compartment is the distribution volume for citrate V_{DCi}.

The principle of mass balance is applied to calculate the amount of citrate (number of moles) n_{Ci} that is dissolved in the single compartment with the volume V_{DCi} (see fig. 2).

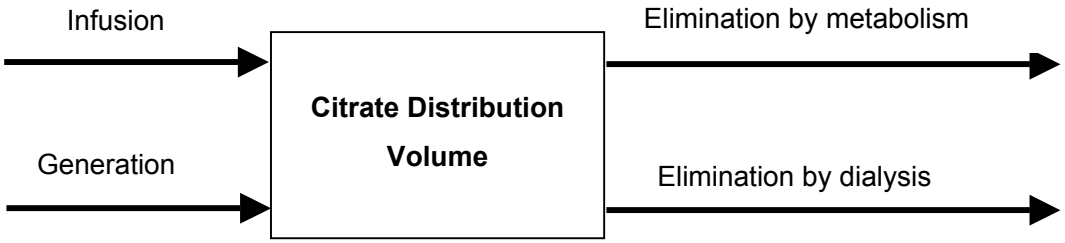

Figure 2 One compartmental model showing the input and the output of citrate mass for the calculation of the systemic citrate concentration.

The difference between the input rate dn_{in}/dt and the output rate dn_{out}/dt is the net flux of citrate dn_{Ci}/dt, see equation 1:

$$\frac{dn_{Ci}}{dt} = \frac{dn_{in}}{dt} - \frac{dn_{out}}{dt}$$

Equation 1

This equation is true, if input and output rate are both positive rates. Output rates are often described as negative rates. However, in this document all rates are positive!

The systemic concentration of citrate $c_{Ci}(t)$ is the amount of citrate divided by the distribution volume $V_{DCi}(t)$:

$$c_{Ci}(t) = \frac{n_{Ci}(t)}{V_{DCi}(t)}$$

Equation 2

As shown in figure 2 the citrate input is the infusion and the natural generation of citrate, while the output can be separated into the elimination by metabolism and the elimination by dialysis. The elimination by kidneys is zero, assuming that the patient is anuric. The calculation of the input and the output rate is described below.

2.1.1.2 Input of citrate

The input rate dn_{in}/dt is the sum of the infusion rate dn_{inf}/dt and the metabolic generation rate g:

$$\frac{dn_{in}}{dt} = \frac{dn_{inf}}{dt} + g$$

Equation 3

Citrate is infused into the arterial blood line with the rate $I_{vol}(t)$. This is the fluid flow rate indicated by volume of the used citrate solution. The model accepts every concentration of the citrate solution. The concentration of the solution is one of the initial input data.

A considerable fraction of the infused citrate is cleared by the dialyzer, and therefore the amount of the infused citrate entering the systemic circulation is much lower than the amount infused into the arterial line.

The molar infusion rate of citrate into the arterial line $I_n(t)$ is the product of the rate of citrate solution indicated by volume $I_{vol}(t)$ and the citrate concentration in this solution, $c_{Ci\text{-}sol}$:

$$I_n(t) = I_{vol}(t) \cdot c_{Ci-sol}$$

Equation 4

Because citrate does not enter red blood cells (Whitfield and Levy 1981), the infused citrate is only dissolved in the plasma water. The concentration of infused citrate in the plasma water at the arterial line $c_{Ci\text{-}inf\text{-}art}(t)$ is the molar infusion rate divided by the plasma water flow rate Q_{Pl} and multiplied with a dilution factor f, because the total volume is the sum of the plasma water volume and the infusion volume:

$$c_{Ci-inf-art}(t) = \frac{I_n(t)}{Q_{Pl}} \cdot f$$

Equation 5

The dilution factor f is calculated as follows:

$$f = \frac{Q_{Pl}}{Q_{Pl} + I_{vol}(t)}$$

Equation 6

Plasma water flow, Q_{Pl}, can be calculated from the blood flow rate, Q_B, hematocrit, Hct and plasma water fraction, f_p:

$$Q_{Pl} = Q_B \cdot (1 - Hct) \cdot f_P$$

Equation 7

As already mentioned, the infusion rate into the body, dn_{inf}/dt, is the difference between the molar infusion rate into the arterial line and molar removal rate into the dialysate:

$$\frac{dn_{inf}}{dt} = I_n(t) - K_{Ci} \cdot c_{Ci-inf-art}(t)$$

Equation 8

A certain level of citrate is always found in the blood. It can be assumed that prior to dialysis citrate is in a steady state, i.e. metabolic generation rate g equals the metabolic elimination rate $dn_{out\text{-}met}/dt$:

$$g = \frac{dn_{out-met}}{dt}$$

Equation 9

The metabolic elimination rate depends on the metabolic rate constant, k, and the systemic citrate concentration. Assuming a constant volume it is:

$$\frac{dn_{out-met}}{dt} = k \cdot c_{Ci}(t) \cdot V_{DCi}$$

Equation 10

If k and the initial concentration of citrate (before the start of the infusion = $c_{Ci}(0)$) are known, the generation rate can be calculated. $c_{Ci}(0)$ is the plasma water concentration of citrate. Plasma concentration is always already corrected for plasma protein content by division by f_P.

$$g = k \cdot c_{Ci}(0) \cdot V_{DCi}(0)$$

Equation 11

It is assumed that the metabolic generation rate always remains constant.
Inserting equations 11 and 8 into equation 3 gives the total citrate input rate = infusion rate + generation rate:

$$\frac{dn_{in}}{dt} = I_n(t) - K_{Ci} \cdot c_{Ci-inf-art}(t) + k \cdot c_{Ci}(0) \cdot V_{DCi}(0)$$

Equation 12

2.1.1.3 Elimination of citrate

The elimination rate is the sum of the dialyzer elimination rate $dn_{out\text{-}dial}/dt$ and the metabolic elimination rate $dn_{out\text{-}met}/dt$:

$$\frac{dn_{out}}{dt} = \frac{dn_{out-dial}}{dt} + \frac{dn_{out-met}}{dt}$$

Equation 13

The **dialyzer elimination** of the systemic citrate delivered by the patient's blood is described by equation14. The systemic citrate concentration prior to the citrate bottle infusion port (cci-sys) is diluted by the carrier solution infused at the citrate infusion port, f is correcting for this dilution.

$$\frac{dn_{out-dial}}{dt} = K_{Ci} \cdot c_{Ci}(t) \cdot f$$

Equation 14

It is assumed that the **metabolic elimination** is a first order kinetics, i.e. the rate of the elimination is proportional to the concentration:

$$\frac{dc(t)}{dt} = k \cdot c(t)$$

Equation 15

If k (metabolic rate constant) is known the amount of citrate removed per min by metabolism (flux of citrate metabolized, $dn_{out\text{-}met}/dt$, in mmol/min) can be calculated. In the current study metabolic rate constant was estimated from the data obtained during the clinical trial. It was calculated using the decline in citrate levels after stopping dialysis and citrate infusion, see chapter 2.3.2.7.

In equation 1 the flux (dnci/dt) of citrate (amount of citrate in the body (dnci) over time (dt)) is calculated. In order to use the results of equation 15 in equation 1 we now have to calculate dn/dt from dc/dt:

$$\frac{d\left(\frac{n(t)}{V(t)}\right)}{dt} = k \cdot c(t)$$

Equation 16

If volume of distribution V(t) is constant, the result is equation 10. However, if V(t) is not constant as it occurs during HD when ultrafiltration decreases the distribution volume, the result is following:

$$\frac{V(t)\cdot\frac{dn(t)}{dt}-n(t)\cdot\frac{dV(t)}{dt}}{V(t)^2}=k\cdot c(t)=k\cdot\frac{n(t)}{V(t)}$$

$$\Leftrightarrow\frac{dn(t)}{dt}=k\cdot n(t)+\frac{n(t)}{V(t)}\cdot\frac{dV(t)}{dt}$$

Equation 17

Since dV/dt is the negative ultrafiltration rate, UF, the metabolic elimination rate can be written as follows:

$$\frac{dn_{out-met}}{dt}=k\cdot n(t)-\frac{n(t)}{V(t)}\cdot UF$$

Equation 18

Insertion of equations 14 and 18 into equation 13 results in:
total elimination rate = dialyzer elimination rate + metabolic elimination rate

$$\frac{dn_{out}}{dt}=K_{Ci}\cdot\frac{n(t)}{V(t)}\cdot f+k\cdot n(t)-\frac{n(t)}{V(t)}\cdot UF=(K_{Ci}-UF)\cdot\frac{n(t)}{V(t)}+k\cdot n(t)$$

Equation 19 Total elimination of citrate.

2.1.1.4 The systemic citrate concentration

Insertion of equations 12 and 19 into equation 1 results in the following differential equation:
citrate flux = citrate input rate - total citrate elimination rate

$$\frac{dn(t)}{dt}=I_n(t)-K_{Ci}\cdot c_{Ci-inf-art}(t)+k\cdot c_{Ci}(0)\cdot V_{DCi}(0)-(K_{Ci}\cdot f-UF)\cdot\frac{n(t)}{V(t)}-k\cdot n(t)$$

Equation 20

The solution of this differential equation is the total citrate amount dissolved in the distribution volume. The division by the distribution volume results in the plasma water concentration. This

has to be multiplied with the factor for plasma water fraction f_P in order to compare it with the measured patient plasma or serum concentrations.

2.1.2 Model of calcium concentration

2.1.2.1 One-compartmental model

Ionized calcium Ca_{ion} in the extracellular fluid is in equilibrium with bound calcium and calcium complexed with small anions e.g. citrate. It is well known that the plasma concentration of ionized calcium is precisely controlled and the control mechanisms are quite rapid. A fast shift of calcium to stores or release from these stores enables the maintenance of a quite constant ionized calcium plasma level.

In the model described here it is assumed that total calcium is evenly distributed in a certain volume of distribution, and is increased or decreased by input or output of calcium introduced by the dialysis procedure. Since internal calcium shifts between plasma and calcium stores can not be measured directly they are not considered in this model. With the described assumption, a **one-compartmental model** like the citrate model is employed here. The input of calcium is only by calcium infusion, the output is via dialytic removal of ionized calcium and of citrate-calcium complexes (fig. 3).

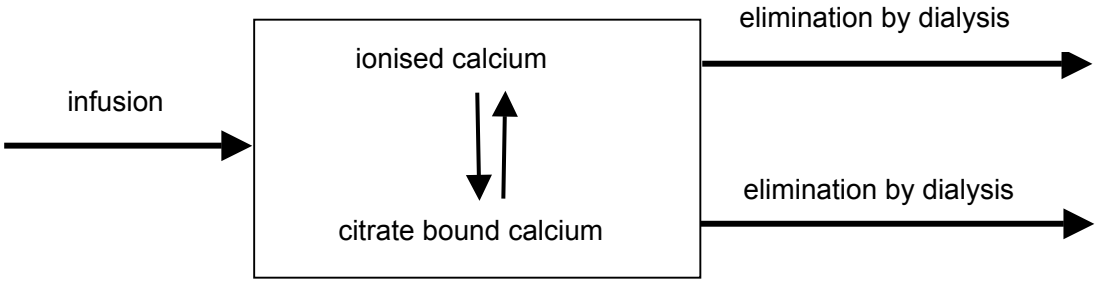

Figure 3 One compartmental model showing the input and the output of calcium mass for the calculation of the total and ionized calcium concentration.

The principle of mass balance is applied for total calcium. The difference between the input rate $dn_{Ca\text{-}in}/dt$ and the output rate $dn_{Ca\text{-}out}/dt$ is **the net flux of total calcium dn_{Ca}/dt:**

$$\frac{dn_{Ca}}{dt} = \frac{dn_{Ca-in}}{dt} - \frac{dn_{Ca-out}}{dt}$$

Equation 21

2.1.2.2 Systemic concentration of total calcium

Systemic concentration of total calcium $c_{Ca}(t)$ is the amount of calcium, $n_{Ca}(t)$, divided by the distribution volume $V_{DCa}(t)$, e.g. extracellular water.

$$C_{Ca}(t) = \frac{n_{Ca}(t)}{V_{Ca}(t)}$$

Equation 22

The amount of calcium, $n_{Ca}(t)$, is the sum of the initial amount of calcium, $n_{Ca}(0)$, and the integration of the infusion and elimination (the integration of the net flux of calcium dn_{Ca}/dt).

$$n_{Ca}(t) = n_{Ca}(0) + \int \frac{dn_{Ca}}{dt}$$

Equation 23

The initial amount of calcium, $n_{Ca}(0)$, is the product of the initial total calcium concentration and the initial distribution volume:

$$n_{Ca}(0) = C_{Ca}(0) * V_{Ca}(0)$$

Equation 24

Inserting equation 24 into 23 and then into equation 22 results in:

$$C_{Ca}(t) = \frac{C_{Ca}(0) * V_{Ca}(0) + \int \frac{dn_{Ca}}{dt}}{V_{Ca}(t)}$$

Equation 25

2.1.2.3 Concentration of calcium bound to the proteins

There are two kinds of calcium binding proteins: albumin (binding 75-90% of protein bound calcium) and globulin. However, in this model the assumption is made, that all plasma proteins have the physico-chemical properties of albumin.

Assume that the one molecule of albumin, A, has N binding sites for the ionized calcium molecule. The reaction equation is: $A + NCa \leftrightarrow Ca_NA$, and according to the law of mass action:

$$K_{CaA} = \frac{[A] * [C_{Ca-ion}]^N}{Ca_N A}$$

$K_{CaA:}$ dissociation constant for the ionized calcium and protein (albumin).

Equation 26

The equilibrium concentrations can be calculated from equation 26 only if all or none of the protein binding sites are occupied.

The calculation of the equilibrium concentrations is easier if it is assumed that all binding sites are *identical* and *independent*. *Identical* means, that the calcium ions bind to every binding site with the same affinity. *Independency* means, that the binding of one calcium ion to one binding site does not affect the binding of calcium to another site.
With these assumptions, the reaction equation between a calcium binding site and ionized calcium can be written: P + Ca ↔ CaP According to the law of mass action:

$$K_{CaP} = \frac{C_{Pfree}(t) \times C_{Ca-ion}(t)}{C_{CaP}(t)}$$

K_{CaP}: dissociation constant between calcium binding site and calcium, 0.011 mol/l (Pedersen 1971; Pedersen 1972).

Equation 27

Concentration of total calcium binding sites, C_P is the sum of free binding sites C_{Pfree} plus calcium saturated binding sites, $C_{CaP}(t)$:

$$C_p(t) = C_{Pfree}(t) + C_{CaP}(t)$$

Equation 28

Total concentration of calcium binding sites can be calculated using the data from the studies by Pedersen (Pedersen 1971; Pedersen 1972): the mean molar weight of albumin is 69000 g/mol, the number of binding sites per albumin molecule, N, is 12. With these assumptions the total concentration of protein binding sites, $C_p(t)$, can be calculated as follows:

$$C_p(t) = \frac{n_P(0)}{V_{Ca}(t)}$$

Equation 29

$$n_p(0) = N * \frac{p[g/l]}{69000} * V_{Ca}(0)$$

p: plasma concentration of proteins [g/l]

Equation 30

Insertion of the equation 30 into the equation 29 results in:

$$C_P(t) = N * \frac{p[g/l]}{69000} * \frac{V_{Ca}(0)}{V_{Ca}(t)}$$

Equation 31

2.1.2.4 Concentration of ionized calcium and calcium complexed by citrate

Concentration of total calcium is the sum of: the ionized calcium concentration [$C_{Ca\text{-}ion}(t)$], concentration of calcium bound to the blood proteins [$C_{CaP}(t)$], and the concentration of calcium-citrate complexes [$C_{Caci}(t)$]. If the concentration of total calcium is given, ionized calcium concentration can be calculated from the following equation:

$$C_{Ca}(t) = C_{Ca-ion}(t) + C_{CaP}(t) + C_{CaCi}(t) \Rightarrow$$
$$\Rightarrow C_{Ca-ion}(t) = C_{Ca}(t) - C_{CaP}(t) - C_{CaCi}(t)$$

Equation 32

The effect of other small anions that occur in the human blood and may complex ionized calcium (e.g. phosphate, lactate, sulfate, malate) is neglected since their normal concentration is relatively low 0.2 mmol/l and accounts for approx. 9% of total calcium concentration.

Since one molecule of citrate chelates one molecule of calcium the equation of this reaction can be written as follows: Ca + Ci ↔ CaCi

According to the law of mass action:

$$K_{CaCi} = \frac{C_{Ca-ion}(t) * C_{Ci-free}(t)}{C_{CaCi}(t)}$$

Equation 33 K_{CaCi}: dissociation constant between calcium and citrate, $7.76 * 10^{-4}$ mol/l (Toffaletti et al. 1976).

Concentrations in equilibrium depend on total citrate concentration, which was calculated in the chapter “Modelling of citrate concentration”:

$$C_{Ci}(t) = C_{Ci-free}(t) + C_{CaCi}(t)$$

Equation 34

According to the thermodynamic theory the concentrations of: ionized calcium, citrate bound calcium and protein bound calcium can be regarded as coupled equilibriums. Using this method the concentrations of: ionized calcium, citrate bound calcium and protein bound calcium can be calculated using the set of the equations: 32, 28, 34, 33, 27.

$$C_{Ca}(t) = C_{Ca-ion}(t) + C_{CaP}(t) + C_{CaCi}(t)$$

Equation 35

$$C_p(t) = C_{Pfree}(t) + C_{CaP}$$

Equation 36

$$C_{Ci}(t) = C_{Ci-free}(t) + C_{CaCi}(t)$$

Equation 37

$$K_{CaCi} = \frac{C_{Ca-ion}(t) * C_{Ci-free}(t)}{C_{CaCi}(t)}$$

Equation 38

$$K_{CaP} = \frac{C_{Pfree}(t) \times C_{Ca-ion}(t)}{C_{CaP}(t)}$$

Equation 39

From equation 36:

$$C_{Ci-free}(t) = C_{Ci}(t) - C_{CaCi}(t)$$

Equation 40

Inserting equation 40 into equation 38 results in:

$$K_{CaCi} = \frac{C_{Ca-ion}(t) * [\ C_{Ci}(t) - C_{CaCi}(t)\ ]}{C_{CaCi}(t)}$$

Equation 41

Solving eq. 41 for $C_{CaCi}(t)$ results in:

$$C_{CaCi}(t) = \frac{C_{Ca-ion}(t) * C_{Ci}(t)}{K_{CaCi} + C_{Ca-ion}(t)}$$

Equation 42

From eq. 35:

$$C_{P-free}(t) = C_P(t) - C_{CaP}(t)$$

Equation 43

Inserting eq. 35 into eq. 38 and solving it for $C_{CaP}(t)$ results in:

$$C_{CaP}(t) = \frac{\left[C_P(t) * C_{Ca-ion}(t)\right]}{K_{CaP} + C_{Ca-ion}(t)}$$

Equation 44

Inserting eq. 42 and eq. 41 into eq. 35 and solving it for $C_{Ca\text{-}ion}(t)$ results in a cubic equation in the normal form:

$$(C_{ca-ion}(t))^3 + (C_{Ca-ion}(t))^2\left[\ K_{CaCi} + K_{CaP} + C_{Ci}(t) + C_P(t) - C_{Ca}(t)\ \right] +$$
$$+ C_{Ca-ion}(t)\left[\ K_{CaCi} * K_{CaP} + K_{CaP} * C_{Ci}(t) + K_{CaCi} * C_P(t) - K_{CaCi} * C_{Ca}(t) - K_{CaP} * C_{Ca}(t)\ \right] -$$
$$- K_{CaCi} * K_{CaP} * C_{Ca}(t) = 0$$

Equation 45

This equation has following coefficients:

$$a = 1$$

$$b = K_{CaCi} + K_{CaP} + C_{Ci}(t) + C_P(t) - C_{Ca}(t)$$

$$c = K_{CaCi} * K_{CaP} + K_{CaP} * C_{Ci}(t) + K_{CaCi} * C_P(t) - K_{CaCi} * C_{Ca}(t) - K_{CaP} * C_{Ca}(t)$$

$$d = - K_{CaCi} * K_{CaP} * C_{Ca}(t)$$

Any cubic equation can be transformed in the following way. Cubic equation is divided by a, and a substitution is made:

$$C_{Ca-ion}(t) = y - \frac{b}{3a}$$

Equation 46

This leads to the next equation:

$$y^3 + 3py + 2q = 0$$

Equation 47

This new equation has following coefficients:

$$3p = \frac{3 \cdot a \cdot c - b^2}{3 \cdot a^2}$$

$$2q = \frac{2 \cdot b^3}{27 \cdot a^3} - \frac{b \cdot c}{3 \cdot a^2} + \frac{d}{a}$$

Any cubic equation has at least one real solution, but three real solutions are also possible.
The number of real solutions is dependent on the sign of the discriminant D.
The discriminant D is defined as:

$$D = q^2 + p^3$$

If D>0, there is only one real solution.
If D=0 and p=q=0 then there is one triple solution equal to 0.
If D=0 and $p^2=-q^3 \neq 0$, then there are two real solutions.
If D<0 there are three real solutions.

Only a positive real solution is interesting for the calculation of concentrations, negative solutions are ignored.

There are various approaches to solve a cubic equation, (for more details the reader is referred to (Bronstein and Semendjajew 1962)). Here it is solved using a method with auxiliary variables φ and r as proposed by Bronstein et al. (Bronstein and Semendjajew 1962).

An auxiliary variable r is defined as:

$$r = \pm\sqrt{|p|}$$

The sign of r is so chosen that it is the same as the sign of q. Then the auxiliary variable φ and the solutions of equation (y_1, y_2, and y_3) are calculated in dependence on the sign of p and D according to the table 1. The solutions in the red box in table 1 are complex solutions, which are ignored.

From the table 1 the positive, real solutions for y can be found. Once y is known, concentration of ionized calcium can be calculated from the equation 46:

$$C_{Ca-ion}(t) = y - \frac{b}{3a}$$

Again only positive values of ionized calcium are accepted. If the concentration of ionized calcium is calculated, the concentration of calcium complexed by citrate can be estimated according to the equation 42:

$$C_{CaCi}(t) = \frac{C_{Ca-ion}(t) * C_{Ci}(t)}{K_{CaCi} + C_{Ca-ion}(t)}$$

$p < 0$		$p > 0$
$q^2 + p^3 \leqq 0$	$q^2 + p^3 > 0$	
$\cos \varphi = \frac{q}{r^3}$	$\operatorname{ch} \varphi = \frac{q}{r^3}$	$\operatorname{sh} \varphi = \frac{q}{r^3}$
$y_1 = -2r \cos \frac{\varphi}{3}$	$y_1 = -2r \operatorname{ch} \frac{\varphi}{3}$	$y_1 = -2r \operatorname{sh} \frac{\varphi}{3}$
$y_2 = +2r \cos \left(60° - \frac{\varphi}{3}\right)$	$y_2 = r \operatorname{ch} \frac{\varphi}{3} + i\sqrt{3}\, r \operatorname{sh} \frac{\varphi}{3}$	$y_2 = r \operatorname{sh} \frac{\varphi}{3} + i\sqrt{3}\, r \operatorname{ch} \frac{\varphi}{3}$
$y_3 = +2r \cos \left(60° + \frac{\varphi}{3}\right)$	$y_3 = r \operatorname{ch} \frac{\varphi}{3} - i\sqrt{3}\, r \operatorname{sh} \frac{\varphi}{3}$	$y_3 = r \operatorname{sh} \frac{\varphi}{3} - i\sqrt{3}\, r \operatorname{ch} \frac{\varphi}{3}$

Table 1 Solutions of a cubic equation depending on auxiliary variable.

2.1.2.5 Input of calcium

The infusion of calcium is the only calcium input into the model. A metabolic generation of calcium by release from calcium stores is ignored as already mentioned. Since only dialysis and a short postdialytic period (up to 30 minutes) are analyzed, calcium in the diet is also ignored. Therefore the input rate is equal to substitution into the venous line expressed as the molar infusion rate $I_{Ca\text{-}n}(t)$:

$$\frac{dn_{Ca-in}}{dt} = I_{Ca-n}(t)$$

Equation 48

The molar infusion rate of calcium is the product of the infusion rate (volume) $I_{Ca\text{-}vol}(t)$ and the calcium concentration in the infusion solution $c_{Ca\text{-}sol}$:

$$I_{Ca-n}(t) = I_{Ca-vol}(t) \cdot c_{Ca-sol}$$

Equation 49

2.1.2.6 Calcium output

Calcium is removed from the plasma by the dialyzer in the form of ionized calcium and in the form of calcium-citrate-complexes:

$$\frac{dn_{Ca-out-dial}}{dt} = K_{Ca} \cdot c_{Ca-ion-art}(t) + K_{Ci} \cdot c_{CaCi-art}(t)$$

Equation 50

In equation 50 it is assumed, that the clearance of the calcium-citrate-complexes is the same as the one for citrate. This is a reasonable assumption since there is no relevant difference between the molecular weight of citrate (189) and the molecular weight of the citrate-calcium complex (229). Calculations of the concentrations of ionized calcium and calcium complexed by citrate in the extracorporeal circuit are calculated as shown above in the chapter 2.1.2.4.

2.2 In vitro tests

2.2.1 Procedure

Clearance of citrate was measured for the polysulfone hollow fiber dialyzer F60S (Fresenius Medical Care, Bad Homburg, Germany, technical data see table 3) using a 4008-dialysis machine (F Fresenius Medical Care, Bad Homburg, Germany). Dialysis solution SK-F 219/0 (Fresenius Medical Care, Bad Homburg, Germany) with dissolved trisodium citrate was pumped through the blood compartment of the dialyzer serving as an artificial “blood”. The temperature of this “artificial blood” was maintained at around 36.6° to simulate the normal core temperature of dialysis patients. The same dialysis solution SK-F 219/0 (Fresenius Medical Care, Bad Homburg, Germany) but without the addition of citrate was used in the dialysate compartment. Specification of the contents of the dialysis solution is shown in the table 2.

Clearance of citrate was measured for the following flows in the blood compartment: 400ml/min, 300ml/min, 200ml/min, 150ml/min, 100ml/min, and 50ml/min. These flows were controlled using direct ultrasound measurement (Transonic® Flowprobe). The dialysis solution flow was set at 500ml/min. In addition the dialysis solution flow rate was verified by the manual measurement of the spent dialysate.
Samples for the citrate measurements were taken from the arterial and the venous line. Citrate concentration was determined using an enzymatic assay (Boehringer Mannheim/R-Biopharm).

Substance	Na	K	Ca	Mg	Bicarbonate	Glucose	Cl	Acetate
Concentration	138	2	0	0.5	33	1 g/l	106	2.0

Table 2 Components of the dialysis solution (in mmol/l) used for in vitro tests (SK-F 219/0).

	FX 40	FX 50	FX 60	FX 80	FX 100
Clearance: QB 200 (ml/min)					
Urea	170	189	193	197	198
Creatinine	144	170	182	189	194
Phosphate	138	165	177	185	189
Vitamin B12	84	115	135	148	161
Inulin	54	76	95	112	125
Clearance: QB 300 (ml/min)					
Urea	209	250	261	276	278
Creatinine	168	210	230	250	261
Phosphate	160	201	220	239	248
Vitamin B12	91	130	155	175	192
Inulin	56	81	104	125	142
Ultrafiltration coeff. (ml/h · mm Hg)	20	33	46	59	73
Membrane material			Helixone®		
Effective surface (m2)	0.6	1.0	1.4	1.8	2.2
Wall thickness (µm)	35	35	35	35	35
Inner diameter (µm)	185	185	185	185	185
Priming vol. (ml)	32	53	74	95	116
Housing material			Polypropylene		
Potting compound			Polyurethane		
Sterilisation method			INLINE Steam		
Form of treatment	HD	HD	HD/HDF	HDF/HF	HDF/HF

	F40S	F50S	F60S	F70S	HF80S	H*d*F100S
Clearance QB 200 (ml/min)						
Urea	165	178	185	190	192	195
Creatinine	140	160	172	177	180	190
Phosphate	138	158	170	174	177	185
Vitamin B12	80	100	118	127	135	160
Inulin	54	75	88	98	110	127
Clearance QB 300 (ml/min)						
Urea	200	225	242	245	248	271
Creatinine	165	195	215	220	225	252
Phosphate	158	190	210	216	220	240
Vitamin B12	86	112	134	145	155	190
Inulin	58	83	97	109	120	145
Ultrafiltration coeff. (ml/h · mmHg) (1)	20	30	40	50	55	60
Membrane material			Fresenius Polysulfone®			
Effective surface (m2)	0.7	1.0	1.3	1.6	1.8	2.4
Wall thickness (µm)	40	40	40	40	40	35
Inner diameter (µm)	200	200	200	200	200	185
Priming vol. (ml)	42	63	82	98	110	138
Housing material			Polycarbonate			
Potting compound			Polyurethane			
Sterilisation method			INLINE Steam			
Form of treatment	HD	HD	HD/HDF	HD/HDF	HDF/HF	HDF/HF

Table 3 Manufacturer technical data on in vitro dialyzers performance using human blood (Hct 32%, total protein concentration 6 g/l), QD 500 ml/min, temperature=37°C and Q_F 0 ml/min.

2.2.2 Calculations

2.2.2.1 Calculation of citrate clearance and extraction coefficient

Citrate clearance, *K*, and extraction coefficient, *E* were calculated according to the formulas 51 and 52:

$$K = \frac{C_{in} - C_{out}}{C_{in}} * QE$$

K: citrate clearance [ml/min]

C_{in}: citrate concentration in the arterial line, dialyzer blood inlet [mmol/ml]

C_{out}: citrate concentration in the venous line, dialyzer blood outlet [mmol/ml]

QE: fluid flow in the blood compartment [ml/min]

Equation 51 Clearance of citrate.

$$E = \frac{C_{in} - C_{out}}{C_{in}} * 100$$

Equation 52 Extraction coefficient of citrate.

2.2.2.2 Calculation of KoA (mass transfer area coefficient) for citrate

The efficiency of the dialyzer in removing any solute can be also described by a constant named: dialyzer mass transfer area coefficient, KoA (Daugirdas and Stone 2001). In contrast to the clearance and the extraction coefficient, KoA is independent from the flows in the blood and in the dialysate compartment.

KoA for citrate was calculated using the measured clearance of citrate in accordance with the standard procedures using the equation 53 (Sargent and Gotch 1996; Waniewski et al. 1993; Werynski 1979):

$$K = \frac{e^{\left(\frac{1}{QE} - \frac{1}{QD}\right) * KoA} - 1}{e^{\left(\frac{1}{QE} - \frac{1}{QD}\right) * KoA} - \frac{QE}{QD}} * QE \quad \Rightarrow \quad KoA = \frac{Ln\left(QE * \frac{K - QD}{QD * (K - QE)}\right)}{\frac{1}{QE} - \frac{1}{QD}}$$

K: clearance of citrate [ml/min]

QE: flow in the blood compartment [ml/min]

QD: dialysate flow [ml/min]

Equation 53

2.3 Description of the clinical trial

2.3.1 Study design

2.3.1.1 Patients

A total of 15 haemodialysis treatments using citrate anticoagulation were carried out in 10 patients with renal failure requiring dialysis. The participants of the study were recruited from the patients of the Munich University Hospital (Klinikum rechts der Isar) and were judged to be at increased risk of bleeding. Citrate anticoagulation was performed as a routine procedure and the decision to apply citrate anticoagulation followed standard operating rules of the department. The clinical characteristics of the patients, including the indication for citrate anticoagulation, are summarized in table 4. At the time of the study none of the patients had clinical evidence of severe liver failure. Study was approved by the local ethics committee. Written informed consent was obtained from all participants.

Patient Nr	Age /Sex	Diagnoses	Indication for citrate anticoagulation	Numbers of dialysis
1.	70/F	CRF, M. Wegener	hemoptysis	2
2.	72/F	CRF, M. Wegener	after surgery	2
3.	53/M	CRF; hypertension	after surgery	1
4.	52/M	ARF, M. Wegener	hemoptysis	3
5.	79/F	CRF; hypertension, diabetes mellitus	after surgery	1
6.	55/M	CRF, polycystic kidney disease	after surgery	1
7.	64/M	CRF, M. Wegener	history of heparin-induced bleeding	1
8.	68/M	CRF, state after right nephrectomy, hypertension, chronic pyelonephritis	after thyroid biopsy	1
9.	69/M	ARF due to heart surgery	after surgery	2
10.	51/M	CRF, chronic glomerulonephritis	immediately before surgery	1

Table 4 Clinical characteristics of patients.

2.3.1.2 Haemodialysis

Haemodialysis was performed with the use of a modified 4008 hemodialysis machine providing semiautomatic citrate application (Prometheus®), which is shown in figure 4. During the study polysulfone hollow fiber dialyzers (FX50, F60S, Fresenius Medical Care, Bad Homburg, Germany, technical data see in table 3) and calcium free, bicarbonate-based dialysis solutions (SK-F 419/1 or SW 415 A) were used.

Bicarbonate concentration was adjusted to meet the patients' needs. Haemodialysis started with the bicarbonate concentration on average set at 32 mmol/l. During haemodialysis acid-base parameters were monitored (see sampling protocol and laboratory tests) and bicarbonate concentration was changed if it was clinically indicated.

Blood flow (183 ml/min ±2) and ultrafiltration rate (492 ml/h ±76) were chosen according to the patients' clinical status and the blood access condition. Dialysis solution flow was set at 500 ml/min.

The dialysis system used (Prometheus®, Fresenius Medical Care, Bad Homburg, Germany) can also be applied as a liver support system. In this operating mode an albuflow filter is separating plasma, which is cleaned from toxins by adsorption columns. In the current study there was no albuflow filter or plasma circuit, the Prometheus® unit was used only for standard haemodialysis with citrate anticoagulation.

Access type consisted either of a-v fistula (8 patients) or of Shaldon triple lumen catheter (7 patients). 13 haemodialysis sessions lasted 4 hours. Two patients required longer dialysis time, i.e. 5 and 6 hours.

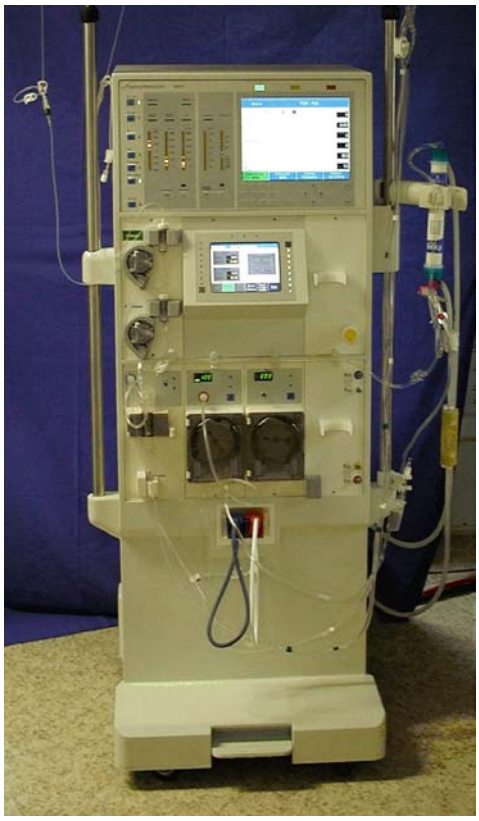

Figure 4 Prometheus® unit with semiautomatic citrate application.

2.3.1.3 Anticoagulation

Citrate anticoagulation was carried out with the use of the citrate anticoagulation module of the Prometheus® device. The citrate solution containing 0.5 mol/l trisodium citrate (Fresenius Medical Care, Bad Homburg, Germany) was infused into the arterial line. In order to recalcify the blood before it was returned to the patient, a solution of 0.5 mol/l calcium chloride (Fresenius Medical Care, Bad Homburg, Germany) was infused into the venous line. Citrate infusion rate was calculated automatically by citrate module using a fixed ratio of citrate to blood flow aiming for a final concentration of 3.3 mmol citrate / 1 L of blood. The calcium solution flow at baseline was in

proportion to the blood flow and was subsequently adapted to the ionized calcium, which was measured periodically according to the algorithm incorporated into the citrate anticoagulation module with the resulting mean rate of 0.4 ± 0.01 ml/min.

2.3.1.4 Sampling Protocol

Blood of the patients was sampled before the treatment (time: “0”) and periodically during the treatment from the arterial line of the extracorporeal circuit (“arterial samples”) at the time points: +15min, +45min, +165min, +225min to measure: citrate concentration, electrolytes (total and ionized calcium, magnesium, sodium and chloride) and acid base parameters. In addition, in order to calculate clearance of citrate and clearance of total calcium three samples from the venous line (+45min, +165min, +225min) were taken simultaneously with the arterial samples. After the termination of the treatment three additional samples (post 0min, +post 15min, +post 30min) were taken to measure citrate concentration and electrolytes levels. Concentrations of citrate from these three samples were used to calculate the metabolic rate constant of citrate.

Samples for blood gas analysis and ionized calcium were collected in lithium-heparin syringes and immediately processed in the dialysis unit. Hematocrit and plasma protein concentrations, taken prior to the treatment, were measured using standard laboratory technique. Citrate concentrations were determined using an enzymatic assay (Boehringer Mannheim/R-Biopharm).

2.3.1.5 Data Collection and Anticoagulation Monitoring

Blood flow and ultrafiltration rate were recorded hourly. All technical problems, clinical adverse effects as well as clotting episodes were recorded if encountered.

After the termination of the haemodialysis session blood lines and filters were inspected visually for thrombus formation.

All laboratory and technical (blood flow, ultrafiltration rate) data were entered into MS-Excel® spreadsheets.

2.3.1.6 Statistical analysis

Data are presented as mean ±SD unless indicated otherwise. The normal distribution of samples was checked by Kolmogorov-Smirnov test with Lilliefors' correction. The *t* test for paired samples or Wilcoxon‘s signed–rank test were used as appropriate to compare differences between pre and post HD values. Other independent data samples were evaluated with *t* test or Wilcoxon‘s rank-sum test. Correlations were measured with the use of Pearson’s and Spearman’s correlation coefficients. Liner regression model was employed to assess whether the loss of calcium into the dialysate can be predicted from the citrate clearance calculated using KoA and the total calcium concentration. P value < 0.05 was considered statistically significant (two-tailed tests). Statistical analysis was performed using SigmaStat software version 2.03.

2.3.2 Pharmacokinetic calculations from patient data

2.3.2.1 Citrate extraction coefficient

Citrate extraction coefficient, *E*, was calculated according to the formula 54:

$$E = \frac{C_{in} - C_{out}}{C_{in}} * 100$$

C_{in}: citrate concentration in the dialyzer blood inlet, [mmol/ml]

C_{out}: citrate concentration in the dialyzer blood outlet, [mmol/ml]

Equation 54

Citrate concentration in the outlet of the dialyzer was measured directly from the venous line. Since clearance across the dialyzer membrane occurs for the solutes dissolved in plasma water, the citrate concentrations measured in plasma have to be converted to plasma water concentration dividing by plasma water fraction, f_p.

$$f_p = 1 - \frac{P}{100}$$

P: total proteins concentration expressed in [g/dl].

Equation 55

Citrate concentration at the blood inlet of the dialyzer was calculated as a sum of the amount of citrate coming from the systemic circulation and the infused amount of citrate, divided by the sum of the volume of plasma water and the infusion volume of citrate solution.

$$C_{in} = \frac{C_{art} * BF * (1 - Hct) + C_{sol} * SF}{PF + SF}$$

C_{sol}: concentration of citrate solution was equal to 0.5 mol/ml

SF: was registered hourly

C_{art}: citrate concentration in samples taken from the arterial line prior to citrate infusion port, [mmol/ml]

Equation 56

Plasma water flow at the arterial sampling site, *PF,* is calculated from the blood flow, *BF*, and haematocrit, *Hct*, with the correction for plasma water fraction, f_p, as follows:

$$PF = BF * (1 - Hct) * f_p$$

Equation 57

2.3.2.2 Clearance of citrate

Total plasma water clearance of citrate, *K*, was calculated according to the equation: (Petitclerc 1998; Waniewski et al. 1993; Werynski 1979):

$$K = \frac{C_{in} - C_{out}}{C_{in}} * PF_{in} + UF\frac{C_{out}}{C_{in}}$$

C_{in}: citrate plasma water concentration in the inlet of the dialyzer (calculated as shown in chapter 2.3.2.1)

C_{out}: citrate plasma water concentration in the venous line (obtained as described chapter 2.3.2.1)

UF: ultrafiltration rate [ml/min]

PF_{in}: plasma water flow in the inlet of the dialyzer, which is the sum of the plasma water flow at the arterial sampling site (see above) plus the volume of the infused citrate solution [*SF*].

Equation 58

2.3.2.3 Increase in systemic citrate concentration

Velocity of an increase in citrate concentration v_c was obtained from the formula 59:

$$v_c = \frac{dc}{dt}$$

dc: the change in the citrate concentration that occurred in the time interval *dt*.

Equation 59

2.3.2.4 Mass removal of citrate into the dialysate

Mass removal of citrate was calculated according to the formula 60:

$$\frac{C_{in} * PF_{in} - C_{out} * PF_{out}}{C_{in} * PF_{in}} * 100$$

C_{in}: citrate plasma water concentration in the inlet of the dialyzer line (calculated as shown in chapter 2.3.2.1)

C_{out}: citrate plasma water concentration in the venous line (described in chapter 2.3.2.1)

PF_{in}: plasma water flow in the inlet of the dialyzer (see equation 58)

PF_{out}: plasma water flow in the outlet of the dialyzer

Equation 60

Plasma water flow in the outlet of the dialyzer [PF_{out}] was calculated as a difference between the plasma water flow in the inlet of the dialyzer [PF_{in}] and the ultrafiltration rate [ml/min]:

$PF_{out} = PF_{in} - UF$

Equation 61

2.3.2.5 Total amount of citrate eliminated into the dialysate

The removal of citrate into the dialysate, Cit_{rem}, was estimated using fluxes of citrate into the dialysate, F_{d1}, F_{d2}, F_{d3}, according to the formula 62. The assumption was made that the flux does not change until the next measurement.

$$Cit_{rem} = F_{d1} * t_2 + F_{d2} * (t_3 - t_2) + F_{d3} * (t_{end} - t_3)$$

$F_{d1:}$ flux of citrate into the dialysate in the first hour of HD

$F_{d2:}$ flux of citrate into the dialysate in the third hour of HD

$F_{d3:}$ flux of citrate into the dialysate in the fourth hour of HD

t_2: time of the flux measurement in the third hour of HD

t_3: time of the flux measurement in the fourth hour of HD

t_{end}: time of the end of HD

Equation 62

Fluxes of citrate into the dialysate, F_{d1}, F_{d2}, F_{d3}, were calculated as: $C_{in} * PF_{in} - C_{out} * PF_{out}$. F_{d1}, F_{d2}, F_{d3} refer to the values at the time points: +45min, +165min, +225min, respectively.

2.3.2.6 Net balance of citrate

Net balance of citrate is the difference between the amounts of citrate infused into the arterial line and the total amount eliminated into the dialysate in accordance with the equation 63:

$$Cit_{net} = C_{\inf} - C_{rem}$$

Equation 63

The number of citrate mmols infused into the arterial line was calculated according to the equation:

$$Cit_{\inf}(t) = \int_0^{t_{end}} C_{sol} * SF(t)dt$$

$C_{sol:}$ citrate solution concentration equal to 0.5 mmol/ml

$SF(t)$: citrate solution flow was registered hourly, for the calculation the assumption was made that the flow is constant between the measurements.

Equation 64

2.3.2.7 Metabolic rate constant

Metabolic rate constant and half time was calculated from the first-order kinetics equation 65:

$$c = c_o \times e^{-k*t}$$

Equation 65

Where c_o is citrate baseline concentration after the termination of HD and c is citrate concentration measured 15 and 30 minutes after HD. After the linearization of the equation 66 and the interpolation of the results citrate metabolic rate constant was estimated.

2.3.2.8 Distribution volume of citrate

A model developed with the use of software VisSim 4.5 and described in the chapter 2.1.1 was used to assess the distribution volume of citrate. For every patient the laboratory parameters (hematocrit, proteins, clearance of citrate, baseline citrate concentration) and the haemodialysis settings (citrate flow, blood flow, and ultrafiltration rate) were implemented into the model. The concentration of citrate calculated by the model was checked for the span of the distribution volumes to find out the best fit with the measured systemic citrate concentration. The best-fitting distribution volume was chosen for the calculations of the mean distribution volume.

2.3.2.9 Total calcium extraction coefficient

Total calcium extraction coefficient was calculated in analogy to the citrate extraction coefficient, see chapter 2.3.2.1.

2.3.2.10 Measured total calcium flux into the dialysate

Total calcium flow into the dialysate, $F_{Ca\text{-}d}$, was calculated as the difference between the flux of total calcium in the blood entering the dialyzer, $F_{Ca\text{-}in}$, and the flux of total calcium in the blood leaving the dialyzer, $F_{Ca\text{-}out}$,:

$$F_{Ca-d} = F_{Ca\_in} - F_{Ca\_out}$$

Equation 66

Fluxes of total calcium in the dialyzer blood inlet and outlet were calculated with the formulas 67 and 68:

$$F_{Ca\_in} = Ca_{in} * PF_{in}$$

Equation 67

$$F_{Ca\_out} = Ca_{out} * PF_{out}$$

Equation 68

Ca_{in}: total calcium concentration in the arterial line [mmol/ml]
PF_{in}: plasma flow rate at the inlet of the dialyzer = *BF*(1-Hct)*
PF_{out}: plasma flow rate at the outlet = PF_{in} – *UF rate* [ml/min]
Ca_{out}: total calcium concentration at the outlet of the dialyzer (in the venous line before the calcium infusion port)

2.3.2.11 Net balance of total calcium

The net balance of total calcium was calculated in analogy to the net balance of citrate, see chapter 2.3.2.6.

2.3.2.12 Expected change in total calcium concentration

The expected change of total calcium concentration due to a positive or negative net calcium balance was calculated by dividing the net balance of calcium by the extracellular water. Extracellular water was assumed to constitute 21% of the body weight (Guyton 1984).

2.3.2.13 Mobile fraction of calcium in the dialyzer

"Mobile fraction" of total calcium (Ca_d) is the part of total calcium which is not bound to proteins, thus either free ionized calcium (Ca_{ion}) or calcium complexed with small anions ($Ca_{Ca\text{-}Ci}$) e.g. citrate. Only this fraction can be removed through dialysis membranes impermeable to proteins, it is also called diffusive calcium or dialyzable calcium.

$$Ca_d = Ca_{ion} + Ca_{Ca-Ci}$$

Equation 69

During citrate anticoagulated haemodialysis the total flux of calcium into the dialysate, $F_{Ca\text{-}d}$, can be regarded as the sum of the flux of citrate-calcium complexes, $F_{Ca\text{-}Ci,}$ plus the flux of ionized calcium, $F_{Ca\text{-}ion}$.

$$F_{Ca-d} = F_{Ca-ion} + F_{Ca-Ci}$$

Equation 70

The flux of a solute (ionized calcium or calcium-citrate complex) is the product of the solute concentration and the clearance of the solute (equations 71 and 72). In this estimation the assumption is made that the clearance of citrate-calcium complex and of ionized calcium is equal to the citrate clearance. This is a rational assumption since the size and the structure of citrate and citrate-calcium complex are very similar (molecular mass: 189 and 229 respectively). Since the majority of diffusible calcium exists as citrate-calcium complexes, the contribution of the flux of ionized calcium to the overall flux of diffusible calcium is very small and the difference in the clearance between ionized and complexed calcium is therefore practically negligible.

$$F_{Ca-ion} = K * Ca_{ion}$$

Equation 71

$$F_{Ca-Ci} = K * Ca_{Ca-Ci}$$

Equation 72

Inserting the equations 71 and 72 into equation 70 results in:

$$F_{Ca-d} = K * (Ca_{ion} + Ca_{Ca-Ci})$$

Equation 73

After the rearrangement the equation 73 can be written as follows:

$$Ca_{ion} + Ca_{Ca-Ci} = F_{Ca-d} / K$$

Equation 74

$(Ca_{ion} + Ca_{Ca-Ci})$ = concentration of the diffusible calcium in the blood compartment of the dialyzer, Ca_d.

F_{Ca-d}= total calcium flux into the dialysate, see chapter 2.3.2.10.

K = citrate clearance, calculations see in the chapter 2.3.2.2

Thus equation 74 can be written as follows:

$$Ca_d = F_{Ca} / K$$

Equation 75

From this equation a concentration of diffusible calcium can be calculated. Dividing this concentration by total calcium concentration in the dialyzer inlet brings the fraction of total calcium that is not bound to proteins (diffusible fraction of total calcium).

2.3.2.14 Equation used to predict calcium flux into the dialysate

For the calculation of the expected calcium flux into the dialysate we used an equation proposed by Sargent and Gotch (Sargent and Gotch 1996) describing the flux, J, as a function of the solute clearance, K, and the diffusible solute inlet concentration, C:

$$J = K * C$$

Equation 76

We assumed that during high-flux dialysis calcium loss is entirely due to diffusive transport since the contribution of the convective transport to the overall flux of small molecules (like the citrate-calcium complex or ionized calcium) is very small.

Therefore, we used a diffusive clearance of calcium-citrate complexes estimated on the basis of KoA using equation 53 (chapter 2.2.2.2). The value of KoA was assumed to be equal to KoA for citrate measured using the dialyzer F60S (in vitro experiments, chapters: 2.2.2.2 and 3.1). This is a reasonable assumption since the majority of diffusible calcium is present as citrate-calcium complexes. Since red blood cells contain only trace amounts of calcium (estimated as 0.02 mmol per kg of erythrocytes) and are regarded as impermeable to plasma calcium {Marx, 1987 763 /id} the plasma flow of the blood was regarded as the effective solute flow for calcium. The solute inlet concentration was equal to the diffusible calcium concentration, which was estimated in the study to be equal to 80% of total calcium concentration (chapter 3.2.4.3). Thus, the final equation to predict calcium loss can be written as follows:

Ca loss = 0.8 * total Ca conc. [mmol/ml] * Citrate clearance [ml/min].

Equation 77

3 Results

3.1 In Vitro Clearance of citrate

The clearance of citrate measured in vitro and the citrate extraction coefficients induced by the filter F60S are shown in the table 5 and in figure 5. At a flow of 100 ml/min in the blood compartment citrate extraction coefficient was 95 % resulting in a clearance of 95 ml/min. The mean value of KoA was equal to: 337 ml/min.

Flow in blood compartment [ml/min]	Citrate clearance [ml/min]	Citrate extraction coefficient [%]
50	49.3	99
100	95	95
150	129	86
200	155	78
300	181	60
400	211	53

Table 5 Citrate clearances [ml/min] and extraction coefficients [%] measured in vitro using electrolyte solution. Dialyzer F60S with surface area of 1.3 m². Flow in the dialysate compartment of 500 ml/min.

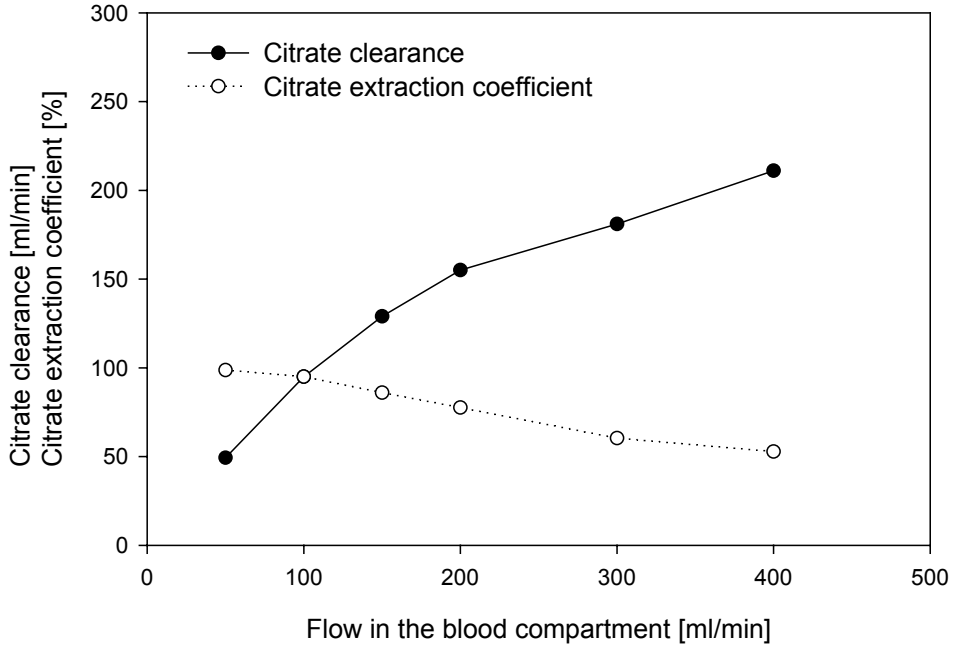

Figure 5 Citrate clearances [ml/min] and extraction coefficients [%] measured in vitro using electrolyte solution. Dialyzer F60S with surface area of 1.3 m² and counter current flow in the dialysate compartment of 500 ml/min.

3.2 Clinical trial

15 haemodialysis sessions were carried out according to the experimental study protocol described above. All haemodialysis treatments were completed successfully with no adverse effects.

3.2.1 Completeness of the data sets

In one patient abnormal high citrate levels were present prior to study and unusual citrate pharmacokinetics were observed. Therefore citrate parameters of this patient are discussed and evaluated separately (see chapter below: “Unusual case of citrate” 3.2.3.4). In another dialysis session the 15 min post dialysis sample was lost in the laboratory and therefore first order kinetics to calculate metabolic rate constant was not carried out. Consequently, 13 HD sessions were evaluated for metabolic rate constant. Of 15 dialysis treatments samples 4 h after the treatment were available from 13 treatments, 2 patients were discharged from the dialysis ward prior to blood sampling. All other data sets are complete.

3.2.2 Anticoagulation

The extracorporeal circuit was effectively anticoagulated during all treatments. No occlusion of the system occurred. Minimal thrombus formation was seen in 5 out of 15 haemodialysis sessions. All clots observed were located in the “arterial” part of the extracorporeal circuit. In the treatments affected post-filter ionized calcium was always within the target range (< 0.2 mmol/l) and not different compared to clot free sessions.

3.2.3 Citrate

3.2.3.1 Systemic citrate concentration

Systemic citrate concentration increased steadily during HD from a baseline value of 0.12 ± 0.05 mmol/l to 0.31 ± 0.15 mmol/l in the fourth treatment hour, see figure 6. It rose most rapidly in the first 40 minutes of HD (first 10 min=0.3 mmol/l*h, next 30 min=0.08 mmol/l*h) and then increased relatively slowly by approximately 0.03 mmol/l*h. Values of systemic citrate concentration using FX50 on average exceeded values measured during the treatments with the dialyzer F60S, see figure 7. The highest systemic citrate concentration was 0.72 mmol/l. Systemic citrate concentrations measured 4 hours after the haemodialysis treatment were comparable to the baseline values (0.10 ± 0.04 mmol/l) indicating that citrate did not accumulate between HD sessions.

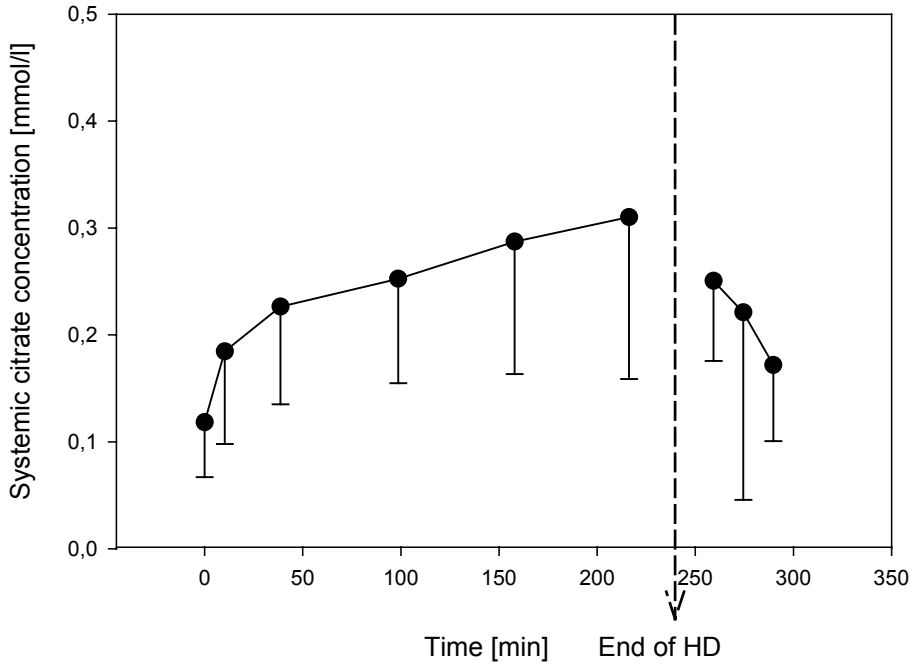

Figure 6 Systemic citrate concentrations [mmol/l] during and after haemodialysis.

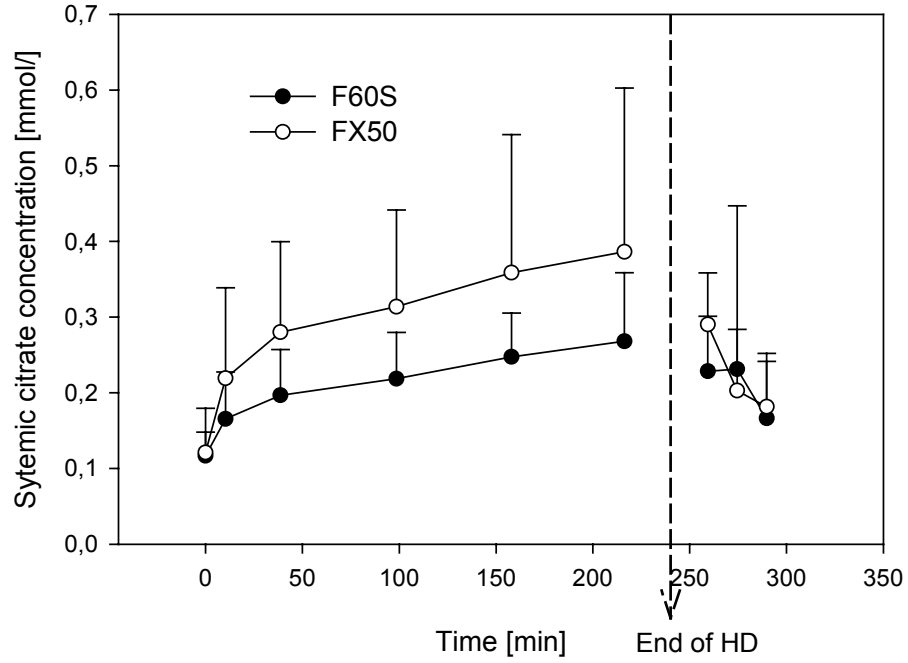

Figure 7 Systemic citrate concentration in the group of patients treated with FX50 and F60S.

3.2.3.2 Removal of citrate by the dialyzer

During the study the removal of citrate into the dialysate was very efficient for both dialyzers. Therefore only a small portion of the citrate infused into the arterial line of the extracorporeal circuit reached the body of the patient (approx. 17%). The mass removal of citrate by the dialyzer was on average 83 ± 5%. The total amount of citrate removed into the dialysate was 83% of the infused citrate and 83% of the citrate coming from the systemic blood. Thus, the total amount of citrate removed into the dialysate comprised of 88 ± 5% of the citrate that was infused into the extracorporeal circuit from the citrate bottle. This removal resulted in the mean net load of citrate delivered to the body equal to 17 ± 7 mmol/ 4 hours of HD, which was consistent with 0.2 ± 0.1 mg/min/kg (0.0011 ± 0.0006 mmol/min/kg).

Citrate plasma water clearance and extraction coefficient were on average 98 ± 10 ml/min and 82 ± 6%, respectively. However, in the current study the clearances and the extraction coefficients of citrate correlated with the type of the used dialyzer. The mean extraction coefficients and the plasma water clearances were higher in the group of patients treated with F60S (mean: F60S 84 ± 0.05%, 102 ± 7 ml/min; FX50 77 ± 0.02%, 91 ± 7 ml/min) using similar effective blood flow (183 ± 2 versus 182 ± 2 ml/min). The differences were significant in the first and third hour of HD. In the fourth treatment hour the differences were too small to reach significance (figures: 8, 9). However, there was also a small difference in hematocrit and thus plasma flow between these two patients groups (F60S: 0.3 versus FX50: 0.34) which could partly contribute to the observed results. Extraction coefficients and clearances tended to decrease during the course of the HD treatment see figure 8 and 9.

3.2.3.3 Metabolic removal of citrate

Citrate metabolic rate constant ranged from 0.0053 to 0.0382 min^{-1} (mean 0.0145 min^{-1}) resulting in mean citrate half time 60 ± 29 min (range: 18 – 131 min). Metabolic rate constant did not correlate with body weight (Pearson: r=-0.175, p=0.568; Spearman: ρ=0.0634 p=0.821).

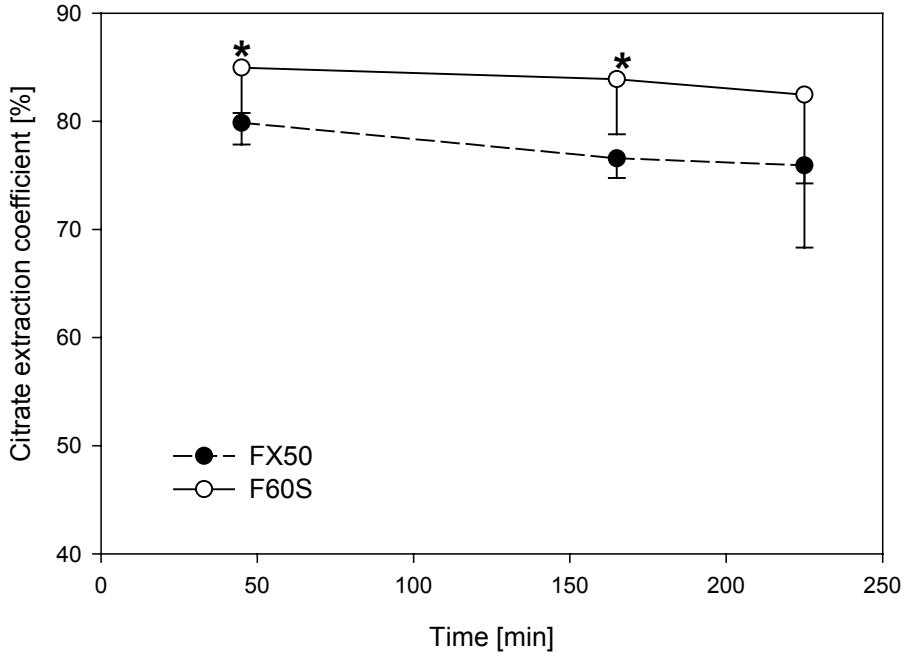

Figure 8 Citrate extraction coefficients [%] measured during haemodialysis, * p < 0.05 between FX50 and F60S.

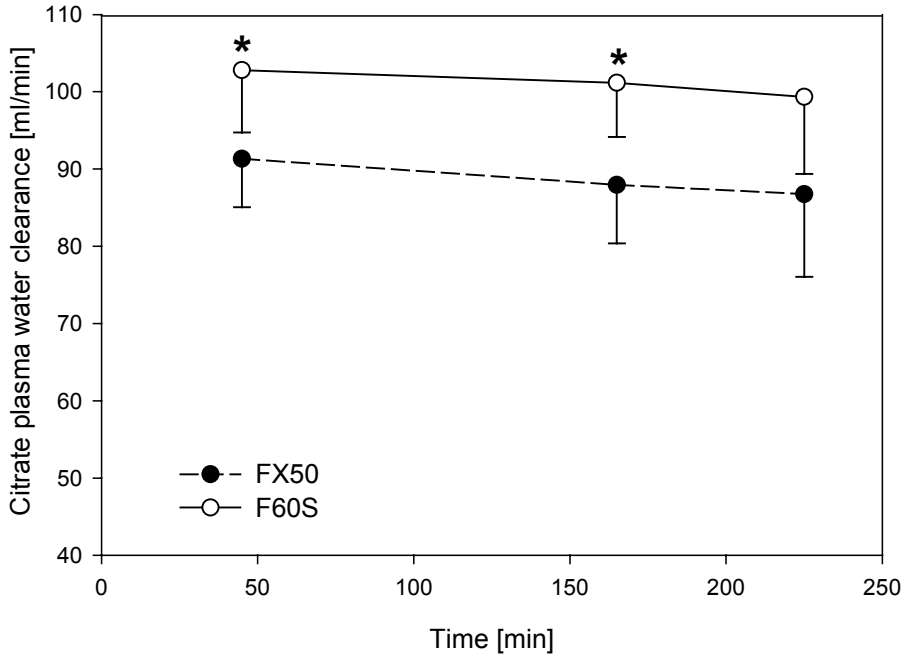

Figure 9 Citrate plasma water clearances [ml/min] measured during haemodialysis, * p < 0.05 between FX50 and F60S.

3.2.3.4 Unusual case of citrate

In one patient abnormal citrate pharmacokinetics was observed and therefore this patient was evaluated separately. In this patient concentration of citrate already at baseline was five-fold higher than the average (0.63 mmol/l versus average: 0.12 mmol/l) and remained relatively constant during and after the treatment, see figure 10.

This case was also characterized by a very efficient removal of citrate into the dialysate. Concentration of citrate in the blood leaving the dialyzer was reduced by 88-90%. Consequently, the total load of citrate for the body of the patient was also small amounting to approx. 1mmol/HD (mean load: 17 mmol). Therefore, the “expected” increase in systemic citrate concentration in this patient was also low. Thus, the relatively stable citrate concentration in this case could result from the higher than average removal of citrate into the dialysate.

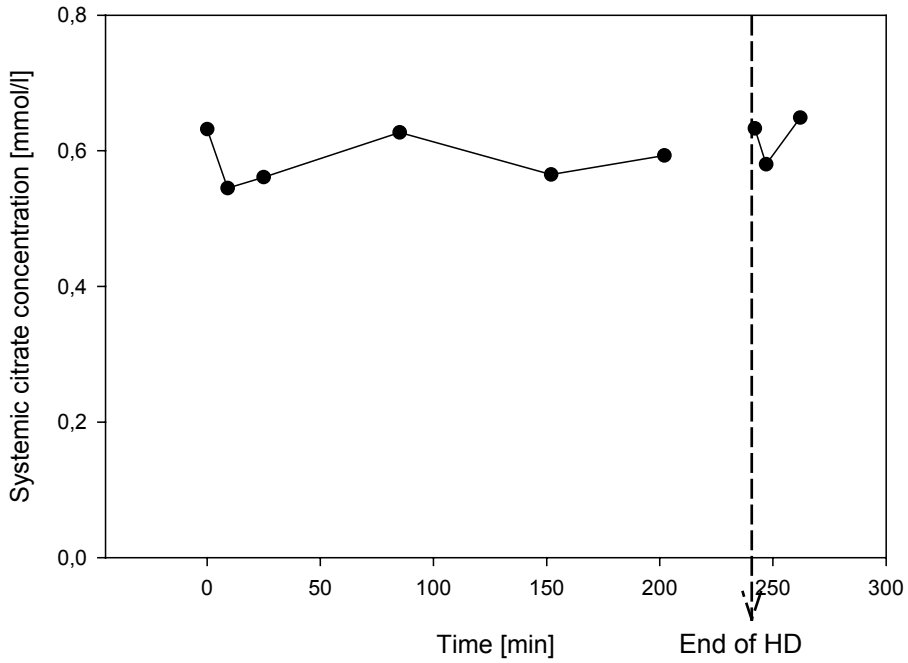

Figure 10 Unusual case of high baseline systemic citrate concentration [mmol/l], measurements prior, during and after haemodialysis.

3.2.4 Calcium

3.2.4.1 Ionized and total calcium

Mean ionized calcium increased during HD treatment by 0.09 mmol/l, from the pre-HD 1.1 mmol/l to post-HD 1.19 mmol/l, [95% CI= 0.03 to 0.14, p=0.006], see figure 11. Although this difference is statistically significant, it seems to be clinically irrelevant, since the majority of ionized calcium values were within the normal range and no clinical symptoms occurred.

During citrate anticoagulation the incidence of asymptomatic ionized hypocalcemia or hypercalcemia was low. Of all ionized calcium measurements during HD 91% [71/78] were within the range of 1.0 to 1.3 mmol/l. The lowest detected ionized calcium level during citrate anticoagulation was 0.9 mmol/l and the highest was 1.38 mmol/l.

The first 30 minutes after the dialysis were associated with higher incidence of ionizsed calcium levels ranging from 1.3 to 1.38 mmol/l (16%=7/45 after HD versus 5%=4/78 during HD).

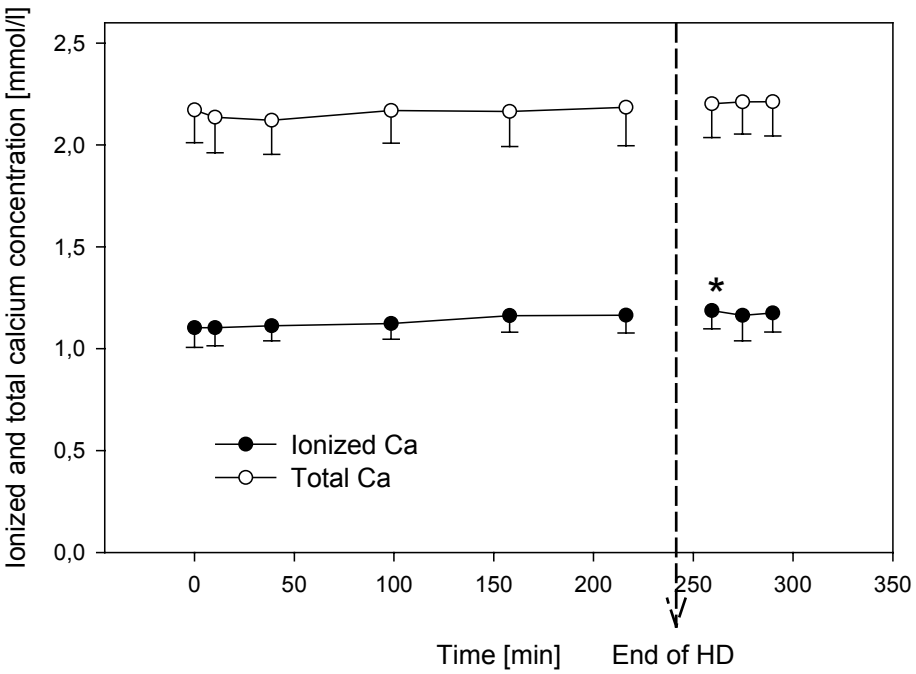

Figure 11 Ionized and total calcium concentrations [mmol/l] during and after haemodialysis. * p < 0.05 in comparison to the pre treatment value (at time point "0").

Total calcium was stable and did not change significantly during HD (pre-HD: 2.17 ± 0.16 mmol/l; directly post-HD (2.20 ± 0.17 mmol/l; p=0.294) or up to 4 h after HD, see figure 11.
Thus, during and after citrate anticoagulation dangerous hypo- or hypercalcaemia was not observed.

3.2.4.2 "Mobile" fraction of calcium - release of calcium ions from proteins

Calcium not bound to proteins (e.g. ionized calcium or calcium complexed by small anions like citrate) can diffuse through the dialyzer membrane. Diffusible calcium in the human systemic blood constitutes of approx. 60% of total calcium (Marx and Bourdeau 1987). In this study we have calculated the concentration of diffusible calcium after the infusion site of citrate i.e. in the inlet of the dialyzer, which we named "mobile fraction". After adding citrate into the arterial line the mobile fraction of calcium represented 80 ± 3% of total calcium indicating a substantial release of calcium from proteins. The proportion of diffusible to total calcium in the dialyzer tended to be remarkably constant between patients (mean: 80%, 95%CI=79 to 81%).

3.2.4.3 Total calcium loss into the dialysate

Total calcium concentration was markedly decreased in the blood leaving the dialyzer (61% FX50 and 66% F60S). The loss of calcium was calculated as a flux of total calcium into the dialysate. It was on average higher in the group of patients treated with the filter F60S (F60S: 0.18 mmol/min, FX50: 0.17 mmol/min) but its range overlapped with the filter FX50 (FX50: 0.13 - 0.19 mmol/min; F60S: 0.15-0.22 mmol/min), emphasizing the interindividual variability.

3.2.4.4 Correlation between the measured and the calculated flux of calcium

A predicted loss of calcium calculated using equation 77 (chapter 2.3.2.14), was compared with the measured calcium flux.

In the first treatment hour there was a strong, statistically significant correlation between these two values, (Pearson: $r = 0.803$; $p<0.001$) see figure 12. The predicted flux on average exceeded the measured flux, see figure 13. The mean difference between the values in the first HD hour was 0.1 mmol/min. Only in one patient the measured flux was slightly higher than the predicted one and differed by 0.009 mmol/min. This patient had the lowest protein concentration (4.3 g/dl) which was associated with the highest fraction of diffusible to total calcium, 84%. All other participants had protein concentration over 5.0 g/dl.

In the third hour of HD the correlation was weaker but still statistically significant: $r = 0.788$, $p<0.001$. In the fourth treatment hour the significance was lost but the power of the performed test (0.307) was also below the desired power of 0.800.

As expected, a strong and statistically significant correlation was also found between the loss of calcium and the product of the clearance of calcium (citrate-calcium), K, and the total calcium concentration, Ca_{tot}, expressed in mmol/ml: $r = 0.832$, $p <0.001$. The linear regression yielded the equation, which was strikingly similar to the theoretically developed equation 77:

$Ca_{loss} = -0.0170 + (0.831 * Ca_{tot} * K)$

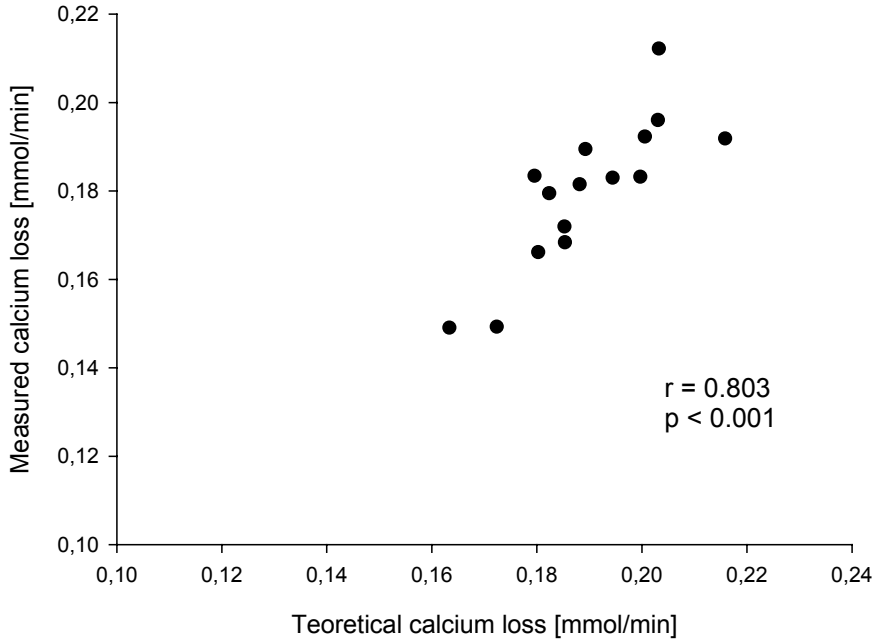

Figure 12 Correlation between theoretically calculated loss of calcium into the dialysate and the flux of calcium measured in the first hour of the HD treatment.

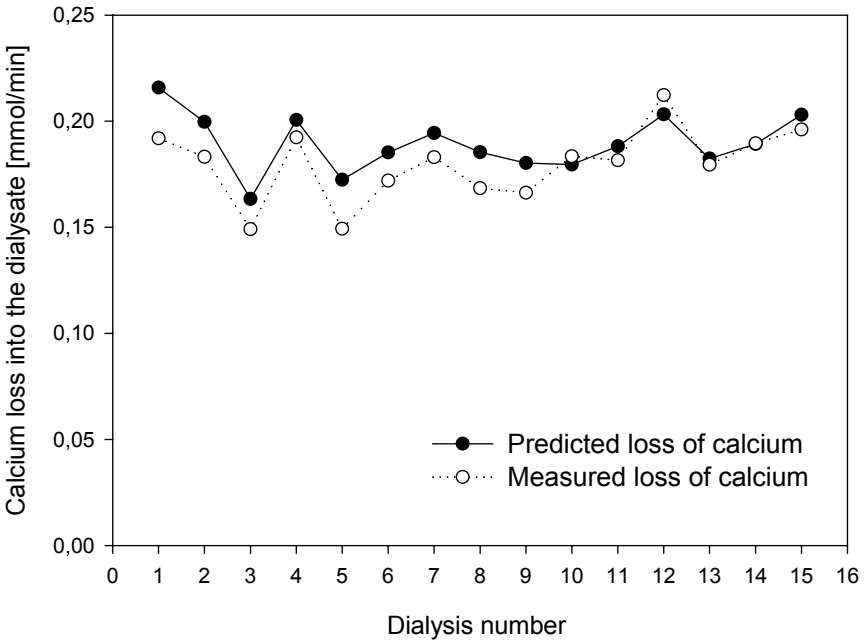

Figure 13 Calcium loss [mmol/min] predicted from the calculations and measured in the first hour of haemodialysis.

3.2.4.5 Net balance of calcium

Calcium loss during four hours of dialysis treatment amounted on average to 43 ± 4 mmol. It was counterbalanced by calcium supplementation equal to 48 ± 1 mmol. This led to a positive net balance of total calcium during the majority of the HD treatments (13 out of 15 HD sessions) with a mean value of: 5 mmol/per HD, range: –1 to 17 mmol/per HD.

Positive net balance of calcium did not correlate with increases of total calcium concentration (Pearson: r=0.383, p=0.159), see figure 14, or of ionized calcium concentration (Pearson: r=-0.114, p=0.685. In contrast, modest negative calcium balance observed in two patients was associated with a small post-dialytic decrease in total calcium level, and in one patient also in a lower post-dialysis ionised calcium level.

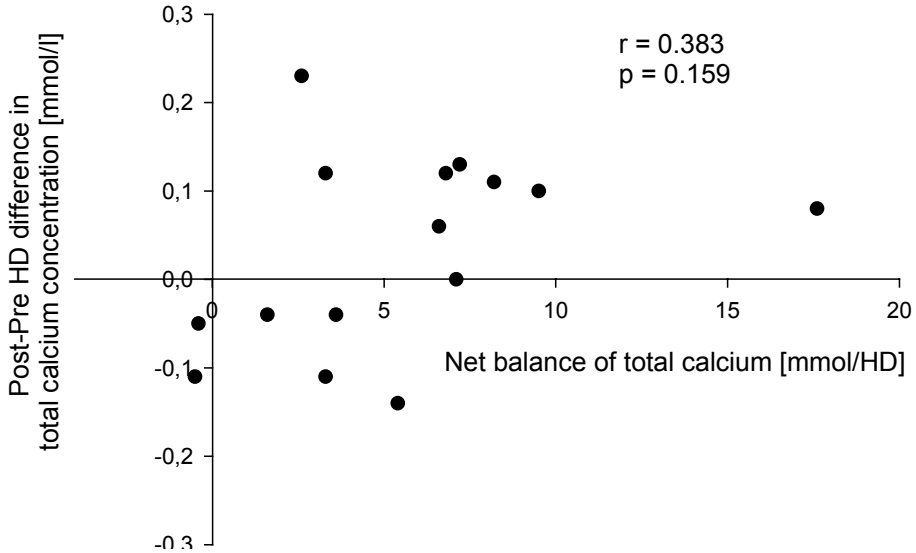

Figure 14 Correlation between the net balance of total calcium [mmol/HD] and the difference between the pre dialysis and the post dialysis total calcium concentration [mmol/l].

3.2.5 Effects on other electrolytes and acid base status

3.2.5.1 Magnesium

The application of the dialysis solution containing 0.5 mmol/l of magnesium resulted in an asymptomatic decrease in magnesium concentration from 0.9 ± 0.16 mmol/l (pre-HD) to 0.8 ± 0.09 mmol/l (post-HD), p=0.006, see figure 15.

More pronounced decrease concerned six patients with the initial magnesium level over 0.9 mmol/l. In these patients mean magnesium level dropped from 1.1 mmol/l to 0.9 mmol/l (95% CI=0.1 to 0.2; p=0.002).

Two patients had an initial mild hypermagnesaemia (magnesium level up to 1.28 mmol/l), which was corrected to normal values during the haemodialysis treatment. All other measured values of total magnesium were within normal range (0.7-1.1 mmol/l).

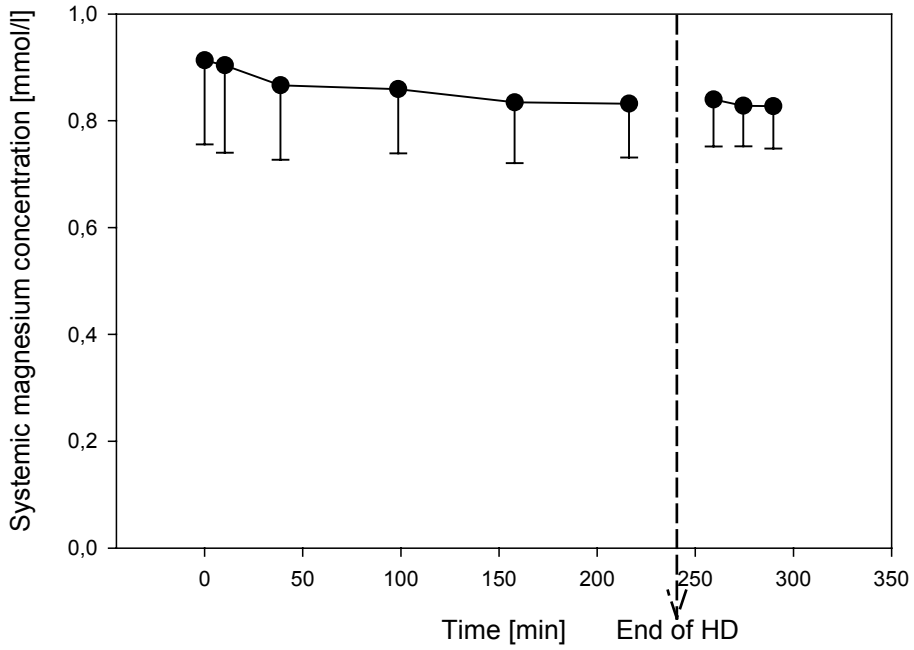

Figure 15 Systemic magnesium concentrations during and after haemodialysis [mmol/l].

3.2.5.2 Sodium

Infusion of sodium citrate bears the risk to induce hypernatremia. In this study, hypernatremia was not observed. Sodium concentration in the systemic blood did not change significantly (pre-HD: 142 ± 4 mmol/l; post-HD: 139 ± 2 mmol/l, p =0.7), see figure 16.

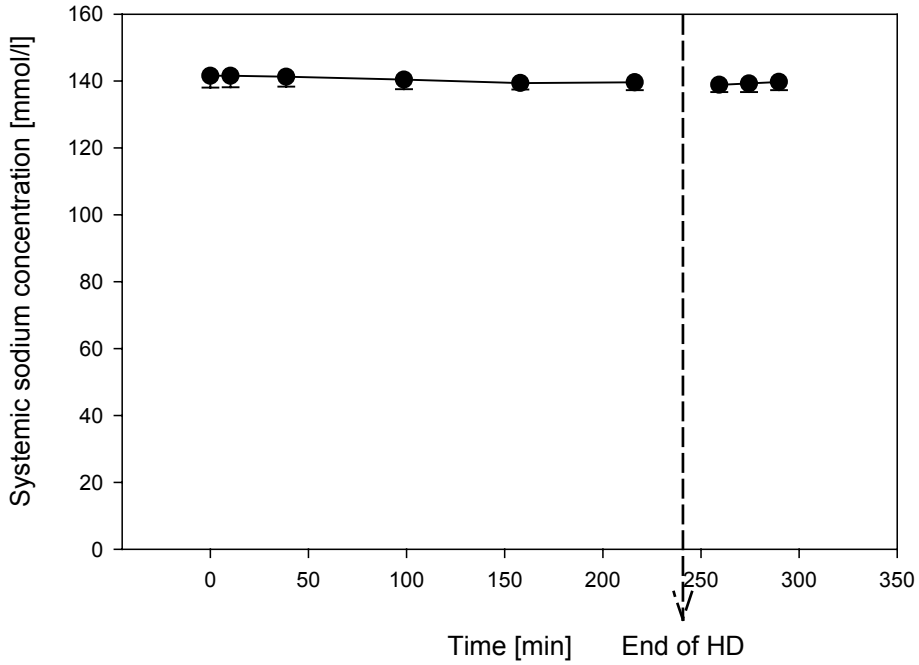

Figure 16 Systemic sodium concentrations during and after haemodialysis [mmol/l].

3.2.5.3 Chloride

Systemic chloride concentration remained almost unchanged during and after citrate anticoagulation (mean pre-HD: 100 mmol/l, mean post-HD: 101 mmol/l), see figure 17.

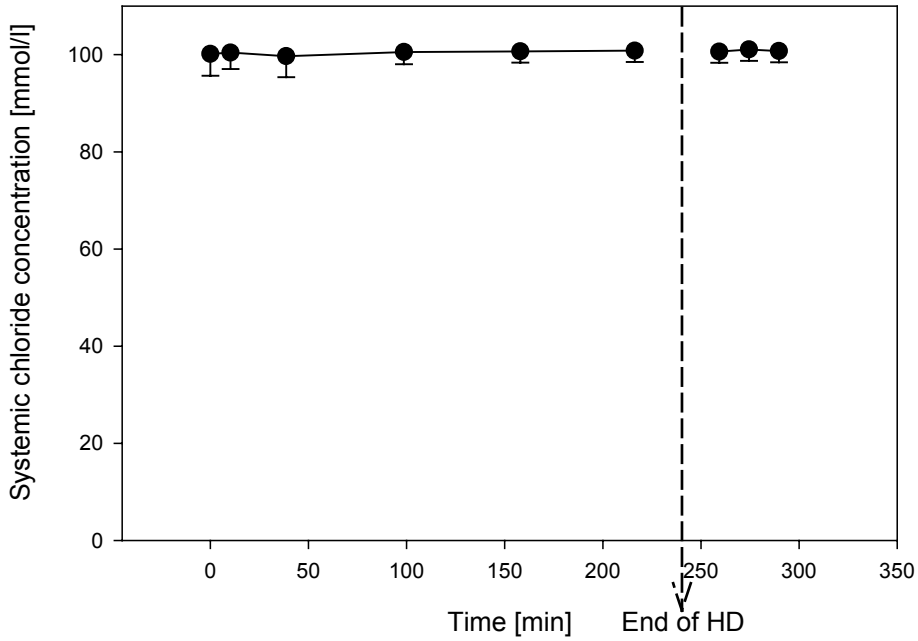

Figure 17 Systemic chloride concentrations during and after haemodialysis [mmol/l].

3.2.5.4 Acid-base parameters

No episodes of clinically relevant alkalosis or acidosis complicated the course of the study.

Because of the differences in the acid-base parameters of the arterial and the venous blood, these parameters were evaluated separately for patients with different vascular access type (fistula or catheter). Bicarbonate concentrations and pH increased during the treatment as typical for bicarbonate haemodialysis (av-fistula patients: mean pH from 7.41 to 7.46, mean bicarbonate concentration from 21 mmol/l to 24 mmol/l; catheter patients: mean pH from 7.41 to 7.43, mean bicarbonate from 25 mmol/l to 26 mmol/l).

3.3 Modelling

3.3.1.1 Initial input data for modelling

If not indicated otherwise all simulations are performed using initial parameters chosen based on the average data from the clinical trial as follows:

body weight: 72 kg

baseline citrate concentration: 0.12 mmol/l

hematocrit: 0.3

protein concentration: 6 g/dl

initial ionized calcium concentration: 1.1 mmol/l

initial total calcium concentration: 2.17 mmol/l

metabolic rate constant: 0.0145 min^{-1}

citrate infusion rate adjusted to achieve 3.33 mmol of citrate per 1 litre of whole blood

calcium infusion rate: 0.2 mmol/min

blood flow: 183 ml/min

plasma water clearance of citrate and citrate-calcium complex: 98 ml/min

clearance of ionized calcium calculated using equation 53 (KoA=603 ml/min from internal Fresenius Medical Care data)

distribution volume of citrate: 54% of the body weight

distribution volume of ionized calcium: 21% of the body weight {Guyton, 1984 765 /id}

net ultrafiltration rate: 6 ml/min

3.3.2 Citrate modelling

3.3.2.1 Systemic citrate concentration calculated by the model

In this chapter it is described, how the laboratory parameters of every patient (Hct, protein concentration, initial citrate concentration, citrate clearance) and the treatment settings (blood and dialysate flow, citrate and calcium flow) were implemented into the model separately. For every patient the plasma concentrations of citrate calculated by the model were compared with the measured citrate concentrations. The curve of the time-dependent increase in citrate concentration calculated by the model had a similar shape as those observed during the patient study (see example at figure 18). However, citrate concentration calculated by the one-compartmental model consistently underestimates citrate concentration in the first hour of the HD treatment.

3.3.2.2 Apparent volume of distribution of citrate

At the last treatment hour the mean apparent volume of distribution of citrate was equal to 54 ± 18% of the body weight (40 ± 22 litres).

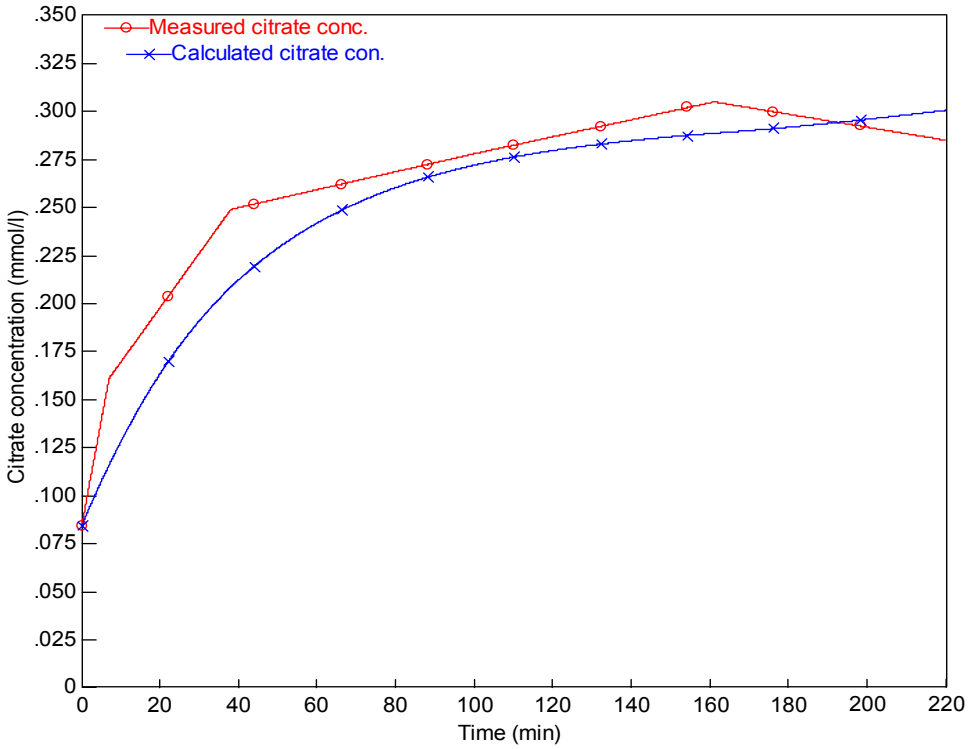

Figure 18 Citrate concentration [mmol/l] measured during haemodialysis (red line with open circules) and calculated by the model (blue line with crosses).

3.3.2.3 Influence of ultrafiltration on systemic citrate concentration

In agreement with the measured data, citrate concentration calculated by the model increased steadily during the HD treatment.

According to first order kinetics, the metabolic removal of citrate increases proportionally to the systemic citrate concentration. Thus, theoretically, a plateau phase in systemic citrate concentration should be detected, when the metabolic removal reaches the rate of citrate delivery to the body. However, initially a plateau phase was not observed even when long-lasting (10 h) dialysis sessions were simulated. Instead of reaching a steady state, citrate concentration showed a slow but steady increase over time. Surprisingly, this increase in citrate concentration turned out to be dependent on ultrafiltration rate and the resulting reduction in distribution volume. Only when a constant distribution volume for citrate was assumed, a plateau phase in citrate concentration was observed in the model, see figure 19.

3.3.2.4 Influence of the metabolic rate constant on systemic citrate concentration

As expected, metabolic rate constant determines the maximum of systemic citrate concentration. It also influences the time point, at which a steady state of systemic citrate concentration (in case of a constant distribution volume) or alternatively a phase of slow increase in citrate concentration (due to decreasing of distribution volume), is reached. Higher metabolic rate constant results in a lower maximum concentration of citrate which is, in addition, reached faster, see figure 20.

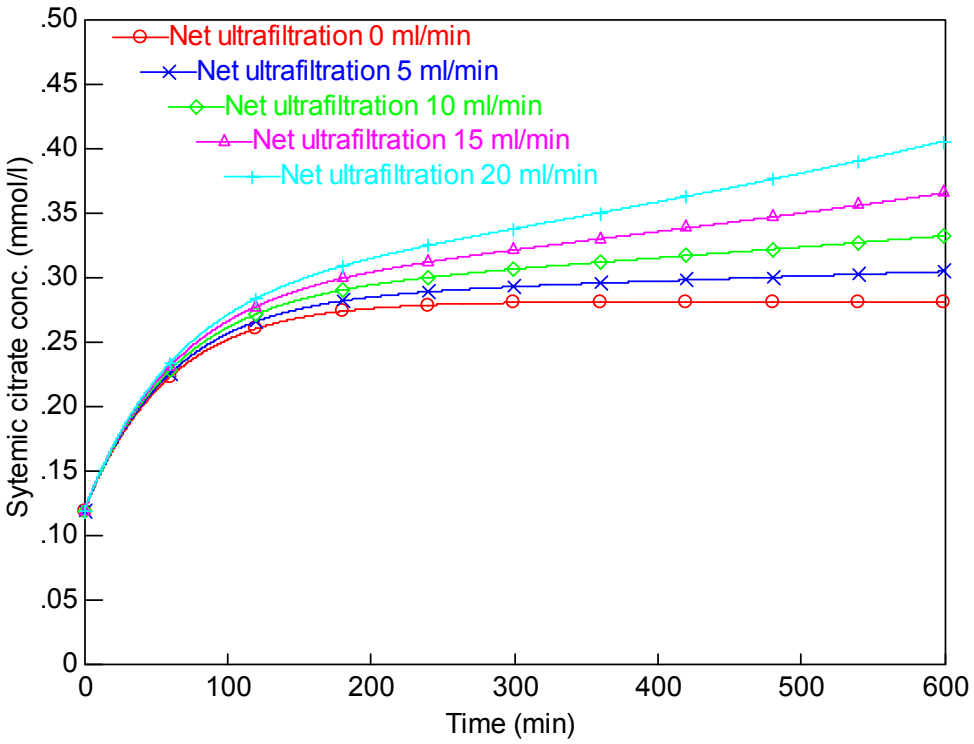

Figure 19 Increases in the ultrafiltration rate from 5 to 25 ml/min result in the parallel increases in systemic citrate concentration due to continuously decreasing volume of distribution. The plateau in systemic citrate concentration is observed only when net ultrafiltration rate is equal to 0 ml/min.

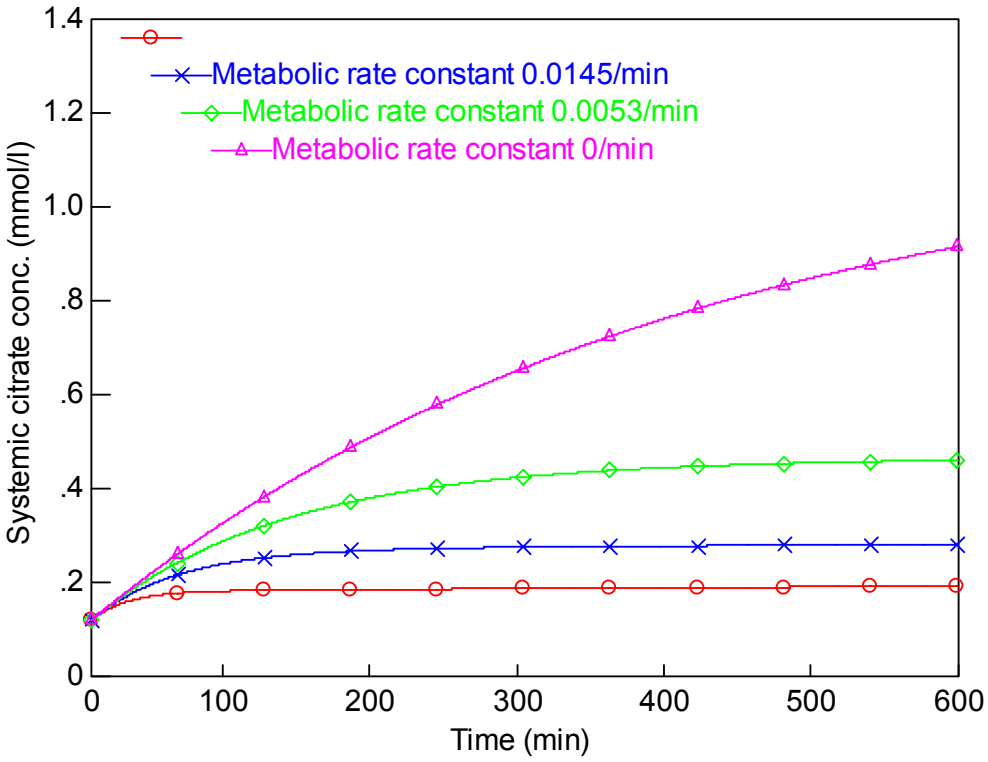

Figure 20 Influence of the metabolic rate constant on the systemic citrate concentration during high-flux dialysis. The concentration of citrate is calculated using the max, mean and min metabolic rate constant from the patient study (0.0382/min 0.0145/min and 0.0053/min, respectively) and 0/min metabolic rate constant as in complete liver failure.

3.3.2.5 Influence of the citrate infusion rate on systemic citrate concentration

The dose of citrate reaching the systemic circulation of a given patient is related to the amount of citrate infused into the arterial line of the extracorporeal circuit, assuming use of the same dialyzer, blood flow and dialysate flow. In the majority of published protocols the amount of citrate infused ranges from 2.5 to 7.5 mmol of citrate per litre of whole blood. According to the modelling 4 hours of dialysis using 7.5 mmol of citrate / litre of whole blood increases systemic citrate concentration by 0.44 mmol/l whereas the use of 2.5 mmol of citrate / 1 litre of whole blood increases plasma levels by 0.12 (blood flow = 183 ml/min), see figure 21.

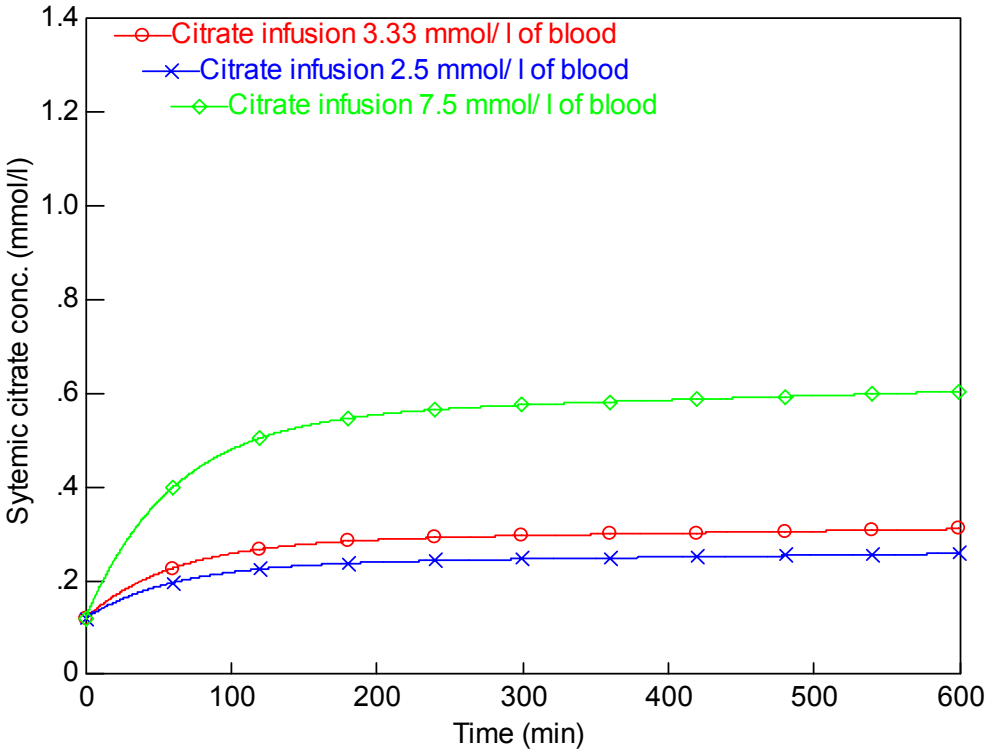

Figure 21 Influence of the citrate dose infused into extracorporeal circuit on the systemic citrate concentration during high-flux dialysis.

3.3.2.6 Influence of citrate clearance on systemic citrate concentration

The results of the modelling demonstrate that systemic citrate concentration depends more on dialyzer performance than on the efficiency of the body metabolism, see figure 22.

Lower citrate clearances in chronic haemodialysis are associated with distinctly higher systemic citrate concentrations, see figure 23. Systemic citrate concentration after four hours of high-flux haemodialysis (citrate clearance 98 ml/min using a blood flow of 183 ml/min) is expected to rise by about 0.2 mmol/l. In these settings a decrease in filter performance only of about 10 ml/min leads to an increase in systemic citrate concentration in the range of 0.1 mmol/l.

As calculated by the model, four hours of the slow continuous renal therapies using lower blood flow (150 ml/min) and lower clearances (25 ml/min), results in a concentration of citrate threefold higher in comparison to high-flux haemodialysis (0.75 – 1.75 mmol/l), see figure 24.

According to the model, the application of 4 hours of high-flux haemodialysis in patients with complete lack of citrate metabolism (metabolic rate constant equal to 0 min^{-1}) would probably not induce systemic citrate concentration higher than 1.0 mmol/l due to the efficient removal of citrate into the dialysate, figure 22. In contrast slow renal therapy in a patient with a low normal metabolism (min value from the clinical trial 0.0053 min^{-1}) could generate markedly higher citrate concentration (1-2 mmol/l) during a treatment lasting 10 hours, figure 24.

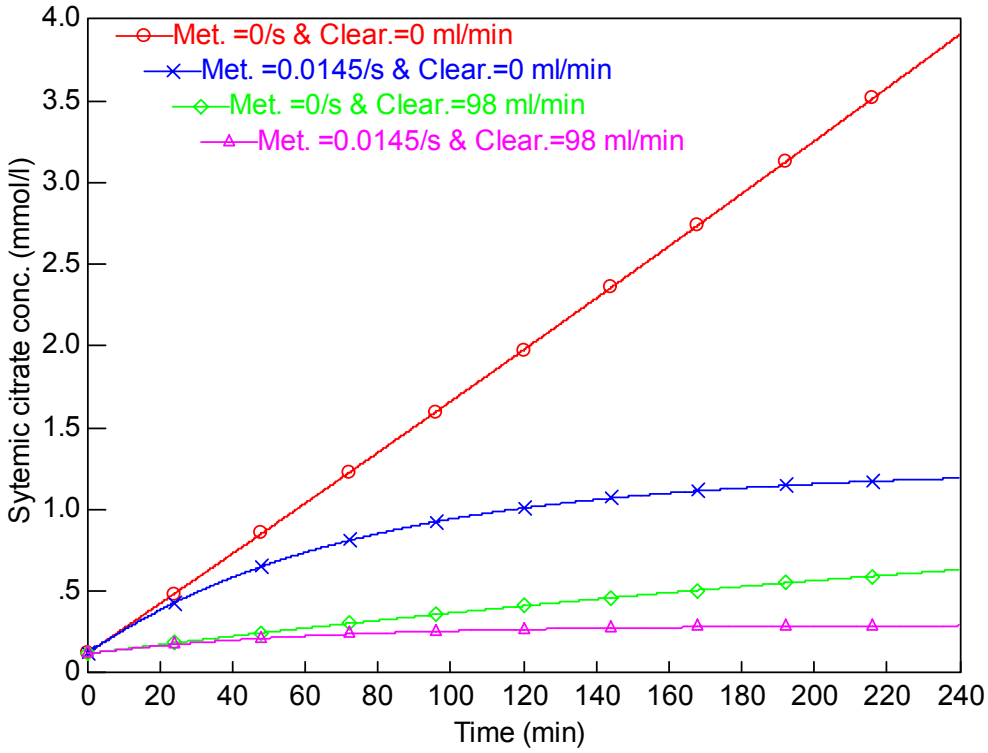

Figure 22 Effect of the metabolic and the dialyzer elimination on the systemic citrate concentration.

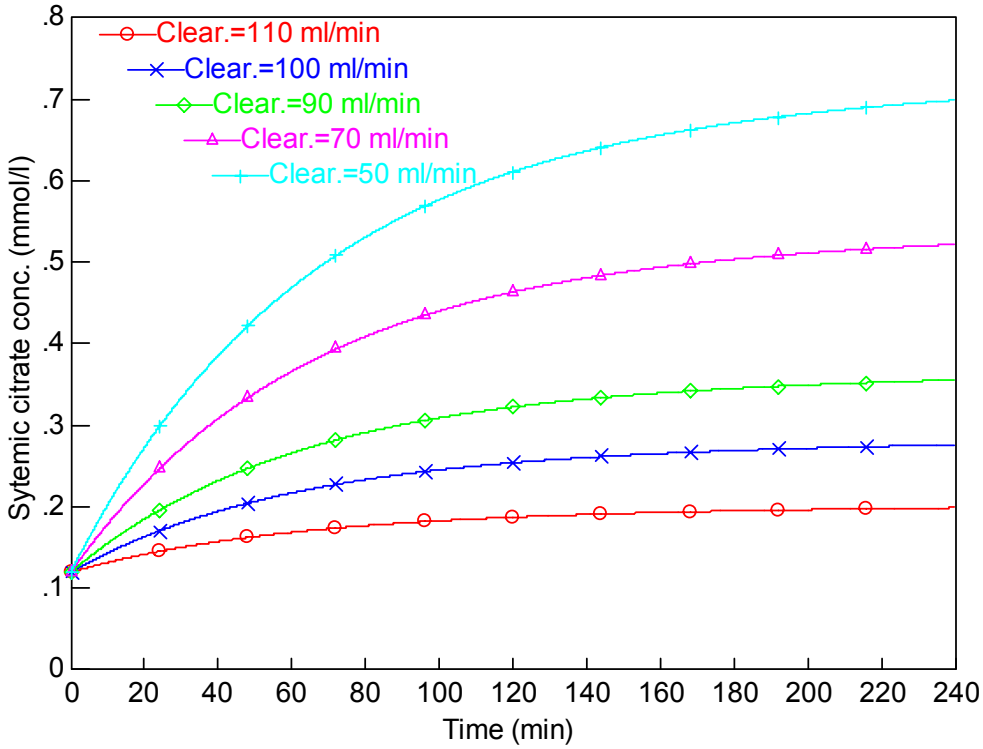

Figure 23 Effect of dialyzer clearance on the systemic citrate concentration during 4 hours long high-flux haemodialysis, blood flow: 183 ml/min.

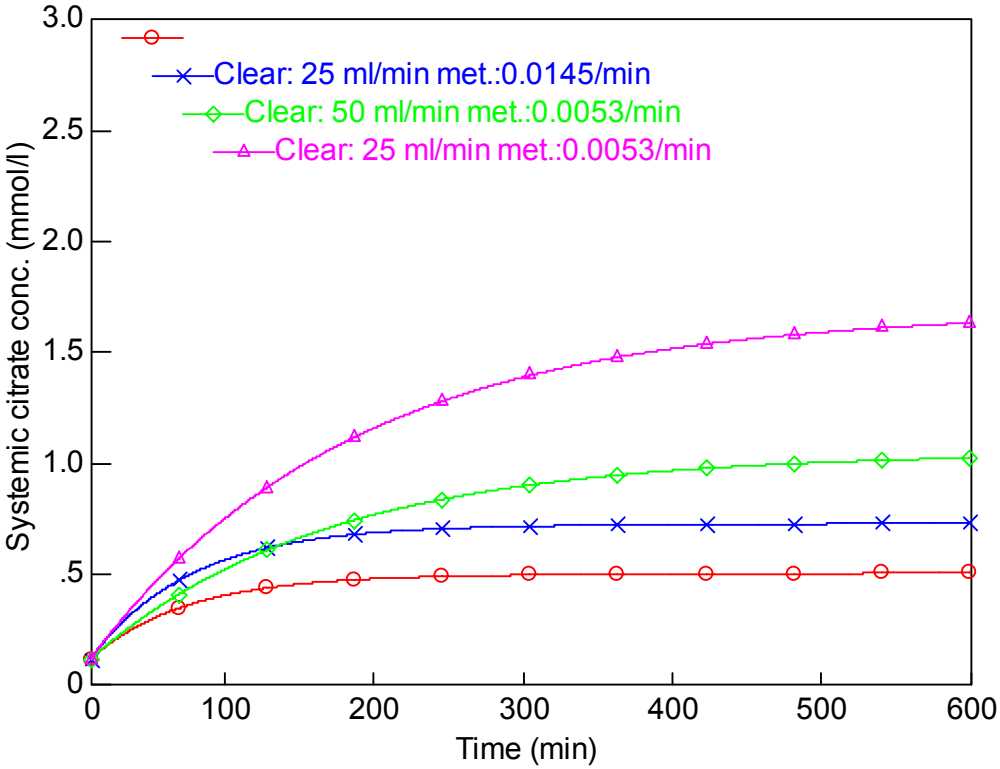

Figure 24 Expected effect of the dialyzer clearance and the metabolic elimination of citrate (mean: 0.0053/min and min: 0.0145/min from the patient study) on the systemic citrate concentrations during slow continuous renal therapies, blood flow: 150 ml/min.

3.3.2.7 Influence of the body weight & distribution volume on systemic citrate concentration

The volume of distribution is closely linked to the body weight. Both these parameters influence the systemic citrate concentration, see figures 25 and 26. Assuming a distribution volume equal to 54% of body weight the difference in citrate concentration in patients weighing 70 kg and 40 kg is around 0.1 mmol/l, increasing the baseline citrate concentration level from 0.1 to 0.3 mmol/l or 0.4 mmol/l, respectively.

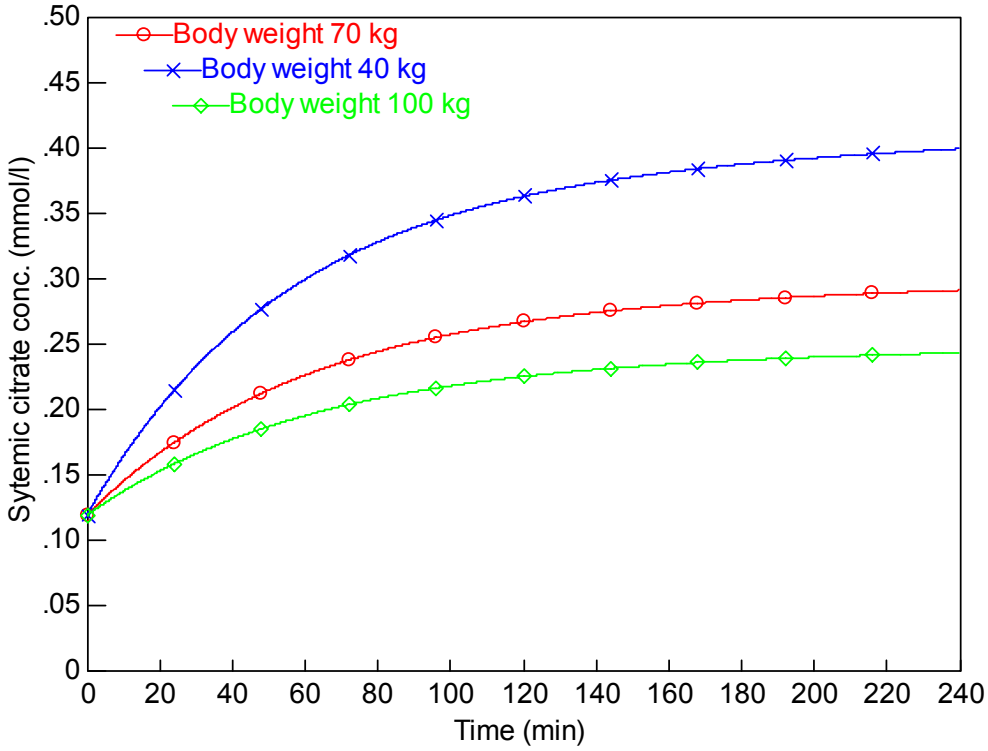

Figure 25 Effect of the body weight on systemic citrate concentration during high-flux haemodialysis.

However, theoretically this difference may increase further if the distribution volume in a small patient is additionally reduced due to the impairment of the blood circulation and the exclusion of citrate from deep compartments, see figure 26.

Continuous renal therapies (blood flow 150ml/min, citrate clearance 25ml/min) in a small patient may induce dangerously high systemic citrate concentration (over 2mmol/l) in 6 hours of the treatment if the distribution volume is equal to the data reported for critically ill patients (34% of the body weight, mean distribution volume =23l for the mean body weight of 70kg), see figure 27.

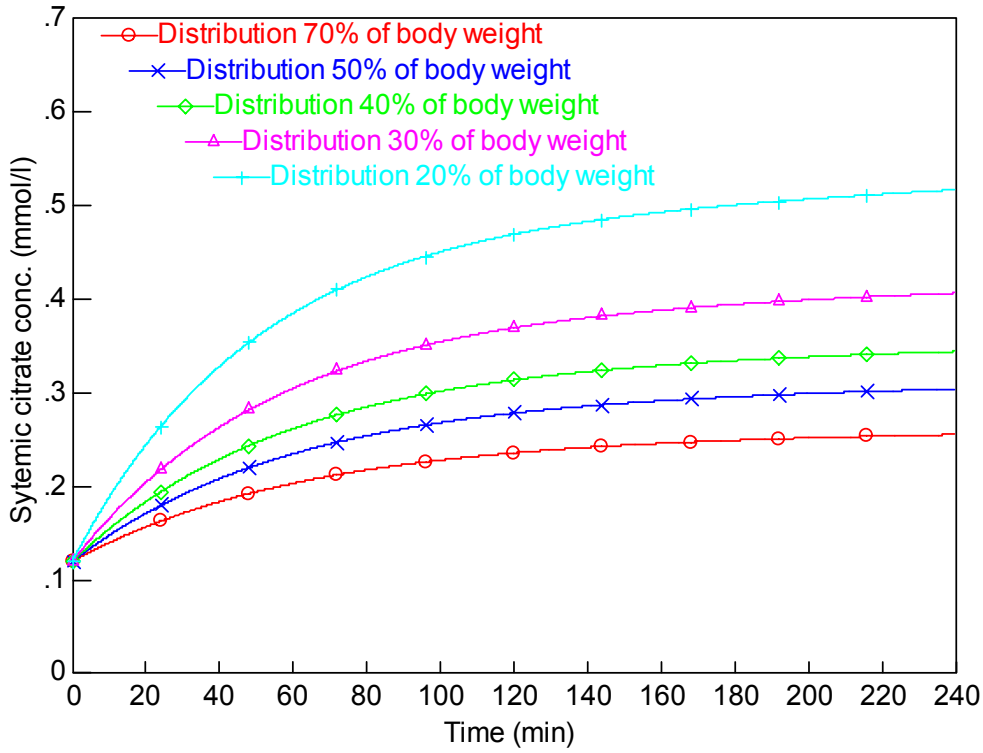

Figure 26 Effect of the distribution of citrate on the systemic citrate concentration during high-flux haemodialysis.

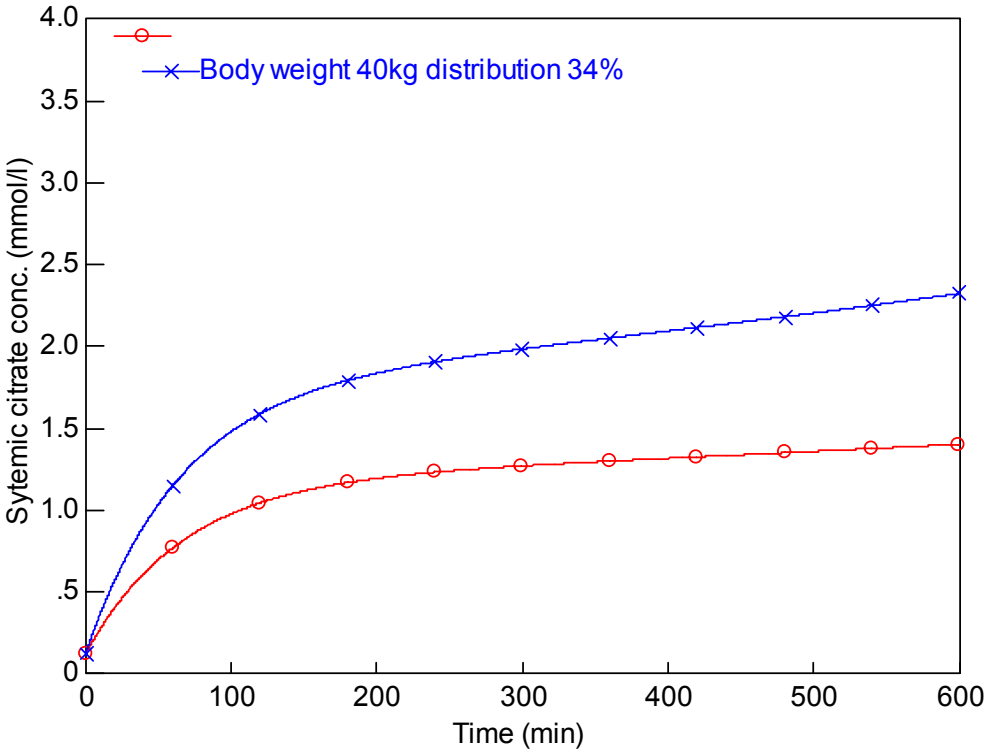

Figure 27 Minor distribution volume of citrate (distribution volume: approx. 34% of the body weight according to the data on the critically ill patients (Kramer et al. 2003) in a patient with small body weight (40kg) could cause very high systemic citrate concentration during 10 hours of slow dialysis treatment, blood flow: 150ml/min, clearance: 25ml/min as in CVVHD.

3.3.2.8 Influence of the blood flow on systemic citrate concentration

When citrate solution flow rate is fixed to the blood flow rate, a higher blood flow carries a higher load of the citrate to the body of the patient, which translates into higher systemic citrate concentration. Based on the model, a blood flow of 300 ml/min over 4 hours of high-flux haemodialysis doubles the systemic citrate concentration in comparison to a blood flow of 200 ml/min reaching 0.6 and 0.3 mmol/l, respectively (figure 28).

Clearance used in this simulation was calculated using the equation 53 (chapter 2.2.2.2) and KoA measured in vitro for F60S (KoA = 337 ml/min) and then reduced by 10% to account for the difference between the in vitro and in vivo measurement of clearance.

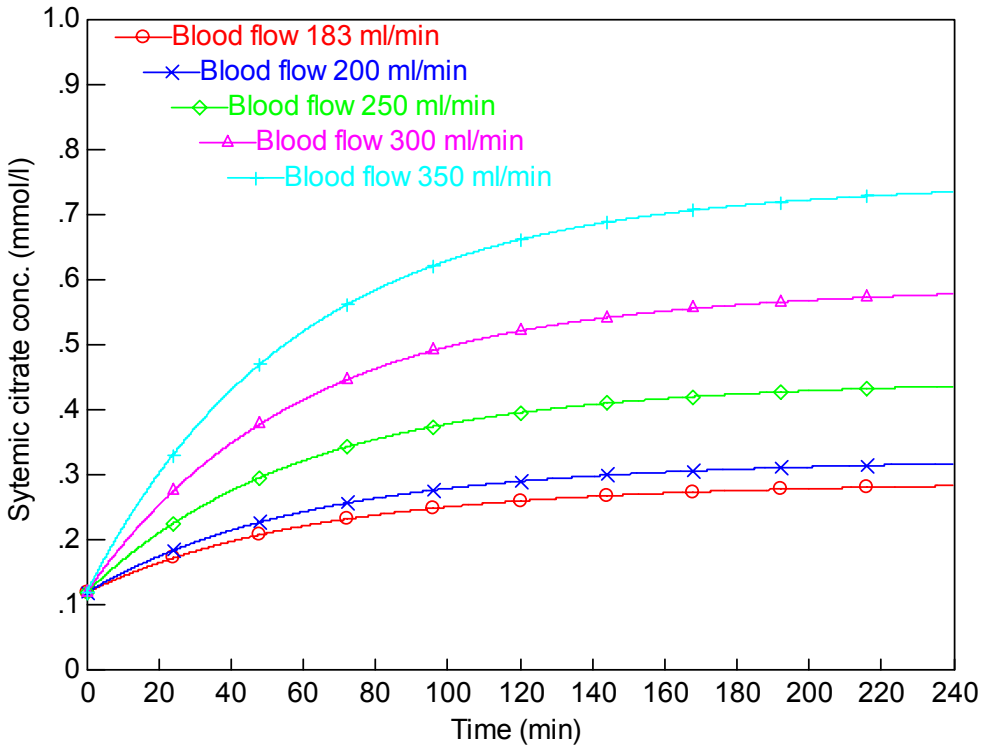

Figure 28 During high-flux haemodialysis an increase in the citrate infusion rate proportional to the increase in the blood flow results in the rise in the systemic citrate concentration, since diffusive removal into the dialysate increases less than the citrate infusion rate.

3.3.2.9 Disposition of the infused citrate

Using the results of the patient study it was possible to calculate the mass removal of citrate into the dialysate (83%, see chapter 3.2.3.2). However, the evaluation of the disposition of the total amount of the infused citrate was not possible. Therefore, the disposition of the infused citrate was estimated using modelling, as is shown in figure 29. According to the modelling approx. 80 % of citrate infused into the arterial line of the extracorporeal circuit was immediately removed into the dialysate and never reached the systemic circulation of the patient, which is slightly smaller

than calculated previously citrate mass removal (83%). This discrepancy results probably from the observed in the modelling underestimation of citrate concentration in the first two hours of the treatment and consequently smaller removal of citrate into the dialysate.

As calculated by the model, an additional 3% of the initially infused citrate entered the body of the patient but later was removed into the dialysis solution when the blood was again circulating through the dialyzer. The remaining 17 % of the infused citrate was available for metabolic removal. Most of this (about 11%) was degraded already during the haemodialysis treatment. Only about 6% of the citrate originally infused into the arterial line was present in the body at the end of the haemodialysis session.

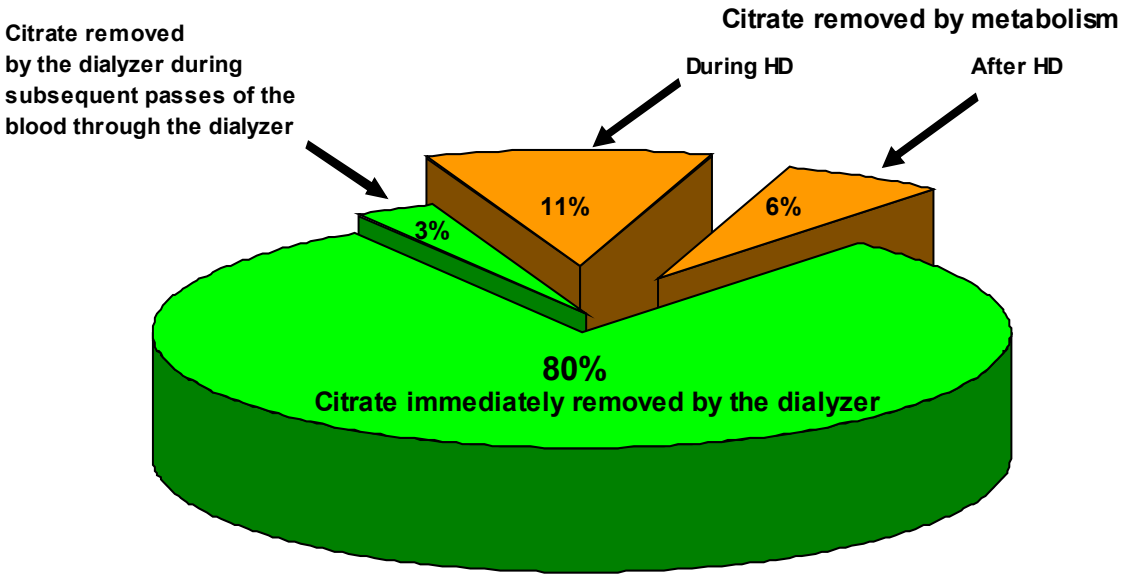

Figure 29 Disposition of the infused citrate during and after high-flux haemodialysis of four hours duration (dialyzers: FX50 and F60S).

3.3.3 Calcium modelling: risk of hypocalcaemia

3.3.3.1 Hypothetical dialysis (1): Citrate dialysis without calcium supplementation.

In contrast to apheresis procedures, in haemodialysis settings citrate concentration is not the sole factor that influences the concentration of ionized calcium. In haemodialysis anticoagulated with citrate the use of calcium free dialysis solution removes large amounts of ionized and citrate bound calcium to the effect that the blood that leaves the dialyzer is deprived of a substantial

amount of calcium (the reduction rate of total calcium concentration is approx. 60%). Infusion of citrate solution in pre filter mode induces a shift of ionized calcium from proteins increasing the concentration of diffusible calcium. Therefore during citrate anticoagulated dialysis the loss of calcium into dialysate is higher than during heparin anticoagulated dialysis against calcium free bath. Assuming that calcium cannot be released from intracellular stores, a 4 hours haemodialysis of citrated blood without calcium supplementation would lead to systemic hypocalcaemia (figure 30). However, the development of systemic hypocalcemia can be even more rapid in a patient with small body mass and consequently a small distribution volume.

When transport of calcium from and to the bones is ignored, mathematical calculation suggests that during citrate anticoagulated dialysis without calcium supplementation ionized calcium would decrease to 0.6 mmol/l within 60 minutes if the patient is weighing 45 kg (with hematocrit of 0.3). At this level cardiac symptoms are very frequent.

Thus, patients with small body mass are at higher risk to develop systemic hypocalcaemia in case of malfunction of calcium supplementation.

In this study one treatment was complicated by a technical problem with the substitution of calcium during the first 15 minutes. This lack of calcium delivery for 15 min resulted in a drop of ionized calcium in the arterial line from 1.04 to 0.94 mmol/l and of total calcium from 2.18 to 2.0 mmol/l. The observed decrease in calcium is in perfect agreement with the results of the mathematical model, if calcium release from the bones was ignored.

3.3.3.2 Hypothetical dialysis (2): Dialysate flow in bypass mode

Citrate infused into the arterial line is completely reaching the body of the patient (no removal of citrate or calcium into the dialysate.

In order to compare the influence of a citrate infusion with the effect of calcium removal by the dialyzer on ionized calcium levels a hypothetical “dialysis” without any dialyzer clearance (dialysate bypass mode, no filtration or diffusion of citrate and calcium into the dialysate) was simulated in the mathematical model. Since the total dose of citrate infused into the extracorporeal circuit would enter the systemic circulation of the patient, systemic citrate concentrations in a patient weighing 70 kg (hematocrit=0.3) would increase as shown in figure 31. Despite the chelation of calcium by citrate, the normal calcium supplementation rate used in this study (0.2 mmol/l) would by far exceed the concentration of ionized calcium bound to citrate. The model indicates that an increase in ionized calcium concentration would occur. Therefore, if the dialysis machine switches into bypass mode during a citrate dialysis, this will not result in hypocalcemia.

3.3.3.3 Hypothetical dialysis (3): Complete lack of metabolic elimination.

A complete lack of citrate metabolic elimination is unlikely in a stable haemodialysis patient without identified liver failure. However, even under this extreme condition, citrate levels will stay relatively low, since citrate is efficiently removed by the dialyzer and serious disturbances in ionized calcium level are not to be expected, see figure 32

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3.3.3.4 Hypothetical dialysis (4): Bypass mode and lack of calcium supplementation

Complete dose of citrate infused into the arterial line enters the body of the patient (no dialysate flow) but in addition lack of calcium supplementation occurs.

In this case citrate concentration will increase up to approximately 1.1 mmol/l and would be expected to reduce ionized calcium to approx. 0.8 mmol/l, see figure 33. This influence of a citrate concentration of 1.1 mmol/l on the ionized calcium level in the blood predicted by the model is consistent with empirical data (Bolan et al. 2001; Bolan et al. 2002) correlating the relationship between the concentration of citrate and ionized calcium.

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3.3.3.5 Hypothetical dialysis (5): Bypass mode and lack of citrate metabolism

Although the coincidence of the lack of the filter and the metabolic removal of citrate can be associated with very high systemic citrate concentrations the infusion of calcium at the average rate of 0.2mmol/min into the venous line can readily maintain ionized calcium concentration, as shown in figure 34. In this clinical situation systemic citrate concentration increases significantly but the lack of the filter removal of calcium allows to maintain normal ionized concentration if the typical calcium supplementation is continued.

3.3.3.6 Hypothetical dialysis (6): Bypass mode, lack of metabolic elimination and lack of calcium supplementation

Only the coincidence of an ineffective calcium supplementation (e.g. calcium pump failure) with lack of citrate elimination by dialysis and metabolism would result in critically lowered ionized calcium values, see figure 35. However, such conditions although theoretically possible are rather unlikely to occur in practice. Ionized calcium in such a case would be expected to drop to life threatening values (0.6 mmol/l) in around 140 minutes. The time to achieve this hazardous level of ionized calcium is longer compared to an isolated stop of the calcium pump, because calcium is not removed by dialysis.

3.3.3.7 Hypothetical dialysis (7): Uncontrolled delivery of citrate into the arterial line (citrate pump failure).

Another risk for the development of hypocalcaemia would be the uncontrolled rapid infusion of citrate. This can hypothetically occur if the pump segment of the infusion tubing for citrate protrudes out of its roller pump. A case of such a citrate pump failure has been reported during an

aphaeresis procedure and was associated with cardiac manifestations of hypocalcaemia after 15 minutes of treatment. The reported ionized calcium level was 0.64 mmol/l (Uhl et al. 1997). Model simulation suggests that the citrate dose described in this report would be associated with a systemic citrate concentration of 2.5 mmol/l after 15 minutes, which is likely to induce the reported ionized calcium level. However, in haemodialysis settings most of the infused citrate is removed into dialysate. In order to parallel the situation of aphaeresis the coincidence of a concomitant bypass mode of the dialysate and the simultaneous lack of calcium supplementation would be necessary. Such a coincidence, although theoretically possible, is very improbable to occur in reality.

Thus, the failure of calcium supplementation appears to represent the most probable risk for the rapid development of ionized hypocalcaemia during citrate anticoagulated haemodialysis, see table 6.

Hypothetical dialysis		**Time to reach Ca^{+2} of 0.6 mmol/l**	
Number/ Figure	*Malfunction of the system*	*Patient weighing 72kg*	*Patient weighing 45kg*
1/ 30	No calcium infusion (Calcium pump stop)	110 min	70 min
2/ 31	Dialysate flow in bypass mode	Ionized calcium may increase	Ionized calcium may increase
3/ 32	Lack of citrate metabolism (Complete liver failure)	Ionized calcium not lower than 1.0 mmol/l	Ionized calcium not lower than 0.9 mmol/l
4/ 33	Dialysate flow in bypass mode and no calcium infusion	Ionized calcium not lower than 0.8 mmol/l	Ionized calcium not lower than 0.7 mmol/l
5/ 34	Dialysate flow in bypass mode and lack of citrate metabolism	Ionized calcium concentration may increase	Ionized calcium concentration may increase
6/ 35	Dialysate flow in bypass mode, lack of calcium infusion and lack of citrate metabolism	150 min	100 min

Table 6 Time (in minutes) required to reach a life threatening decrease of ionized calcium from 1.1 mmol/l to 0.6 mmol/l during intermittent, high-flux dialysis (dialyzers FX50, F60S) using blood flow: 183 ml/min, dialysate flow: 500 ml/min, and citrate dose: 3.33 mmol/ per litre of whole blood in the extracorporeal circuit.

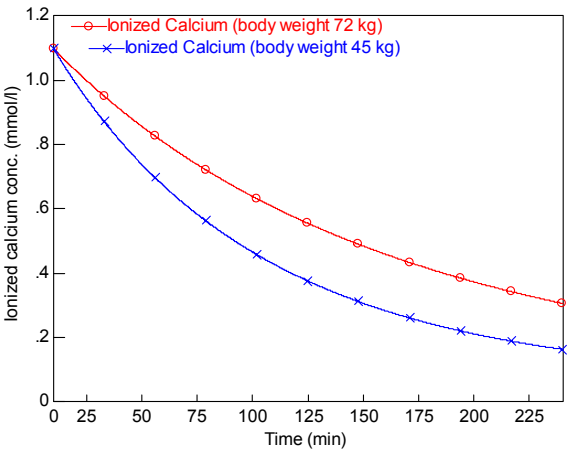

Figure 30 Lack of calcium supplementation decreases systemic ionized calcium concentration more rapidly in patient with smaller body mass (45kg).

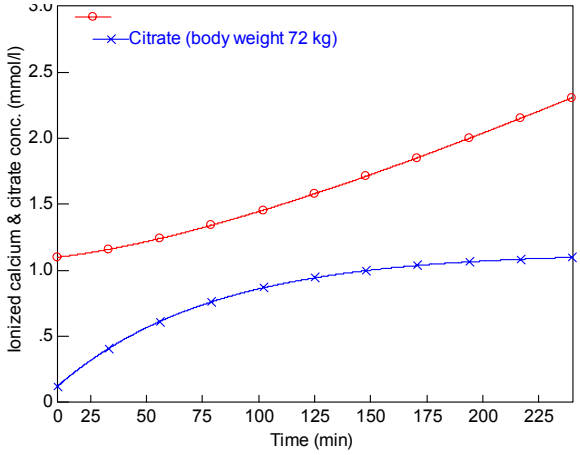

Figure 31 Bypass mode (no removal of citrate and calcium into the dialysate) increases systemic citrate concentration but the calcium infusion rate of 0.2 mmol/min exceeds the concentration of citrate-calcium complexes leading to a hypothetical increase in ionized calcium concentration

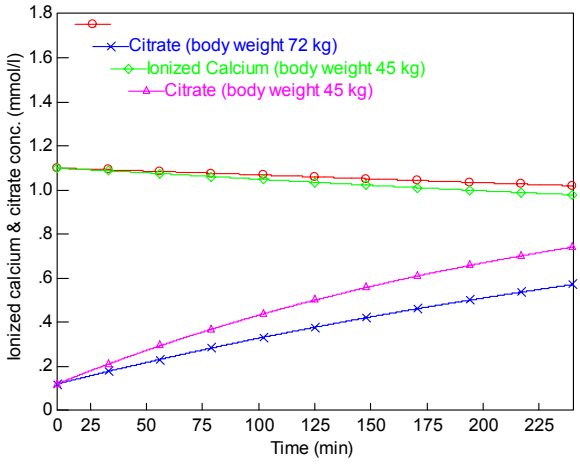

Figure 32 Complete lack of citrate metabolism increases systemic citrate concentration but ionized calcium remains at a safe level (not lower than 0.9 mmol/l if initial concentration of calcium was at least 1.1 mmol/l and the weight of patient was not below 45 kg).

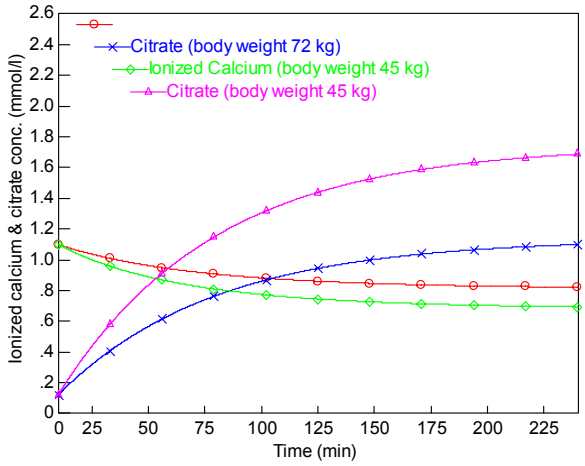

Figure 33 Bypass mode (no removal of citrate and calcium into the dialysate) and lack of calcium supplementation decreases ionized calcium concentration to 0.7-0.8 mmol/l in 4 hours of treatment.

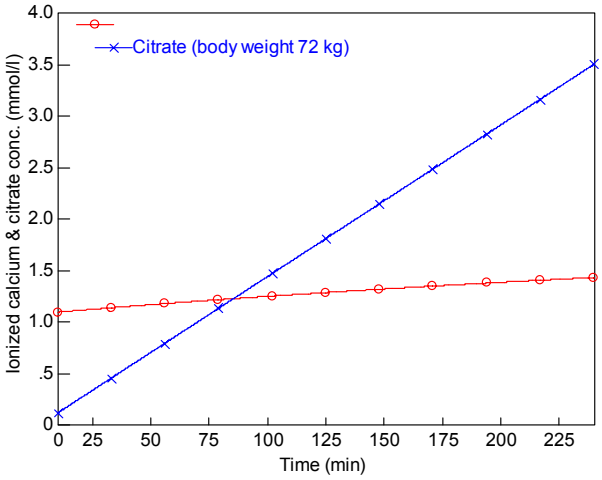

Figure 34 Bypass mode (no removal of citrate and calcium into the dialysate) and absence of citrate metabolism produce high systemic citrate concentrations. Ionized calcium concentration is not affected if calcium supplementation is continued.

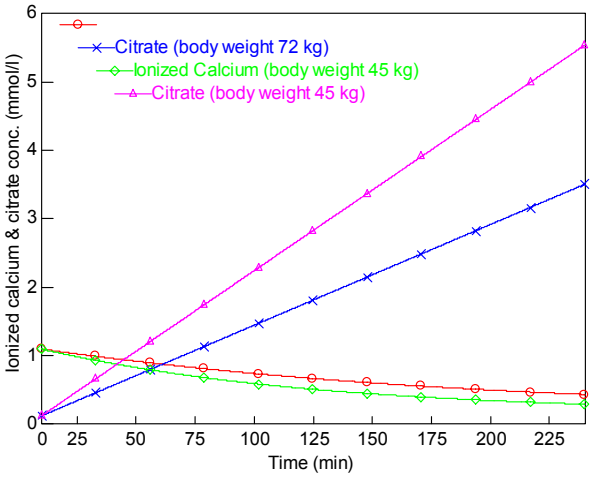

Figure 35 Bypass mode (no removal of citrate and calcium into the dialysate) combined with lack of metabolism of citrate and lack of calcium supplementation (calcium pump failure). Ionized

calcium is lowered to dangerous levels (0.6 mmol/l) within 100-150min of treatment.

4 Discussion

The most controversial issues in the use of regional citrate anticoagulation are the prescription of the doses for citrate and calcium as well as the necessity of ionized calcium monitoring. In particular, the potential toxicity of citrate and the safety of this method require solid data. The study presented here is addressing these issues by analyzing pharmacokinetic parameters of regional citrate anticoagulation. The data from this investigation may help to use this effective anticoagulation method more safely.

4.1 Efficacy of citrate anticoagulation

4.1.1 Empirical concepts of citrate dose and anticoagulation monitoring

For the achievement of satisfactory anticoagulation the dose of citrate infused into the extracorporeal circuit and its effect on ionized calcium is the most important parameter. Several approaches have been used to assure sufficient extracorporeal anticoagulation:

- monitoring of clotting parameters
- monitoring of ionized calcium
- no monitoring of the anticoagulation effect
- fixed ratio of citrate to blood flow

The relationship between the citrate concentration and the prolongation of whole blood clotting time were measured in vitro by Pinnick et al. in 1983 and the results of these experiments became the basis to guide the dose of citrate for the next years (Pinnick et al. 1983). Later on, after the devices to measure ionized calcium were introduced, the level of ionized-calcium inside the extracorporeal circuit was more commonly used to monitor the antithrombotic effect of citrate. Led by experience the dose of citrate was calibrated to induce ionized calcium level not higher than 0.1 to 0.5 mmol/l in the extracorporeal circuit. Most authors recommend monitoring of antithrombic effect of citrate, both in intermittent and in continuous therapies. However, in order to reduce the complexity of citrate anticoagulation there were also attempts to abandon monitoring of the citrate effect. Intermittent haemodialysis treatments without anticoagulation monitoring were conducted successfully, premature termination of the treatment due to system clotting did not occur (Apsner et al. 2001; Janssen et al. 1993). During continuous renal replacement therapies a satisfactory anticoagulation without monitoring is more difficult to obtain. Palsson et al. varied the dose of citrate depending on the ultrafiltration requirement of the patient without monitoring the antithrombotic effect of citrate. This approach resulted in a substantially shorter filter life time

(29,5 h) in comparison to the protocols that included the monitoring of anticoagulation, i.e. measurement of the clotting tests or ionzed calcium level, (44 to 82 h) (Bunchman et al. 2003; Chadha et al. 2002; Cointault et al. 2004; Dorval et al. 2003; Gupta et al. 2004; Hofmann et al. 2002; Kutsogiannis et al. 2000; Mehta et al. 1990; Mehta et al. 1991; Monchi et al. 2003). The use of a constant ratio of citrate to the blood flow represents an alternative to the anticoagulation monitoring and proved to be effective both in the intermittent and in the continuous settings (Janssen et al. 1993; Thoenen et al. 2002; van der Meulen et al. 1992). The above discussion, whether to monitor anticoagulation efficacy or not, results in a large part from the insufficient knowledge about the calcium and citrate pharmacokinetics. Data from this study suggest that the anticoagulant response to citrate dose can precisely be predicted, which may obviate the need to monitor ionized calcium levels in the venous line in order to document the low ionized calcium level.

4.1.2 Current study - citrate dose and anticoagulation

In our study, prevention of clotting by the delivery of a constant ratio of citrate to blood flow proved to be effective. The dose of citrate (3.33 mmol/ litre of blood) was chosen on the basis of experiences from the literature (Apsner et al. 2001; Chadha et al. 2002; Hofbauer et al. 1999; Hofmann et al. 2002; Janssen et al. 1993; Janssen et al. 1996; Kutsogiannis et al. 2000; Mehta et al. 1990; Mehta et al. 1991; Meier-Kriesche et al. 2001; Pinnick et al. 1983; von Brecht et al. 1986; Wiegmann et al. 1987). No complete occlusion of the system occurred, but in 5 out of 15 haemodialysis sessions small clots were found in the “arterial” part of the extracorporeal circuit.
Since in the treatments affected post-filter ionized calcium was always within the target range (< 0.2 mmol/l) the observed clots could be a result of an insufficient mixing of the blood and the anticoagulant. It is also possible that the clots were formed at the vascular access site (before the infusion site of citrate) and were then transported to the arterial part of the circuit. It is also well known that the individual predisposition for clotting is highly variable which may explain different requirement for ionised calcium levels to interrupt the clotting cascade sufficiently. The hypothesis that some patients may need a lower target ionised calcium level (e.g. < 0.1 mmol/l) was not investigated in this study.

4.1.3 Kinetic concept of citrate dose and efficient anticoagulation

There is general agreement that the degree of anticoagulation with the use of citrate is only related to citrate concentration and to the level of ionised calcium in the extracorporeal circuit. The current study on citrate and calcium kinetics suggests that a better prediction of the degree of anticoagulation in the extracorporeal circuit would have been obtained, if citrate dose had been adjusted not only to the blood flow but instead to plasma water flow and possibly also to total calcium level at the beginning of the treatment.

4.1.3.1 Hematocrit and protein concentration

We observed a high variability in extracorporeal citrate plasma concentration from 4.9 to 7.2 mmol/l, which resulted from the different levels of hematocrit and of proteins. Considering that citrate does not enter the red blood cells, we can more precisely estimate expected citrate concentration in the extracorporeal circuit. Patients with higher hematocrit and protein concentration developed higher concentration of citrate in the extracorporeal circuit. However, while the effect of hematocrit on plasma water citrate concentration is strong, the influence of proteins is very small and can be neglected.

4.1.3.2 Calcium

Another important parameter to consider for the anticoagulation efficacy is the initial total calcium concentration. The results from this study demonstrate that a substantial amount of protein bound calcium is released from its protein binding sites during citrate anticoagulation in the extracorporeal circuit. Thus, an additional important parameter affecting the efficiency of citrate anticoagulation is the degree of calcium release from proteins, which in turn depends on the initial total calcium level.

Figure 36 shows the relationship between concentration of citrate and ionised calcium in the extracorporeal circuit. The concentrations were calculated using the law of mass action with the assumption that the diffusible calcium concentration is equal to 80% of total calcium concentration. These results are compared with the correlation described from the empirical measurements for ionized calcium and citrate concentrations up to 5.0 mmol/l. Interestingly the linear correlation described by Diaz et al (Diaz et al. 1995) for the citrate concentration up to 5 mmol/l is very close to the logarithmic correlation calculated from the law of mass action. However, for higher concentrations of citrate the linear correlation used by Diaz et al. predicts unrealistic ionized calcium concentrations, even below 0. In contrast to data by Diaz et al. our calculations show that at high concentrations of citrate (>5.5 mmol/l) the concentration of ionized calcium is not further decreased significantly. In addition at high citrate levels unbound calcium has only little influence on the concentration of ionised calcium, whereas at lower concentrations it plays an important role for the efficacy of citrate to lower ionised calcium. Thus, if citrate anticoagulation is operated using low infusion rates of citrate (< 3 mmol/l) total calcium needs to be taken into account.

Citrate also chelates magnesium, but the ability of citrate to bind magnesium is much smaller than that for calcium. For lower dosages of citrate the concentration of magnesium may also need to be considered, because some of the capacity of citrate to bind calcium will be diverted to the binding of magnesium und thus less citrate is available for calcium binding. These considerations are particularly important in continuous dialysis therapies, when a low infusion rate of citrate could be beneficial to avoid citrate accumulation. The more detailed estimation of citrate dose in

continuous dialysis should therefore include the influence of total calcium, the effect of magnesium as well as the expected increase in systemic citrate concentration.

In intermittent dialysis with high flux dialyzers citrate concentrations in the extracorporeal circuit in the range of 5.5-7.5mmol/l are common and this is not supposed to result in citrate accumulation due to the efficient removal of citrate into the dialysate. In addition, the time dependent increase in systemic citrate concentration is relatively small and negligible in comparison to the extracorporeal concentrations. Therefore in the intermittent setting higher infusion rates of citrate aiming for higher extracorporeal levels are acceptable and the calculation of the right dose of citrate to obtain the desired ionised calcium level can be based only on hematocrit and the blood flow, without the necessity to adjust citrate infusion rate during the course of the treatment.

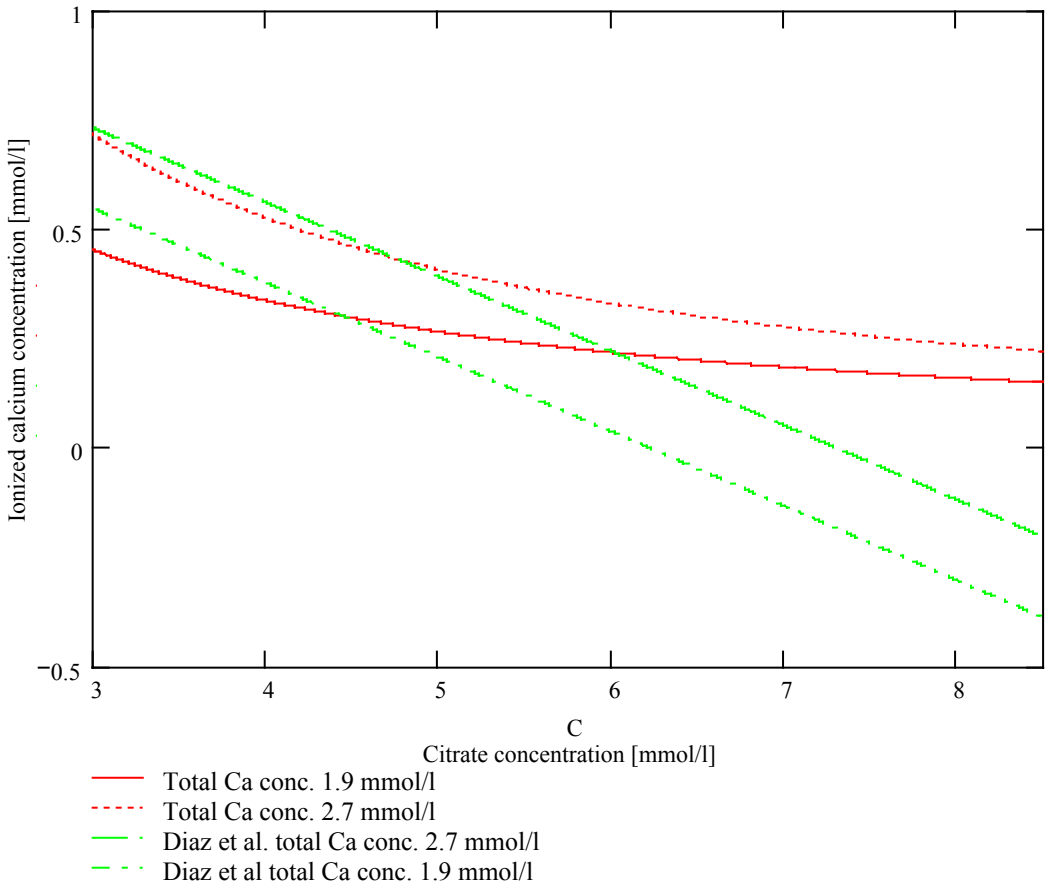

Figure 36 Solid and dotted red lines: correlation between citrate and ionized concentration in the blood calculated using law of mass action assuming concentration of diffusible calcium equal to 80% of total calcium (solid line: total calcium concentration of 2.7 mmol/l and dotted line: total calcium concentration of 1.9 mmol/l). Dashed and semi-dashed green lines: correlation described by Diaz et al. (Diaz et al. 1995) for total calcium of 2.7 mmol/l and 1.9 mmol/l respectively. For concentrations of citrate up to 5 mmol/l results of both correlations are equivalent. High citrate concentrations (above 5 mmol/l) in the correlation by Diaz et al. give negative unrealistic ionized calcium concentrations.

Summarizing, because in intermittent haemodialysis high citrate concentrations (>5.5 mmol/l) in the extracorporeal circuit are safe, it is possible for every patient to calculate from his hematocrit the required dose of citrate to achieve a target concentration of citrate in the extracorporeal circuit. The required dose of citrate (mmol/min) for patients with different hematocrit levels is shown in figure 37. These doses aim for a citrate concentration in the circuit of 7.0 mmol/l which will reliably achieve a target concentration of ionised calcium. Since the effect of citrate on ionised calcium can be estimated very precisely, measurement of ionised calcium or other anticoagulation parameters to confirm efficacy of citrate anticoagulation are not necessary.

In contrast, in continuous dialysis a low dose citrate anticoagulation may be preferable (extracorporeal citrate concentration <5.5 mmol/l) to avoid citrate accumulation. In this case, in addition to hematocrit the calculation of the citrate infusion rate probably requires to also consider total calcium and magnesium concentrations as well as the expected increase in systemic citrate concentration.

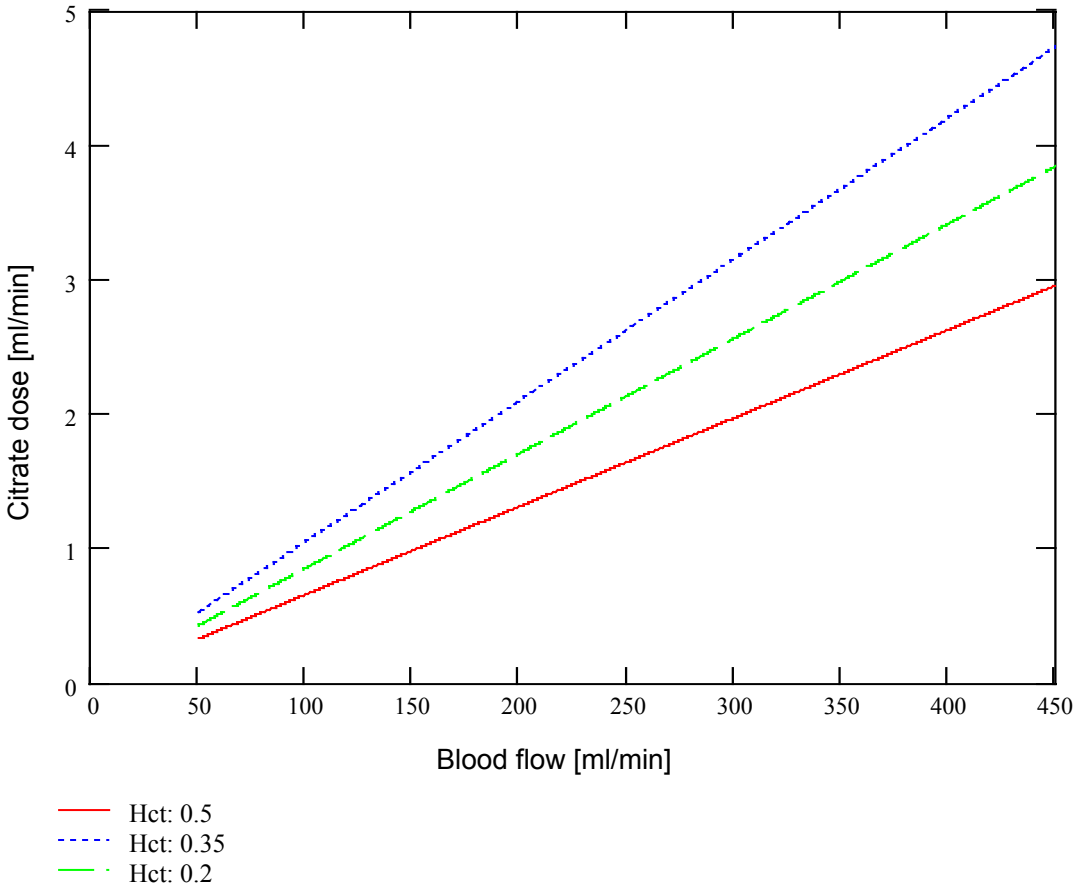

Figure 37 Nomogram of citrate dosing at a given blood flow (ml/min) in patients with different hematocrits. Citrate dose is given in ml/min assuming use of 0.5 mol/l citrate solution. Concentration of citrate in the extracorporeal circuit 7.0 mmol/l.

4.2 Safety aspects of citrate anticoagulation

4.2.1 Pathophysiology of citrate side effects

Since a portion of the infused citrate enters the body of the patient, the toxicity of citrate needs to be considered. As citrate is a ubiquitous substance in the metabolic pathways of the human body immunological reactions are not likely to occur. From the long experience with the use of citrate as an anticoagulant in transfusion medicine there are numerous evidences that citrate-related hypocalcaemia but not toxicity of the substance itself constitutes the most important risk for this anticoagulation method (Abbott 1983; Bolan et al. 2001; Bolan et al. 2002; Bunker et al. 1962; Denlinger et al. 1976; Diaz et al. 1994; Diaz et al. 1995; Hester et al. 1983; Kost et al. 1986; Ladenson et al. 1978; Marquez et al. 1986; Olinger et al. 1976; Perkins et al. 1971; Schmitt and Gotz 1988; Toffaletti 1983).

4.2.1.1 Clinical symptoms of hypocalcaemia

Concentration of calcium ions in the human body is maintained within narrow limits and is subject to tight control by several mechanisms. Calcium ions are essential for a normal cell function and play a key role in muscle contraction and nerve cell excitability. Since the electrical and contractile heart performances are calcium dependent processes, disturbances in ionized calcium levels (both hypo and hypocalcaemia) can cause fatal cardiac collapse. Mild hypocalcaemia (ionized calcium above 0.8 mmol/l) is usually asymptomatic. Typically the first symptoms of hypocalcaemia comprise signs of the excessive neuromuscular irritability such as perioral and peripheral paresthesia, uneasiness, shivering, headache, lightheadedness, bitter taste, nausea, cramping and tetany (Bolan et al. 2001; Bolan et al. 2002; Dzik and Kirkley 1988; Hester et al. 1983; Zaloga 1992). Neuromuscular symptoms such as perioral paresthesia, cramping, and nausea are frequently observed during blood cell aphaeresis procedures. Usually they are not treated with calcium infusion, resolving spontaneously after the cessation of the blood withdrawal. Cardiovascular insufficiency develops at severely depressed levels of ionized calcium (<0.5-0.6 mmol/l) and may be preceded by neuromuscular manifestations or can occur without prodromal signs. The prolongation of QT interval is the most commonly recognized electrocardiographic sign of hypocalcaemia. QT interval prolongation is known to promote life-threatening arrhythmias such as ventricular fibrillation and heart block. These cardiac phenomena were reported after massive transfusion of citrated blood and were ascribed to hypocalcaemia. The decline in the left ventricular performance and hypotension represent other signs of hypocalcaemia-related cardiac depression. The study by Marquez et al carried out in liver transplant recipients monitored by Swan-Ganz catheter has conclusively shown that the hypocalcaemia-induced left ventricular insufficiency, that follows massive transfusions of citrated blood, is not accompanied by the change in left or right cardiac filling pressure or systemic vascular resistance (Marquez et al. 1986). This suggests that the depressed myocardial

contractility was the main mechanism of the hypocalcaemia-induced left cardiac insufficiency. The early recognition of cardiac manifestations of hypocalcaemia is critical, since the intravenous infusion of calcium can quickly abolish these life-threatening disturbances. Kost et al suggested that the development of cardiovascular phenomena could start already at 0.4 mmol/l below the mean of the reference range (Kost et al. 1986). However, the individual sensitivity to the depression of ionized calcium is highly variable and depends on many factors. In aphaeresis settings it has been found that women are more prone to develop neuromuscular symptoms than men (Bolan et al. 2002). Intensive care patients have a higher incidence of preexisting hypocalcemia and in addition are at higher risk to develop the symptoms of low ionized calcium due to the underlying diseases (Tan et al. 2002; Zaloga 1992). Nevertheless, Mehta et al reported good tolerance of ionized calcium level of 0.61 mmol/l during citrate-anticoagulated CAVHD in critically ill patients (Mehta et al. 1990).

4.2.1.2 Treatment of hypocalcaemia

The intravenous infusion of ionized calcium is an effective antidote for citrate-induced hypocalcaemia. However, the overdosing of calcium is also detrimental and can result in cardiac arrest. The management of hypocalcaemia is particularly problematic in intensive care settings. Data from studies in intensive care patients indicate the harmful impact of calcium on cellular function in ischemic or septic states (Zaloga 1992) and on the progression of acute renal failure (Tan et al. 2002). Therefore, some authors recommend a conservative approach to the treatment of hypocalcaemia in critically ill patients, i.e. rather to monitor ionized calcium than to infuse calcium. The administration of calcium is only justified in the presence of cardiac symptoms or when ionized calcium drops below 0.8 mmol/l (Zaloga 1992).

4.2.1.3 Mechanisms of hypocalcaemia during citrate infusion

The clinically observed hypocalcaemia that follows citrate infusion can be explained by the disturbance of the chemical equilibrium. It is well established that the concentrations of citrate, citrate-calcium complexes and ionized calcium are closely related and always create a chemical equilibrium, described by the equation below (K = dissociation constant).

$$K = \frac{\left(\ Citrate^{-} \ \right) * \left(Calcium^{+} \right)}{Citrate - Calcium}$$

Thus, the delivery of an additional amount of citrate to the body fluids induces changes in the concentrations of these substances resulting in a new chemical equilibrium. The increase in citrate concentration is accompanied by an increase in calcium-citrate complexes due to the chelation of calcium ions by citrate which is decreasing the ionized calcium concentration.

It has been postulated that the release of calcium from proteins and the calcium mobilization from bones may participate in the maintenance of ionized calcium levels after the infusion of citrate

(Apsner et al. 1997; Dzik and Kirkley 1988). During transfusion of blood products or plateletpheresis parathyroid hormone (PTH) levels were measurably elevated within 2 to 4 minutes and reached peak levels between 5 and 15 minutes of blood donation(Dzik and Kirkley 1988). However, the influence of these mechanisms on systemic ionized calcium concentrations is not known.

The reduction in concentration of citrate and citrate-calcium complexes due the metabolism in Krebs cycle and urinary excretion restores the chemical equilibrium and results in an increase in ionized calcium concentration. Thus, the effectiveness of the metabolic degradation plays a crucial role in limiting the toxicity of citrate. On the other hand, bicarbonate production that follows citrate degradation in Krebs cycle can lead to alkalosis that is known to increase the capacity of proteins to bind ionized calcium. Therefore, alkalosis can worsen ionized hypocalcaemia. The induction of alkalosis alone can produce symptoms typical for hypocalcaemia as seen in tetany due to hyperventilation.

4.2.1.4 Safety of citrate anticoagulation during non-haemodialysis extracorporeal treatments

During non-haemodialysis treatments citrate-induced hypocalcaemia may occur exclusively due to citrate infusion. Studies on transfusion and aphaeresis procedures have shown a high correlation between citrate dose, systemic citrate concentration and the occurrence of hypocalcaemia (Bolan et al. 2001; Bolan et al. 2002; Hester et al. 1983). These studies have also demonstrated the safety of this method using doses typical for the transfusion of citrated blood without the mandatory supplementation of calcium. Nevertheless, these results cannot be transferred to haemodialysis setting because pharmacokinetics of citrate during dialysis are more complex.

4.2.1.5 Special features of haemodialysis contributing to hypocalcaemia

In contrast to e.g. the transfusion of blood products, during dialysis the dose of citrate reaching the patient is usually unknown. In haemodialysis two processes occur simultaneously: a constant infusion of citrate into the extracorporeal circuit and a continuous elimination of citrate into the dialysate. The use of a wide range of filters is associated with different citrate clearances increasing the complexity of the citrate dose assessment. In addition, the clearance of citrate is not a parameter provided by the manufacturers of dialyzers.

Moreover, during haemodialysis citrate infusion is not the only mechanism causing hypocalcaemia. The majority of citrate anticoagulation protocols require the application of calcium free dialysis solution. This leads to a substantial removal of calcium in the form of ionized calcium and calcium-citrate complexes into the dialysate. In order to preserve the body balance of calcium any regimen using calcium free dialysis solution requires calcium supplementation.

4.2.2 Citrate toxicity – clinical observations in RRT

4.2.2.1 Hypocalcaemia in haemodialysis

Empirical dosage schemes for citrate and calcium supplementation in haemodialysis settings frequently resulted in complications attributed to citrate-induced hypocalcaemia. However, the level of ionized calcium was usually not reported due to the lack of the appropriate laboratory devices. The majority of authors described only mild symptoms such as: paresthesia, malaise, cramps or hypotension (Faber et al. 1990; Flanigan et al. 1996; Hocken and Hurst 1987; van der Meulen et al. 1992; von Brecht et al. 1986; Wiegmann et al. 1987). However, a cardiac arrest due to ventricular fibrillation was also reported in two haemodialysis patients after citrate anticoagulation (Charney and Salmond 1990). The citrate anticoagulation protocol used in these patients was based on the approach proposed by Brecht et al., which included the infusion of a highly concentrated solution of citrate together with the use of calcium containing dialysate. The influx of ionized calcium from the dialysate was believed to alleviate the need for the separate calcium supplementation (Flanigan et al. 1987; von Brecht et al. 1986). The cardiac arrest observed by Charney et al. did not respond to standard cardiac support therapy until the intravenous infusion of calcium was administered. Since calcium reversed these phenomena, they were presumed to result from citrate induced hypocalcaemia. Despite the missing documentation of electrolyte and acid-base parameters during the occurrence of cardiac arrest this report labeled citrate anticoagulation unsafe. Subsequently in April 2000 the FDA warned on the use of highly concentrated solutions of citrate and discouraged their application in haemodialysis.

4.2.2.2 Hypocalcaemia in CRRT

Continuous renal replacement therapies carry a higher risk of citrate accumulation and toxicity due to the prolonged delivery of citrate accompanied by lower citrate clearances. In addition, ischemia, sepsis or initial hypocalcaemia, which are frequently observed in critically ill patients, increase the risk of citrate-related complications. Therefore the low reported values of ionized calcium during citrate anticoagulation in continuous treatments are not surprising. However, all described episodes of severe hypocalcaemia (minimum reported values of ionized calcium 0.61-0.73 mmol/l) induced no cardiac symptoms (Cointault et al. 2004; Gabutti et al. 2002; Gupta et al. 2004; Mehta et al. 1990; Meier-Kriesche et al. 2001; Palsson and Niles 1999; Tolwani et al. 2001). While some authors decided to terminate the infusion of citrate in cases of profound hypocalcaemia (Gabutti et al. 2002; Palsson and Niles 1999) others continued the treatment and successfully managed hypocalcaemia applying higher doses of calcium (Cointault et al. 2004; Meier-Kriesche et al. 2001; Tolwani et al. 2001).

4.2.2.3 Association between alkalosis and hypocalcaemia in haemodialysis

Neuromuscular symptoms of hypocalcaemia were frequently reported in early publications on citrate anticoagulation in haemodialysis. In these studies citrate anticoagulation was carried out with higher dose of citrate in comparison to the current protocols and with use of standard buffer concentrations of the dialysis solutions. These early approaches often resulted in the development of alkalosis. Although the majority of patients experienced only mild alkalosis (pH < 7.5) severe alkalosis (pH up to 7.58) was also described (Kelleher and Schulman 1987). Subsequent studies have demonstrated that in intermittent haemodialysis alkalosis can effectively be prevented by reducing the buffer content of the dialysis solution by 3-6 mmol/l in comparison to the standard concentration used with heparin anticoagulation (Apsner et al. 2001; Janssen et al. 1993; Janssen et al. 1996; van der Meulen et al. 1992). The effective prevention of alkalosis by reducing the buffer content of the dialysis solution together with the reduction of citrate dose improved patients' comfort during citrate anticoagulation and decreased the incidence of paresthesias.

4.2.2.4 Alkalosis in CRRT

Alkalosis was also frequently observed in continuous renal therapies (Chadha et al. 2002; Gabutti et al. 2002; Hofmann et al. 2002; Kutsogiannis et al. 2000; Mehta et al. 1990; Mehta et al. 1991; Thoenen et al. 2002; Tolwani et al. 2001; Ward and Mehta 1993). Critically ill patients may receive a considerable amount of buffers with parenteral nutrition contributing to the development of alkalosis (Chadha et al. 2002; Hofmann et al. 2002). Therefore in intensive care patients the reduction of the bicarbonate content should not only concern the dialysis solution but also the parenteral alimentation. However, alkalosis in critically ill patients can resolve spontaneously due to the subsequent development of metabolic acidosis (Kutsogiannis et al. 2000). Nevertheless, some cases may need infusion of hydrochloric acid (Mehta et al. 1990; Mehta et al. 1991; Ward and Mehta 1993).

4.2.2.5 Citrate toxicity in liver failure

The presence of end-stage liver failure in critically ill patients treated by citrate-anticoagulated continuous therapies represents a particular risk for the development of citrate toxicity. Meier-Kriesche et al reported severe hypocalcaemia (0.68 mmol/l) in a patient with ARF and fulminant hepatic failure treated by citrate anticoagulated CVVHD (Meier-Kriesche et al. 1999). This severe hypocalcaemia was reversed with extremely high doses of calcium and with the reduction of citrate flow. During the occurrence of citrate toxicity an increased ratio of total to ionized calcium was observed. This could be explained by the accumulation of citrate-calcium complexes due to insufficient liver metabolism. The authors of this publication have proposed to monitor the ratio of total to ionized calcium as an early sign of severe liver failure, which predisposes to increased requirements of calcium supplementation and to the reduction of citrate dose. Thus, citrate anticoagulation seems to be applicable even in severe liver failure patients treated by continuous

renal replacement therapies but only when the higher requirements for calcium infusion and smaller dose of citrate are anticipated.

4.3 Safety of the current patient study

4.3.1 Citrate: systemic concentration

4.3.1.1 Changes of systemic citrate concentration

In our study systemic citrate concentration at baseline (0.12 mmol/l) was comparable to that observed by other authors in haemodialysis (0.1 to 0.18 mmol/l) (Evenepoel et al. 2002; Faber et al. 1990; Hocken and Hurst 1987; Janssen et al. 1993; Janssen et al. 1996; Lohr et al. 1988; Lohr et al. 1989). However, during haemodialysis the values measured were much lower than previously reported (see table 7). With a similar dose of citrate infused into the extracorporeal circulation during IHD the concentration of citrate reported by Janssen et al (Janssen et al. 1993; Janssen et al. 1996) was twice as high.

The differences in systemic citrate concentrations may result from the use of low-flux filters (Evenepoel et al. 2002; Janssen et al. 1993; Janssen et al. 1996) in contrast to the use of high-flux dialyzers in this study. Even in our study, the difference of about 10 ml/min in the dialyzer clearances (F60S: 102 ml/min versus FX50: 91 ml/min) was associated with a difference between systemic citrate concentrations of approx. 0.1 mmol/l, see figure 7. Such an influence of the dialyzer clearance on the systemic citrate concentration is in perfect agreement with the results of the modeling, see chapter 3.3.2.5. Comparable doses of citrate (3.2 mmol/l) applied in continuous treatment can generate much larger systemic citrate concentration: concentrations up to 2.95-3.71 mmol/l were reported (Mehta et al. 1990; Mehta et al. 1991).

All measured values of systemic citrate concentration in our study were far less than the toxic level, which was suggested by Bunker et al at 2.5 mmol/l (Bunker et al. 1962; Janssen et al. 1993), where citrate was infused without giving calcium and cardiac side effects were recorded. We observed the highest systemic citrate concentration equal to 0.72 mmol/l and only in two patients it exceeded 0.5 mmol/l. These concentrations are also much lower than the typical levels during apheresis procedures (1 to 2 mmol/l) (Bolan et al. 2001; Bolan et al. 2002).

During dialysis, ultrafiltration to remove excess fluid was applied, steadily decreasing the volume of distribution for citrate. Therefore, we failed to observe a plateau phase of citrate concentration. Data from the mathematical model suggest that citrate concentration would have stabilized after approximately 220 minutes of the procedure, if no fluid had been removed. This prediction is in a good agreement with the observations from apheresis studies, which demonstrated a steady

increase in systemic citrate concentration during procedures lasting 90 to 200 min (Bolan et al. 2001; Bolan et al. 2002).

Systemic citrate concentration [mmol/l]				Dose of citrate [mmol] per litre of blood	Author
Pre-HD	2nd HD hour	4th HD hour	Post-HD		
0.1	0.73	0.76	Not reported	Not reported	(Lohr et al. 1988)
0.12	0.5	0.55	Not reported	Not reported	(Lohr et al. 1989a)
0.1	Not reported	Not reported	0.75	4-4.3	(Hocken and Hurst 1987)
0.14	1.87	1.3	0.42	5.5- 7.0	(Faber et al. 1990)
0.1	Not reported	Not reported	0.6	3.4	(Janssen et al. 1996)
0.18	Not reported	Not reported	1.13	4.33	(Evenepoel et al. 2002)

Table 7 Systemic citrate concentration during citrate anticoagulated haemodialysis reported in the literature.

The velocity of the increase in systemic citrate concentration is particularly high during the first 40 minutes, whereas during the next three hours the increase is comparable to that in the first 40 minutes. Data from the apheresis studies confirm this observation (Bolan et al. 2001; Bolan et al. 2002). Therefore, the first 40 minutes of citrate anticoagulation may be critical for the development of citrate side effects.

4.3.1.2 Prediction of systemic citrate concentration

Mathematical model of citrate concentration paralleled well the real citrate concentrations. This indicates that systemic citrate concentrations are predictable and results only from the mechanisms of citrate delivery, distribution and elimination. Interestingly, for every patient the curve of citrate accumulation obtained using one compartment model underestimated the systemic concentration in the first half of the treatment (approximately first 120 minutes). This may suggest a distribution of citrate into two compartments.

However, this discrepancy could also result from the insufficient knowledge about the metabolic elimination of citrate which may be dependent not only on the first order kinetics assumed in our model. On one hand, the majority of the basic metabolic pathways in the body follow first order kinetics, thus this rule should be also applicable to the enzymes of the archaic Krebs cycle. But on the other hand, citrate has to be transported to the mitochondria before it enters the Krebs cycle. This transport requires crossing the cellular and mitochondrial walls. Since at physiological pH of the blood, citrate and citrate-calcium complex are anions, they are not freely diffusing from

the extracellular to the intracellular space. Thus, they may require transport processes different from first order kinetics of the enzymes.

4.3.1.3 Unusual case of high baseline citrate concentration

High baseline citrate concentrations have been reported so far in liver failure patients (NORDMANN and NORDMANN 1961) (Kramer et al. 2003). In this study we observed an unusually high citrate concentration at baseline in one patient with normal liver function. The reason for this high citrate concentration remains elusive. Therefore, as citrate concentration is not routinely measured, every patient that receives citrate anticoagulation for the first time should be observed more carefully.

4.3.2 Citrate: elimination

4.3.2.1 Citrate extraction coefficient of the dialyzer

The low systemic citrate concentration during citrate anticoagulation resulted mostly from the very efficient removal of citrate by the dialyzers. Approximately 83% of the citrate mass infused into the arterial bloodline was immediately removed into the dialysis solution and never reached the patient. The citrate extraction coefficients of both dialyzers used in this study were much higher (F60S 84%, FX50 77%) than the reported ones so far (30-69%) (Evenepoel et al. 2002; Janssen et al. 1993; Morita et al. 1961). This is due to high-flux properties of the dialyzers used in this study.

A unique feature of dialysis is that citrate removed into the dialysate (100%) is coming from three sources:
(1) citrate that has been infused into the arterial line immediately before the blood reaches the dialyzer (94%)
(2) citrate that has previously been infused and reached the patients circulation and returns to the arterial line (3%)
(3) citrate produced by the metabolism of the body (3%).

As a result during a high efficient dialysis only very small percentage of the infused amount of citrate (approximately 17% according to the modelling) is left for metabolic removal.

4.3.2.2 Citrate clearance of the dialyzer

Although we used two kinds of dialyzers the clearance of citrate was not expect to differ since both filters have similar in vitro clearances for urea which like citrate belongs also to small class molecules (see table 3, chapter 2.2.1). Thus, the observed small difference in clearances could partly result from the difference in hematocrit. However, the difference in extraction coefficients between dialyzers is an argument for a better removal of citrate using the dialyzer F60S. In addition to the clearance, the efficiency of the dialyzer to remove a given solute can also be

described by a constant called mass transfer coefficient, KoA. In contrast to the clearance, this parameter does not depend on the flow in the blood or the dialysate compartment. Therefore, the in vitro estimation of citrate KoA for the dialyzer FX50 could best answer the question whether the dialyzers differ in removing citrate.

In the current study we have estimated the citrate KoA for F60S. The in vitro measurement of clearances and KoA typically results in an overestimation of the dialyzer performance in comparison to the clinical data. The difference between in vitro and in vivo values of clearances usually increases with the time course of HD treatment as usually more blood proteins and blood cells adhere to the dialyzer membrane. We have also observed these phenomenon in the current study. The clearance calculated using KoA was higher than the measured value on average by 13±5 ml/min, 15±6 ml/min and 17±10ml/min in the first, third and fourth hour, respectively.

Clearance of citrate appears to constitute 63-64% of total urea clearance for blood flow of 180-200 ml/min, see figure 38, which is in a good agreement with the published data (Pinnick et al. 1983). The higher clearance of urea is not surprising since citrate is dissolved only in the plasma water in contrast to urea, which also enters erythrocytes. In addition the diffusion coefficient of citrate is smaller than that of urea ($0.632 * 10^{-5}$ cm^2/s versus $1.808 * 10^{-5}$ cm^2/s). It is worth mentioning, that the clearance of citrate (or urea) does not increase proportionally to the blood flow. Thus, the use of high blood flow which requires a higher citrate flow for an adequate anticoagulation carries a higher load of citrate to the body and increases the risk of citrate accumulation.

4.3.2.3 Citrate dose for metabolic elimination during high-flux haemodialysis

Noteworthy, the dose of citrate reaching the patient increasing the body pool and eventually requiring metabolism was much smaller in this study using FX50 (0.2mg/min/kg) or F60S (0.16mg/min/kg with) dialyzers compared to aphaeresis procedures (1-1.7mg/min/kg). According to the UK Blood Transfusion Guidelines the maximum reinfusion rate of citrated blood during aphaeresis procedures should not exceed 1.9 mg of citrate/kg/min, which is also much faster than the current citrate protocol during haemodialysis.

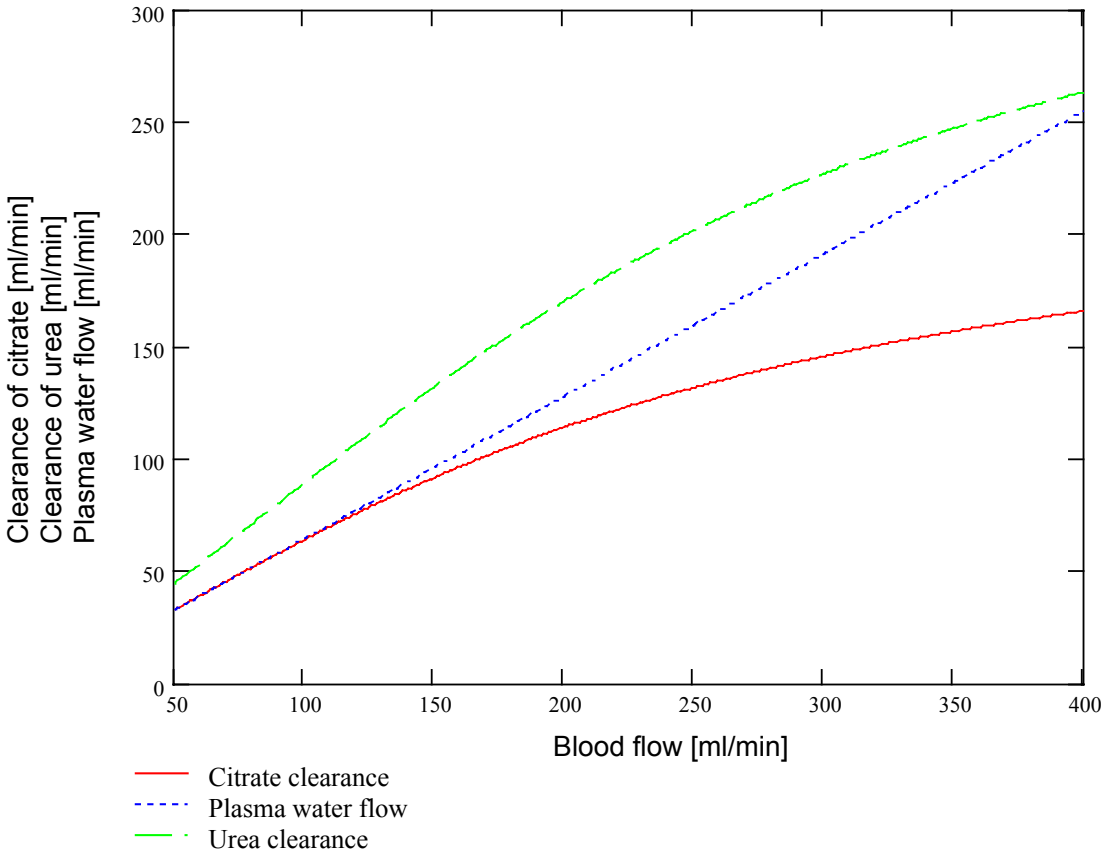

Figure 38 Citrate clearances (solid red line) and urea clearances (dashed green line) using dialyzer F60S in comparison to the plasma water flow (dotted blue line) while increasing blood flow. Clearances of urea and citrate calculated with KoA: 735 ml/min (Fresenius Medical Care data) and 337 ml/min (data from the current study). Effective flow for urea: sum of the plasma water flow and 80% of the red blood cells flow (Daugirdas and Stone 2001). Plasma water flow calculated for hematocrit: 0.3 and protein concentration: 6 g/dl.

4.3.2.4 Citrate metabolism and half time

The current study demonstrated a huge variability in citrate metabolic half time ranging from 18 to 131 minutes. The mean citrate half time (60 minutes) in our patients was longer than in immunopheresis recipients (32.9 minutes) with normal renal and liver function (Apsner et al. 1997). Our results are similar to that obtained in patients with liver cirrhosis or acute hepatic failure (69 ± 33 minutes, 49.7 ± 5.4 minutes) (Apsner et al. 1997; Kramer et al. 2003).

Liver metabolism is commonly regarded as the main component of citrate elimination in a human body. This is known from the experiences with massive transfusion of hemoderivates during the anhepatic phase of liver transplantation. In this situation dramatic increases in systemic citrate concentrations (to about 5.0 mmol/l) are observed together with profound hypocalcaemia (values 0.5 mmol/l to 0.18 mmol/l). Importantly, after hepatic graft reperfusion these parameters normalize spontaneously (Marquez et al. 1986). Under normal conditions renal clearance of citrate is believed to constitute only a small portion of the total body clearance (19 ml/min versus

481 ml/min), but it is also known to increase if bicarbonate concentration is elevated (Baruch et al. 1975; Hamm 1990; Marangella et al. 1991; NORDMANN and NORDMANN 1961). The data from the current study indicate that the renal elimination of citrate may substantially contribute to the overall citrate elimination.

Nevertheless, the metabolism of citrate in haemodialysis patients was sufficient to prevent citrate accumulation if low dose of citrate was delivered using high-flux dialyzers. The systemic citrate levels returned to baseline within 4 hours after the treatment limiting the effect of citrate anticoagulation on citrate levels to the day of the dialysis, as also seen by Wiegmann et al (Wiegmann et al. 1987).

4.3.2.5 No correlation between citrate half time and the body mass

It is widely agreed that citrate can be metabolised by all cells containing mitochondria. Thus, the differences in the body size may have in theory an impact on the metabolic rate constant. Chadha et al. pointed to the higher risk of citrate toxicity in paediatrics in particular in children with small body size. He raised the question about the contribution of other tissues apart from liver to the metabolic elimination of citrate (Chadha et al. 2002). In the current study there was no correlation of metabolic rate constant and body weight. Therefore, it is more likely that in patients with small body size the smaller distribution volume plays the predominant role in increasing systemic citrate concentration. Although in adults the differences in body size are smaller than in children this nevertheless may influence systemic citrate concentration and could contribute to the development of citrate toxicity as indicated by the results of the modelling, see chapter 3.3.2.7.

4.3.2.6 Safety of citrate anticoagulation during high-flux HD in liver failure patients

Since the load of citrate for the body is very small and the metabolic elimination constitutes only a small part of the total removal, citrate anticoagulation during 4 hours of high-flux dialysis seems to be safe even for patients with moderate liver failure. This conclusion is supported by the in vitro modelling (see chapters 3.3.2.4 and 3.3.2.6). In addition, citrate metabolic elimination in patients with liver failure is not much reduced when compared to haemodialysis patients.

A complete absence of metabolic citrate elimination could pose a danger for citrate intoxication but is unlikely to occur frequently, since Krebs cycle is a basic metabolic pathway necessary for cell function. An indirect evidence on the paramount importance of Krebs cycle in a human being is the lack of genetic defects concerning the enzymes of this cycle. Such genetic defects are probably lethal and therefore not observed in people. Thus, only in end-stage liver failure a severely diminished metabolic elimination of citrate can be anticipated. The study by Meier-Kriesche et al corroborates this prediction demonstrating that not all patients with severe liver failure have impaired citrate metabolism (Meier-Kriesche et al. 2001). In this study poor citrate metabolism defined as an increased ratio of total to ionized calcium was an independent risk

factor for patient mortality. This suggests that the inability to degrade citrate might be another indicator of critical metabolic impairment.

4.3.3 Citrate: distribution volume

This study also allowed estimating citrate distribution volume in stable dialysis patients. Mean distribution volume assessed in the fourth hour of the treatment corresponded to the common percentage of the total body water in adults (54% of body weight, 40 litres). This confirms that citrate is easily distributed and transported through the walls of the cells, which is mainly due to its good solubility in aqueous media. The good solubility of citrate and citrate-calcium complex is associated with their small molecular sizes and the presence of the free carboxyl group.

However, as it has already been mentioned (chapter 4.3.1.2) citrate distribution may be two-compartmental. The smaller distribution volume assessed in a shorter time by the group of Apsner is in favour of this hypothesis. The distribution volume in immunopheresis recipients and critically ill patients measured after 120 minutes (current study 240 minutes) was reduced by approx. 40 % in comparison to our data (17.55 to 23 litres) (Apsner et al. 1997) (Kramer et al. 2003). This difference could result from the shorter observation time and be due to the incomplete distribution into the deeper compartment.

4.3.4 Calcium: risk of hypocalcaemia during high-flux dialysis

From the data obtained in this study one can conclude that during a 4 hours long high-flux dialysis the risk of hypocalcemia is mainly due to a large calcium removal into the dialysate. In contrast, chelation of calcium in the patients' systemic blood due to the presence of citrate is less important. In the current study ionized and total calcium concentrations in the patient circulation were not affected by the increased level of citrate-calcium complexes (see chapter 3.2.4.1 and 4.3.5). In addition, total substitution of calcium (mean: 48 mmol in 4 h) was close to total removal (mean: 43 mmol in 4 h) preventing effectively the development of hypocalcaemia.
This conclusion is also in line with the results of the modelling simulating hypothetical malfunctioning of the citrate anticoagulation apparatus during dialysis (chapter 3.3.3).

4.3.5 Calcium: systemic concentration

In agreement with many previous studies on citrate anticoagulation during intermittent dialysis total calcium concentration was on average not notably affected (Apsner et al. 2001; Faber et al. 1990; Janssen et al. 1993; Lohr et al. 1988; Pinnick et al. 1983; Seaton et al. 1983; van der Meulen et al. 1992). Although some authors reported an increase in total calcium after haemodialysis with citrate anticoagulation the results are difficult to compare to the current study due to the large differences in the citrate anticoagulation protocols. Therefore an increase in total calcium due to the accumulation of citrate-calcium complexes cannot be excluded in these

studies. None of the studies calculated the net balance of calcium. Therefore, it is also possible that a larger calcium supplementation reached the patients exceeding the capacity of calcium elimination from the extracellular water to a deeper compartment (Flanigan et al. 1996; Janssen et al. 1996; Wiegmann et al. 1987). In addition, some studies intended to increase total calcium levels in patients with initial hypocalcemia (Ashouri 1985; Lohr et al. 1989b; von Brecht et al. 1986).

In the current study the presence of the increased concentration of citrate-calcium complexes did not introduce a relevant change in the ionized calcium concentration. Since, both total and ionized calcium remained unaffected; one can conclude that calcium complexed by citrate was compensated by a release of calcium from protein binding sites. This study has already demonstrated the occurrence of the release of calcium from proteins in the extracorporeal circuit, and it is not surprising that the same phenomenon appears to contribute to the maintenance of ionized calcium level in the systemic circulation. The release of calcium from proteins is proportional to citrate concentration. Since the systemic citrate concentration was much smaller than the extracorporeal concentration, the degree of this release in the patient's body must also have been smaller but sufficient to preserve normal ionized calcium concentration. The measurement of the diffusible calcium would allow estimating the magnitude of this effect precisely. Studies in transfusion medicine give additional evidence of a notable release of calcium from proteins in the systemic blood when citrated blood products are transfused. Diaz et al noted that the strength of the correlation between citrate and ionized calcium concentration was increased from r = -0.63 to r = -0.93 when total calcium was included into the regression equation (Diaz et al. 1995).

The above data emphasize that systemic citrate concentration in the current study was safe and low enough not to decrease ionized calcium concentration probably because of effective buffering by calcium released from proteins.

4.3.6 Calcium: release of calcium ions from proteins

Up to now, only little attention has been paid to the change of diffusible calcium concentration after the infusion site of citrate.

During citrate anticoagulated high-flux haemodialysis the loss of calcium into the dialysate accounts for the main risk of hypocalcaemia. This loss is dependent on the diffusible calcium concentration. Thus, the estimation of the diffusible calcium concentration would help to more precisely predict the loss of calcium and to improve the safety of the citrate procedure.
Therefore this phenomenon has been investigated in this study in detail.

Calcium in the blood exists in three forms: calcium bound to proteins, ionised calcium and calcium complexed with small anions like lactate or citrate (Marx and Bourdeau 1987). Under physiological conditions approx. 40% of calcium is bound to proteins, about 10-15% of calcium is complexed by small anions and 45-50% of calcium is remaining in ionized form. There are two kinds of calcium binding proteins: albumin (binding 75-90% of protein bound calcium) and globulin (Marx and Bourdeau 1987). The number of calcium molecules that can be bound to one molecule of a protein is variable, e.g. pH influences the capacity of proteins to bind calcium ions, increasing during alkalosis. At steady state, the concentrations of ionised calcium, protein bound calcium and calcium complexed by small anions create chemical equilibrium dependent on the dissociation constants.

In the extracorporeal circuit during citrate anticoagulation the fractions of calcium undergo substantial transformation to reach a new steady state. In order to understand these changes it is helpful to follow calcium on the way from the blood access site to the filter. After citrate is infused into the arterial line of the extracorporeal circuit a large portion of ionised calcium is chelated by citrate, reducing the percentage of ionised calcium to 10 –15% of total calcium. Citrate-induced and subsequently a dialyzer-induced decrease in ionized calcium concentration disturb the equilibrium between calcium and proteins and results in a release of calcium from proteins. This leads to a new steady state of chemical equilibrium that can again be described by dissociation constants.

However, the exact calculation of concentrations of diffusible calcium after the infusion of citrate solution based on the equilibrium theory is cumbersome due to the following factors: (Fogh-Andersen 1977; Moore 1970; Pedersen 1971; Toffaletti et al. 1977).

- The two kinds of calcium binding proteins (albumin and globulin) are present at varying concentrations in the individual patient.
- The number of binding sites on the given protein is varying.

Therefore, in this study we have estimated the release of calcium ions in the extracorporeal circuit in a semi-empirical approach.

In the first step we compared the flux of calcium into the dialysate with the initial theoretically possible flux of diffusible calcium before the infusion site of citrate. For every patient the flux of calcium into dialysate exceeded the initial flux of diffusible calcium (mean initial flux: 0.16 mmol/min, calculated as 60% of the plasma water flux of total calcium, mean flux into dialysate: FX50-0.17 mmol/min, F60S-0.18 mmol/min). This observation indicated that the release of calcium ions from proteins occurs in the extracorporeal circuit to a significant degree and cannot be ignored.

In the second step, we calculated the hypothetical concentration of the diffusible calcium in the dialyzer that would enable to achieve the measured fluxes of calcium into the dialysate. For this

estimation we assumed that the clearance of ionized calcium is equal to the clearance of citrate and of citrate-calcium complexes. Clearances of citrate and of citrate-calcium complex can be regarded as equal due to similar molecular weight: 189 and 229. Difference in ionized calcium clearance is negligible since its concentration is very low as the majority of diffusible calcium exists as citrate-calcium complexes.
This detailed analysis demonstrated that the infusion of citrate in the pre-filter mode during high-flux dialysis increases notably the concentration of diffusible calcium from approx. 60% to 80%. Noteworthy, the diffusible fraction of calcium after citrate infusion was relatively constant between the patients (95% CI 79-81%). Consequently, the increase in the diffusible calcium concentration and the subsequent loss of calcium is proportional to the total calcium concentration but not to ionized calcium concentration.

4.3.7 Calcium: removal by the dialyzer

The mean measured values of calcium flux over the dialyzer membrane (F60S: 0.18 mmol/min, FX50: 0.17 mmol/min) are comparable to the loss of 7mg of elemental calcium per minute (0.175 mmol/min) as reported by previous investigators (Pinnick et al. 1983; von Brecht et al. 1986).

However, it should be emphasized that the loss of calcium into the dialysis solution had a very high interindividual variability ranging from 0.13 to 0.22 mmol/min despite similar effective blood flows (179 – 187 ml/min).

4.3.7.1 Importance of hematocrit for calcium removal

The variability in calcium flux over the dialyzer could partly result from different clearances of calcium-citrate complexes and ionized calcium associated with different hematocrit levels, see figure 39. Since red blood cells contain only trace amounts of calcium (estimated as 0.02 mmol per kg of erythrocytes (Marx and Bourdeau 1987) and are regarded as impermeable both to plasma calcium or plasma citrate the effective solute flow for calcium (calcium-citrate complexes) is equal to plasma flow which is dependent on hematocrit level. It is well established that during intermittent dialysis clearances are proportional to the effective flow of the solute. In conclusion, low hematocrit results in higher plasma flow which induces bigger clearance and consequently larger loss of calcium into the dialysate.

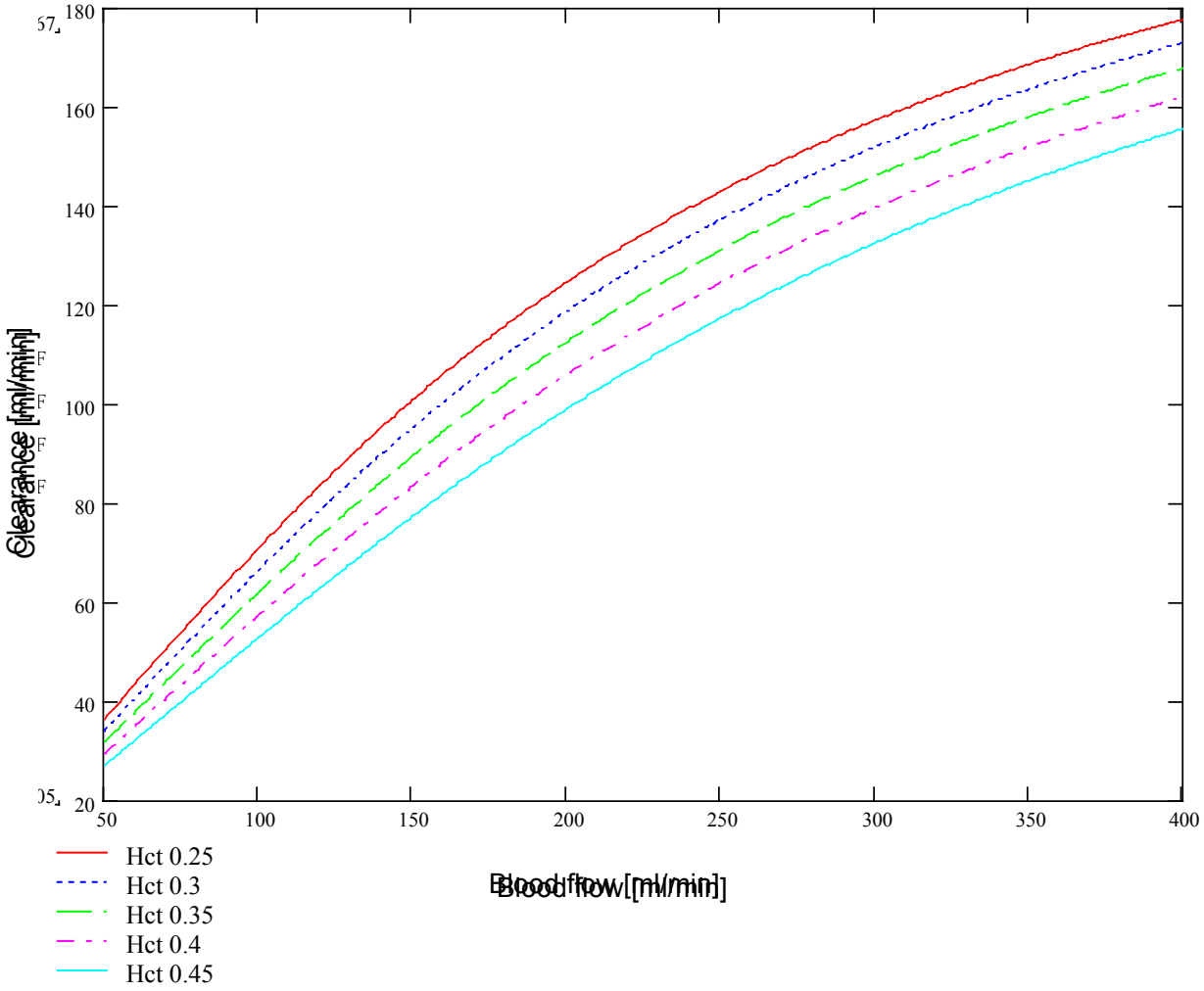

Figure 39 Clearance of citrate (and of citrate-calcium complexes) that is dissolved only in plasma water depends on the blood flow and the level of hematocrit.

4.3.7.2 Importance of total calcium concentration for calcium removal

In addition, according to the law of diffusion, the loss of calcium is linked to the concentration of diffusible calcium in the dialyzer i.e. concentration of ionized calcium and calcium bound to small anions e.g. citrate. This concentration is a driving force for the calcium transfer over the semipermeable membrane. Our results show that the concentration of diffusible calcium increases after the infusion site of citrate amounting to 80% of total calcium concentration. The proportion of diffusible to total calcium was relatively constant making the removal of calcium proportional to the systemic total calcium concentration. Thus, patients with higher total calcium concentration have bigger fluxes of calcium across the dialyzer membrane and greater calcium removal than patients with lower total calcium concentration.

4.3.7.3 Pre-dialysis estimation of the expected flux of calcium into the dialysate

In this study we attempted to predict the loss of calcium based on the patient total calcium concentration and calcium clearance (citrate-calcium complex clearance). Using the theoretically developed equation 77 we obtained the values of calcium flux strongly correlating with the measured calcium loss. The correlation was most distinct in the first treatment hour. The reduction of the correlation coefficient observed in the later haemodialysis phase could partly result from the use of the pre-dialysis value of hematocrit. During the dialysis the ultrafiltration is usually applied increasing hematocrit and reducing the effective flow of calcium. Another limitation of the calculation of the expected calcium flux is the use of a constant value of clearance. The clearance can change during the course of the treatment and it typically decreases due to the increasing amount of proteins and blood cells adhering to the dialysis membrane.

The calculated loss of calcium was on average higher than the measured one by approx. 0.1 mmol/min. This could be partly due to the use of KoA value which was measured in electrolyte solution but not in the blood. It is well known that the value for KoA obtained in the blood and the resulting clearances are smaller, e.g. due to protein layer on the dialyzer membrane. Only in a one patient the predicted calcium removal was underestimated in comparison to the measured value. This was associated with the lowest protein concentration (4.3 g/dl) and the highest fraction of diffusible to total calcium, 84%. All other study participants had protein concentration over 5.0 g/dl. This may indicate than in patients with a very low proteins concentration (below 5 g/dl) the diffusible fraction of total calcium requires correction to 0.84 when expected calcium loss is estimated using the equation 77.

Summarizing, the initial loss of calcium during citrate anticoagulated high-flux dialysis can be predicted using the equation 77. As demonstrated during the current study hypocalcaemia but not hypercalcaemia poses a danger for acute, life-threatening complications during citrate anticoagulation. The use of an in vitro value of KoA in the equation 77 results in an acceptable overestimation of calcium loss which adds to the safety of this evaluation.
This equation (77) identifies patients with expected high losses of calcium and consequently higher requirement for calcium supplementation. The calculated loss of calcium may also serve as a minimum recommended dose for calcium supplementation for all patients aiming at a neutral or positive calcium balance.

4.3.8 Calcium: net balance

In the current study the flux of calcium across the filter membrane was variable; in contrast, calcium substitution rate was almost constant: 0.2 ± 0.005 mmol/min. The application of a similar calcium substitution rate for patients with different fluxes of calcium into the dialysate resulted in a large variability in calcium net balance (from –1 to 17 mmol/HD).

A positive net balance of calcium did not correlate with higher systemic ionized calcium concentration, e.g. the patient with a positive Ca balance of 17mmol/HD did not show a corresponding increase in ionised calcium level. The positive calcium net balance in 10 patients was expected to increase total calcium by at least 0.2 mmol/l. However, this did not occur, probably because added calcium was transported from the extracellular water to another compartment (e.g. bones or intracellular space). It is unclear, whether this transport occurred spontaneously or was induced by calcium supplementation. The concentration of total calcium remained stable (+/- 5%) in all patients throughout the study period.
In this study only two patients experienced modest negative calcium balance. In both of them there was a small decrease in post dialytic total calcium level.

Positive calcium balance was rapidly “buffered” probably by moving calcium to a deeper compartment.
In contrast, a lower calcium concentration due to negative calcium balance was not rapidly reversed indicating that calcium movement from the deeper compartment is not as fast as the calcium movement in the opposite direction. This hypothesis is supported by observations known from anhepatic phase of liver transplantation showing strong correlation between citrate and ionized calcium concentration ($r = -0.93$; $p<0.01$) (Diaz et al. 1994; Diaz et al. 1995). During liver transplantation large amounts of the blood products are transfused and induce particularly high systemic citrate concentrations due to the lack of citrate metabolism. This in parallel decreases significantly ionized calcium concentration leading to a profound hypocalcaemia. Thus, the transport of calcium from the bones is not rapid enough to prevent the occurrence of hypocalcaemia in this clinical situation.

4.3.9 Calcium: importance of systemic ionized calcium measurements

Ionized calcium measurements are indispensable in citrate anticoagulation but should not be used to draw conclusions about calcium balance. Since calcium is readily distributed into the body stores (e.g. bones) neither the measurement of ionized calcium nor of total calcium in plasma are reliable parameters of calcium net balance during citrate anticoagulation.

Nevertheless, the importance of ionized calcium measurements for the monitoring and prevention of hypocalcaemia cannot be overemphasized. Hypocalcaemia can result from a dose of calcium lower than the removal into dialysate as well as from the high systemic citrate concentration.
Ionized hypocalcaemia due to the lower calcium substitution in comparison to the loss can develop relatively fast. In the current study an inadequate calcium delivery lasting only 15 minutes was associated with a 0.1 mmol/l decline in ionized and total calcium concentrations. This decrease is consistent with the expected theoretical decline that is a function of calcium net balance and the distribution volume equal to the extracellular water.

In contrast, hypocalcaemia associated with high systemic citrate concentration is not expected to develop rapidly. From the empirical nomograms it can be predicted that a citrate concentration of approximately 1.0 mmol/l is required to induce a measurable decrease of ionized calcium (from 1.2 to around 1.0 mmol/l) (Bolan et al. 2001; Bolan et al. 2002; Hester et al. 1983). During haemodialysis it takes some time to accumulate this relatively high level of citrate concentration in the patient's circulation.

As opposed to aphaeresis and transfusion of blood products, in haemodialysis treatments systemic citrate concentrations equal or higher than 1.0 mmol/l may be present without notable ionized hypocalcaemia due to calcium supplementation. In case of sufficient calcium infusion "the excess citrate" can bind calcium increasing total calcium concentration but not ionized calcium concentration and therefore leading to the increased ratio of total to ionized calcium. The increased ratio of total to ionized calcium has also been reported during citrate anticoagulation applied for continuous renal therapies. In 33% of CVVHD patients with liver failure an increased ratio of total to ionized calcium was detected but not in CVVHD patients with normal liver function. These data emphasize the likelihood of high systemic citrate concentrations in patients with liver failure treated by continuous renal therapies.

4.4 Kinetic concepts to reduce the risks

The safety of citrate anticoagulation is closely linked with maintaining an adequate concentration of ionized calcium in the patient's circulation. A sufficient calcium supplementation guarantees the safety for this anticoagulation method. During citrate anticoagulation calcium homeostasis is challenged by two mechanisms: filter removal of calcium and calcium binding by the increased citrate concentration in the systemic blood.
Thus, the requirement for calcium dose inevitably increases if (1) high losses of calcium into the dialysis solution occur, see table 8, or if (2) high systemic citrate concentration develops, see table 9.

Risk factors for high calcium removal into the dialysate
• High initial total calcium level
• High filter clearance high-flux filters high plasma water flow due to: high blood flow, low hematocrit
• High citrate concentration in the extracorporeal circuit or low protein concentration (a higher mobile fraction of calcium can be expected)

Table 8 Risk factors for high calcium removal into the dialysate during citrate anticoagulated haemodialysis.

Risk factors for high systemic citrate concentrations
• high citrate dose infused into the arterial line high blood flow long duration of citrate delivery-continuous treatments • low filter clearance for citrate low-flux filters low plasma water flow (high hematocrit, high proteins) low dialysate flow dialysate bypass mode decrease in the filtration area due to clotting of the dialyzer fibers • low distribution volume of citrate small body weight = small lean body mass = small total body water possible slow entry of citrate into deep compartments of distribution volume • impaired citrate metabolism (severe liver failure)

Table 9 Risk factors for high systemic citrate concentration during citrate anticoagulated haemodialysis.

It is worth noting that despite many possible influences on the citrate level the data of this study show that systemic citrate concentration can reliably be predicted from the mathematical equations. For every patient the approximate loss of calcium can also be anticipated using equation 77 (see chapters: 2.3.2.1, 3.2.4.4 and 4.3.6).

The complex regulation of calcium level by protein buffering and by the transport to the body stores makes the prediction of systemic ionized calcium concentration imprecise in particular due to the insufficient knowledge about the pharmacokinetics of calcium transport to the body stores. On the other hand the tight control of calcium metabolism may contribute to the good tolerance of citrate anticoagulation and to the safety of this procedure in the majority of patients. However, in theory the calcium maintaining processes can be impaired in some clinical states such as:

- Low initial total calcium
- Disturbances in pH of the blood (alkalosis)
- Critical states with ischemia or sepsis

For intermittent haemodialysis the pharmacokinetic data obtained during this study help to more precisely predict the “safe dose” of calcium supplementation in order to prevent hypocalcaemia or hypercalcaemia during citrate anticoagulation.

The current study highlights that the risk of hypocalcaemia in citrate anticoagulation with the use of high-flux filters is mainly associated with the calcium loss into the dialysate. In contrast, in all patients studied the systemic citrate concentration was too low to disturb the systemic ionized calcium concentration due to the effective release of calcium from buffering proteins. Therefore substituting calcium to the amount that equals calcium removal into the dialysate may be sufficient to prevent hypocalcaemia in most cases. Early publications concerning citrate anticoagulation in intermittent haemodialysis corroborate this suggestion. In these reports citrate

anticoagulation was performed successfully with calcium doses aiming to restore calcium removal by the dialyzer (Pinnick et al. 1983) (von Brecht et al. 1986).

The most probably malfunction of the citrate anticoagulation system that may induce a rapid hypocalcaemia in intermittent haemodialysis is associated with the occurrence of an unnoticed interruption of calcium supplementation. However, this risk can be minimized by the integration of the calcium pump into the set of alarms. For example; in the Prometheus® device there is a direct link between the calcium pump, the drop chamber in the calcium infusion line and the blood pump. In addition the occurrence of hypocalcaemia can most effectively be detected by frequent monitoring of ionized calcium. Monitoring should be particularly frequent during the first 40 minutes of the procedure when calcium homeostasis is challenged by the rapid increase in systemic citrate concentrations. Since some patients may have higher systemic citrate concentrations already at baseline every patient that undergoes citrate anticoagulation for the first time should be observed more carefully.

In the past, when the devices to measure ionized calcium were not readily available, citrate anticoagulation in intermittent haemodialysis was performed successfully without calcium monitoring (Faber et al. 1990; Janssen et al. 1993; Janssen et al. 1996; Lohr et al. 1988; Lohr et al. 1989; Pinnick et al. 1983; Seaton et al. 1983; van der Meulen et al. 1992; Wiegmann et al. 1987). However, such an approach is not advisable nowadays.

Since alkalosis can enhance calcium binding with proteins and therefore increases the risk of hypocalcaemia it is also important to avoid alkalosis by decreasing bicarbonate concentration in the dialysis solution. It is best to monitor ionized calcium together with the blood gas analysis. In the current study blood pH was kept within the normal range by decreasing bicarbonate concentration of the dialysate from a mean of 32 to 28mmol/l. This procedure is in a good agreement with other investigators (Apsner et al. 2001; Janssen et al. 1993; Janssen et al. 1996; van der Meulen et al. 1992).

With a “safe dose“ of calcium supplementation, i.e. at least the amount removed into the dialysate, a rapid development of hypo- or hypocalcaemia is not likely. In the current study the average dose of calcium (0.2 mmol/min) was higher than the filter removal for the majority of patients (13 out of 15 HD treatments). Despite the theoretical “overdosing” of calcium, hypercalcaemia was not observed. Therefore it can be concluded that infusing more calcium than is lost into the dialysate does not carry a risk of immediate hypercalcaemia in a stable patient. However, a calcium supplementation higher or lower than needed to maintain neutral calcium balance may lead to deleterious long-term effects in chronic dialysis patients. While a negative balance of calcium can result in bone resorption, a positive balance of calcium may lead to the vascular calcifications. Therefore, the optimum dose of calcium merits further consideration.

In liver failure patients pharmacokinetic calculations suggest that citrate anticoagulation during 4 hours of haemodialysis can also safely be used if high-flux filters are employed. The load of citrate for the body with the use of high-flux filters is relatively small and therefore the systemic concentration of citrate should not increase much in patients with severe liver insufficiency without citrate metabolism. Much higher citrate levels are expected if the liver works but the dialyzer doesn't eliminate citrate, (see figure 20 in chapter 3.3.2.6). However, the metabolic derangement typical for severe liver failure e.g. initial hypocalcaemia and hypoproteinemia may induce poor tolerance of citrate anticoagulation despite low citrate levels. If other risk factors for high systemic citrate levels, in particular a lower filter clearance for citrate coexists, citrate-induced hypocalcaemia may develop. The exact data on the calcium dose requirement in end-stage liver failure can only be elucidated by another pharmacokinetic investigation in this special patient population. At present careful monitoring of the patient with liver failure and the adjustment of the calcium dose according to systemic ionized calcium concentrations is mandatory.

Critically ill patients treated with continuous renal replacement therapies face numerous risks for the development of high systemic citrate concentrations that may outpace the body's compensatory mechanisms. The comorbid conditions of the critically ill patients can predispose to impaired citrate metabolism. A well-designed pharmacokinetic study evaluating citrate anticoagulation in continuous treatments has yet to be undertaken.

5 Conclusions

Regional citrate anticoagulation in combination with high-flux filters can be regarded as an ideal and safe method for haemodialysis patients with chronic or acute renal failure who are at increased risk of bleeding. However, the number of patients that can benefit from citrate anticoagulation is certainly higher. The excellent control of thrombus formation and the non-immunogenicity of citrate offer advantages over heparin for all haemodialysis patients. With the emerging evolution of new extracorporeal procedures (liver support therapy, LDL-apheresis) the number of patients that can be effectively anticoagulated by citrate anticoagulation is expected to increase further.

It should be emphasized that the kinetics of citrate and calcium concentration may differ considerably depending on the clinical features of the patient and on the treatment modalities. The knowledge of the pharmacokinetic principles of citrate anticoagulation is a useful tool for the safe and effective performance of this anticoagulation method. Therefore, new information on citrate fate, its metabolism and distribution volume should be of interest to all involved in the care of patients receiving different forms of extracorporeal therapies.

6 Summary

Regional citrate anticoagulation is an important alternative anticoagulation method for haemodialysis patients at high risk of bleeding. In order to better define the optimal dose of citrate and calcium infusion and to identify patients at risk for side effects, the kinetics of citrate and calcium were studied in vivo during and after 15 haemodialysis treatments and in vitro assessing the clearance of citrate-calcium complexes by F60S dialyzers. In addition, a mathematical model was developed to identify the parameters affecting calcium and citrate kinetics in order to predict citrate and calcium requirements in individual patients to avoid side effects.

Citrate and calcium doses for effective anticoagulation

Citrate dose should be adapted not only to blood flow (approx. 3 mmol per litre of blood) but also to hematocrit. Patients with low hematocrit need more citrate, because they have more plasma water per litre of blood. Sodium concentration in the dialysate of 138 mmol/l does not require any adaptation. Bicarbonate in the dialysate should be reduced by 4 mmol/l (e.g. from 32 to 28 mmol/l). Calcium supplementation should be at least as high as calcium removal by the dialyzer. Calcium removal is higher in patients with high initial total calcium, with use of effective high flux dialyzers, and with high blood flow. With use of high flux dialyzers previous recommendations of calcium supplementation in the literature may no longer be valid, because of the more efficient removal of calcium into the calcium free dialysate. Ionized calcium has to be monitored in the arterial line prior to citrate infusion for safety reasons, in order to detect any trend towards hypocalcemia.

Risk assessment

Citrate toxicity within the citrate concentration ranges that can be reached in intermittent haemodialysis is limited to the disturbances of ionized calcium concentration, mainly to the induction of hypocalcemia. Hypercalcemia rarely develops, probably due to calcium uptake e.g. by the bones. Hypocalcemia is potentially lethal and is the most important risk with use of citrate anticoagulation. During intermittent high-flux haemodialysis it is primarily due to calcium removal into the dialysate. Hypocalcemia develops rapidly (even within 15 minutes), if calcium substitution is interrupted and can reach life-threatening levels already after 1 hour in a small patient (45kg). All other possible causes for hypocalcemia are less important and less dangerous.

Special patient groups and continuous dialysis

Patients with liver failure have an increased risk to accumulate citrate, but removal by high-flux dialysis effectively limits accumulation. Slow continuous dialysis is more risky because of low citrate clearance. In this situation patients with liver failure may experience severe hypocalcemia. Patients with small body weight and thus smaller volume of distribution for citrate (e.g. children) can develop high citrate levels but can probably be managed safely with citrate anticoagulation if ionized calcium is closely monitored. The more precise estimation of the calcium requirements in these special patient groups needs further kinetic studies.

7 Summary in German

Die regionale Citrat-Antikoagulation ist eine wichtige Alternative für diejenigen Hämodialysepatienten, die ein hohes Blutungsrisiko haben. Um die Dosis von Citrat und Calcium besser definieren, und damit Komplikationen vermeiden zu können, wurde die Kinetik vom Citrat und Calcium sowohl in vivo während und nach 15 Dialysebehandlungen als auch in vitro durch die Clearancemessungen von Citrat-Calcium-Komplexen mit dem Dialysator F60S untersucht. Zusätzlich wurde ein mathematisches Modell der Citrat-Antikoagulation entwickelt. Mit diesem Model wurden die Faktoren, die die Kinetik beeinflussen, identifiziert, damit die verabreichten mengen von Citrat und Calcium zur Vermeidung von unerwünschten Wirkungen an die individuellen Bedürfnisse der Patienten angepasst werden können.

Citratdosis für die effektive Antikoagulation

Die Citratdosis sollte nicht nur an den Blutfluß (etwa 3 mmol/ pro Liter Blut), sondern auch an den Hämatokrit angepasst werden. Die Patienten mit niedrigem Hämatokrit benötigen mehr Citrat, weil sie mehr Plasma-Wasser pro Liter Blut haben. Eine übliche Natriumkonzentration im Dialysat von 138 mmol/l muss nicht geändert werden. Der Bikarbonatgehalt der Dialysatlösung sollte um 4 mmol/l reduziert werden (z. B. von 32 auf 28). Die Calciuminfusion sollte wenigstens den durch die Dialyse verursachten Calciumverlust ersetzen. Ein höherer Gesamtcalciumspiegel im Patientenkreislauf, die Verwendung eines high-flux Dialysators oder ein höherer Blutfluss sind mit einer grösseren Clearance und einem grösseren Verlust von Calcium verbunden. Die in der Literatur empfohlenen Calciumverabreichungen könnten nicht ausreichend sein, wenn ein high-flux Dialysator verwendet wird, weil mehr Calcium entfernt wird. Ionisiertes Calcium sollte durch die Messungen im arteriellen Schlauch überwacht werden um die Entwicklung einer Hypokalzämie frühzeitig erkennen zu können.

Riskoanalyse

Die Citratnebenwirkungen sind in der intermittierenden Dialyse auf Calciumstörungen, insbesondere auf Hypokalzämie beschränkt. Hyperkalzämie tritt sehr selten auf, vermutlich wegen der Calciumaufnahme z.B. in die Knochen. Hypokalzämie ist eine potentiell lebensgefährliche Komplikation, die in der Hämodialyse durch die Verwendung einer calciumfreien Dialysatlösung verursacht werden kann. Wenn bei der Hämodialyse mit Citrat-Antikoagulation die Calciuminfusion unterbrochen ist, tritt die Hypocalcemia sehr schnell auf (innerhalb von 15 Minuten) und kann bei kleinen Patienten (45 kg) schon innerhalb von 1 Stunde lebensbedrohliche Werte erreichen. Alle anderen möglichen Ursachen der Hypokalzämie sind weniger wichtig und weniger gefährlich.

Besondere Patientengruppen

Bei den Lebererkrankungen besteht ein besonderes Risiko der Citratakkumulation im Patientenkreislauf, wird jedoch durch die effektive Citratentfernung während der Hämodialyse begrenzt. Wegen der niedrigen Citratclearance ist die kontinuierliche Dialysetherapie riskanter. Bei diesem Verfahren kann Hypokalzämie bei Patienten mit Leberversagen auftreten. Eine geringe Körpergröße und das dadurch bedingte kleinere Citrat-Verteiligungsvolumen (z. B. bei Kindern) verstärkt das Risiko der Citratakkumulation. Trotzdem ist eine sichere Durchführung der Citrat-Antikoagulation unter Calciumüberwachung zu erwarten. Die genauere Schätzung des Calciumbedarfs in diesen Patientengruppen erfordert eine gezielte klinische Studie.

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