How to reduce the exposure to anticoagulants when performing haemodialysis in patients with a bleeding risk.

A study of methods used in clinical practise.

Malin Skagerlind
Runt flyger jorden i vått och torrt. Dess hastighet är förkräckande. Men inte en fluga tycks blåsa bort vilket är häpnadsväckande.

-Alf Henrikson 1995-
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Abstract

When a patient suffers from kidney failure and also has an enhanced risk of bleeding, the standard haemodialysis (HD) treatment becomes a problem. When human blood comes in contact with artificial material, as in the tubing system and in the dialyser (the extra corporeal circuit, ECC), the coagulation system is activated. If there is no increased risk of bleeding a bolus dose of anticoagulation is given intravenous to the patient before HD to avoid clotting. The most common anticoagulants used during HD are unfractionated heparin (UFH) and low molecule weight heparins (LMWH). Without anticoagulants there will be a total coagulation (clotting) of the blood in the ECC, an interrupted treatment and a blood loss of up to 300 ml for the patient. With an ongoing bleeding or an increased risk of bleeding in a patient that also needs HD, there are various alternatives that can be used to avoid or lower the need of anticoagulation. However, there is no golden standard, neither in Sweden or worldwide.

The overall aim of this Thesis was to evaluate the safety and the efficacy of various models of anticoagulation that may be used in patients with a bleeding risk.

The first study examined a low-dose anticoagulation model that was locally developed in Umeå, Sweden in the 1980s. The primary aim was to clarify to what extent this priming model was safe and efficient during intermittent HD for patients with a bleeding risk. Consecutive acute HD treatment protocols (248 procedures in 68 patients) were included. There were 178 patients with an increased bleeding risk who had their ECC (tubes, chambers and dialyser) flushed through (priming) with Heparin-Albunin-priming (HA-priming). There were 70 patients with no increased bleeding risk who received
standard intermittent HD (priming with saline); these patients also received a bolus dose of anticoagulation intravenous before dialysis.

The low-dose method entailed priming of the ECC with HA-priming with the intention to coat the surfaces with the solution and protect from blood to attach to it. Comparisons were made to dialysis in patients with no increased bleeding risk, who had received standard anticoagulation (SHD) with UFH or LMWH. The priming solutions were always discarded before HD was initiated. None or limited doses of UFH were added during the HD. There was no difference in extent of prematurely interrupted HA-primed dialysis compared to SHD (2.2 vs. 4.3%, p = 0.62). No secondary bleeding due to anticoagulation was reported in the protocols.

**Study 2** was performed to further clarify data in an extended group of acute intermittent HD using either HA-priming (885 treatments in 221 patients at risk of bleeding) or SHD (523 treatments in 100 patients with no bleeding risk who had received standard anticoagulation). In this extended study there was no difference in the extent of prematurely interrupted HA-dialysis (0.8%) compared to SHD (1%, p = 0.8). The results also showed less clotting for dialysers with a membrane area ≤ 1.7 m². No secondary bleeding due to anticoagulation was reported in the protocols.

**Study 3** was an experimental *in vitro* study. The aim was to compare the anticoagulation effect of priming the ECC with different concentrations of albumin and/or heparin in saline. Priming with saline only was also evaluated. The priming fluids were always discarded after priming. Fresh whole blood from healthy human donors was used to perform *in vitro* dialyses in a recirculation system. The donated blood was equally divided into two bags, whereas one bag represented the control group and the
other the intervention group. Priming with saline only and priming with albumin in saline resulted in rapid clotting of the blood in the ECC. These experiments indicated that HA-priming or priming with heparin in saline enabled fulfilment of all the in vitro dialyses.

**Study 4** was a clinical randomized cross-over study. The aim was to minimize the use of anticoagulant during HD in patients with a bleeding risk. Four different low-dose anticoagulation models were compared to SHD. Stable chronic HD patients participated in the study. The patients were their own controls. Aside from SHD, the four models of low-dose anticoagulation used were Heparin priming (H), HA-priming (HA), HA-priming in combination with a citrate containing dialysate (HAC), and a dialyser manufactured with a heparin-grafted membrane (Evodial®). The H-model was least suitable with 33 % interrupted treatments and the most extra doses of UFH needed. The HAC and Evodial® models were most preferable, both with an activated partial thromboplastin time (APTT) within references and with the least amounts of UFH needed. Evodial® had a lower urea reduction rate compared to the other models. HAC was the only model with no interrupted treatment. One patient suffered from a severe hypersensitivity reaction using Evodial®. No other side-effects were reported during the study.

**In conclusion** an acute kidney injury is a life-threatening situation that also includes patients with an increased bleeding risk and in need of HD for survival. If intermittent HD is the selected option, a priming of the ECC with a HA-solution in combination with a citrate containing dialysis fluid (HAC) is a safe and sufficient option for anticoagulation. Another option could be the heparin-grafted dialyser (Evodial®), although with a lower clearance coefficient and with a caution for a risk for hypersensitivity reaction or anaphylaxis.
Sammanfattning på svenska

När njurarnas funktion sviktar kan dialysbehandling behövas. Vid dialysbehandling utan någon förestående blödningsrisk administreras en dos blodförtunnande läkemedel intravenöst innan behandlingen startas. Detta minskar risken för att blodet ska koagulera i systemet.

Om det däremot finns en ökad risk för blödning uppstår ett dilemma: patienten behöver dialys utan att en blödning uppstår eller att en pågående blödning förvärрас.

När blod kommer i kontakt med främmande material aktiveras koagulationen. Det material som finns i de slangar, kamrar och det dialysfilter som blodet ska passera många varv genom under dialysen är just ett, för blodet, främmande material. Om blodet koagulerar i systemet kan det leda till att behandlingen måste avbrytas i förtid, effekten av dialysen blir sämre än planerat och patienten kan dessutom förlora 300 ml blod som inte går att återföra från slangarna.

Det finns olika metoder för att genomföra dialys utan, eller med en reducerad dos, blodförtunnande läkemedel. Det finns däremot ingen uttalad 'golden standard'.

Syftet med den här avhandlingen var att klargöra säkerheten och effekten, både vad gäller blödningsrisk och dialyseffekt, vid användande av Heparin-Albumin-priming (HA) - en metod som är utformad vid Norrlands universitetssjukhus i Umeå på 1980-talet.
Syftet var också att undersöka olika priming-lösningar in vitro varvid de med bästa resultat prövades i en klinisk studie där de jämfördes mot andra metoder.
Studie 1 utvärderade data från 248 behandlingsprotokoll från akuta dialysbehandlingar av 68 patienter. Jämförelse gjordes mellan dialyser av:  
- Patienter med en ökad blödningsrisk som därför fått dialyssystemet igenomspolat (primat) med HA-priming (HA, 178 st)  
- Patienter utan blödningsrisk som fick dialyssystemet primat med koksalt och fick en dos blodförtunnande läkemedel intravenöst (SHD, 70 st).  
Resultatet visade signifikant lägre heparindoser vid behandling efter HA-priming jämfört med SHD (medianvärden: HA 2000 enheter (E) mot SHD 5500E, p < 0.001).  
Det var inte någon skillnad i antal avbrutna behandlingar på grund av clotting: HA 2.2 % och SHD 4.3 % (p = 0.62).  
Ingen nytillkommen eller tilltagande blödning hade noterats på protokollen.

Studie 2 var en utvidgad studie av data från behandlingsprotokoll från 1408 akuta dialysbehandlingar av 321 patienter mellan åren 1997-2013. Syftet var att utvärdera resultatet från studie ett och att även utöka mängden data. Genom att inkludera flera olika dialysatorer kunde en eventuell variation över tid upptäckas. Jämförelser gjordes mellan:  
- HA-priming - 883 behandlingar med totalt 221 patienter som fick ingen eller minimal dos antikoagulation efter att systemet spolats igenom med heparin och albumin-priming.  
- SHD - 221 behandlingar med totalt 100 patienter som fick systemisk antikoagulation.  
Jämförelser gjordes också mellan olika material i dialysatorernas membran, membranens storlek (m²) och porstorlek, samt den bakomliggande orsaken till akuta dialysbehandling.
Resultatet visade att dialys efter HA-priming genomfördes med signifikant lägre doser heparin i genomsnitt (1200E) jämfört med SHD (5000E, medianvärden, p < 0.001). Trots signifikant lägre heparindoser med HA sågs ingen skillnad i antalet avbrutna behandlingar på grund av clotting (HA 1 % och SHD 0.8 %, p = 0.8).

Studien visade att det är större risk för clotting med en dialysator vars area är ≥1.7m². Det var ingen skillnad i clotting mellan olika membranmaterial eller mellan high-flux dialysatorer och low-flux-dialysatorer.

**Studie tre** var en experimentell in vitro-studie med syfte att utvärdera effekten av priming med:

- Olika koncentrationer av Heparin och Albumin tillsammans i koksalt.
- Heparin i koksalt
- Albumin i koksalt
- Priming med endast koksalt


Studien visade att priming med enbart heparin i koksalt samt HA-priming var goda alternativ. Priming med albumin i koksalt eller endast koksalt resulterade i total clotting av filer och dialysslang efter i genomsnitt 20 minuters dialys.

**Studie fyra** var en klinisk randomiserad cross-over studie. Syftet var att jämföra fyra olika metoder för att minimera tillförsel av blodförtunnande läkemedel.

Slangar, kamrar och dialysfilter primades med:
1) Heparin i koksalt (H-priming)
2) Heparin och Albumin i koksalt (HA-priming)
3) Heparin och Albumin i koksalt kombinerat med dialysvätska innehållande Citrat (HAC)
4) Koksalt i kombination med en dialysator som har heparin fixerat på membranytan (Evodial®).

Studiedeltagare var 23 personer med kronisk njursvikt som behandlades med regelbunden hemodialys. Deltagarna var sina egna kontroller genom hela studien.

För att få utgångsvärden genomfördes också en behandling med deltagarnas vanliga dos av heparin eller tinzaparin inom ramarna för studien.


Vid behandling med Evodial® fick en patient en kraftig överkänslighetsreaktion som medförde att behandlingen avbröts. Inga andra komplikationer tillstötte under studien.
Populärvetenskaplig sammanfattning.


En person som behöver akut dialysbehandling kan nyss ha genomgått en operation, varit med om en olycka eller fått en stor blödning (t.ex. hjärnblödning).

Personer med kronisk njursvikt som behöver regelbunden dialys kan också drabbas av en ökad blödningsrisk.

Vid ökad risk för blödning är det olämpligt att ge de vanliga doserna av blodförtunnande läkemedel eftersom det kan förvärra en pågående blödning eller kan framkalla en blödning som precis upphört.

I den vetenskapliga litteraturen finns ett antal metoder beskrivna för att minska eller helt undvika blodförtunnande läkemedel när en person med ökad blödningsrisk behöver dialys. Alla metoderna har olika fördelar och nackdelar. Det har inte gått att hitta någon samstämmighet för om någon metod är mer fördelaktig.

I Umeå utvecklade professor Bernd Stegmayr på 1980-talet en metod för att undvika avbrutna behandlingar och minimera mängden blodförtunnande läkemedel vid dialys av personer med ökad blödningsrisk. Metoden innebar att man löser albumin och heparin i koksalt (s.k. Heparin-Albumin-priming) och sedan spolar igenom slangar och filter med
den lösningen innan dialysbehandlingen. Vid behandling utan blödningsrisk används bara koksalt.

Hypotesen är att albuminet fäster på insidan av slangar och filter och att heparinet i sin tur fäster på albuminet och skyddar mot att blodet klibbar fast i systemet. Metoden har använts sedan dess men har inte tidigare jämförts med andra metoder.

De två första studierna i den här avhandlingen visade att de som fått systemet genomspolat med HA-priming behövde mindre än hälften så mycket blodförtunnande läkemedel för att behandlingen skulle kunna genomföras, jämfört med de som fått vanlig behandling. Cirka 20 % klarade behandlingen helt utan något blodförtunnande läkemedel då systemet spolats igenom med HA-priming före behandlingen. Det var inte heller fler avbrutna behandlingar fast de fick lägre doser blodförtunnande läkemedel.

I den tredje studien gjordes experiment med dialys av donerat blod. Systemet spolades igenom med olika koncentrationer av heparin och albumin i koksalt för att se om någon variant skulle vara mer fördelaktigt. En av de koncentrationer som ingick i försöken var HA-priming. Man provade också att spola igenom systemet med bara koksalt innan dialysen.

Det visade sig att bara koksalt eller koksalt med albumin inte fungerade. Systemet täpptes då till av koagulerat blod inom 20 - 30 minuter. De övriga olika koncentrationerna av heparin och albumin i koksalt samt heparin i koksalt klarade dialys i upp till tre timmar.

Med de tidigare studierna som bakgrund designades sedan en klinisk studie där man jämförde fyra olika metoder för att att minimera behovet av blodförtunnande läkemedel vid dialysbehandling.

Ett så kallat bifynd i studien: Det visade sig att heparin läcker ut i blodet då dialyskatetern hanteras före och efter behandlingen (i katetern lägger man heparin eller citrat mellan behandlingarna för att det inte ska bli koagulerat blod i den). En patient med ökad blödningsrisk bör ha något som inte är blodförtunnande i sin kateter mellan behandlingarna.
Abbreviations and definitions

Apoptosis: Cell death
AKD: Acute kidney disease
AKI: Acute kidney injury
APTT: Activated Partial Thromboplastin Time
AT: Antithrombin – inactivates enzymes that contributes to coagulation
ATP: Adenosine triphosphate – contributes to energy metabolism in cell processes
ADP: Adenosine diphosphate – contributes to energy metabolism in cell processes
AVF or AV-fistula: arterio-venous fistula
AVG or AV-graft: arterio-venous graft
Ca2+: ionized calcium
CDC: central dialysis catheter
Cl- : Chloride
CKD: Chronic kidney disease
CLS: catheter locks solution
CVVHD: continuous venous to venous haemodialysis
ECC: extra corporeal circuit
ESAO:
Factor X or Xa: A protein involved in the coagulation cascade
Factor XII: A protein involved in the coagulation cascade
Gauge: In this Thesis; outer diameter measure for needles
GFR: Glomerular filtration rate
H-priming: heparin-priming
HA-priming: heparin-albumin-priming
HAC: heparin-albumin-priming combined with a citrate-based dialysate
Hb: haemoglobin
HCO\textsubscript{3}: Bicarbonate
HD: haemodialysis:
HIT: Heparin induced thrombosis
HRQOL: Health related quality of life
ICU: Intensive care unit
INR: International Normalized Ratio – a measure for coagulation time
K+: Potassium
KDIGO: Kidney Disease Improving Global Outcomes
LMWH: low molecular weight heparin
LPL: lipoprotein lipase
Mg: Magnesium
Na+: Sodium
NSAID: Non-steroid anti-inflammatory drugs
PD: Peritoneal dialysis
PGI2: prostacyclin, a sort of prostaglandin that inhibits platelet aggregation and has a vasodilating effect.
Predilution: exchange fluid is added before the dialyser during the treatment
Priming: Rinsing of the ECC with saline or other fluids before treatment to remove as much air as possible from the system
RCA: Regional citrate anticoagulation
QB: Quantity of blood per minute - the blood flow in the ECC counted in ml/min
SHD: standard haemodialysis
TG: triglycerides
UF: ultrafiltration, removing water from the blood
UFH: unfractionated heparin
vWF: von Willebrands Factor
Original papers

This Thesis is based on the following papers:

I Fransson F, Kyrk T, Skagerlind M and Stegmayr B. Rinsing the extra corporeal circuit with a heparin and albumin solution reduces the need for systemic anticoagulant in haemodialysis. *Int J Artif Organs* 2013; 36 (10): 725-729

II Skagerlind M and Stegmayr B. Heparin albumin priming in a clinical setting for hemodialysis patients at risk for bleeding. *Hemodialysis international, September* 2016; 00:00-00 Accepted for publication.


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INTRODUCTION

To take care of a patient with an increased bleeding risk during haemodialysis is challenging. Since there is no established common standard for this procedure, there may be doubts about what method is the most safe considering the bleeding risk. There may also be doubts about what method results in least clotting and gives the most efficient removal of uraemic toxins and balancing high potassium levels. Ethical considerations may arise when a nurse performs dialysis in a patient with a bleeding risk since there is no consensus about the used methods. These concerns led to the studies that underlie this Thesis. Also ambitions were hopes of getting closer to universal guidelines and the possibility for providing the best care for patients with an acute kidney failure and bleeding risk, no matter where they live.
The kidneys
Most people have two kidneys that are positioned on each side of the spine and partly behind the lowest ribs on the back. The kidneys mainly filtrate waste products and concentrate fluid into urine. In this process the kidneys regulate the levels of fluid and ions, remove urea, creatinine and uric acid, and excrete other toxins and drugs. The hormone renin is produced by the kidneys and helps to regulate the water and salt levels as well as the blood pressure. The released hormone erythropoietin stimulates the bone marrow to produce erythrocytes that support increased haemoglobin levels and thereby ensures oxygen transport (Rippe 2004).

Chronic kidney failure
Chronic kidney failure (CKD) can proceed without pronounced symptoms down to a decreased kidney function of more than 50%. CKD is defined in five different stages Table 1, and the most common diagnoses are glomerulonephritis, diabetic nephropathy, nephrosclerosis and polycystic kidney disease. Symptoms for kidney failure can vary but common problems are hypertension, lower haemoglobin, retention of water and disturbed salt- and pH-balance (El Nahas and Arif 2015).

When there is < 10% of the kidney function left, a need for artificial cleaning of the blood is initiated to avoid death from uraemic toxins and water retention. This is performed by removing uraemic solutes from the body by dialysis or by a kidney transplant from a living or deceased donor.
Table 1: Stages for chronic kidney disease

<table>
<thead>
<tr>
<th>CKD</th>
<th>GFR (ml/min)</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;90</td>
<td>Normal and healthy</td>
</tr>
<tr>
<td>2</td>
<td>60 - 90</td>
<td>Decreased function without symptoms</td>
</tr>
<tr>
<td>3</td>
<td>30 - 60</td>
<td>Retention of phosphate Lower haemoglobin</td>
</tr>
<tr>
<td>4</td>
<td>15 - 30</td>
<td>Symptomatic kidney failure Deranged electrolytes Disturbance in calcium and phosphate levels Anaemia Metabolic acidosis Uraemic symptoms</td>
</tr>
<tr>
<td>5</td>
<td>GFR &lt; 15</td>
<td>Pronounced uraemic symptoms Fluid retention</td>
</tr>
<tr>
<td>5D</td>
<td></td>
<td>Dialysis/Transplantation</td>
</tr>
</tbody>
</table>

The stages defined by filtration rate and the definition of symptoms for each stage
CKD = chronic kidney disease
GFR = glomerular filtration rate and.

Acute kidney injury

Acute kidney injury (AKI) was first mentioned in the beginning of the 19th century by Heberden. In the beginning of the 20th century AKI was mentioned by Osler where it appeared during or after burning injuries, pregnancy, trauma, poisoning and surgery. During world war one AKI was called “war nephritis”. During world war two, 10% of the most severely injured patients developed AKI and more than 90% of these patients died. With time, the care of the injured was improved, which led to less developed AKI (Clyne 2015).
Today AKI often occurs when patients are within the hospital care. Advanced age, nephrotoxic drugs, sepsis, heart failure and endovascular interventions are known risk factors for AKI (Clyne 2015). The main reasons (Jefferson et al. 2015) for acute renal failure are divided into three main categories according to the location (Table 2).

**Table 2: Categories and location for acute kidney injury**

<table>
<thead>
<tr>
<th>Location</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre renal</td>
<td>In the course of septic shock, dehydration, pathologically low blood pressure and heart failure.</td>
</tr>
<tr>
<td>Renal</td>
<td>Damages in one or more parts of the kidneys structure. Hypertension Toxic substances Thrombotic angiopathy Vasculitis Epidemic nephropathy</td>
</tr>
<tr>
<td>Post renal</td>
<td>Obstructions in urinary tracts or external obstruction such as kidney stones or insufficient emptying of the ureter or urinary bladder.</td>
</tr>
</tbody>
</table>

**Poor survival in patients with AKI**

The survival of AKI has been reported to be low (Åhlström et al. 2005). In a large prospective multi-centre study of 1600 patients, it was reported that 35.5% of the patients with AKI in the ICU died within six months (Nisula et al. 2013). One study reported that of 172 included patients, 61 died in the ICU, 12 died on the ward, 5 died within 3 months and one patient died within 6 months; this resulted in 46% deaths in that study (Hofhuis et al. 2013). Rimes-Stigare et al. reported a 5-year-survival of 15-35% and that 10% of the survivals remained dialysis dependent (Rimes-Stigare et al. 2012). Noble et al. reported similar ICU-deaths (approximately 72%) in the AKI-group.
treated with intermittent HD vs. the AKI-group treated with continuous HD (Noble et al. 2006).
Dialysis in history

In 1854 Graham introduced the knowledge of diffusion and osmosis that was the base for the principle of dialysis (Stegmayr 2015). In 1913 Abel and Rowntree performed dialysis on animals (Kolff and Berk 1944). In 1924 and 1928 Necheles and Haas performed dialysis on humans. The treatment resulted in 2 grams of urea removed after a whole day of dialysis (Kolff and Berk 1944) (compared to today approximately 30 grams per 4 h HD for a 70 kg patient). In 1938 Thalheimer performed dialysis with cellophane tubes as a membrane and heparin as an anticoagulant (Kolff and Berk 1944). In 1942 Kolff performed the first dialysis in a young man. The machine was a dialyser in the shape of a large horizontal cylinder placed in a tank. Kolff wrapped the cylinder with cellophane that had been industrial manufactured. Heparin was used as anticoagulant, both in the machine and as given to the patient to prevent clotting in the ECC. In 1945 Kolff saved a woman’s life by treating her acute kidney failure with an 11-hour dialysis treatment (Vienken 2009).

The dialysis lowered the urea and the blood pressure, and reversed oedemas, but other problems occurred. After several treatments the patient had no veins available for blood access. Quote: “We believe we are able to keep patients that are suffering from uraemia and anuria alive so long as blood vessels for punctures are available” (Kolff and Berk 1944). In 1958 Jackson et al. reported several stable dialysis treatments (regarding complications) with the Kolff twin coil artificial kidney, using heparin as anticoagulant (Jackson 1958).
**Nils Alwall and the first artificial kidney.**

In Sweden, at Lund University, experiments *in vitro* started in 1942 by Alwall, Lembit, Norviit and colleagues. In 1946, in the department of medicine at the University hospital in Lund, Nils Alwall tested the stationary drum artificial kidney in rabbits. That same year the first acute dialysis was performed; heparin was used for anticoagulation. The blood was led through tubes of cellophane that were wrapped around a net. An artery and a vein on the patient’s lower arm were used as the blood access. However, this access could not be used more than a couple of times though (Stegmayr 2015). In 1948 Alwall created an arterial venous shunt as blood access for dialysis. The disadvantage was that it clotted after a few treatments due to the thrombogenicity of the materials available. In 1949 the first artificially manufactured kidney (a dialyser) in Sweden, was produced in the rubber factory Trelleborgs Gummifabrik, with support from the ironworks Avesta Jernverk that produced the cover of the dialyser. The dialyser consisted of stainless steel and cellophane tubes. In 1949 the first Swedish dialysis ward was established in Lund. The second dialysis ward was established 1958 in Umeå where patients from the entire northern part of Sweden, Norway and Finland came for treatment (Stegmayr 2015). In 1960 Alwall initiated a long-term haemodialysis program, but the patient survival was short – less than five months. When the idea of the arterio-venous fistula came up in 1966 by several researchers, dialysis treatments increased dramatically, and in 1969 there were 100,000 treatments per year in Sweden, compared to 3,500 in 1965 (Kurkus et al. 2007).
Dialysis today

There are two main dialysis paths – haemodialysis (HD), which requires access to the patient’s blood, and peritoneal dialysis (PD), which requires access to the patient’s abdominal cavity through a permanent catheter.

Haemodialysis

Most uraemic toxins, such as creatinine and urea, are removed by dialysis (Arund et al. 2016). Aside from uraemic toxins and water, potassium and phosphate are important substances to remove. Today the efficacy of HD is roughly measured with the urea reduction rate (URR) – the percentage $(\text{Urea}_{\text{after}}/\text{Urea}_{\text{before}})$ change in urea during a HD session - with a wish of a reduction of at least 70%. There is also an efficacy equation expressed as $Kt/V$: $K$ represents the urea clearance and $t$ represents the duration of dialysis. These ($K$ and $t$) are divided with $V$ that represents the volume of urea distribution and is equal to the patient’s total body water mass. A recommended $Kt/V$ is > 1.2 today (KDIGO 2012; Sridharan et al. 2016). Techniques for evaluating removal of toxins and other substances during HD have improved, and the availability of a direct effect measure from the waste water may be possible in the future (Enberg et al. 2012; Holmar et al. 2015; Uhlin et al. 2006).

The blood access

The blood access is the patients “life line” and must be carefully taken care of to maintain its function. It must also be protected from contamination of bacteria; this is done by using aseptic techniques when being handled (Böhler and Fischer 2004).
To lead the blood from the patient into the extra corporeal circuit (ECC), an access to the blood is needed. The access needs to enable a high blood flow (QB) within the ECC for an efficient dialysis treatment, i.e. a QB of 200 - 400 ml/min is needed. The blood access alternatives are either a central dialysis catheter (CDC) or an arterio-venous fistula (AVF).

Central dialysis catheter

A so-called short time central dialysis catheter (CDC) is the most common access during acute intermittent HD. The CDC is placed in a large vein (the jugular, femoral or subclavian vein) using local anaesthesia, and can be used immediately after insertion. The CDC has two lumens lying side by side - one called the arterial lumen the other called the venous lumen (Image 1).

After the treatment the lumens are flushed with saline and an antithrombotic and/or antiseptic catheter lock solution (CLS) is injected into each lumen. The CLS in most cases consists of heparin or a citrate solution (with or without heparin) and is left within the lumens of the CDC between the treatments to protect from blood clot formations and infections (Böhler and Fischer 2004).

Image 1: A CDC with the red arterial lumen and the blue venous lumen side by side.
**Arterio-venous fistula**

Patients with chronic intermittent HD preferably have an arterio-venous fistula (AVF). An AVF is a vein and an artery that are surgically connected to each other on the lower or upper part of the patient's arm. After approximately two months the venous part of the fistula has grown in size and has a larger diameter and a high internal blood flow. Before HD the AV-fistula is punctured with 17-15 gauge (≈ 1.4 - 1.8 millimetre) dialysis needles - one needle in the “artery part” (the distal part of the vein, toward the artery) and one needle in the proximal venous part (toward the heart). After the treatment the needles are removed and light pressure with sterile swabs is applied until the puncture site bleeding stops. For the next treatment a new puncture is made either in the same place using a blunt needle (so-called button hole technic) or with a sharp needle in almost the same place.

An alternative to AVF is an arterio-venous graft (AV-graft) where a loop formed synthetic vessel is surgically connected to an artery and a vein. The AV-graft can be used within two weeks after surgery. The haemodialysis treatment with an AV-graft is the same as with an AVF; the difference being that there is a new puncture site for each treatment using a so-called rope ladder technique.

**The blood in the extra corporeal circuit**

From a CDC or an AV-fistula the blood is sucked out from the arterial lumen/needle and into the ECC where it is pumped through the arterial part of the ECC and into the dialyser where it is filtered. Then the filtered blood goes out from the dialyser into the venous part of the ECC and back to the patient through the venous lumen/needle (**Figure 1**).
Since a CDC is narrower than an AVF, the achieved blood flow is primarily less. An AVF can have an internal blood flow of more than 1500 ml/min and allowing dialysis with a QB of up to 400 ml/min and sometimes higher. A higher QB rate gives better clearance over a shorter time (Böhler and Fischer 2004). A higher QB has also been associated with less clotting problems (Locatelli et al. 2004). The QB rate is mainly limited by the venous and arterial pressures that usually are set to a limit of ± 200 mm Hg (Polaschegg and Levin 2004).

**Figure 1: A schematic illustration of the extra corporeal circuit**
Blood goes from the patient, driven by a rotating pump, through the dialyser, and back to the patient. The dark blue arrow represents the inflow of dialysate and the light blue arrow the outflow of dialysate.

**The dialyser**

The dialyser is a semipermeable membrane with blood flowing on one side of the membrane and dialysate on the other side of the membrane. Thereby uraemic toxins and electrolytes are removed with diffusion (the striving for equal concentrations of substances on both sides) through the hair thin capillaries the membrane consists of (Image 2). These capillaries have pores, microscopic holes, through which the molecules seep (Figures 2 and 3).
Figure 2: Diffusion and diffusion + convection
A dialyser is shown in the middle. The red arrows represent the in- and outflow of the blood. Diffusion is illustrated on the left; molecules (pink dots) wander through the membrane striving for equal concentrations on both sides. Convection is illustrated to the right; molecules follow the excess water (blue dots) that is pressed through the membrane pores. This figure is used with kind permission from Fresenius Medical Care, Sweden.

Figure 3: Schematic illustration of one of the hair-thin capillaries in the dialyser
The blood passes through the capillary and waste products (molecules like urea, creatinine and potassium) seep through the pores and are carried with the dialysate out to the waste.

Excess fluid in the blood is removed with ultrafiltration (UF). The mechanism of UF is a difference in pressure between the blood and the dialysis fluid. With a higher pressure on the “blood side”, water molecules are forced away from the blood and out into the ‘dialysate side’ of the membrane and goes out into the waste. This is called the transmembrane pressure. During UF there is also a convection that causes substances
from the plasma to pass through the membrane with the water molecules (Furuland and Wikström 2008; Weiss 2015). UF can also be made without diffusion, and is then called isolated UF. Isolated UF is used if there is a large amount of excess fluid to remove.

Image 2: A dialyser sawed in half
Showing the membrane material – the hair thin capillaries that the blood flows through.

Quote translated: "When a sausage that is very salty is immersed in a liquid, preferably a running liquid, the salt seeps out through the casing. A requirement for this is the concentration difference of salt on the inner vs. the outer surface of the casing. If placed in a solution with a very high salt concentration, the salt from the liquid migrates into the sausage. This well-known process that is common in kitchens of many housewives is called dialysis in scientific language. Dialysis is a combination of the Greek words dia (through) and lysis (loosening). The term was coined in the second half of the 1800s and was based on laboratory studies that showed substances seeping through thin membranes of various materials."
**The semipermeable membrane**

At the time that Nils Alwall described the principle for dialysis, the skin of sausages was made from pig intestines. The intestines functioned like today’s semipermeable membranes in dialysers – molecules up to a certain size are allowed to pass through the membrane pores in both directions (Figure 4).

**Figure 4: The principle of a semipermeable membrane**

On the left side the blood flows (red arrow). On the right side the dialysate flows (blue arrow) in an opposite direction. There is a higher concentration of molecules (uraemic toxins, electrolytes) on the left side. The blood and the dialysate flows on separated sides of the semipermeable membrane through which the molecules can move in both directions (purple arrow), in this case from left to right striving for equal concentrations on both sides (diffusion).

Dialysers exist in different sizes and with different membrane materials. Synthetic materials are most common nowadays, whereas previously cellulose and modified cellulose were used (Ronco et al. 2004a).

**Biocompatibility of the dialyser**

The complement system and the coagulation system acknowledge contact with artificial material as when tissue is injured. The activations that these systems induce are aimed at preventing blood loss and infection. The complement system is activated via the lectin pathway (a cascade of activated proteins) as well as leucocytes such as granulocytes when blood gets in contact with the dialyser (Wiegner et al. 2016).

There is a significant neutropenia that occurs during the first 15-30 minutes of intermittent HD (Mares et al. 2010; Stegmayr et al. 1992). Some neutrophils migrate
and affect the lungs, causing a mild pulmonary dysfunction during the treatment. The coagulation system is activated via the intrinsic pathway - so-called contact activation. This reaction is also strongly related to inflammation (Wiegner et al. 2016).

Uraemic neutrophils have an increased tendency for apoptosis (cell death). This apoptosis was shown to decrease when switching from a dialyser with a hemophan membrane to a dialyser with a polysulfone membrane (Hörl 2007; Lundberg et al. 1994; Stegmayr et al. 1992; Uhlenbusch-Körwer 2004a). A less complement activating membrane is preferable, especially during HD of severely ill patients. In addition, Mares et al. showed that proteins adsorb to polysulfone dialysers through the lectin pathway (Mares et al. 2009). Studies have shown a close interaction between the complement activation system and the coagulation system (Wiegner et al. 2016). For an average patient approximately 10 000 litres of blood are exposed to 300 m² of artificial membrane during more than 600 hours each year in dialysis (Mares et al. 2009).

The dialysate

The dialysate (dialysis fluid) is partly composed to adjust for some essential electrolytes (Table 3). The concentration of the dialysate may vary based on the prescription. The dialysate enables removal of waste products and adjusts the substance levels in the patient’s blood toward the levels of these substances in the dialysate. The choice of dialysis fluid is based on the patient’s needs and also on laboratory data. For example, a patient with a high potassium level is prescribed a dialysate with a low potassium concentration so as to achieve a more substantial removal of potassium. The final concentration of the dialysis fluid is mixed in the dialysis machine by blending the fluid with highly purified water (e.g., proportions 1:44) (Bonnie-Schorn et al. 1998; Grassman et al. 2000).
### Table 3: Example of concentrations of contents in a dialysate after mixing with bicarbonate and purified water

<table>
<thead>
<tr>
<th>Content</th>
<th>Concentration after mixing with bicarbonate concentration and 8.4% purified water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$ (mmol/L)</td>
<td>138</td>
</tr>
<tr>
<td>K$^+$ (mmol/L)</td>
<td>2</td>
</tr>
<tr>
<td>Ca$^{2+}$ (mmol/L)</td>
<td>1.25</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cl$^-$ (mmol/L)</td>
<td>108.5</td>
</tr>
<tr>
<td>HCO$_3^-$ (mmol/L)</td>
<td>32</td>
</tr>
<tr>
<td>Acetate (mmol/L)</td>
<td>3.0</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$Na^+ = $ sodium, $K^+ = $ potassium, $Ca^{2+} = $ ionized calcium, $Mg = $ magnesium, $Cl^- = $ chloride, $HCO_3^- = $ bicarbonate

### Different types of haemodialysis treatments

**Continuous haemodialysis**

In the intensive care units (ICU) haemodialysis is often performed by using a continuous treatment called *continuous venous to venous haemodialysis* (CVVHD). The principle for the blood transport is the same as described above with blood access and an ECC including a dialyser. The CVVHD treatment proceeds for 24 hours/day and if necessary continues for days. Kramer was first to describe this method in 1977. At that time it was used as a principle for removing fluid when a diuretic treatment had no effect. The patient's own blood pressure was the mechanism for pumping the blood through the ECC and the dialyser. Nowadays the machines have been developed to function in both monitoring and as a blood pump (Furuland and Wikström 2008).
CVVHD treatment is often referred to as a good alternative for patients with haemodynamic failure (Paganini and Marshall 2007; Ronco et al. 2004b).

**Intermittent haemodialysis**

This treatment gives a more effective filtration and fluid removal within a shorter period of time than for CVVHD. The principle for the treatment is the same as described above. In a patient with end stage kidney failure intermittent HD is performed at a dialysis centre 2 - 5 times a week and 2 - 5 hours per time depending on the patient’s need. There are also patients that manage their own intermittent HD either in a dialysis unit or in their own home. These patients often choose to do treatments more frequently to keep the toxins, electrolytes and excess fluid at a constant low level. Some patients prefer nocturnal dialysis so as to have their days free (Furuland and Wikström 2008).

**CVVHD and intermittent HD – what are the differences?**

CVVHD and intermittent HD both have its pros and cons. Intermittent HD gives a more effective removal of uraemic waste products and excess fluid (in relation to a shorter duration of treatment) and the patient is not bound to the machine for more than 3 – 5 hours. If hypotension appears due to extensive ultrafiltration, within a short period the intermittent HD sessions can be scheduled more frequently, and the treatment time can be lengthened. It is also possible to counteract hypotensive episodes by regulating the sodium concentration in the dialysate. Newer dialysis machines can monitor the patient’s blood volume and adjust both UF rate and sodium levels to those required so as to avoid hypotension. There are also new techniques that may help to prevent
episodes of hypotension (Ahlstrom et al. 2005). A higher extent of UF per hour causes a higher haematocrit and thereby an increased risk for clotting.

CVVHD gives a slower continuous waste removal by filtration, dialysis or a combination of both methods. The fluid removal is not as extensive per hour as for intermittent HD, and continuous removal over 24 hours is allowed. A slower fluid removal rate may reduce the risk for hypotensive episodes. CVVHD also allows adding more replacement fluid continuously and keeping a controlled balance between added and removed fluid (Clyne 2015).

Studies have shown that there is no difference in mortality between the two treatments (Paganini and Marshall 2007; Truche et al. 2016; Vinsonneau et al. 2006). A meta-analysis of 15 randomised controlled trials of 1550 treatments in patients with AKI showed no differences in mortality, hospitalization time or renal recovery when comparing intermittent HD and CVVHD (Rabindranath et al. 2007).

Truche et al. reported that CVVHD might be more beneficial for patients with excess fluid and an instable blood pressure. These authors also concluded that intermittent HD is suggested for patients with haemodynamic instability (Truche et al. 2016). However, according to Vinsonneau et al. such a restrictive attitude toward intermittent HD is not warranted. These authors compared two groups of patients with multiple-organ failure who received either CVVHD or intermittent HD. Their conclusion was that almost all of these patients could be treated with intermittent HD. They did not observe any differences in haemodynamic tolerance between the two treatment types (Vinsonneau et al. 2006).
The work group Kidney Disease Improving Global Outcomes (KDIGO) concluded that there was no ideal general choice for patients with AKI, and that the total individual circumstances must be considered in each case (KDIGO 2012). The blood-membrane interaction causes a stress on the inflammatory physiological systems during a long-term interaction - such as during CVVHD. Neutrophils, macrophages (these secrete TNF-α, a cytokine that causes inflammation and promotes coagulation) and platelets are affected more by CVVHD than by intermittent HD due to the longer period of time for CVVHD (Opatrný et al. 2002).

Peritoneal dialysis

Peritoneal dialysis (PD) is a method where the peritoneum (a smooth tissue membrane that covers most of the organs in the abdomen) is the dialyser. Through a permanent catheter a dialysate is led into the abdomen where it remains for 3 - 4 hours. When the dialysate is tapped out, it contains uraemic toxins and excess fluid.

The concentration of the dialysate, the amount of dialysate (1.5 - 3 L) and how often the fluid has to be changed are individually prescribed by the needs of the patient. The patients mainly manage the treatment themselves at home and can also perform the fluid changes at work or other locations. Most patients do cyclic fluid exchanges three to five times/day. Some patients keep fluid in the abdomen overnight. Another option is a machine that controls for automatic fluid exchanges during the night. Some patients need so-called “assisted PD” if for a reason they cannot take care of the dialysis themselves for a period of time.
**Peritoneal dialysis in patients with acute kidney injury**

PD can be a choice of dialysis in patients with AKI (Stegmayr 2008). There is not sufficient data for examining differences in mortality when comparing PD to blood dialysis methods in these patients (Chionh et al. 2013). During PD there is no need of anticoagulation, which makes this choice of therapy suitable for patients with a bleeding risk. A direct treatment start after operation/insertion is possible with only a few complications documented such as leakage or obstruction (Stegmayr et al. 2015).

**Transplantation**

A kidney transplantation (a kidney from a living or a deceased donor) enables a more permanent or long-term treatment to remove uraemic waste products (Makaroff et al. 2013). An acute kidney transplantation usually requests a living donor, mainly relatives to children that are prepared in advance. The transplantation unit in addition needs to be on alert. Acute kidney transplantations are therefore rare, i.e. cannot be taken for granted.

Studies have concluded a higher quality of life in the group of patients that received a transplant compared to those who undergo intermittent HD (Landrenau et al. 2010). However, the period of waiting for a transplant that can be up to four years or longer is challenging for the patient. The need of support, and help in maintaining hope is a major task. The patients also need to be prepared for life after transplantation. After several years with regular dialysis treatments this can be somewhat of a crisis for these patients. The process does not end immediately with the transplantation (Yngman-Uhlin et al. 2016).
Epidemiology in Sweden 2015

In the end of 2015, the prevalence for CKD in active uraemic care (dialysis and transplanted) was 953 per million inhabitants in Sweden. The incidence during 2015 was 114 per million inhabitants.

The mean age for patients in active uraemic care has increased every year (Table 4).

Table 4: The mean age for patients in active uraemic care

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean age men</th>
<th>Mean age women</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>2000</td>
<td>57</td>
<td>56</td>
</tr>
<tr>
<td>2010</td>
<td>59</td>
<td>58</td>
</tr>
<tr>
<td>2015</td>
<td>60</td>
<td>59</td>
</tr>
</tbody>
</table>

The mean age for patients on intermittent HD has increased over the years, e.g. 60 years in 1990 compared to 66 years in 2015. Overall, there is an unequal distribution between men and woman among patients in active uraemic care; 64% are men and 36% are women. The reason for this has not been explained, but more men suffer from diagnoses such as IgA nephritis.

Of the 9391 patients in active uraemic care, 3090 (33%) were treated with HD, 813 (9%) with PD, and 5488 (58%) were living with a transplanted kidney. The number of persons living with a transplanted kidney has increased with 64% over the last 20 years. There were 724 new patients in HD during 2015, while the patients treated with PD decreased with 7 patients. The mortality among patients in dialysis care each year is almost 20%, and the main cause of death is cardiovascular.
Over many years diabetic nephropathy has been the most common diagnosis leading to a need of HD (prevalence in 25% for recent years). While diabetes mellitus is now less frequent for causing uraemia, about 40% of the new patients have diabetes as a dominating or contributing cause of uraemia (Svenskt njurregister 2016). Table 5 shows the distribution of diagnoses of patients in active uraemic care.

Table 5: The mean distribution of diagnoses for the patients in active uraemic care in Sweden 2015

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulonephritis</td>
<td>25</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>18</td>
</tr>
<tr>
<td>Adult polycystic kidney disease</td>
<td>10</td>
</tr>
<tr>
<td>Hypertonia</td>
<td>4</td>
</tr>
<tr>
<td>Uraemia with no further explanation</td>
<td>11</td>
</tr>
<tr>
<td>Other diagnoses</td>
<td>23</td>
</tr>
</tbody>
</table>
**Coagulation**

The coagulation system is activated by either the extrinsic or the intrinsic pathway.

The intrinsic pathway is activated when the blood gets in contact with artificial materials and is also related to inflammatory processes. Both pathways activate factor X and prothrombin. Factor X and prothrombin activate fibrin that forms clots together with activated platelets. On an artificial surface this results in a fibrin layer (Wiegner et al. 2016).

**Coagulation under influence of uraemia**

Uraemic coagulation disorder was first mentioned in 1877 by Bright (Winchester and Lindsay 2004). Bleeding is not uncommon among chronic dialysis patients and has been reported in 40 – 50% of cases. Uraemia results in platelet dysfunction. This can lead to petechiae, nose bleeding, gastrointestinal and gingival bleeding, and prolonged haemorrhage after needle puncture and from postoperative sites. The platelets in uraemic patients contain higher levels of nitric oxide (as compared to healthy people) that inhibit platelet aggregation. There is also an increased platelet breakdown in patients with renal failure. HD contributes to an increased platelet dysfunction via the complement activation during the treatment. But HD also results in lower levels of uraemic toxins and thereby less platelet dysfunction caused by the toxins (Lutz et al. 2014; Wiegner et al. 2016).

The von Willebrand factor (vWf) is elevated in uraemic patient, and it has a disturbed metabolism, structure and function. The vWf is needed to bind platelets to collagen in the skin in case of a skin bleeding. The uraemic vWf elevation results in a shortened bleeding time (Lutz et al. 2014; Wiegner et al. 2016).
In the pre-dialysis phase a correlation between serum creatinine and risk of bleeding has been seen (Hörl 2007).

The Hageman FXII that is activated by negative loaded surfaces is rapidly adsorbed on artificial material and induces the coagulation cascade (Wiegner et al. 2016).

Erythrocytes lead to a concentration of platelets along the vessel walls within the blood flow along with a stimulation of platelet ADP release and an inactivation of PGI2, thus stimulating platelet function. Thereby, anaemia may contribute to platelet activation (Lutz et al. 2014).

Drugs may also interact with the coagulation. Some antibiotics like beta-lactam can contribute to a disturbed platelet function. Aspirin is common in patients with CKD and can lead to a longer bleeding time. This prolonged bleeding time is more pronounced in patients with kidney failure compared to those with normal kidney function (Lutz et al. 2014).
**Anticoagulation during intermittent haemodialysis**

In 1912-1913 when Abel, Rowntree and Turner performed dialysis on animals, clotting was prevented with an extract from leeches that was added to the blood when it passed the tubes (Alwall 1984). In 1944 Kolff et al. stated that clotting could be prevented by heparin. In 1955 Kolff debated about the need of finding a way to eliminate the danger of heparin. He suggested sodium citrate or a calcium-binding resin as an anticoagulant alternative (Kolff 1957).

There is no consensus about anticoagulation during haemodialysis among the available guidelines. Kessler et al. concluded this after investigating the guidelines from nephrology societies in Europe, United Kingdom, United States, Canada, Australia, and Japan. The recommended substances as well as dosage differ between these guidelines (Kessler et al. 2015).

**Unfractionated heparin**

The most common anticoagulant drug used during acute intermittent HD is unfractionated heparin (UFH) (KDIGO 2012). In 2016 an article celebrated 100 years since Heparin was discovered (Torri and Naggi 2016). In 1916 McLean discovered it, but it was then called cephalin (McLean 1916). In 1918 Howell renamed the substance to heparin (Howell and Holt 1918). The clinical use of heparin as anticoagulant was developed in Sweden by Jorpes and Crafoord, and in Toronto, Canada by Murray and Best during the 1930s (Hirsh et al. 2001; Mätzsch 2010).

Heparin is a polysaccharide of glycosaminoglycan type with a molecular weight of 15,000 Da (3000-30000) (Yates and Rudd 2016). UFH is distributed as an intravenous injection. When used during intermittent HD a bolus injection is given at treatment start.
and intermittent infusion is needed during the treatment since the half-life of UFH is 60 - 120 minutes. UFH binds to the plasma co-factor antithrombin (AT). Without UFH, AT is a slow inhibitor of coagulation. When UFH binds to AT, AT is converted into a fast inhibitor. The heparin-AT-complex inactivates a number of coagulation enzymes. The most extensive inhibition is on thrombin – the ‘key clotting enzyme’ that activates platelets and factor Xa. Inhibited thrombin prevents fibrin formation. The lab test activated partial thromboplastin time (APTT) monitors how UFH inhibits thrombin, factor Xa and factor IXa. The relation between APTT level and the effect of heparin has been questioned lately, but is still the most convenient way to monitor the effect of heparin mainly due to the lack of alternatives. The bleeding risk increases with the dose of heparin since the half-life of heparin rise with the dose size (Hirsh et al. 2001).

**Low molecular weight heparins**

Low molecular weight heparin (LMWH) is a group of more selective anticoagulants with up to double half-life compared to UFH and with a 90% bioavailability. The mean molecular weight is one third of UFH (Dorobantu and Bogdan 2016). LMWH is eliminated by 70% through the kidneys (UFH by 40%). This gives a greater risk of accumulation in a patient with a severely decreased kidney function according to KDIGO. Other authors report that LMWH tinzaparin was shown to be eliminated in ways other than through the kidneys, and that it is not likely that tinzaparin will accumulate in patients with renal failure (KDIGO 2012; Lutz et al. 2014).

LMWH affects the platelet function less and has a lower affinity toward endothelial cells, macrophages and plasma proteins in comparison to UFH. Also, LMWH has a more predictable dose response compared to UFH (Torri and Naggi 2016). The disadvantage
is that there is no existing antidote for LMWH. The antidote for UFH, protamine, partly helps to reduce the effect of LMWH (Carlsson 2013; Mätzsch 2010).

**There is no golden standard for anticoagulation during acute HD**

Although studies are lacking, many European centres are using LMWH instead of UFH when performing HD in patients with AKI (KDIGO 2012). There is no consensus in the guidelines (Kessler et al. 2015).

‘The European Best Practice Guidelines’ advocates LMWH as a first choice while the ‘British Renal Association’ recommends UFH. ‘The National Kidney Foundation’ claims that there are limited data for clinical use of LMWH. ‘The Caring for Australians with Renal Impairment’ points out uncertainties in differences between LMWH and UFH regarding haemorrhage, thrombosis and HD (Kessler et al. 2015). A British study reported no difference in bleeding rates between two groups of patients using UFH and LMWH. For UFH and LMWH, respectively, the incidence of major bleeding was reported to be 1.33% and 1.92%, and for clinically significant bleeding to be 3.33% and 3.96% (Nadarajah et al. 2015).

In the US LMWH is not at all approved for medical use (Kessler et al. 2015).
Side effects from heparin

Besides bleeding, heparin-induced thrombocytopenia (HIT) is a complication of heparin therapy that appears with an incidence of 3% in all patients that receive UFH or LMWH (Greinacher 2015). HIT must be suspected when a patient who receives heparin has a decreasing platelet count, particularly if the fall is by more than 50% of the baseline count, even if the platelet count nadir remains above 150 x 10⁹/L. Clinically, HIT may manifest as skin lesions at heparin injection sites or by acute systemic reactions (e.g., chills, fever, dyspnoea, chest pain) after administration of an intravenous bolus of heparin (Warkentin et al. 2003).

There are two types of HIT; type 1 HIT presents within the first two days after the exposure to heparin and is a non-immune disorder that results from the direct effect of heparin on platelet activation. The platelet count normalizes with continued heparin therapy (Greinacher 2015; Warkentin and Greinacher 2004).

Type 2 HIT is an immune-mediated disorder that occurs four to ten days after the exposure to heparin. It is accompanied by life- and limb-threatening thrombotic complications (Antovic and Holmström 2013; Hörl 2007; Warkentin and Greinacher 2004). In general medical practice, the term HIT refers to HIT type 2.

Another side effect of heparin administration is the induction of release of lipoprotein lipase and hepatic lipase from its binding sites. This results in a temporary decreased ability of the body to metabolize triglyceride into free fatty acids and energy (Stegmayr 2014).
Regional citrate anticoagulation

Regional citrate anticoagulation (RCA) was first mentioned in 1961 by Morita et al. (Buturovic-Ponikvar 2016). The citrate binds to calcium ions, which results in a reduced function of the coagulation system. During intermittent HD, RCA is performed with an infusion of trisodium citrate (half-life 30 - 90 minutes) on the arterial side of the ECC. Before the blood is returned to the patient, calcium is infused into the blood on the venous side of the ECC, after the dialyser, to reverse the anticoagulant effect of the citrate and to avoid hypocalcaemia (Buturovic-Ponikvar et al. 2008; Paganini and Marshall 2007). During CVVHD RCA is often performed with an infusion of trisodium citrate or anticoagulant citrate dextrone A. These substances are continuously infused on the arterial side of the ECC. Calcium is infused on the venous side, after the dialyser, to avoid hypocalcaemic side effects. RCA requires frequent monitoring and intermittent substitution of calcium - a careful and frequent surveillance by the nurses is demanded to avoid hypocalcaemia (Brandl et al. 2012; Kozik-Jaromin et al. 2009; Paganini and Marshall 2007). The common citrate levels in RCA-protocols are 3 - 5 mmol/L with an ionized calcium level at 0.1 - 0.4 mmol/L. At citrate levels from 6 mmol/L, ionized calcium is 0.1 mmol/L or less and coagulation is totally inhibited (Fiaccadori et al. 2015). Metabolic complications (alkalosis, acidosis, hypocalcaemia and hypernatremia) as a side effect from the citrate infusion may be avoided since the citrate has a molecular weight of 294 Da. This allows up to 70% of the citrate to be dialyzed away from the ECC when using a high-flux dialyser (Fiaccadori et al. 2015). The sodium and bicarbonate levels can be adjusted in modern dialysis machines and hypocalcaemia is prevented with a calcium infusion as described above. There are special modules available for RCA during CVVHD. These are not yet available for intermittent HD machines. If RCA is used for intermittent HD, there is a need of availability of infusion pumps, calcium free dialysate and ion meters in combination with
good anticoagulation protocols and trained nurses (Buturovic-Ponikvar 2016). A recent *in vitro* study showed that the target for Ca\(^{2+}\) levels during RCA should be 0.2 - 0.25 mmol/L for efficient anticoagulation. That study also reported that magnesium (Mg\(^{2+}\)) might support the coagulation when the Ca\(^{2+}\) decreased. Furthermore, a decrease in cytokine release when the citrate concentration increased was also reported (Strobl et al. 2017).

In summary, there is no golden standard for anticoagulation in patients on chronic haemodialysis.
Haemodialysis treatment in patients with a bleeding risk

A recent review study concluded that there is no consensus for which is the better choice of anticoagulant for haemodialysis. Also, there is no golden standard for how to perform dialysis in patients with a bleeding risk (Kessler et al. 2015).

The KDIGO international guidelines for dialysis of patients with a bleeding risk recommends the following: keep a good blood access function; reduce the blood’s viscosity and hemoconcentration by saline flushes and predilution, keep a high QB, use a diffusive treatment, reduce the blood-air-contact in the air-trap, and assure that all alarms are functioning. According to Kessler et al., the guidelines from Europe and the United Kingdom suggest 100 - 300 ml saline flushes each 15 - 30th minute or to use a prostacyclin infusion. The latter comes with side effects such as flushing and hypotension. Both states that RCA is not an option during intermittent HD. ‘The National Kidney Foundation’ suggests using RCA, saline flushes or a citrate-based dialysate (KDIGO 2012; Kessler et al. 2015). The KDIGO also presented results from five large randomised controlled studies that compared heparin as anticoagulant to RCA during CVVHD. Two of these studies reported an improved dialyser survival and less interrupted treatments with RCA compared to heparin. A few patients had side effects with RCA such as metabolic acidosis and hypocalcaemia in. One of the studies reported a heparin induced bleeding in one patient. Another study reported a greater bleeding incidence with heparin. Two of the five studies showed no significant differences in the survival of the ECC when comparing RCA and heparin (Betjes et al. 2007; Hetzel et al. 2011; KDIGO 2012; Kutsogiannis et al. 2005; Monchi et al. 2004; Park et al. 2011).
What are the options for dialysing patients with a bleeding risk?

The literature described some alternatives to minimize or eliminate the use of anticoagulants when dialysing patients with a bleeding risk. The results varied.

**Regional citrate anticoagulation** was mentioned to being used mainly during CVVHD treatment. There was a need for careful surveillance of ionized calcium to avoid hypo- or hypercalcemia, alkalosis and clotting tendency of the ECC (Opatrný et al. 2007; Zimbudzi 2013). Citrate lowers the activation of the coagulation cascade, reduces inflammatory activity and decreases platelet activity in the ECC. However, the dose needs to be adjusted by the patient’s haemoglobin level, i.e. a low haemoglobin level demands a higher citrate dose. Since citrate is mainly metabolized by the liver, this method cannot be used in patients with multiple organ failure (Monchi et al. 2004).

**Citrate containing dialysate** resulted in frequent clotting if no other anticoagulant was added (Stegmayr et al. 2013). **Saline flushes** resulted in extensive clotting and interrupted treatments (74%) while **intermittent saline infusion** resulted in 48% interrupted treatments (Zimbudzi 2013). **Saline with argatroban** as priming fluid and for flushing resulted in few clotting events but caused a prolonged bleeding time (Yixiong et al. 2010). **Dialysers with coating** from albumin or heparin required extra doses of heparin to avoid clotting (Evenepoel et al. 2007; Kessler et al. 2013; Richtrova et al. 2007; Sagedal et al. 2011).
The Heparin-Albumin-priming
At the University Hospital of Umeå, in Sweden, an in-house low-dose model was developed in the middle of the 1980s for anticoagulation during dialysis of patients with a bleeding risk. There was an urgent need of dialyzing patients with a bleeding risk and priming with heparin in saline was tested but was not sufficient. The heparin did not seem to fixate on the surface of the ECC. The local model consists of heparin and albumin in saline (HA-priming: 5000 IU UFH/L and 1g Albumin/L). The solution was used to flush the ECC before dialysis. The hypothesis was that the priming solution attached to the inside of the ECC and thereby protected against clotting. The theory was based on the old reuse concept where dialysers were used several times since there was a fibrin layer created during the patient’s treatment. The remaining fibrin layer protected the dialyser from clotting (Pereira et al. 1996; Uhlenbusch-Körwer 2004b).

The HA-priming model has not been established in other centres, and the lack of consistency in how patients with a bleeding risk are best dialysed remains an unsolved point.

In summary, the models above indicate that there is no golden standard for anticoagulation during intermittent HD in patients with kidney failure and an increased bleeding risk. It is important to obtain knowledge that the method used for anticoagulation is based on evidence that ensures a minimized risk for the patient undergoing dialysis.
The dialysis nurse

Behind these technically advanced treatments is a person, a human being in an acute, and many times life threatening, situation – in this Thesis referred to as “the patient”. Intermittent HD is a technically advanced treatment that is performed by nurses in response to a physician’s prescription. For a dialysis nurse it is challenging to superintend a patient that does not receive standard anticoagulation before intermittent HD. Many times these patients are in the ICU and are not able to be transported to the dialysis ward.

Preparations for an intermittent haemodialysis treatment

(The preparation process described below is an example from preparations of the Fresenius 4008 dialysis machine. The principle also applies to other machines.)

The preparations for the treatment, performed by the nurses, start approximately one hour before the patient arrives at the dialysis ward. A prescribed dialysis fluid and a bicarbonate bag are attached to the machine. The machine undergoes an automatic self-control program. When the self-control is successfully completed, the tubes and dialyser prescribed are attached to the machine. All the prescribed settings – treatment time, sodium and bicarbonate levels, type of treatment and amount of water to eliminate from the patient, are now adjusted. After the approved self-test by the machine the nurse starts the priming. For SDH this is made with saline and the purpose is to flush and fill the whole ECC, including the dialyser, with saline to remove all air that otherwise would induce immediate clotting.

If HA-priming is prescribed, the priming solution is first mixed manually by adding heparin and albumin into a 2 litre saline bag. HA-priming requires a slower blood pump speed than regular priming, to avoid troublesome foam from the albumin in the ECC, that entail difficulties to remove all the air from the system. During the priming the nurse
also manually tests the pressure alarm by pinching the tubes on the arterial and on the venous side of the ECC. The tubes are pinched until the alarm sets off to ensure that it works (Dialysis protocol ‘prescription page’ is available as appendix on pages 120-123).

**Patient preparation**

The patient’s weight is of importance before dialysis. A typical chronic dialysis patient has a so-called “dry weight” as starting point and the excess weight is considered to be water that needs to be eliminated during dialysis. This water elimination depends on whether the patient still has a residual function or not. The nurse calculates the need of fluid removal and, in most cases jointly with the patients, decides about the amount that should be eliminated during the treatment.

The handling of the blood access before and after the treatment is a delicate procedure that demands aseptic technique to avoid infection and malfunction of the access.

After the treatment has started the nurse monitors all machine settings again according to the prescription. The arterial and venous pressures, the transmembrane pressure, and the conductivity of the dialysate are noted on the treatment protocol. All parameters are then double-checked by a nursing colleague. All settings and the monitoring of the entire treatment are documented on the standardised protocol. A summary of this is also later documented in the digital patient log (Dialysis protocol ‘nurses documentation page’ is available as appendix on pages 120-123).

During the treatment the machine monitors the pressures with an automatic alarm system, but the nurses also check these regularly and if necessary adjustments of the blood access and the treatment parameters are made.
When caring for a patient with a bleeding risk, who did not receive standard anticoagulation, monitoring is more frequent. The ECC is visually examined for formations at the bottom and around the walls of the chamber. Rising venous or transmembrane pressures are also signs of increased clotting. If a dose of UFH is prescribed ‘as needed’ on the protocol, the nurse has three choices if signs of clotting appear: let the treatment proceed, give the prescribed dose of UFH, or call the physician for advice. These all depend on the total situation and the situation can change over time during a 3-hour dialysis treatment. If no anticoagulation is given and clotting proceeds, the treatment is highly likely to be interrupted by total clotting. The patient then loses about 300 ml of blood since a totally clotted ECC is not possible to reverse to give the blood within the ECC back to the patient. Depending on the duration to when, or if, total clotting occurs, the physician has to consider if a new treatment should be performed immediately or not. If a new treatment should be performed, the dialysis machine has to be prepared from the beginning again.

Between dialyses the machine is disinfected both inside and outside. Inside disinfection is performed with an automatic disinfection program, chemicals and high temperature. Outside disinfection is performed manually with a cleansing disinfection solution and cloth.
Performing haemodialysis in another ward

In cases where the patient is on another ward and cannot be moved, the nurses, in pairs, transport the dialysis machine and the portable water cleaning machine, to that location, for example, the intensive care unit (ICU). At that location the dialysis nurses performs some plumbing work of various difficulties to connect the water-cleaning machine to the tap. Often a converter has to be connected to the tap. Operating this system can sometimes be challenging depending on the space and scope of the ward. At the dialysis ward, colleagues are always nearby to counsel if technical problems emerge. In contrast to the dialysis ward, performing HD in, for example, the ICU the dialysis nurse is on her/his own as the specialist of the treatment if problems arise.

An experienced dialysis nurse continuously evaluates whether to watchful wait or to administer a bolus dose of heparin. The focus is always on what is best for the patient, in this case to avoid starting or increase an ongoing bleeding, and at the same time perform a HD without pronounced clotting in the ECC for the best cleaning result. This is a real challenge and requires knowledge and experience to not expose the patient to danger. Even though the nurse is able to have dialogue with the nephrologist, many decisions have to be made instantly with the physician’s prescription as guidance. Signs of clotting constantly have to be evaluated and the patient’s clinical condition has to be considered when a decision is made about a bolus dose of heparin. This is a balancing act that cannot be learned in ways other than by experience.

Acute HD is based on daily laboratory test results but as well as on the overall clinical picture. The nurse meets the same patient in different situations and settings with the same aim, to perform dialysis without adding any extra risk and at the same time maintain a presumption for good dialysis quality. For a dialysis nurse, performing a
treatment in another ward also includes communication and cooperation with the nurses and physicians at that location. The nurse becomes the expert on the dialysis situation there and then, - and must be able to report how the treatment proceeds at that moment. For a dialysis nurse it is important to be able to focus on the patient’s needs and also to perform a good treatment. In fragile patients, like those with AKI, close monitoring and surveillance is important. Each patient is unique with unique needs. All this must be considered when caring for these critically ill patients.

An evaluated and safe treatment method that is also efficient is of high importance in situations like those described above. This is important for the patient to be treated with the method and it is also important for the nurses working with the method. Knowing that a method is evaluated and safe strengthens and facilitates the performance of HD.
Rationale

Patients in acute or chronic need of HD for survival have various technical options. Intermittent HD is the most available and is therefore mostly used. To prevent clotting events during HD anticoagulation is used and the procedures used for this vary between centres and countries. There is a lack of an established golden standard.

The clinical situation is further complicated when a patient has a bleeding risk. Conventional doses of anticoagulation are not possible to use in these situations. Various alternatives to perform intermittent HD with none or limited doses of anticoagulation have been tried. The methods described in the literature still demands a close monitoring, and additions of anticoagulants, since clotting increases the risks for an interrupted treatment and a completed treatment may be lifesaving. The situation is challenging for both the physicians and the dialysis nurses. The highly technically advanced treatment puts forth many parameters to consider. It is a balancing between satisfying treatment results and to not increase the bleeding risk. The lack of an established method that offers efficacy and safety during intermitted HD of patients with a bleeding risk is a fact.

The international ethical guidelines for nurses from the International Council of Nurses (ICN) express that nurses and national nurses associations have a responsibility to lobby for standardised treatment policies and protocols that minimise errors. In the “Code of ethic for nurses” ICN further formulates that the nurse, when providing care, shall ensure that the use of technology and scientific advances are compatible with the safety, dignity and rights of people. The ICN also says that the nurse should be active in developing a core of research-based professional knowledge that supports evidence-based practice (International Council of Nurses 2012).
Aims

The aim of this Thesis was to investigate alternatives to standard anticoagulation when dialysing patients with an increased bleeding risk.

Study 1
The aim was to investigate, in a further perspective, if the Heparin-Albmin-priming (HA-priming) was a safe method that reduced the need for intravenous anticoagulant without an extended clotting incident during intermittent HD treatment in patients with a bleeding risk.

Study 2
The primary aim was to clarify if HA-priming was a safe and efficient procedure during intermittent HD in a large group of patients with a bleeding risk. The secondary aim was to investigate if specific variables were associated with clotting tendencies of dialysers.

Study 3
The aim of this study was to design a controlled in vitro environment to systemically examine the potency of the components in the priming solution. This would enable investigation of different priming methods to clarify which of them have a beneficial outcome, and thus motivating their use in HD patients with a bleeding risk.

Study 4
The aim of this randomized, clinical cross-over study was to clarify which of four different low-dose anticoagulant models was preferable in reducing the exposure to anticoagulants and in preventing clotting in HD patients with a bleeding risk.
Ethics

Studies 1 and 2 were retrospective studies as part of a quality assessment.

Study 3: Heparin and Albumin as part of the priming solution limits exposure to anticoagulation during hemodialysis: In vitro studies, was approved 2012-232-31M by the Regional Ethical Review Board in Umeå, Sweden.

Material and methods

Malin Skagerlind's (MS) participation in the studies.

Study 1: MS participated in the manuscript progress and contributed with input from a dialysis nurse perspective.

MS presented the results at the European society of artificial organs congress (ESAO) in Glasgow, Scotland, 2013.

Study 2: MS registered the data from the 1408 acute treatment protocols and performed the basic statistical calculations and interpreted all the statistical results. MS discussed the results with the co-author and formulated it in manuscript.

MS presented the results on the National Nephrology Spring meeting in Umeå, Sweden 2016

Study 3: MS participated in the study design and in the pilot experiments to form the definitive experimental study. MS established contact with different wards to receive hemochromatosis blood that was tapped for being able to perform primary experiments before starting with the donated blood. MS supervised T. Kyrk and A. Bechara (on separate occasions) in the principles of blood tapping, blood sample collection, priming preparation, dialysis machine preparation, dialysis machine settings, dialysis machine function and use. MS performed measurements of the machines to eliminate sources of error when a fluid loss during recirculation was detected. MS conducted a continuously follow-up of the progression of the experiments. MS arranged the compensation to the donors. MS participated continuously in analysis of the statistics and influenced the manuscript from a dialysis nurse perspective.
Study 4: MS participated in planning and design – calculating the assumed number of participants in communication with the dialysis nurses, prior to asking the patients about participation, read earlier studies of the models included, participated in discussion of which parameters and lab test that should be included to evaluate the results. MS arranged and performed the study, mainly by coordinating the day and time for each participant’s treatment, preparing the study protocol and instructions for each treatment, and ordered prescriptions for lab sample collection via the electronic database for each specific treatment. MS functioned as support at the location or by telephone if the dialysis nurses had any questions (mainly about clotting and extra doses of heparin) or if any problems encountered during any treatment. MS performed basic statistical calculations and interpreted all the statistical results. MS wrote the main part of the manuscript with support from the co-author.

MS presented the results at the ESAO congress in Leuven, Belgium, 2015 and at the National Nephrology Spring meeting in Umeå, Sweden 2016.
Study 1

A retrospective study of data from acute intermittent HD treatment protocols was performed. A total of 248 treatments in 68 patients were included. Of these, 14 patients made a cross-over since they were treated with both standard intermittent HD and HA-priming. For this design these patients were counted as a ‘new’ patient. This resulted in a total of 82 patients when summarise, calculating and interpreting the data. The data from the protocols were analysed retrospectively as a quality assessment study of the HA-priming method.

The treatment protocols were divided into two groups:

The **HA-group** with a total of 178 treatments in 59 patients at a risk for bleeding. The ECC was primed with a saline solution (0.9 mg/ml) containing unfractionated heparin (UFH, 5000 IU/Litre) and albumin (1 gram/Litre).

The **S-group** with a total of 70 treatments in 23 patients with no increased risk for bleeding. The ECC was primed with saline and the patients received regular anticoagulation.

The preparations of the dialysis machine and the dialysis system were performed by dialysis nurses and based on prescription from the physician in charge.

The preparation in the HA-group: To prevent clotting when dialyzing the patients with a bleeding risk the ECC was flushed through with the HA-priming solution. The priming solution was pushed forward and into a sterile waste bag when the patient’s blood was pumped into the ECC. The priming solution was discarded after treatment start. No recirculation was made.
Some of the patients in the HA-group were prescribed to receive a smaller dose of UFH before treatment started. Other patients were prescribed a small dose of UFH that was administered by the nurses if signs of clotting showed. The nurses continuously evaluated whether this dose was needed by visually examining the ECC, and by monitoring changes in venous pressure and transmembrane pressure. Administration of extra UFH could be repeated if necessary, depending on the patient’s total condition. There were also patients with a severely high bleeding risk or an ongoing bleeding. For these patients no extra doses were allowed. All prescriptions and eventual extra doses given were noted on the acute treatment protocols.

The preparation in the S-group: The ECC was primed with saline to remove all air. The priming solution was pushed forward and into a sterile waste bag when the patient’s blood was pumped into the ECC. The priming solution was discarded after treatment start. No recirculation was made.

In both groups the system was primed according to the machines automatic priming program, with the difference being that the pump pace was set to a lower pace during the HA-priming to avoid foam and bubbles from the albumin.
Clotting was also visually evaluated after the treatments and noted on the treatment protocols by the nurses. The evaluation of clotting was based on the clinics standard protocol that consists of a four level graded scale:

0 = clean dialyser
1 = a few pink stripes on the dialyser
2 = the dialyser was red all over
3 = total clotting and interrupted treatment.

Clot formations in the chambers and eventual side-effects were separately noted on the protocol.

There were six different types of dialysers within the 248 protocols:

F7HPS\textsuperscript{1} (material: polysulfone, area: 1.6m\textsuperscript{2})
F60\textsuperscript{2} (polysulfone, 1.3 m\textsuperscript{2})
GFS12\textsuperscript{3} (hemophan, 1.2m\textsuperscript{2}), GFS16\textsuperscript{4} (hemophan, 1.6m\textsuperscript{2})
GFS20\textsuperscript{5} (hemophan, 1.8m\textsuperscript{2})
PRO600\textsuperscript{6} (polycarbonate polyether, 1.3m\textsuperscript{2}).

The dialysis machines used were mainly Fresenius 4008\textsuperscript{7} and Gambro AK200\textsuperscript{8}.

\textsuperscript{1} Fresenius Medical Care, Bad Homburg, Germany
\textsuperscript{2} Fresenius Medical Care, Bad Homburg, Germany
\textsuperscript{3} Gambro, Lund, Sweden
\textsuperscript{4} Gambro, Lund, Sweden
\textsuperscript{5} Gambro, Lund, Sweden
\textsuperscript{6} Gambro, Lund, Sweden
\textsuperscript{7} Fresenius Medical Care, Bad Homburg, Germany
\textsuperscript{8} Gambro, Lund, Sweden
Study 2

Included were 1408 acute intermittent HD treatment protocols from 321 patients. The protocols were retrospectively investigated as an extended quality assessment study and covered the years 1995 - 2002, 2006 - 2007 and 2012 - 2013. The distribution over the years was a decision based on the knowledge that the material in tubes and dialysers were developed and changed over this period of time. Thus, these time periods were chosen to evaluate differences of such changes.

The protocols were divided into two groups:

The HA-group: A total of 885 treatments including 221 patients with a bleeding risk. The ECC was primed with a saline solution (0.9 mg/ml) that contained unfractionated heparin (UFH, 5000 IU/Litre) and albumin (1 gram/Litre).

The S-group: A total of 523 treatments including 100 patients with no increased bleeding risk. The ECC was primed with saline (0.9 mg/ml) and the patients received regular anticoagulation.

In both groups the ECC was primed according to the machines automatic priming program. The pump pace was manually set to a lower pace during the HA-priming to avoid foam and bubbles from the albumin. The machines performed self-control during the priming. Both priming solutions were pushed forward and into a sterile waste bag when the patients’ blood was pumped into the ECC. The priming solution was discarded after treatment start. No recirculation was made.

According to an individual’s prescription in the HA-group (by the physician in charge) the nurses were allowed to administer smaller doses (100 - 500 IU) of UFH. The nurses
evaluated and monitored the ECC and administered the prescribed dose of UFH if signs of clotting were seen. This could be repeated if necessary. In patients with a severely high bleeding risk or an ongoing bleeding, extra doses were not allowed. All prescriptions and eventual extra doses given were noted on the treatment protocol.

Some patients were treated with both SHD and HA (n = 102). A paired comparison was made for the first intermittent HD of each model.

There were 16 different dialysers with the membrane materials polysulfone, hemophan, polyamide, polyamix and polycarbonate-polyether and with areas between 1.3 - 2.2 m². The manufacturers were Gambro, Lund, Sweden and Fresenius Medical Care, Bad Homburg, Germany.
Study 3

This was two studies merged into one based on the publishing journals request. Medical student Tobias Kyrk performed Sub-Study one. Medical student Alex Bechara performed Sub-Study two.

The *in vitro* experiments were performed with blood from 30 healthy donors. The donated blood was used for dialysis in a recirculating ECC after priming with different solutions. The criteria for blood donators were a peripheral haemoglobin > 125 g/L for women and > 135 g/L for men, and a blood pressure of <180 / <100. The donors should not have used any type of anticoagulation (e.g., NSAID, ibuprofen) within 48 hours before the tapping. The tapping was made according to the routines at the Blood Centre at the University Hospital of Umea. The bags for blood tapping were modified - the leucocyte filter was removed and there were three parallel bags instead of four. To avoid coagulation, there was 63 ml of a citrate-phosphate-dextrose solution\(^9\) in the first tapping bag. A total of 450 ml blood was tapped from each donor with the blood bag on a rock with a scale. The blood was tapped into the first of the three parallel bags, carefully mixed with the citrate, and thereafter immediately equally divided into the second and the third bag. The *in vitro* dialysis was performed immediately after tapping. The two blood bags were then connected to one dialysis machine each (both machines of model Fresenius 4008\(^{10}\)). The two machines were primed differently - one as control and one as the intervention. The dialysate flow was set to 500 ml/h during the entire study and the ultrafiltration was set to zero.

\(^9\) Per 1000 ml water for injections: citric acid monohydrate 3.27 g, sodium citrate dihydrate 26.3.

\(^{10}\) Fresenius Medical Care, Bad Homburg, Germany
An unexpected fluid removal respectively of 30 and 60 ml/h was detected in the dialysis machines. The exact fluid loss was measured by using a scale during recirculation of a 2 L saline bag. The scale measure was noted from start and every 5 minutes for a total of 60 minutes. This was made separately for each machine (Figure 5). The machines were old and no longer used in treatment of patients. The fluid loss was probably caused by micro leakage in the fluid balancing system small enough not to be detected by the machines self-check-program.

To compensate for the fluid loss during the experiments, a continuous saline infusion was established. An external injection pump was connected to the venous chamber of each machine. Each injection pump was set to a pace corresponding to the earlier measured fluid loss for each machine.

Figure 5: The fluid loss measured during 60 minutes for each dialysis machine
Since the blood tapping bags contained a citrate solution, initial tests were made to measure ionized calcium as a marker for when the citrate had been dialyzed out of the blood in the ECC. Ionization calcium was analysed at 0, 3, 6, 9, 12 and 15 minutes (Figure 6). These tests showed normalized ionized calcium levels (reference 1.08 – 1.29 mmol/L) within 12 minutes, which was set to be “minute 0” for blood sampling during the study.

There was no systemic anticoagulant added during any of the sessions.

**Figure 6: Analyze of ionized calcium**
Ionized calcium ($Ca^{2+}$) was used as a marker for when the citrate solution had been dialyzed away from the blood. $Ca^{2+}$ normalized within 12 minutes of dialysis (reference 1.08 – 1.29 mmol/L).

Sub-Study 1

Sub-Study 1 included three different interventions.

The control group had the ECC primed with:

1 L saline with 1 g Albumin and 5000 IU Heparin (HA, n = 18).

The intervention group had the ECC primed with:

1. 1 L saline (0.9 mg/ml) with 1 g Albumin (A, n = 6)
2. 1 L saline with 5000 IU unfractionated Heparin (UFH, n = 6)

3. 1 L saline (0.9 mg/ml, n = 6)

The dialyser used was F4HPS\textsuperscript{11}, a low-flux membrane consisting of Fresenius Polysulfone\textregistered{} with a membrane surface of 0.8m\textsuperscript{2}. The tubing system was DiaLine A/V set\textsuperscript{12}. The blood flow was kept at 200 ml/min during all sessions (Table 6).

Sub-Study 2

Sub-Study 2 included two different interventions.

The control group had the ECC primed with:

1 L saline with 1 g Albumin and 5000 IU of Heparin (HA, n=12).

The intervention group had the ECC primed with:

1. 1 L saline with 1 g Albumin and 20,000 IU of Heparin (4H+A, n=6)
2. 1 L saline with 4 g Albumin and 5000 IU of Heparin (H+4A, n=6)

The dialyser used was FX-50\textsuperscript{13}, a high-flux membrane consisting of Helixone\textregistered{} (polysulfone based) with a surface of 1.4m\textsuperscript{2} (Table 7).

To enable more blood sampling narrower ECC tubes were used during Sub-Study 2, i.e. AV-Set FMC Pead/Baby\textsuperscript{14}. This limited the blood flow to 100 ml/min to keep the artery and venous pressures within ≤ 200 mm Hg. The dialysate used was SmartBag\textsuperscript{15} 311.25 (K\textsuperscript{+}: 3.00 mmol/L, Glucose: 1.00 g/L, Ca\textsuperscript{2+}: 1.25 mmol/L). The machines had a separate inflow for bicarbonate bags and the bags used were 900 g 5008 BicBag\textsuperscript{16}.

\textsuperscript{11} Fresenius Medical Care, Bad Homburg, Germany
\textsuperscript{12} F.M. S.p.A., Cigliano, Italy
\textsuperscript{13} Fresenius Medical Care, Bad Homburg, Germany
\textsuperscript{14} Fresenius Medical Care, Bad Homburg, Germany
\textsuperscript{15} Fresenius Medical Care, Bad Homburg, Germany
\textsuperscript{16} Fresenius Medical Care, Bad Homburg, Germany
Table 6 and 7: Machine settings and dialyser in Sub-Studies 1 and 2

<table>
<thead>
<tr>
<th>Dialysis during Sub-Study 1</th>
<th>Dialysis during Sub-Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mmol/L)</td>
<td>Na⁺ (mmol/L)</td>
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<tr>
<td></td>
<td>136</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
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<tr>
<td>Temperature (°C)</td>
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<tr>
<td>Dialysate flow (ml/min)</td>
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</tr>
<tr>
<td>Blood flow (ml/min)</td>
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</tr>
<tr>
<td>Dialyser</td>
<td>F4HPS</td>
</tr>
</tbody>
</table>

Na⁺ = sodium

Blood analyses in Sub-Study 2

Blood samples for albumin and blood count were collected and analysed. Plasma APTT\textsuperscript{17} (reference 22 - 36 sec) samples were analysed to monitor the effect of heparin in the priming solution. All blood samples were coded and were analysed at the accredited Department of clinical chemistry, at University Hospital of Umeå, Umeå, Sweden.

The dialysis was performed in parallel with the two machines running at the same time. The parted blood was used as control in one machine and as intervention in the other machine. Blood samples were collected from the arterial side of the ECC with the start after 12 minutes, and then within 10-minute intervals up to 192 minutes or until an eventual interrupted treatment caused by clotting. After the treatment the ECC was flushed through with saline according to the machines regular program for when blood

\textsuperscript{17} Lab method: SP Liquid Hemosil IL on ACL Top 700 LA
is returned from the ECC to the patient. A light sensor on the machine detects when there is no blood left in the system and the machine automatically stops.

After the treatment clotting was visually evaluated in the dialyser on a five-graded scale:

0 = the dialyser was clean
1 = a few visible stripes
2 = moderate amounts of stripes
3 = the dialyser was red coloured all over
4 = total clotting and interrupted treatment.

Clot formations in the chambers were separately noted as “slight to moderate clots on the bottom” or “fully clotted chamber”.

Another pre-investigation experiment was performed to detect an eventual albumin loss since the hypothesis of the HA-priming was that the solution attaches to the surface of the ECC.

Sub-Study 1: Samples for measurement of albumin (mg/L) were collected every 15th minute during recirculation and dialyzing of an albumin solution with a concentration of 1 g/L in a 2 L saline bag for 180 minutes. The high-flux dialyser FX50\textsuperscript{18} with a polysulfone membrane and an area of 1.8m\textsuperscript{2} was used.

Sub-Study 2: Samples for measurement of albumin (mg/L) were collected every 15th minute during recirculation and dialyzing of the HA-priming solution with an albumin concentration of 1 g/L and heparin concentration of 5000 IU/L in a 2 L saline bag for

\textsuperscript{18} Fresenius Medical Care, Bad Homburg, Germany
180 minutes. The wet dialyser APS-18U with an Asahi Polysulfone membrane and an area of 1.8m² was used.

During both studies samples were collected from a membrane after the dialyser (before the fluid went back to the blood bag). A tubing system of the model AV-Set SRB-R 2008/4008 was used during these experiments.

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20 Fresenius Medical Care, Bad Homburg, Germany
Study 4

Demography

Participants were 23 chronic HD patients (16 males) in a stable condition. Excluded were patients with cognitive failure, difficulties with fluid control (more than 3 kg body weight gain between dialysis), access problems or acute infection. None of the patients had an enhanced risk for bleeding. The patients were informed verbally and received written information. Thereafter, those who wanted to participate wrote their consent on a separate form (Patient information available as appendix on pages 120-123). All patients except one were on some type of daily anticoagulant medication (Table 8).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Patients n=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylic acid</td>
<td>14</td>
</tr>
<tr>
<td>Warfarin</td>
<td>6</td>
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<tr>
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</tr>
<tr>
<td>Dalteparin subcutaneous</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>23</strong></td>
</tr>
</tbody>
</table>

There were four low-dose anticoagulant models in the study (Table 9). The models were compared to each other and to the patients’ standard intermittent HD treatment that was included as a baseline treatment. The patients were their own control in the study. The study was performed in two steps, Step 1 and Step 2. Each study treatment was performed on the patient’s mid-week dialysis.
Table 9: The four low-dose anticoagulant models

<table>
<thead>
<tr>
<th>Study model abbreviation</th>
<th>Priming solution concentration</th>
<th>Solution use</th>
<th>Dialyser Area Material</th>
<th>Dialysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>UFH (5000 IU/L) Saline (0.9 mg/ml)</td>
<td>Solution flushed through the ECC before treatment; then wasted.</td>
<td>FX 80 1.8 m² Helixone®</td>
<td>Smartbag® 211.25 or SmartBag® 311.25</td>
</tr>
<tr>
<td>HA</td>
<td>UFH (5000 IU/L) Albumin (1 g/L) Saline (0.9 mg/ml)</td>
<td>Solution flushed through the ECC before treatment; then wasted.</td>
<td>FX 80 1.8 m² Helixone®</td>
<td>Smartbag® 211.25 or SmartBag® 311.25</td>
</tr>
<tr>
<td>SHD* (Baseline)</td>
<td>Saline (0.9 mg/ml)</td>
<td>Solution flushed through the ECC before treatment; then wasted.</td>
<td>FX 80 1.8 m² Helixone®</td>
<td>Smartbag® 211.25 or SmartBag® 311.25</td>
</tr>
<tr>
<td>HAC</td>
<td>UFH (5000 IU/L) Albumin (1 g/L) Saline (0.9 mg/ml)</td>
<td>Solution flushed through the ECC before treatment; then wasted.</td>
<td>FX 80 1.8 m² Helixone®</td>
<td>SelectBag® CX265G Citrate</td>
</tr>
<tr>
<td>Evodial®</td>
<td>Saline (0.9 mg/ml)</td>
<td>Solution flushed through the ECC before treatment; then wasted.</td>
<td>Evodial® 1.6 m² HeprAN</td>
<td>Smartbag® 211.25 or SmartBag® 311.25</td>
</tr>
</tbody>
</table>

*The patients received their regular start dose of tinzaparin before SHD.
UFH = Unfractioned Heparin, The ECC = the extra corporeal circuit, H = Heparin-priming, HA = Heparin-Albumin-priming, SHD = standard haemodialysis, HAC = Heparin-Albumin-priming and a citrate containing dialysate.
**Priming**

The ECC was primed with at least 1 L of respective priming solution. When the system had been primed the patient’s blood was pumped into the ECC. The priming solutions were then pushed away from the ECC into a sterile waste bag that was discarded. No recirculation of the priming solution was made.

**Dialysers and dialysate**

During intermittent HD with model 1 - 3 and during baseline intermittent HD the dialyser FX80\(^{21}\) was used. The dialyser in model 4 (Evodial\(^\circledast\)\(^{22}\)) had a membrane that was heparin grafted from the manufacturer and the membrane consisted of an anionic copolymer of acrylonitrile and sodium methallyl sulfonate. The patients had their regularly prescribed dialysate during the sessions except for during model 3 where a citrate-based dialysis fluid was used.

The citrate-based dialysate SelectBag\(^\circledast\)\(^{23}\) CX265G Citrate had a potassium concentration of 2.0 mmol/L. Patients with 3.0 mmol/L in their regular treatment dialysate were compensated with a mixture of 1 gram of oral potassium citrate as a single dose before the start of the HAC treatment.

**Anticoagulation**

There was no bolus dose of anticoagulant given from the start during the models 1 - 4. During the standard treatment (baseline) the patients received their regular individual prescribed dose of tinzaparin. Since this was the patient’s weekly regular treatment day

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\(^{21}\) Fresenius Medical Care, Bad Homburg, Germany
\(^{22}\) Gambro Lundia AB, Lund, Sweden
\(^{23}\) Gambro Dasco S.p.A. Modela, Italy
the intention was to avoid interrupted treatments. The nurses were therefore allowed to administer smaller doses of UFH if signs of clotting arose.

If a patient needed more than 20 IU of UFH per kilo body weight to avoid clotting during a treatment, that treatment was counted as interrupted. This occurred in two patients during one treatment each.

The dialysis machines used were Fresenius 4008, Fresenius 5008 and Gambro Artis™. For all treatments with the HAC model, the Gambro Artis™ was used since the dialysate bags were only compatible with that machine.

**Blood sampling**

Blood samples were collected at 0, 30 and 180 minutes. To study the efficacy of the treatments creatinine and urea was analysed. To correct for the effect of ultrafiltration albumin, haemoglobin, erythrocytes, erythrocyte volume fractions were analysed. To study the effect of biocompatibility platelets, leukocytes, granulocytes, lymphocytes, monocytes, basophils and eosinophils were analysed. To study the interference of UFH on the intrinsic clotting system Activated Partial Thromboplastin Time (APTT) was analysed. To study how UFH affected the release of lipoprotein lipase from the endothelial surface, triglyceride concentration was analysed.

Blood samples at 0 minutes were collected from the patient’s blood access. If the access was a CDC, 5 ml of fluid was aspirated from each lumen. The first part of this fluid included the catheter lock solution (1.6 - 2.5 ml, depending on catheter length) and the rest was blood, to assure that all CLS fluid was removed. Then the lumens were

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24 *Fresenius Medical Care, Bad Homburg, Germany*
25 *Baxter, Gambro, Lund, Sweden*
flushed with saline, backward and forward several times, before the blood sample was collected. At 30 and 180 minutes the samples were collected from a silicone sampling membrane on the arterial side of the ECC. No separate venous puncture for blood sampling was made. If a treatment was interrupted no further blood samples were collected. The lab results were adjusted for differences in hemoconcentration due to ultrafiltration by using changes in albumin and erythrocyte volume fraction.

**Randomisation**

The study was performed in two steps where step one also included the standard dialysis treatment (baseline, SHD). In step one the patients were randomised to the order of treatments, i.e. with models: H, HA or SHD. In step two the patients were randomised to start with either HAC or Evodial® and received the other treatment at the next study session. A nurse with no connection to the patients made the randomisation by lots.

**Drop-outs**

Five participants dropped out during the study period. The reasons for drop out were due to change of treatment regime (n = 1), impaired health (n = 1), and no given reason (n = 2). One of the participants dropped out after having suffered from a side-effect with Evodial®. Due to the drop out a few patients did not participate in all treatments, but all underwent standard haemodialysis (SHD) and were treated with one or more of the models.

The total number of participants in each model was as follows:

H-priming n = 20, HA-priming n = 21, HAC n = 19, Evodial® n = 19.
**Evaluation of clotting**

Experienced dialysis nurses visually evaluated the clotting of the dialyser in for grades based on the clinics standard protocol:

0 = clean dialyser

1 = a few pink stripes on the dialyser

2 = the dialyser was red all over

3 = total clotting and interrupted treatment.

The chambers were graded clean, collar around the inside, clot formations, and total clotting.

Other data collected were treatment time, patient weight, need of ultrafiltration, blood pressure before and after treatment, venous and artery pressure in the ECC, QB (blood speed in ml/min), and quantity of blood dialysed.
Statistics

The software used was IBM SPSS editions 21 and 23 (Chicago, USA) and Epi Info™ Version 7, Centres for Disease Control and Prevention (Atlanta, USA; http://www.cdc.gov). In all the studies a two-tailed significance level less than 0.05 was considered significant.

Study 1: Statistical analyses were performed using Fishers test and Students t-test.

Study 2: Group comparisons were made by Students t-test for normally distributed data and Mann-Whitney U-test for not normally distributed data, using ranked Wilcoxon paired test. Spearman non-parametric test was used for bivariate correlation analysis to avoid the influence of outliers. Multi-regression analysis included grade of clot as dependent factor and the variables age, blood pressure, blood pump speed, artery pressure, UF-rate, TMP and total anticoagulation in the model.

Study 3: Statistical analysis was performed using Wilcoxon paired non-parametric rank test. Comparisons between relations and groups were made using Fishers test.

Study 4: Differences between models were calculated using the paired non-parametric Wilcoxon rank sum test. Group comparisons were calculated using Mann Whitney U-test. Student t-test was used for normally distributed data.
Results and discussion

Study 1

Demography
A total of 248 treatments in 68 patients were included. The mean age of the patients was 63 years (range 18-82) and 70% were male. The reasons for AKI that required intermittent HD are given for each group in Table 10. Blood accesses were CDC (80.5%), femoral catheter (6.1%) and fistula 8.5%. There was no significant difference in blood flow between the groups: 213 ml/min for the HA-group vs. 206 ml/min for the S-group (p = 0.053). There was a difference in creatinine before treatment: 441 (±207) µmol/l for the HA-group vs. 515 (±220) µmol/l for the S-group (p = 0.027).

Safety
There was no treatment related extended bleeding reported in the protocols. A total of 18% (n=32) of the treatments in the HA-group completed the treatments without any UFH. A total 24% (n=42) of the treatments in the HA-group did not receive a starting dose. Five of these patients received 600 – 1500 IU of heparin during the treatment. There was a significant difference in the mean amount of UFH dose when comparing the groups (Table 11). In the HA-group there were a more than 50% lower mean UFH dose from start, during the treatment and when summarizing the doses.
Table 10: Diagnosis in percent for acute kidney injury (AKI) requiring intermittent HD

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>HA n=178</th>
<th>S n=70</th>
<th>Total n=248</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKI*</td>
<td>8</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>ATN**</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Haematology</td>
<td>20</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Chronic dialysis</td>
<td>3</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Other***</td>
<td>15</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Postop</td>
<td>46</td>
<td>37</td>
<td>43</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*AKI = Acute kidney injury, not further specified  
**ATN = Acute tubular necrosis  
***Other = Cancer, Nephrostomy-stenosis, rhabdomyolysis, thrombotic microangiopatia, multiple trauma, multi organ dysfunction syndrome, nephropathia epidemica, hepatorenal syndrome, mixed connected tissue disease

Table 11: The amount of unfractionated heparin (UFH) within the groups

<table>
<thead>
<tr>
<th></th>
<th>HA Units of UFH in mean</th>
<th>S Units of UFH in mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start dose</td>
<td>1174</td>
<td>2521</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Maintenance dose/h</td>
<td>268*</td>
<td>909</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total dose</td>
<td>2156</td>
<td>5689</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

P-value for significance when comparing differences between the groups.  
*Calculated dose of boluses  
HA = Heparin-Albumin-priming and small or no bolus dose of heparin  
S = Standard haemodialysis with saline priming and a bolus dose of heparin
Efficacy

Although the HA-group had a significantly lower dose of heparin, there was no difference in total clotting. Total clotting was reported in 2.25% of the treatments in the HA-group and in 4.29% in the S-group (4 and 3 treatments, respectively, p = 0.62, Figure 7). The treatment time was significantly longer in the S-group with 215 minutes vs. 197 minutes in the HA-group (p = 0.01).

There were indications that clotting might be different with different dialysers but the study material was too small for sufficient statistical analysis.

In conclusion, this study clarified that in this limited number of dialyses there was no evident risk for an aggravated bleeding during dialysis for the protocol used. This motivated an extended study to increase safety analysis and to estimate variables that may interfere with clotting.

Figure 7: Clotting in % for each grading level for both treatment groups
There was no significant difference in total clotting between the groups (p=0.62).
HA = Heparin-Albumin-priming and small or no bolus dose of heparin
S = Standard haemodialysis treatment with saline as priming and a bolus dose of heparin before treatment start.
Study 2

Demography

The 321 patients treated with acute intermittent HD had a mean age of 62 (±16) years (range 15 - 98). There was no difference in mean age between HA-group and S-group (62.2 vs. 61.6 years, p = 0.8). The indications for acute intermittent HD are shown in Table 12. The blood access was CDC (75%), femoral catheter (11%), fistula (8%) and arterio-venous graft (5%). The total treatment time differed between the groups. The S-group had a mean time of 197 minutes and the HA-group had a mean time of 190 minutes (p < 0.01).

Table 12: Indications for acute intermittent haemodialysis in %.

<table>
<thead>
<tr>
<th>Dialysis indication</th>
<th>HA  n = 221</th>
<th>S  n = 221</th>
<th>Total n = 321</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Medicine</td>
<td>38</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>Post-operative</td>
<td>38</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>HD*</td>
<td>15</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Trauma</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Intoxication</td>
<td>0.5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Contrast media</td>
<td>0</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Unknown**</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*HD= Haemodialysis (patients on chronic dialysis in need of acute dialysis).
**No diagnosis was noted on the protocol.
HA = Heparin-Albumin-priming and small or no bolus dose of heparin.
S = Standard haemodialysis with saline priming and a bolus dose of heparin.
Safety

Heparin free dialyses were completed in 24% of the 885 treatments in the HA-group.

The total dose of UFH was significantly lower in the HA-group vs. the S-group with a median of 1200 IU vs. 5000 IU (p < 0.001). This corresponded to less than 25% of the S-groups. The means UFH amounts between the HA-group and the S-group are given in Table 13. There was no extended bleeding attributed to the treatment reported in the protocols in either group.

Table 13: The mean value of heparin doses given

<table>
<thead>
<tr>
<th></th>
<th>HA Units of UFH in mean</th>
<th>S Units of UFH in mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start dose</td>
<td>825</td>
<td>1777</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Maintenance dose/h</td>
<td>221</td>
<td>612</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Total dose</td>
<td>1838</td>
<td>14913</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Total dose* (median)</td>
<td>1200</td>
<td>5000</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

All values in the HA-group were significantly lower.

*To avoid influence of outliers a median value was also calculated. The difference was still significant.

HA = Heparin-Albumin-priming and small or no bolus dose of heparin.

S = Standard haemodialysis with saline priming and a bolus dose of heparin.
**Efficacy**

Although there were significantly lower doses of UFH in the HA-group, there were not higher incidences in interrupted treatments when comparing the groups: HA (1%) vs. S (0.8%) \((p = 0.8\), Figure 8). Among the interrupted treatments \((n=9)\) the clotting occurred around 120 minutes. There were too few patients to do a statistical comparison between the groups.

<table>
<thead>
<tr>
<th>Clotting in %</th>
<th>Clean</th>
<th>Medium</th>
<th>Severe</th>
<th>Total clotting</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>49</td>
<td>37</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>S</td>
<td>59</td>
<td>26</td>
<td>12</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Figure 8: Clotting in % for each grading level for both treatment groups.
There was no difference in total clotting between the HA and the S \((p = 0.8)\).
There were more severe clotted dialysers in the HA-group \((p < 0.001)\).
HA = Heparin-Albumin-priming and small or no bolus dose of heparin
S = Standard haemodialysis with saline priming and a bolus dose of heparin

There were more severe clotted dialysers in the HA-group \((p < 0.001)\).
The time of first notation of signs of clotting leading to extra dose did not differ between the groups; HA-group: 113 \((±49)\) minutes \((n=49)\), S-group: 106 \((±56)\) minutes \((n = 22)\).
In many of the protocols there was a lack of notes about time for when the extra doses of UFH were given. There were no differences in clotting between the membrane
materials (p = 0.3). Also, there were no differences in clotting when comparing the same material and the same surface area (p = 0.2).

When comparing all different dialysers and treatments, the low-flux dialyser F7HPS (also the most frequently used) had most clean dialysers (62%) after treatment. The high flux dialysers had a worse outcome with fewer amounts of clean dialysers:

- FX10 (39% clean, p < 0.001)
- FX80 (32% clean, p < 0.001)
- GFS20 (33% clean, p = 0.02)

F7HPS had a better result compared to the low-flux dialyser F8HPS (52%, p = 0.02).

A dialyser with a membrane area of ≤1.7m$^2$ resulted in less clotting (61%) than those with a larger membrane area (43%, p < 0.001).

When comparing clotting according to the diagnoses that led to a need of dialysis, the most “clean” dialysers were found in the group with a medical reason. Medical diagnoses were, for example, severe infections, rhabdomyolysis, or dehydration. Those who had thoracic or aortic surgery had more clotting (any grade) compared those who had a medical diagnosis (p = 0.032). Patients on chronic dialysis that has had acute dialysis had higher clotting tendencies compared to patients with a medical diagnosis leading to acute dialysis (p = 0.021).

In conclusion, this study showed that the HA-priming was a safe anticoagulation model. However, there were still a considerable number of patients that needed additional heparin. The risk for interrupted dialyses due to clotting was small. Data indicated that a
smaller surface area of the dialyser was preferable. There were no differences between high-flux vs. low flux dialysers when comparing dialyser with the same membrane surface area. A question arises as to whether a change in heparin or albumin concentration or other models could be more beneficial. Here an experimental model would be of value.
Study 3

The method developed for this study easily enabled paired comparisons of the blood from one donor. A schematic sketch of the study setup is presented in Figure 9.

**Figure 9: Flow chart for Study 3**

Pre-experiments, two Sub-Studies with interventions and control group for each Sub-Study.

**Albumin loss**

Whether albumin was either adsorbed to the membranes surface or lost through dialysis was evaluated in both Sub-Studies. The albumin loss when dialyzing a 1000 mg/L albumin solution in a recycling ECC was measured. A total loss of 120 mg after 180 minutes of dialysis with the FX50 dialyser and 100 mg with the APS-18U dialyser was found.
**Sub-Study 1**

In Sub-Study 1, all the HA-priming sessions (n = 18) and the H-priming sessions (n = 6) resulted in 192 minutes of survival of the ECC, with a clotting level at 1 (a few pink stripes on the dialyser). With the Saline-priming and the Albumin-priming all sessions ended prematurely due to clotting within a time range of 18 - 35 minutes. The H-priming resulted in a mean ECC patency of 21 minutes (range 18 - 27) before clotting. This was significantly shorter compared to the HA sessions that all had a patency up to 192 minutes (p = 0.026). The Albumin sessions had a mean time of 26 minutes (range 19 - 35) before clotting, and this was also significantly shorter compared to the HA sessions (p = 0.028).

**Sub-Study 2**

In Sub-Study 2 a high-flux dialyser was used and the tubes were of paediatric size to enable more blood sample collecting. All the sessions primed with HA, 4H+A and H+4A had a patency up to 192 minutes.

HA and 4H+A resulted in clotting grade 1 (a few pink stripes).

During the H+4A sessions a clot formation around the walls of the venous chamber was visible, which resulted in a significantly higher grade of clotting compared to the other solutions (p < 0.003).

A maximum APTT was noted at 10 minutes with HA and H+4A. There were no differences in APTT between HA and H+4A. The APTT thereafter decreased over time. During the HA-priming there was a significant reduction in APTT between 10 and 192 minutes (p = 0.007). The 4H+A-priming had an APTT > 180 seconds during all sessions, from treatment start to 192 minutes. The HA-priming resulted in significantly more reduced APTT compared to the 4H+A-priming (p = 0.01). Figure 9 shows the
APTT for the three different primings. There were no differences in platelet count, not even after adjusting for differences in haemoglobin or haematocrit. The mean baseline value for haemoglobin (±SD) was 145 (±11) g/L, for EVF was 0.42 (±0.03) and for platelets was 217x10⁹ (±47) (Figure 10).

![APTT during the different primings](image)

**Figure 10: The APTT in mean for each priming model**
Based on samples collected with ten-minute intervals from the recirculating priming fluid.
HA = Heparin-Albumin-priming (5000 units of heparin and 1 g of albumin per litre saline).
4H+A = Four times the heparin dose (20 000 units) and 1 g of Albumin per litre of saline.
H+4A = Heparin (5000 units) and four times the albumin dose (4g per litre of saline).

Based on the results in this experimental study it was motivated to keep the concentrations of the HA-priming as used in Studies 1 and 2. In addition, the use of heparin-only as priming was motivated. The use of these concepts should be evaluated further in a clinical study.
Study 4

Demography

Table 1: Baseline data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67</td>
<td>±12</td>
</tr>
<tr>
<td>Treatment time (minutes)</td>
<td>221</td>
<td>±23</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>114</td>
<td>±12</td>
</tr>
<tr>
<td>Platelets (10⁹/L)</td>
<td>219</td>
<td>±71</td>
</tr>
<tr>
<td>Erythrocyte Volume Fraction</td>
<td>0.36</td>
<td>-</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>152/74</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84</td>
<td>±19</td>
</tr>
<tr>
<td>Blood rate (ml/min)</td>
<td>304</td>
<td>±52</td>
</tr>
<tr>
<td>Ultrafiltration achieved (L)</td>
<td>1138</td>
<td>±989</td>
</tr>
<tr>
<td>Total blood volume dialysed (L)</td>
<td>65</td>
<td>±12</td>
</tr>
</tbody>
</table>

The participants in this study were stable patients with dialysis dependent end stage kidney disease (n = 23). The mean age was 67 years (16 males) and the diagnosis that led to HD is shown in Table 15. Nine of the patients had diabetes mellitus, 12 had arterio-venous fistula, 10 had a CDC and one had a femoral dialysis catheter as the blood access. Baseline data are shown in Table 14.
Table 15: Diagnosis that led to intermittent HD

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic nephropathy</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>5 (22)</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>5 (22)</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>3 (13)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>23 (100)</strong></td>
</tr>
</tbody>
</table>

**Safety**

The APTT was at a mean 44 (±36) (median 32, reference 22 - 36 seconds) before treatment. Notably, the APTT was significantly higher from the start in patients with a CDC as blood access. Including all low-anticoagulant dialyses, the CDC group had a significantly higher mean APTT at 61 (±59) seconds from the start compared to those with an AVF who had a start APTT at 30 (±6.7) seconds (p = 0.001). After 30 minutes of dialysis there was no longer a difference in APTT according to blood access (p = 0.082).

The H-priming model was not significantly different in APTT at 180 minutes compared to SHD, whereas all other models had a significantly lower APTT compared to SHD at both 30 and 180 minutes (Tables 16 and 17). When comparing dialyses with 500 or 1000 IU of UFH given vs. no UFH given, APTT did not differ. The mean APTT values at the start for those who received 500 or 1000 IU of UHF vs. no added UFH were 49 (±40) sec vs. 40 (±36) sec, respectively. For respective comparisons the mean APTT values at 30 min was 38 (±12) sec vs. 37 (±6) sec and at 180 min was 32 (±7) sec vs. 33 (±5) sec.
Table 16: Activated partial thromboplastin time (APTT) for each model at 30 and 180 minutes

<table>
<thead>
<tr>
<th>Study mode</th>
<th>APTT above ref. at 30 min</th>
<th>APTT above ref. at 180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>HA</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>HAC</td>
<td>16.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Evodial®</td>
<td>5.3</td>
<td>0</td>
</tr>
</tbody>
</table>

H = Heparin-priming, HA = Heparin-Albumin-priming, HAC = Heparin-Albumin priming and a citrate containing dialysate, SHD = standard dialysis treatment, Sec = seconds.

Table 17: Mean values, SD and range for APTT in seconds for each model at 30 and 180 minutes compared to SHD.

<table>
<thead>
<tr>
<th>Priming model</th>
<th>APTT 30 minutes</th>
<th>vs. SHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sec ±SD (range)</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>37±16 (25-96)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>HA</td>
<td>34±7 (26-50)</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>HAC</td>
<td>34±8 (27-40)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Evodial®</td>
<td>31±4 (25-40)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>SHD</td>
<td>98±39 (49-181)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Priming model</th>
<th>APTT 180 minutes</th>
<th>vs. SHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sec ±SD (range)</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>38±21 (25-110)</td>
<td>ns</td>
</tr>
<tr>
<td>HA</td>
<td>30±3 (23-34)</td>
<td>P=0.001</td>
</tr>
<tr>
<td>HAC</td>
<td>30±4 (24-44)</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Evodial®</td>
<td>29±3 (24-35)</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>SHD</td>
<td>48±15 (30-85)</td>
<td></td>
</tr>
</tbody>
</table>

H = Heparin-priming, HA = Heparin-Albumin-priming, HAC = Heparin-Albumin priming and a citrate containing dialysate, SHD = standard dialysis treatment, Sec = seconds.
Table 18: Mean, SD and range of UFH in IU added during treatment with each priming model

<table>
<thead>
<tr>
<th>Priming model</th>
<th>Units of added UFH mean ±SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>643 ±1442 (0-6500)</td>
</tr>
<tr>
<td>HA</td>
<td>413 ±695 (0-3000)</td>
</tr>
<tr>
<td>HAC</td>
<td>184 ±299 (0-1000)</td>
</tr>
<tr>
<td>Evodial®</td>
<td>184 ±506 (0-2000)</td>
</tr>
<tr>
<td>SHD</td>
<td>4413 ±1838 (2500-9000*)</td>
</tr>
</tbody>
</table>

*During baseline (SHD) the patients regular start dose of tinzaparin was given. H = Heparin-priming, HA = Heparin-Albumin-priming, HAC = Heparin-Albumin priming and a citrate containing dialysate, SHD = standard dialysis treatment.

**Efficacy**

SHD had significantly less clotting compared to all the low-dose models (p < 0.01). Evodial® had less clotting than HA and HAC (p < 0.01). There were no differences in clotting in chambers between the four low-dose models. During the study eleven dialyses in eight individuals resulted in total clotting, and thus interrupted treatments. Seven of these dialyses were with model H (33%), three with HA (15%) and one with Evodial® (5%). There was no interrupted treatment with the HAC model. Compared to SHD H-priming had significantly more clotting (p = 0.008), whereas the other models had no significant differences in clotting compared to SHD. H-priming also had the most interrupted dialyses compared to SHD (p = 0.02, Fisher’s test) and HAC (p = 0.03). Comparing H with the outcome for HA, HAC, and Evodial® together, H-priming resulted in more interrupted dialyses (P = 0.04, RR 3.7, CI 1.2 - 12, **Figure 11**).
The urea reduction rate (URR) was lower with Evodial® compared to the other low-dose models at both 30 and 180 minutes ($p < 0.01$, Table 19). The H model had a significantly shorter mean treatment time compared to SHD (197 ± 64 vs. 220 ± 23 min, $p = 0.025$). The URR was less with Evodial® vs. SHD at 30 and 180 min (22 vs. 27% and 55 vs. 63%, $P < 0.01$).

Table 19: The urea reduction rate (URR) in % (±SD) for each priming model after 30 and 180 minutes dialysis.

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>HA</th>
<th>HAC</th>
<th>Evodial</th>
<th>SHD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>URR 30’</strong></td>
<td>25 (±8)</td>
<td>28 (±6)</td>
<td>26 (±9)</td>
<td>22 (±7)</td>
<td>27 (±6)</td>
</tr>
<tr>
<td><strong>p-values at 30’</strong></td>
<td>$p &lt; 0.01$ vs. Evodial</td>
<td>$p &lt; 0.01$ vs. Evodial</td>
<td>$p &lt; 0.05$ vs. HAC</td>
<td>$p = 0.001$ vs. SHD</td>
<td></td>
</tr>
<tr>
<td><strong>URR 180’</strong></td>
<td>57 (±30)</td>
<td>61 (±9)</td>
<td>61 (±8)</td>
<td>55 (±15)</td>
<td>63 (±7)</td>
</tr>
<tr>
<td><strong>p-values at 180’</strong></td>
<td>$p &lt; 0.01$ vs. Evodial</td>
<td>$p &lt; 0.01$ vs. Evodial</td>
<td>$p &lt; 0.05$ vs. SHD, p &lt; 0.05 vs. Evodial,</td>
<td>$p = 0.001$ vs. SHD</td>
<td></td>
</tr>
</tbody>
</table>

P-values for comparisons that were significant are shown in the table.

Triglycerides

At baseline the triglycerides (TG) was at a mean of 1.77 mmol/L (median 1.65, range 0.93 - 3.98).

There was a reduction in TG after 30 minutes dialysis within all models. With Evodial® that reduction was not significant. The reduction of TG was most pronounced with SHD and the difference was significant compared to H, Evodial® and HAC (p < 0.02). At 180 minutes TG was significantly increased with Evodial® (p = 0.01) and HAC (p=0.031, Table 20). When comparing SHD and HA there was no difference in TG reduction.

Patients with a CDC had a greater reduction in triglycerides at 30 minutes compared to those who had an AV-fistula (-13% vs. -4%, p = 0.034). This was an indication of a spill over of catheter lock solution when the CDC was handled.

Table 20: Mean change (±SD) in % in triglycerides at 30 and 180 minutes, compared to baseline

<table>
<thead>
<tr>
<th>Dialysis mode</th>
<th>30 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>-9 (±15)</td>
<td>-18 (±46)</td>
</tr>
<tr>
<td>HA</td>
<td>-11 (±15)</td>
<td>-18 (±48)</td>
</tr>
<tr>
<td>HAC</td>
<td>-7 (±10)</td>
<td>19 (±29)</td>
</tr>
<tr>
<td>Evodial®</td>
<td>-1 (±12)</td>
<td>32 (±53)</td>
</tr>
<tr>
<td>SHD</td>
<td>-21 (±14.0)</td>
<td>-7 (±48)</td>
</tr>
</tbody>
</table>

Values were adjusted for ultrafiltration before calculation.

SHD = Standard haemodialysis, H = Heparin priming, HA = Heparin-Albumin, HAC = Heparin-Albumin in combination with Citrate containing dialysate.
**Biocompatibility**

There were significant reductions in leukocytes, platelets and lymphocytes at 30 and 180 minutes (Tables 21 and 22).

**Table 21: Paired analyses of mean difference in cell count in % at 30 minutes compared to start**

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>HA</th>
<th>HAC</th>
<th>Evodial®</th>
<th>SHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td>-12</td>
<td>-11</td>
<td>-8</td>
<td>-8</td>
<td>-7</td>
</tr>
<tr>
<td>Platelets</td>
<td>-5</td>
<td>-7</td>
<td>-7</td>
<td>-6</td>
<td>-8</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>-27</td>
<td>-18</td>
<td>-22</td>
<td>-20</td>
<td>-16</td>
</tr>
<tr>
<td>Monocytes</td>
<td>-28</td>
<td>-23</td>
<td>-21</td>
<td>-8</td>
<td>-14</td>
</tr>
<tr>
<td>Basophils</td>
<td>-13</td>
<td>-8</td>
<td>-21</td>
<td>-2</td>
<td>-1</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>-25</td>
<td>-18</td>
<td>-3</td>
<td>-12</td>
<td>0</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>-6</td>
<td>-7</td>
<td>-3</td>
<td>-6</td>
<td>-5</td>
</tr>
</tbody>
</table>

Values were adjusted for ultrafiltration before calculation.

Significant differences between start and 30 minutes *within the same model* are given as a) (p<0.05) or b) (p<0.01).

Significant differences between low-dose-models compared to standard dialysis (SHD) as reference are given as c) (p<0.05), other models compared to H-priming as d) (p<0.05), other models compared to HA-priming as e) (p<0.05), and HAC compared to Evodial® as f) (p<0.05).

H = Heparin priming, HA = Heparin-Albumin, HAC = Heparin-Albumin in combination with Citrate containing dialysate, SHD = Standard dialysis.

**Tables 22: Paired analyses of mean difference in cell count in % at 180 minutes compared to start**

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>HA</th>
<th>HAC</th>
<th>Evodial®</th>
<th>SHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td>-9</td>
<td>-11</td>
<td>-9</td>
<td>-11</td>
<td>-9</td>
</tr>
<tr>
<td>Platelets</td>
<td>-5</td>
<td>-7</td>
<td>-7</td>
<td>-6</td>
<td>-6</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>-29</td>
<td>-26</td>
<td>-33</td>
<td>-27</td>
<td>-17</td>
</tr>
<tr>
<td>Monocytes</td>
<td>-15</td>
<td>-25</td>
<td>-21</td>
<td>-28</td>
<td>-20</td>
</tr>
<tr>
<td>Basophils</td>
<td>-16</td>
<td>-29</td>
<td>-23</td>
<td>-14</td>
<td>-16</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>-1</td>
<td>-3</td>
<td>0</td>
<td>-3</td>
<td>-6</td>
</tr>
</tbody>
</table>

Values were adjusted for ultrafiltration before calculation.

Significant differences between start and 180 minutes *within the same model* are given as a) (p<0.05) or b) (p<0.01).

Significant differences between low-dose-models compared to standard dialysis (SHD) as reference are given as c) (p<0.05), other models compared to H-priming as d) (p<0.05), other models compared to HA-priming as e) (p<0.05), and HAC compared to Evodial® as f) (p<0.05).

H = Heparin priming, HA = Heparin-Albumin, HAC = Heparin-Albumin in combination with Citrate containing dialysate, SHD = Standard dialysis.
Pro and cons in summary for Study 4

<table>
<thead>
<tr>
<th>Treatment model</th>
<th>Pro</th>
<th>Con</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>- Low cost</td>
<td>- High APTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Massive clotting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Extra time for preparation</td>
</tr>
<tr>
<td>Heparin Albumin</td>
<td>- Significantly reduced anticoagulant need.</td>
<td>- Higher cost</td>
</tr>
<tr>
<td></td>
<td>- &gt;50% of the treatments completed without any anticoagulant</td>
<td>- Extra time for preparation</td>
</tr>
<tr>
<td></td>
<td>- No bleeding occurred</td>
<td></td>
</tr>
<tr>
<td>Heparin Albumin + citrate dialysate</td>
<td>- Significantly reduced anticoagulant need.</td>
<td>- Higher cost</td>
</tr>
<tr>
<td></td>
<td>- &gt;50% of the treatments completed without any anticoagulant</td>
<td>- Extra time for preparation</td>
</tr>
<tr>
<td></td>
<td>- No bleeding occurred</td>
<td></td>
</tr>
<tr>
<td>Evodial®</td>
<td>- Significantly reduced anticoagulant need.</td>
<td>- Higher cost</td>
</tr>
<tr>
<td></td>
<td>- &gt;60% of the treatments completed without any anticoagulant</td>
<td>- A risk of hypersensitive reactions</td>
</tr>
<tr>
<td></td>
<td>- No bleeding occurred.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- No extra time for preparation</td>
<td></td>
</tr>
</tbody>
</table>

**Individual differences – individualized treatment models**

It was shown that the different low-dose models were more or less suitable for different individuals. After the study, a personal low-anticoagulant sheet was made for each patient’s file based on the individual results. The sheet contained results for treatment time, extra doses of heparin needed, clotting level in chambers and dialyser, and APTT for each of the treatments for the individual patient. The preferable model for each individual was highlighted on the sheet. Tables 23 and 24 shows examples of the results and “best choice” for two separate participants.
Table 23: Example of one of the study participant's results that can be used in practice if needed

<table>
<thead>
<tr>
<th>Priming</th>
<th>Time</th>
<th>Extra doses</th>
<th>Chambers</th>
<th>Dialyser</th>
<th>APTT0</th>
<th>APTT30</th>
<th>APTT180</th>
<th>Best choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD</td>
<td>180</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>32.5</td>
<td>55.4</td>
<td>34.1</td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>237</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40.7</td>
<td>40</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>179</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>36.8</td>
<td>38.2</td>
<td>33.6</td>
<td>HA</td>
</tr>
<tr>
<td>Evodial</td>
<td>180</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33.5</td>
<td>34</td>
<td>32.5</td>
<td>Evodial</td>
</tr>
<tr>
<td>HAC</td>
<td>179</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>32.5</td>
<td>32.4</td>
<td>32.1</td>
<td>HAC</td>
</tr>
</tbody>
</table>

The ‘best choices’ are marked with bold letters and in the right column. Other data are: Treatment time achieved for each model, extra dose needed, clotting levels in chambers and dialysers, and APTT at 0, 30 and 180 minutes. H = Heparin, HA = Heparin-Albundin, HAC = Heparin-Albundin in combination with Citrate containing dialysate, SHD = Standard haemodialysis. APTT = activated partial thromboplastin time (ref 22-36 seconds).

Table 24: Another example of one of the study participants’ results that can be used in practice if needed.

<table>
<thead>
<tr>
<th>Priming</th>
<th>Time</th>
<th>Extra doses</th>
<th>Chambers</th>
<th>Dialyser</th>
<th>APTT0</th>
<th>APTT30</th>
<th>APTT180</th>
<th>Best choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD</td>
<td>223</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34.8</td>
<td>164.4</td>
<td>55.6</td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>236</td>
<td>1000</td>
<td>2</td>
<td>1</td>
<td>28.5</td>
<td>29.6</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>239</td>
<td>1000</td>
<td>2</td>
<td>2</td>
<td>31.9</td>
<td>31.3</td>
<td>33.8</td>
<td></td>
</tr>
<tr>
<td>Evodial</td>
<td>Clotting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAC</td>
<td>225</td>
<td>500</td>
<td>2</td>
<td>2</td>
<td>29.1</td>
<td>30.9</td>
<td>28.6</td>
<td>HAC</td>
</tr>
</tbody>
</table>

The ‘best choice’ is marked with bold letters and in the right column. Other data are: Treatment time achieved for each model in the study, extra doses needed, clotting levels in chambers and dialysers, and APTT at 0, 30 and 180 minutes. H = Heparin, HA = Heparin-Albundin, HAC = Heparin-Albundin in combination with Citrate containing dialysate, SHD = Standard haemodialysis. APTT = activated partial thromboplastin time (ref 22-36 seconds).

In conclusion, HA, HAC and Evodial® showed beneficial results regarding the need of UFH, and they also had mean APTT levels within reference. Evodial® had less efficacy regarding URR at both 30 and 180 minutes. The hypersensitive reaction in one patient is also a reason for caution. Further studies are recommended to confirm these data.
Discussion

The aim of this Thesis was to investigate alternatives to standard anticoagulation when dialysing patients with an increased bleeding risk.

The studies showed that it is possible to significantly lower the use of anticoagulation with the various models tested although each different model had its advantages and disadvantages. HA-priming was the starting point of the Thesis since this model has been used at the University Hospital in Umeå since the 1980s. Although the clinical experience had been good during the period where there were limited alternatives, the model had not been evaluated in comparison to other models; this encouraged Studies 1 - 3.

The review of the protocols from 1995 - 2013 showed that HA-priming significantly reduced the need of anticoagulant. Despite the lower doses of anticoagulant there were no differences in the numbers of interrupted treatments due to clotting as compared to standard haemodialysis.

There were no reports of aggravated bleeding during the studies.

According to Kontanko et al. synthetic materials provide a superior biocompatibility compared to cellulose membranes (Kontanko et al. 2015). This could not be evaluated with cell count in Studies 1 and 2, but there were no significant difference in clotting when comparing membrane materials, not even when comparing materials of the same size. When comparing the area of the dialyser membranes there was a difference – a
dialyser with an area > 1.7 m$^2$ was more prone to result in clotting than a dialyser with a smaller area.

The *in vitro* study showed that the established HA-priming with the concentration of 1 g of albumin and 5000 IU of heparin per litre saline was a sufficient model. There was no difference between HA compared to those with a greater amount of albumin or heparin during the experiments. Albumin in saline resulted in an interrupted treatment within a mean of 26 minutes. Saline only resulted in even earlier interruption. Heparin in saline was successful according to survival of the ECC. However, there were high APTT values throughout all the *in vitro* treatments with this priming.

In the *in vivo* study (Study 4) H-priming also resulted in high APTT values. But here, the model was associated with a large need of extra doses of heparin. Despite the extra doses of heparin, more than one third of the treatments ended up with total clotting of the ECC. According to these results H-priming is a less preferable model. The results seem reasonable since the manufacturers argue that the ECC and the dialysers are negatively loaded by production, and that heparin should not be able to attach to these surfaces. This counteracts with the results in the *in vitro* study where the heparin priming resulted in > 192 minutes (Kyrk et al. 2014). In that study the blood was recirculated and heparin leftovers were possibly present in the ECC during the whole dialysis. During *in vivo* dialysis heparin is metabolized and the short half-life will not result in a high APTT during the whole treatment unless more heparin is added, which on the other hand may be needed with that priming model.

However, adding albumin to the priming solution seems to help in binding heparin toward the surface of the ECC and in preventing clotting. Such benefit was not found
when priming with heparin only or albumin only (Kyrk et al. 2014). The assumption that the HA-priming creates a coating of the ECC surface was strengthened by the experiment in Study 3 when a 2-litre bag with HA-priming was recirculated during a 3-hour dialysis. Albumin seems to cover the inside of the ECC, although parts of it may have been dialysed away despite it having a high molecular weight (66,000 Da).

The in vivo study showed that HAC was the best choice with no total clotting in any of the 19 treatments performed. Evodial® also allowed most treatments without clotting but showed a lower cleaning coefficient and caused a serious hypersensitive reaction in one participant. The participant had taken ACE-inhibitors in the daily medication. It is known that cytokine release may occur if a patient is on ACE-inhibitors and is treated with a dialyser membrane material consisting of polyacrylonitrile (Desormeaux et al. 2008; Tielemans et al. 1990). The Evodial® dialyser membrane consists of an acrylonitrile and sodium methallyl sulfonate copolymer called polyethyleneimine26. However, such reactions were not reported in another study with Evodial® (Laville et al. 2014). In that study no adverse events at all were reported. The previous study also found no differences in cleaning coefficient compared to the control group. That study had dialysers of same size (1.6 m²) in both groups, while Study 4 in this Thesis used the FX80 dialyser with an area of 1.8m² during all other low-anticoagulant treatments, which could have influenced the results. A French study compared Evodial® to a dialyser with an E-vitamin coated membrane, both without adding anticoagulant before or during the treatment. Both dialysers resulted in a total clotting frequency between 12 and 22% (Islam et al. 2016). Another study (Frasca et al. 2015) investigated the frequency of clotting with Evodial® and postdilution when priming with saline and administering 1000 IU of UFH from the start and a repetitive dose of the same amount

26 Gambro Lundia AB, Lund, Sweden
after two hours of dialysis. They reported a massive clotting in 10% of the treatments. The same study also evaluated Evodial® with postdilution, saline priming and a start dose of LMWH of 47 IU per kilo body weight. With that treatment massive clotting occurred in 1%. However, the anticoagulant doses are high in both these examples and not applicable when considering dialysis of a patient with a bleeding risk.

Evodial® has also been studied in combination with citrate dialysate and the result was 15% premature interrupted treatments. In 4.2% of the treatments the blood was not possible to give back to the patient because of massive clotting. In that study 89% of the patients had CDCs and temporary CDCs were associated with lower blood flow. Further, the lower blood flow was associated with clotting (François et al. 2014). Within the frame of the studies in this Thesis, no inverse correlation between blood flow and clotting was found.

The use of citrate containing dialysate, without any additional anticoagulation, has been shown to require extra doses of anticoagulants (Stegmayr et al. 2013). In a study where predilution was used in combination with citrate dialysate all treatments were successful without any additive anticoagulant, but at the cost of unsatisfying performance and less biocompatibility compared to regular treatment (Richtrova et al. 2016). The combination of HA-priming and citrate containing dialysate seems to reduce the risk for clotting according to the results in Study 4 of this Thesis. Another study (Aniort et al. 2012) used citrate dialysate and postdilution (> 20 litres) which resulted in a 50% reduction of the patients’ standard UFH dose. There was one interrupted treatment reported, due to an AV-fistula stenosis. All patients in that study (n=10) had an AV-fistula, a QB of > 350 ml/min, high postdilution volumes, and a polysulfone dialyser with 2.1 m² area. This is not applicable on a group of patients with AKI where a
temporary CDC, often having a reduced function (François et al. 2014), is dialysed. Further, the patients with an acute need of dialysis cannot be treated with that high blood flows and postdilution volumes (unless they are chronic patients with the acute treatment need and are used to that kind of dialysis). The results, however, may be interesting for patients in chronic dialysis.

The contact between blood and artificial materials causes an activated coagulation and complement system. The reductions in leucocyte cell count that appear strengthen this assumption. The leucocyte and platelet reduction can be interpreted as a sign of inflammatory activity. Monocytes, macrophages and neutrophils are known to release coagulation and fibrinolytic factors. They are also known to interact with the haemostatic system (Swystun and Liaw 2016). In this Thesis, the H-priming model had the most pronounced reduction in leucocytes. This indicates activation of leucocytes that can contribute to the high presence of clotting and interrupted treatments with that model. During the H-priming dialysis, the blood may have been in frequently direct contact with the material in the ECC and thereby been under influence of the activated cascade systems during the whole treatment. The H-priming was included to prove its inconvenience vs. the low cost that had been advocated. The study showed that H-priming is less preferable, and in the long run it might cost even more with new material and pro-longed treatment time if an entirely new treatment must be prepared to achieve the dialysis needed.

Heparin is known to have more than an anticoagulant effect on the blood cells. The levels of triglycerides (TG) were decreased during all low-anticoagulant models besides with Evodial®. Heparin induces a lipoprotein lipase (LPL) release from the surface of the blood vessels and results in a release of free fatty acids (TG) by hydrolysis from
LPL. TG initially gets lower but subsequently increases with a remaining lack of functioning LPL and a catabolic situation may appear during a regular supply of heparin (Mahmood et al. 2010; Stegmayr 2014).

High APTT was noted before the start of dialysis in some patients in the clinical study. The first assumption was contaminated blood samples by heparin leftovers from the catheter lock solution since samples were collected from the CDC before the start of dialysis. The present results later showed that heparin was present in the blood circuit and affected the TG levels in these patients - indicating that the decrease was a sign of a leakage of the heparin containing catheter lock solution into the patient's blood. This was determined since the decrease in TG was no longer significant after 30 minutes in the same patients. At that time the small doses of heparin that leaked into the circulation during preparation had been dialyzed away. This incidental finding of the leakage of catheter lock solution (CLS) is an important issue. It seems like heparin tends to leak from the CDC while it is being handled and prepared both before and after dialysis. Another study reported leakage when injecting the CLS into CDCs. Blood samples were collected by venous puncture before the adding of CLS and 15 minutes after the injection. The authors found high APTT with a mean of 120 seconds at 15 minutes after the solution was added (McGill et al. 2005). This needs to be considered when handling the access both before and after dialysis, and should be a reason to choose a catheter lock solution without heparin when dialysing patients with a bleeding risk.

There were no reductions in clotting between those with or without per-oral anticoagulants in Study 4. Also, no prolonged bleeding was seen. This is in agreement with Nadarajah et al. that also reported no increased bleeding caused by per-oral
anticoagulants (Nadarajah et al. 2015). In another study patients on per-oral anticoagulant were in no need of systemic anticoagulation during HD with Evodial® or with a polysulfone dialyser with the same membrane area (Krummel et al. 2014). Such limited clotting was not confirmed in Study 4 of this Thesis. However, some protective effects towards clotting were reported in another study with patients on warfarin with an average International normalized ratio (INR) of 1.5. These patients were more sensitive to heparin than patients on no per-oral anticoagulant or on dabrigatran (Edrich et al. 2015).

The favour for the patients in the clinical study was the possibility to immediately identify (and when necessary also use) the model of the low-anticoagulant methods that suited the specific individual. The studies in this Thesis indicate that custom made individualized low-anticoagulant models can be recommended, especially in acute dialysis treatment of patients with a risk for bleeding but also in general for patients in chronic dialysis treatment.

**The patients perspective**

This Thesis included studies that aimed to improve the safety and efficacy in the care for patients in need of haemodialysis and with an increased bleeding risk. These patients are vulnerable and might be a challenge for the dialysis nurse. We are not aware of such data and have not reported such results previously, but we have started to examine the patients’ experiences. Preliminary data indicate that we need more knowledge to be able to optimize the care of these patients. There are studies describing the long-term effects for patients who had AKI and had been treated with acute intermittent HD and/or CVVHD.
Quality of life in patients who survived AKI

The patients that had acute AKI and needed intermittent HD had a lower health-related quality of life (HRQOL) than the general population (Hofhuis et al. 2013; Maynard et al. 2003; Nisula et al. 2013; Noble et al. 2006; Åhlström et al. 2005). One study reported that there was no difference in HRQOL when comparing patients with AKI in the ICU to those in the ICU who did not have AKI (Nisula et al. 2013). According to Åhlström et al. the patients were as satisfied with their health as the general population despite the lower HRQOL (Åhlström et al. 2005). Rimes-Stigare et al. reported moderate physical impairment and reduced mental health scores. In that study those who returned to work had better health and graded it as "acceptable to good" (Rimes-Stigare et al. 2012).

For me, who have worked close to these patients the need of a holistic perspective is of importance. How does the patient experience this urgent need of dialysis, and perhaps with an additional risk in regard to an increased bleeding risk? Thereof literature is lacking in descriptions. Besides from studies describing survival and long-term effects the closest one can come in the literature are studies with information about how patients experience a sudden start in chronic dialysis treatment or an average start after long-term chronic kidney failure. These studies showed that for some patients it was a chock that the time to start dialysis had come, even though they knew that it would sooner or later. Studies that investigated patients that recently started their chronic HD described expressions of a major life crisis. Many of them were not prepared for the change in life that regular HD treatments would bring about (Hagren et al. 2001; Hagren et al. 2005; Harwood et al. 2005; Lai et al. 2012).
The side-effects from the dialysis were described as limiting. Many patients suffered from post-dialysis syndrome and were exhausted after the dialysis. Many were also very tired the day after the dialysis, and the following day it was a dialysis day again (Lai et al. 2012; Lee et al. 2015). One study underlined the importance of person-centred information and education and claimed that all patients “should know as much as possible” when they were about to have their first dialysis (Gunnarson 2016).

Can the results above be applicable on the group of patients that are in an acute need of haemodialysis? How can we prepare the patient in need of acute dialysis treatment in a best way? How can we help them in this new situation? Shall we focus on technical information about the dialysis treatment and how the kidney functions, or shall we spare them the information until they are in a better health condition?

This situation is even further complicated in patients with AKI that in need of dialysis and are comprised of an increased risk of bleeding. This has to be considered when dialysis has to be fulfilled with procedures that are insufficiently investigated or not yet established. This may add to the fear of the patient and family before conditions are stabilized. There is a need for further clarification of optimal techniques to use in such treatment conditions and an understanding on how to inform and, from a nursing perspective, take care and treat both the medical and the mental conditions in a comforting way. Would such knowledge give nurses and physicians possibilities to prepare the patient and thereby also avoid or minimize the suffering for the patient? However, the literature is lacking on information about these patients’ experiences of acute kidney injury and the sudden need for dialysis. This has to be further explored in future studies for a holistic perspective in the care of these patients.
Benefits of this Thesis

The benefit of this Thesis was the investigation that started with a smaller and then a larger retrospective clinical study to clarify any differences in heparin need and clotting levels between HA-priming and standard HD. The data were consecutively included which entails coverage for all types of problems leading to a need of acute dialysis. The results from those studies were a base for the experimental studies. This together led to the clinical prospective study. The HA-priming that became a “proven experience” in the clinic and had been used for more than 20 years has now be evaluated and tested against other models. The HAC-combination had not been evaluated previously according to the literature. When the clinical study started Evodial® was a new dialyser without any data yet published. Study 4 was of great benefit for the participants included – they received their own “best choice” sheet in their log. This is an asset if they need a low-anticoagulant-dose treatment in the future.

Limitations of the Thesis

In Studies 1 and 2, any information not noted on the protocols at the time of the treatment could not be evaluated since the researchers did not have the possibility to recreate the situation with the nurses who worked at that time with that specific patient. This could have led to a loss of information. In Study 3 the adding of fluid to compensate for the removal could make the result falsely better since continuous infusion of saline is a known method to reduce clotting (Zimbudzi 2013). The slower blood flow in Study 3, Sub-Study 2, could have contributed to greater clotting, although the results in Study 2 showed the opposite results (Skagerlind and Stegmayr 2016). On the other hand, the dialyser in Sub-Study 2 had a smaller area and the ECCs
bloodlines were narrower in Sub-Study 2. This may have offset the difference, since the total surface area was smaller.

To clarify if the loss of albumin (Study 3) from the ECC was a result of coating inside the ECC or if the albumin was dialysed away, analysis of albumin concentration in isolated ultrafiltrate could have been made. According to the Essential Guide to Blood Coagulation, a rocker for the test tubes should be used after collecting blood samples for coagulation to avoid uneven mixing with the additive. The sample should also have been taken from a venous puncture and not from a heparinised catheter. The patient should also be sitting up when blood for coagulation analysis is collected (Blombäck and Egberg 2013). This was not done in Study 4. The samples were collected from a CDC, femoral catheter or a needle in an AV-fistula. This presented a risk for contamination of the catheter lock solution and result bias.

The handling of the blood coagulation tests may have differed since different nurses collected them. The tubes were tilted by hand after blood collection that may lead to differences in handling. The local laboratory has one hour as the given limit for APTT blood samples counted from the time of sample collection until the start analysis. There may have been samples that were not analysed within that time. Most patients did not sit in an upright position but the same position was presumably used for the same patient every time, which makes it a consistent error. We cannot expect the same results in a group of critically ill patients with acute kidney injury, since they vary in platelet count, bleeding risk and medication compared to chronic patients.

An ambiguity may appear since the grading of clotting is not consistent in the studies. In Study 3 a five level scale was used, while in the other studies a four level scale was
used for evaluation and grading of clotting. The background is that on this hospitals' local standardised treatment protocol uses an evaluation scale with four steps. The five-step scale in Study 3 was based on scales from other studies in the literature.

Study 4 was not blinded because of safety, technical and logistical reasons. This could have contributed to bias when the nurses evaluated the clotting tendencies. There were 20 different nurses involved in the study and they may not have equally evaluated the clotting tendencies and when an urgent need for extra heparin was needed. The statistics in Study 4 was blinded before calculations of the results were made.

**Implications for future research**

The existing studies and guidelines existing all show that there is a need for a consensus about anticoagulation during dialysis in general and anticoagulation during dialysis in patients with a bleeding risk in particular. The clinical study compared four different low-dose models for anticoagulation for haemodialysis in patients with a bleeding risk. None of the models had been compared to each other before as far as we know. Therefore further studies are recommended to strengthen decisions about which - and if - any of these models are most beneficial.

**Ethical discussion**

The HA-priming was designed during a time when a life threatening treatment of acutely ill patients was complicated by frequent clotting. The model used at that time was H-priming or saline priming, both with massive clotting problems. An extra dose of heparin given to a person with acute bleeding could be lethal. It was then the idea of HA-priming came up. This was tested and turned out to allow dialysis with no or low
doses of heparin. Patients of the type included in the studies in this Thesis are vulnerable. The patients with an AKI often also have co-morbidities each of which can be lethal in its own right. The Swedish National Board of Health and Welfare heavily advocate the right to equal care, regardless of gender, age, socio-economic status and domicile. The situation today is not equal when patients receive dialysis. According to the literature, saline intermittent or by flushes each 30th minute results in clotting in a high per cent of the treatments performed. If the same patient instead received HA-priming or HAC, the treatment may have provided a greater removal of toxins and led to a faster recovery and less need of HD. The cost thereby might be lower when summarizing the stay in hospital (an insufficient cleaning may result in a need of extra dialyses).

The participants in Study 4 were all chronic haemodialysis patients with no increased bleeding risk. Since they participated on their regular treatment day, the decision was made not to let the treatment continue to total clotting if it was possible to avoid. We did not want the participants to waste free time (in case of early total clotting a new machine had to be prepared and this could lead to a waiting time up to one hour). Some patients encountered total clotting, and if that took place early in the treatment a new machine with that patient’s regular settings (dialyser, saline priming, start dose) was prepared. The time loss could not be replaced and as a dialysis nurse I know how precious time off the machine are to these patients.

The benefit for the participants was their personal results in the study. The summary of their study results could immediately be used as a guide if any of them was affected by a higher risk of bleeding or an acute bleeding. A follow-up showed that HAC as well as
HA has been effective in most dialyses. If clotting tends to interrupt dialysis predilution (8 litre/3 hours) was added to the HAC or HA model, respectively (unpublished data).

Furthermore there is a possibility that the patients may have felt that they had to participate. They are in a dependency situation since they know us from the clinic. Even though they were informed orally and in writing about the study being voluntary, there might have been a couple of patients participating against their will just to show good intentions. This is not easy to ascertain, but a few patients became very stressed when the nurse frequently checked the ECC for clotting; these patients discontinued their participation after one or two treatments within the study. It is also difficult to know whether all the participants understood what the study meant. Some may have consented to participate without really understanding even if they stated that they did.

The nurses at the dialysis ward know “their” patients’ best and are opposed if something seemed not to be right. They played an important role throughout the study as a spokesperson for some of the patients. This is also beneficial for future patients since we know that the HA-priming is safe, and Study 4 implies that HA in combination with citrate dialysate is even better.
General conclusion

The hypothesis that albumin molecules attaches to the surface of the ECC, and that the heparin molecule attaches to the albumin molecule seem like a good solution when dialysing patients with at risk for bleeding. In combination with a citrate containing dialysis fluid this preparation seems to give even better results in accord with the in vivo study where HAC gave no single interrupted treatment, a satisfying urea reduction rate, a low need of extra anticoagulant doses, and a mean bleeding time within the references values. If HA in combination with citrate dialysate is not available, Evodial® or HA-priming may be other choices to facilitate low dose/no anticoagulant dialyses. A safe and smooth dialysis procedure also facilitates the stressful situation the patient and the nurse are confronted with.
Acknowledgements

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My father who in-between shouted, “go for it” while I sighed and sometimes cried. My mother and my mother in law for taking care of Erik in periods when I needed help.
# Acute dialysis prescription

<table>
<thead>
<tr>
<th>Name: __________________________</th>
<th>Date of birth: __________________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date: __________________________</td>
<td>Ward: __________________________</td>
</tr>
<tr>
<td>Diagnosis: ______________________</td>
<td>Access: _______________________________</td>
</tr>
<tr>
<td>HIV/Hepatitis □ Pos □</td>
<td>Potassium: ______ mmol/l</td>
</tr>
<tr>
<td>If positive specify: ____________</td>
<td>Creatinine: ______ mmol/l</td>
</tr>
<tr>
<td>Date: __________________________</td>
<td>S-Urea: ______ mmol/l</td>
</tr>
</tbody>
</table>

| Type of treatment: HD □ Iso-UF □ | Dialysate: ______ |
| HDF □ HDF-Volym □               | Na / bic: ______ |
| Hemoperfusion □                 | Start dose Heparin □ ______ U |

Dialysor: __________

Treatment time: ______ h

Fluid to remove: ______ L

Heparin maintenance ______ U/h

Other prescriptions:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

Date: __________________________  Doctors sign: __________________________

<table>
<thead>
<tr>
<th>Priming Albumin / Heparin □</th>
<th>Y □ N □</th>
</tr>
</thead>
</table>
**Treatment protocol**

**Name**  
**Date of birth**

**Date**  
**Bloodslag**  
**Batch:**

**Machine**  
**B-Conc/bic:**  
**Batch:**

**Priming:**  
**Batch:**  
**Dialyzer:**  
**Batch:**

**HDF**  
**HD**  
**Treatm.time:**  
**MT**  
**Needle (G):**  
**Tr.cut:**  
**160**  
**170**  
**Supercat**

**Pt weight before**  
**kg**  
**Target:**  
**kg**  
**F:**  
**l**  
**Fluid aim**  
**l**

**BP/Pulse b/HD:**  
**Nurse sign**  
**Nurse ctrl**

<table>
<thead>
<tr>
<th>Time</th>
<th>BP mmHg</th>
<th>Pulse /min</th>
<th>Qb ml/min</th>
<th>Artery pressure mmHg</th>
<th>Vein pressure mmHg</th>
<th>TMP mmHg</th>
<th>Cond. ml/cm</th>
<th>UF-rate ml/h</th>
<th>HDF-rate ml/min</th>
<th>Note</th>
<th>Sign</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td></td>
<td></td>
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<td>*</td>
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</tr>
</tbody>
</table>

**Injections etc**  
**Sign**  
**Injections etc**  
**Sign**

**Nurse sign after HD**  
**UF-vol:**  
**L**  
**Kt/V:**  
**Blood volume:**  
**l**

**Heparin**  
**Innohep**  
**Tot:**  
**HDF-vol:**  
**l**  
**BVM aHD:**  
**Treatment time:**  
**Weight after:**  
**kg**

**BP/pulse aHD:**  
**P-gl eHD:**  
**Filter:**  
**Clean**  
**Striped**  
**Red**  
**Clotted**

* Measure venous pressure after 5 minu  
**Keep filed with patient notes.**
**Study information for patients**

Occasionally the standard dose of anticoagulation medication cannot be administered during haemodialysis. This can be due to the presence of bleeding, or that an operation is planned or was performed just prior to dialysis (resulting in an increased risk of bleeding).

In this study we will investigate which alternative is the best option if you are to undergo dialysis but should avoid being given the full dose of anticoagulation due to an increased risk of bleeding. In most cases we use a priming solution that contains albumin and heparin to rinse the filter and tubing system before treatment.

In this study four different alternatives will be evaluated. These are:

1. Albumin/Heparin
2. Heparin
3. A filter that is produced with heparin coating
4. Albumin/heparin and a citrate solution instead of standard dialysate

Each different experimental occasion will cover approximately 3 hours for a dialysis session (usually necessary in acute dialysis). The same dialysis settings that you normally use will also be used for the study. The dialyser used for the study (FX 80) has a smaller surface compared to the FX 1000 that is usually used during your treatment, because we feel a smaller dialyser is better for these occasions.

Since you will not be receiving the usual anticoagulation medication inserted in your blood, the dialysis filter and the tubing system can turn into somewhat of a striped dialyser where blood is left in the capillaries and the pores, and in the long-term this can cause a less effective filtration. If we notice that the blood is starting to clot, we will add extra anticoagulation (heparin).

We will take blood samples three times (from the venous access before treatment and from the machine during treatment) during each dialysis session to evaluate blood
haemoglobin, efficacy of dialysis (urea) and if a change in the risk for bleeding is developing.

You are free to leave the study at any time and without any needed explanation. Swedish law protects your personal data and the data that are recorded will be registered by a code instead of your personal identification number so that your identity will be protected.

You are free to pose questions at any time throughout the study.

The responsible parties for the study are the Vasterbotten County Council, professor Bernd Stegmayr (co-author) and research nurse Malin Skagerlind (main author).
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