Presence of microemboli during haemodialysis and methods to reduce the exposure to microbubbles

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To my family
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ORIGINAL PAPERS
ABSTRACT

Despite chronic dialysis treatment, patients with end stage renal disease undergoing maintenance haemodialysis (HD) remain at a substantially increased risk of morbidity. Previous reports using Doppler ultrasound (DU) during HD have revealed microembolic signals (ME) in the venous circulation. *In vitro* studies confirm the emergence of microbubbles of air that may pass the security system of the HD circuit without triggering the alarm. The aim of this thesis was to elucidate the presence of ME during HD and examine methods that might reduce exposure to ME *in vivo*.

The first study utilized DU to verify the presence of ME in 40 patients during standard HD. Investigation within 30 minutes after the start of HD and just before the end of session revealed the presence of ME in the venous blood line during both phases. The air trap did not alert for the presence of ME. This indicated that ME may pass into the patient during the entire HD run.

Study 2 analyzed the presence of ME prior to start and during HD when measured at the AV-access and also carotid artery. A total of 54 patients were examined using DU as the investigative technique. ME increased significantly after start of HD in the AV-access, but also at the carotid artery site. These data indicated that ME can enter the body and even pass the lung barrier. The question arose if microbubbles of air are resorbed or may cause ischemic lesions in organs such as the brain.

Study 3 examined whether the amount of ME detected in the AV-access would change by using either a high or a low blood level in the venous air trap/chamber. This was a prospective, randomized and double-blind study of 20 HD patients who were their own controls. After 30 min of standard HD, measurement of ME with DU was performed for two minutes. The chamber setting was changed and after another 30 minutes a new recording was carried out for two minutes. Data showed that setting a high blood level significantly reduced the extent of ME that entered the patient. The results also indicated that ME consisted mainly of microbubbles.

In study 4, twenty patients were randomized in a cross-over setting of HD. Three options were used: a wet-stored dialyzer with high blood
level (WH) and a dry-stored dialyzer using either a high (DH) or a low (DL) blood level in the venous chamber. The exposure of ME, detected by DU, was least when using mode WF, more with mode DH, and most with mode DL. There was a correlation between higher blood flow and more extensive exposure to ME.

Study 5 was an autopsy study of a chronic HD patient with the aim of searching for microbubbles deposited in organs. Microbubbles of gas were verified in the vessels of the lungs, brain and heart. By using a fluorescent stain of anti-fibrinogen it was verified that the microbubbles were covered by clots that had to be preformed before death occurred. This indicated that air microbubbles are not completely absorbed and could result in embolic deposition in the organs of HD patients.

**In conclusion,** these *in vivo* studies showed that ME pass the air trap without inducing an alarm and enter the venous blood line of the patient. The data confirmed the presence of ME in the AV-access and also in the carotid artery. Autopsy data of a deceased HD patient demonstrated the presence of microbubbles in the capillaries of the lungs, but also in the systemic circulation such as in the brain and the heart. A high blood level in the venous chamber and wet-stored dialyzer can reduce, but not eliminate the exposure to microbubbles for patients undergoing HD.
En av njurarnas viktigaste uppgift är att rena blodet från avfallsprodukter, som uppkommer vid kroppens ämnesomsättning (metabolism). Ämnesomsättning är ett sammanfattande namn på de processer där näringsämnen tas upp, omvandlas och bryts ner i kroppen för att till slut omsättas till energi. Vid långt gången njursvikt ansamlas avfallsprodukterna i kroppen. För eliminering av dessa ämnen krävs en speciell blodreningsprocedure som t.ex. hemodialys (bloddialys). Hemodialysapparaten för ut blod från patienten, renar blodet och återför det till patienten. För att förhindra att luft (från t.ex. slangkopplingar) av misstag kommer in i patienten finns speciella luftvakter i dialysapparaten. Enbart i Sverige sker årligen mer än 400.000 hemodialysbehandlingar.

Det är sedan länge känt att mikroskopiskt små embolier (partiklar som följer med blodströmmen), upptäckta med hjälp av Doppler-ulraljudsteknik, förekommer i dialysapparatens blodfylda slangar under pågående hemodialys. I våra tidigare experimentiella studier har resultaten talat för att dessa mikroembolier till största del består av gasbubblor, som passerar luftvakten utan att den larmar. Syftet med avhandlingen var att undersöka förekomst av mikroembolier vid hemodialys och hitta metoder att minska patienternas exponering för mikrobubblor.

I första studien undersökte 40 patienter, med Doppler-ulraljudsapparat (DU), med avseende på mikroembolier under pågående hemodialys (HD). Patienterna undersökes inom 30 minuter efter dialysstart och precis innan avslut. Vid båda mättillfällena fann vi mikroembolier i blodslangarna efter luftvakten. Vid inget tillfälle larmade luftvakten. Detta indikerar att mikroembolier kan passera in i patienten under hela HD behandlingen.

I den andra studien mättes förekomst av mikroembolier i patienternas AV-access (blodkärllet där blodet förs in i och sedan ut från HD apparatens slangar) och halspulsådern (a.carotis). Mätningarna gjordes före och under pågående HD. Totalt undersöktes 54 patienter med DU. Antalet mikroembolier ökades signifikant efter start av HD både i AV-accessen och i halspulsådern. Dessa data talar för att mikroembolier passerar in i patienten och
även passerar lungbarriären. Frågan uppkom om mikrobubblor från HD absorberas i blodet eller om de kan orsaka skador i organ som t.ex. hjärnan.

I den tredje studien undersöktes om antalet mikroembolier, mätningarna skedde i AV-accessen, skulle påverkas av att blodnivån i luftvakten ändrades. Låg nivå jämfördes med hög nivå i luftvakten. 20 kroniska HD patienter lottades till att genomgå en standardbehandling med endera blodnivå i luftvakten. Efter 30 minuters behandling genomfördes en mätning under två minuter med hjälp av DU. Därefter ändrades blodnivån till den motsatta och efter 30 minuter gjordes en till mätning under två minuter. Data visade att antalet mikroembolier som passerade in i patienten minskades signifikant om blodnivån i luftvakten var hög. Resultatet indikerade också att den största andelen av mikroembolierna består av mikrobubblor.

I den fjärde studien lottades 20 patienter till olika sorter HD behandling. Tre olika varianter av HD behandling användes. Behandling med 1) Våtlagrat dialysfilter med hög blodnivå i luftvakten (VH), 2) torrlagrat filter med antingen hög (TH) eller 3) låg blodnivå i luftvakten (TL). Exponeringen av mikroembolier, som registrerades med hjälp av DU, var minst vid behandlingsvariant VH, mer vid TH och mest om behandling med TL användes.


Sammanfattningsvis har dessa studier visat att mikroemobilier passerar in i patienterna under pågående HD, utan att luftvakten larmar. Resultaten bekräftar att mikroembolier finns i patienternas AV-accesser, men också i halspulsådern. Data från obduktion av en avliden HD-patient påvisade mikrobubblor i lungkapillärer, men även i hjärtats och hjärrnans blodcirkulation. En hög blodnivå i luftvakten och våtlagrat dialysfilter kan reducera, men inte eliminera, exponeringen av mikrobubblor för patienter som behandlas med HD.
ABBREVIATIONS

ACM  Arteria cerebri media
AKI  Acute kidney injury
AV  Arterio-venous
CAS  Carotid angioplasty with stent placement
CDC  Central dialysis catheter
CEA  Carotid endarterectomy
CKD  Chronic kidney disease
CT  Computed tomography
DU  Doppler-ultrasound
GFR  Glomerular filtration rate
HD  Haemodialysis
HDF  Haemodiafiltration
MES  Microembolic signals
MRI  Magnetic resonance imaging
PAH  Pulmonary arterial hypertension
PD  Peritoneal dialysis
PFO  Persistent foramen ovale
PTA  Percutaneous transluminal angioplasty
TCD  Transcranial Doppler
TIA  Transient ischemic attack
TMP  Trans-membrane pressure
UF  Ultrafiltration
Qb  Blood flow
Qd  Dialysate flow
This thesis is based on the following papers:


The papers have been reprinted with the permission of the publishers.
INTRODUCTION

The functions of kidney

The kidneys have two main functions, to clean the blood from the main part of the bodies' waste products produced by the metabolism, and to adjust salt and water excretion. The high blood perfusion (1.2 l/min, almost ¼ of the heart’s blood flow) enables 180 l of fluid from the circulation to be filtered daily by the kidney. A large percentage reabsorbs to the blood by the kidneys, and about 1.5 litres of urine will be produced in normal conditions. Other important functions are assisting acid-base balance, blood-pressure regulation, hormone production and metabolism. Although the body is equipped with two kidneys, the function of one reasonably healthy kidney is sufficient for survival. If the function deteriorates below 10% of a normal function, dialysis or kidney transplantation has to be considered for survival⁴.

Chronic versus acute renal failure

Acute kidney injury (AKI) is a clinical syndrome denoted by an abrupt decline in glomerular filtration over days to a few weeks. AKI is often divided into prerenal, renal or postrenal causes. Prerenal AKI may be due to hypovolemia, for example. Renal causes of AKI are, for example, glomerular diseases, acute tubular necrosis due to ischemia, or radio contrast agents. Postrenal renal failure is mostly frequently caused by obstruction of the urinary tract. AKI can be reversible if appropriate action is taken².

The kidney's ability to cleanse the blood is described by the term glomerular filtration rate (GFR). The renal glomerulus contains capillaries specialized for filtration. GFR describes the flow rate of filtered fluid through the kidney and may be measured as the urinary clearance of a filtration marker such as creatinine. Creatinine and other metabolites are retained in the body when kidney function is severely compromised and GFR is low. Clearance rate for a substance is the volume of blood plasma that is cleared of the substance per time unit. Because creatinine is filtered and excreted in the urine, urinary clearance of creatinine is used as an endogenous measure for approximating the GFR. Exogenous substances like iohexol are a better filtrations marker because they do not secrete or reabsorb in
the kidneys. Therefore the urinary excretion equals the filtered load and urinary clearance equals GFR. The normal level of GFR depends on body size, sex, age, physical activity, diet and physiological states, such as pregnancy. The normal level of GFR for a person 20 years of age is approximately 120 ml/min/1.73 m² (adjusted for body surface area)².

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function that are present for more than 3 months, and with implications for health³. Either of following two criteria is needed for CKD diagnosis: (1) presence of at least one marker of kidney damage, for example, albuminuria of more than 30 mg/24 hours, urine sediment, histological abnormalities or structural abnormalities detected by imaging; (2) GFR less than 60 ml/min/1.73m²³.

CKD is divided into categories based on the level of estimated GFR normalized to body surface area (Table 1), and level of albuminuria (Table 2), according to guidelines from KDIGO (Kidney Disease: Improving Global Outcomes)³.

**Table 1. GFR categories in chronic kidney disease**

<table>
<thead>
<tr>
<th>Category</th>
<th>Terms</th>
<th>GFR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Normal or high</td>
<td>≥ 90</td>
</tr>
<tr>
<td>G2</td>
<td>Mildly decreased</td>
<td>60-89</td>
</tr>
<tr>
<td>G3a</td>
<td>Mildly to moderately decreased</td>
<td>45-59</td>
</tr>
<tr>
<td>G3b</td>
<td>Moderately to severely decreased</td>
<td>30-44</td>
</tr>
<tr>
<td>G4</td>
<td>Severely decreased</td>
<td>15-29</td>
</tr>
<tr>
<td>G5</td>
<td>Kidney failure</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

* Glomerular filtration rate (GFR) expressed in ml/min/1.73m²
Table 2. Albuminuria categories in chronic kidney disease

<table>
<thead>
<tr>
<th>Category</th>
<th>Terms</th>
<th>AER*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Normal to mildly increased</td>
<td>&gt;30</td>
</tr>
<tr>
<td>A2</td>
<td>Moderately increased</td>
<td>30-300</td>
</tr>
<tr>
<td>A3</td>
<td>Severely increased</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

*Albumin excretion rate (AER) expressed in mg/24 hours

Together with the cause of CKD and other risk factors and comorbid conditions, knowing the GFR and albuminuria categories is helpful when predicting the prognosis of CKD. The lower GFR and higher category of albuminuria, the higher risk of progress of kidney disease.

Kidney disease

The large number of disorders that can influence the kidneys reflects their wide range of functions, and can lead to many different kidney diseases. When the main function is affected, the filtration rate declines. This leads to retention of, e.g. urea and other toxic metabolites, and accumulation of fluid and volume overload occurs in the body. When the kidneys are no longer working effectively, waste products and fluids build-up and the patient will suffer from a uremic syndrome; this is in spite of adequate management of complications including protein-reduced diet, restricted water intake and medications.

Clinical features of uraemia are, e.g. nausea, vomiting, headache, dizziness, visual impairment, coma or epileptic seizures and subsequent death. To avoid such complications the patient needs renal replacement therapy.

Renal replacement therapy

Renal replacement therapies are treatments for severe kidney failure, and these take over a portion of the function of the failing kidneys to remove the fluid and waste. Renal replacement therapy is typically needed when about 90 percent or more of kidney function is lost.

There are three main types of renal replacement therapy:

- Transplantation is a surgical procedure in which a kidney from a deceased or living donor is transplanted into a patient
with renal failure. This provides the best chance of survival and improves quality of life.

- Peritoneal dialysis (PD) involves infusion of dialysis fluid into the space between the abdominal visceral organs and abdominal wall-peritoneal cavity, allowing movement of uremic solutes between the patient’s blood and dialysis fluid across the peritoneal membrane.

- In haemodialysis (HD), the blood is allowed to flow through a cleansing filter that removes waste products and excess fluid. The cleansed blood is then returned to the body. In my thesis I will focus on this type of dialysis.

The choice of initial treatment for patients with progressive renal failure is influenced by a number of factors that include medical, social and psychological considerations.

**Epidemiology**

The active treatment of uraemia in Sweden has increased in number continuously since treatment options were established in the 1960s. All treatments – transplantation, HD and PD have increased. The Swedish registry for the active treatment of uremics (SNR, Svenskt njurregister) is a computerized, web-based registry for patients with CKD. It is run by the Swedish Kidney Medical Association (SNF, Svensk Njurmedicinsk Förening) and the Swedish Transplantation Society (STF, Svensk Transplantationsförening). The following information is taken from SNR’s latest annual report in 2012 after having obtaining permission. There were more than 3000 patients on HD, approximately 4900 with a functional transplant, and more than 800 having PD treatment. This gives a prevalence of 923 patients in active uraemia treatment per million of inhabitants in Sweden; (the annual growth has declined in recent years and in 2011 it was 2.7%). The number of HD patients increased (Figure 1).
Figure 1. Number of patients in active treatment of uraemia 1991-2011. HD=haemodialysis, HHD=haemodialysis in home, PD=peritoneal dialysis, TRPL=transplantation

Since 1999, the number of dialysis patients increased by 34%. In regard to newly admitted patients for active treatment of uraemia the incidence has remained quite stable at around 125 per million inhabitants per year.

The gender distribution was essentially unchanged over recent years with one-third being women. The average age of those on active treatment or uraemia has risen (Figure 2). HD patients are the oldest group with an average age of over 66 years. The mean age at the start of treatment has increased from year to year and is 64 years for men and nearly 63 years for women.
Glomerulonephritis is the most commonly diagnosed as the cause of end stage renal failure, and this has been the case since the registry was started. Among this group, Immunoglobulin A (IgA) nephritis is the most common type specified. The second most diagnosed is diabetic nephropathy (caused by diabetes type 1 and 2), and the third most common is cystic kidney disease. Diabetes type 1 is the most common individual diagnosis that gives rise to terminal renal failure, including patients on therapy. However, for a number of years diabetes nephropathy was the most common reason for starting active uremia treatment. The proportion of diseases other than glomerulonephritis has also increased, especially nephrosclerosis.

The mortality rate for patients in active uremia treatment has decreased; but despite this, the mortality rate for dialysis patients is still nearly 20% per year. Cardiovascular related death has been the most common representing 41% of the causes.
The history of haemodialysis

In 1854 Thomas Graham, a chemist from Glasgow, described for the first time the principles of solute transporting of substances across a semipermeable membrane (diffusion process). He also realized that successful treatment for kidney failure required that toxins that accumulate be removed. The first artificial kidney was manufactured in 1913 by Abel, Rowntree and Turner in Philadelphia, USA; but at that time it was used for experiments with dogs and not for humans. The first treatment of humans with HD was conducted in 1924 by the German physician George Haas. At that time hirudin was used to prevent blood clotting (coagulation) during dialysis, but it did not work well for longer treatments. Haas solved the problem when heparin was used for the first time in 1927.

In 1943 a clinically useful dialysis machine for acute renal failure was produced for the first time by Willem Kolff from the Netherlands. This was in part thanks to an effective membrane made of cellophane. Kolff once said of his inventions: “The main aim of my endeavors has always been to restore people to an enjoyable existence. If it’s not enjoyable, it should not be done”. Kolff was actively working during the war and his first dialysis machine was built using second-hand materials. The filter consisted of sausage casings, which he got from the local butcher. It was wrapped around a wooden drum rotating in a vat of brine. The drum was driven by an engine from a T-Ford, and the entire dialysis system was placed in a frame taken from a crashed fighter plane. The Norwegian physiologist Kiil introduced the Cuprophan membrane and plate dialyzer, which improved efficiency of dialysis. Nils Alwall, professor at Lund University, developed an improved dialysis machine called the Alwall kidney in 1947. The construction made it possible to get pressure differences across the membrane. For the first time, the excess fluid could be dialyzed away using ultrafiltration. Still, however, HD was only possible for acute renal failure. This was because the patients' veins could only be used for 7-10 treatments before being destroyed. In 1960 Belding Scribner and Wayne Quinton from Seattle designed a well-functioning arterio-venous (AV) shunt made of Teflon. Later, Cimino and Brescia from New York described the first endogenous chronic vascular access. A vein was surgically connected to an artery and formed an anastomosis, AV fistula. The fistula was able to withstand the higher arterial pressure, and a durable safe access to blood flow was created.
Because of this improvement in vascular access, HD became possible even for patients with chronic renal failure\textsuperscript{9} \textsuperscript{6} \textsuperscript{7}.

**Physiological principles of dialysis**

Dialysis treatment is designed to cleanse the blood of toxic metabolites – that are dissolved waste products of metabolism, and also to rid of excess electrolytes, acids and water. For this a membrane is required\textsuperscript{4}. In HD the dialysis filter (see below) contains a membrane, and in peritoneal dialysis the peritoneum acts as the dialysis membrane. Blood flows on one side of the membrane and the clean dialysis fluid flows on the other side. The physical transport mechanisms that can operate across the dialysis membrane are diffusion, ultrafiltration (fluid removal) and convection.

**Diffusion**

Concentration difference of a solute in water leads to equalization of the difference across the membrane; this process is known as diffusion. With dialysis there occurs an equilibration over a semipermeable membrane, which means different permeability for different substances depending on the molecular size. This process is exponential, and the driving force is the concentration differences. Since the dialysis fluid does not contain waste products, there is a transport of these from the blood to the dialysis fluid until the differences in concentration on both sides of the membrane are equalized\textsuperscript{4}.

**Ultrafiltration**

Ultrafiltration (UF) is a fluid flow and a flow of solute across the dialysis membrane due to hydrostatic or osmotic pressure differences across the membrane\textsuperscript{4}.

In HD, a hydrostatic pressure gradient is established due to a difference between the average pressure of the blood on the blood side and a lower average pressure of the fluid on the dialysis side of the membrane. This enables removal of excess fluid from the HD patient. The net pressure across the membrane, which is the driving force for the pressure ultrafiltration, determines the trans-membrane pressure (TMP) in mmHg. The membrane ultrafiltration capacity is determined by the TMP and the membrane properties such as surface
area, thickness and type of membrane material. Ultrafiltration capacity is usually indicated by a membrane ultrafiltration coefficient.

Because water molecules are small they pass easily through a semipermeable membrane and strive to equalize the concentration differences between the blood and dialysate fluid. This concentration difference is determined by a higher concentration of, for example, glucose in the dialysate when compared against the other side of the membrane where there is a lower concentration of dissolved substances in the blood; this leads to an osmotic pressure gradient. The flow of fluid is called osmosis and it is because of this that the excess fluid is removed by peritoneal dialysis. Osmosis is continued until the dilution is equal on both sides of the membrane.

**Convective mass flow**

Water molecules transporting across the membrane during filtration cause passive co-transporting of molecules of dissolved substances due to frictional forces created by the water molecules. This is called convective mass flow, which is proportional to the flow over the membrane. Convection is not dependent on the molecular size to the same extent as for diffusion, which enables the purification of medium-sized molecules corresponding to molecular weights of 500D-12KD.

**Haemofiltration**

In haemofiltration purification occurs by convective mass flow. Blood flow should be relatively high (more than 300 ml/min) to achieve a good effect. A large filtration flux and a highly permeable membrane provide a good elimination of medium-sized molecules from the blood. To prevent dehydration (hypovolemia) fluid must be returned to the blood before it is pumped into the patient. This replacement solution is done either before (pre-dilution) or after (post-dilution) the dialyzer.

Haemofiltration is suitable for patients who have severe heart disease and circulatory instability, i.e. low blood pressure. The disadvantage of haemofiltration is that the method is less efficient at removing small molecules because no diffusion occurs. Large filtration volumes are therefore needed. Dialysis fluid is not used. Because of this, haemofiltration is a rare treatment for long-term dialysis cases.
**Haemodiafiltration**

Haemodiafiltration (HDF) combines HD and haemofiltration. Both diffusion and convection occurs, which means that both the dialysate and replacement fluid are needed. If the amount of replacement fluid infused in the patient is increased, the convective component is also increased. If the replacement fluid is prepared directly through the dialysis machine during treatment (‘on-line’), the bulky bags with substitution fluid can be replaced. Prerequisites for optimal online HDF are good vascular access, high blood flows, high-flux filter and large replacement volumes. This treatment modality is considered as the most effective since both the small (from 50 D) and large (about 1000-20000 D) solutes that accumulate in uraemia can be effectively removed. The online HDF improves haemodynamic stability, which is reflected in less hypotonic episodes during the dialysis as compared with HD. HDF is also believed to induce less inflammation, and oxidative stress, and it reduces the endothelial dysfunction that uraemia causes. A small study (32 patients) indicated that the degree of anaemia in dialysis patients and erythropoietin dosage can be positively affected with HDF compared with HD. Observational studies have indicated that HDF provides an improved survival compared with conventional HD. Expectations were high that randomized, controlled, prospective studies (RCT) would show similar results, but this was not the case. HDF provides a lower level of β2-microglobulin in the blood plasma. The level of s-β2-microglobulin may be important for the development of dialysis-related amyloidosis that is manifested as bone cysts, deposits in tendons, ligaments and carpal tunnel syndrome.

**Vascular access**

Access to the bloodstream is essential and crucial for effective HD. The higher the blood flow the more effective the cleansing. Normal blood flow in the clinic is around 250-400 ml / min. To come to this level larger vessels are used. This can be solved in three ways: (1) construction of an arterio-venous (AV) fistula, (2) AV graft, or (3) insertion of intravenous catheter-central dialysis catheter (CDC).

An AV fistula is created by surgically connecting an artery to a vein. The vein will adapt to the higher blood flow through the artery to expand and the wall will thicken. This allows for punctures with a coarse dialysis needle (connected to the dialysis machine) for repeated dialysis sessions over a long period of time. A common type
of AV fistula is the Brescia fistula. The cephalic vein is surgically connected to the brachial artery on the forearm. After 4-6 weeks, the vein will adapt such that puncture with coarse needles is possible.

If the patient's vasculature, examined with duplex ultrasound preoperatively, is thin, an AV graft can be constructed to provide better conditions for adequate blood flow. An AV graft consists of synthetic material that builds a vessel substitute that connects the vein to the artery. When the above options are not possible the intravenous catheter (e.g. CDC or femoral catheter) remains.

CDC is a catheter placed via the internal jugular or subclavian vein into the superior vena cava. The femoral catheter is placed in the femoral vein via the inferior vena cava. This occurs when the patient needs to start dialysis and acute vascular access is unavailable, or if the patient or his/her vasculature is so fragile that an operation is not appropriate. If possible a vascular access should be chosen since complications such as thrombosis, and above all infections, are minimized.

The best option is often an AV fistula because it is associated with a lower risk of complication as compared with an AV graft, but particular in comparison with CDC. For patients with heart failure, AV access can be a worse alternative because it can cause problems with arterio-venous shunting and increased cardiac load.

The haemodialysis system

The HD machine consists of two main systems – the blood and the fluid systems (Figure 3).

The blood system

Before the patient is connected to the machine, air and residues from sterilization are removed from the tubes and filter. The system is flushed with either saline from a bag or dialysate/replacement fluid if the ‘on-line’ feature is available, and the fluid is recirculated through the system. Most, but not all, of the air is removed by this procedure which is referred to as priming.

The dialysis staff inserts the AV-fistula/graft with large dialysis needles, and the needles are connected to the tubing set. If CDC is
used tubes are connected after the solution (citrate/heparin) to prevent blood clotting (coagulation) is removed. The blood in the tubing is drawn into the dialysis machine with the help of a peristaltic pump that creates a negative pressure. The tubes before the filter are called the artery side, and the tubes after the filter are called the venous side; here the pressure is positive. To prevent blood clots, anticoagulants (heparin or low molecular weight heparin) are added. Blood is pumped into the dialyzer and passes one side of the membrane.

In the tubing set there are techniques for monitoring the pressure before and after the pump. There is also an air trap. It consists of a detector, often based on ultrasound technology, which is coupled to a safety system to alarm and stop the blood flow if there is an air leakage. The air trap also consists of a small chamber (venous chamber) before the blood returns to the patient's vascular access. The purpose of the venous chamber is to allow for any air in the tubes to rise up and pass out of the blood stream before it reaches the air detector.

The fluid system consists of dialysate and replacement fluid during HDF. The fluid contains purified water, and a mixture of electrolytes and dissolved substances (Na, K, Ca, Mg, Cl, bicarbonate, acetate, Glucose) that are present in the blood. The purpose of the system is to restore disturbances in the plasma due to the kidney damage. The fluid temperature can be varied to reduce the risk of treatment complications. For example, at a lower temperature there is a decreased risk for the patient's vessels dilating and thereby contributing to a drop in blood pressure.

The fluid passes the membrane on the side opposite to the blood, which enables the exchange of fluid and solutes.

Large amounts of water come in contact with the patient's blood, and the HDF also infuses fluid to the patient. This places great demands on water quality. Various types of pollutants such as heavy metals, bacteria, and mold should be avoided. This is done with the help of reverse osmosis, chemical disinfection, heating and the use of filters. Advanced water treatment processes the water so that the degree of purity reaches established standards (Svensk läkemedelsstandard).

Other types of contaminants such as air are taken care of by an exhaust system. The fluid is subjected to a negative pressure.
According to Henry’s law the contaminants are released when the fluid dissolved gases. These are then vented via a degassing chamber.

**Figure 3.** The haemodialysis system

**Dialysis filters/dialyzer**

The capillary dialyzer, which is the most common type of dialysis filter, consists of a tube with a membrane and four connections where the blood passes on one side of the membrane, and the dialysate fluid passes on the other side in opposite direction. The reason that blood and fluid flow in the opposite directions is that in this way the blood continually comes in contact with the cleaner dialysis fluid (dialysate). The membrane surface between the blood and the dialysis is maximized being divided into over 10000 pieces of hollow fibers.
(capillaries), hence the name capillary dialyzer. In the capillaries the blood flows surrounded by dialysis solution.

**Biocompatibility**

An important quality of the filter is biocompatibility (tissue compatibility). In particular, the complement system is activated when blood is in contact with the membrane surface, which may lead to allergic reactions and long-term adverse effects of inflammation\(^{29}\) \(^{30}\). Nowadays synthetic membranes of various types of polymers are used, which gives much less activation of the complement system than the previously used cellulose membrane.

**Clearance**

The dialysis membrane’s clearance quality to various substances is used to characterize the dialyzer\(^5\). A substance’s clearance refers to the volume that is completely purified from the substance. The clearance is due to several factors:

- If the blood flow (Q\(_b\)) is increased, it leads to increased clearance up to a certain limit. Blood flow is limited by how well access to the patient’s blood circulation (fistula, graft, CDC) works. Common Q\(_b\) intervals in the clinic are 250-400 ml/min.

- An increase in dialysate flow (Q\(_d\)) increases the diffusion of small molecules from the blood to the dialysate. The clearance of larger molecules is affected very little by Q\(_d\). The normal flow during HD is 500 ml/min.

- Molecular weight determines the degree to which a substance diffuses through the membrane. The dialysis membrane is semi-permeable, and that means that the permeability of substances in solution varies with the molecular size. Characterization of the dialysis membrane’s permeability is indicated by a so-called sieving coefficient (S). S = average concentration in the filtrate/medium concentration in the blood. A membrane with S = 1 for a substance means complete permeability of equivalent molecular size. As a rule, the larger the molecule the lower the membrane permeability. Below are examples of different substances’ molecular weights\(^5\).
Table 3. Molecular weight for different substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>Molecular weight*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>60</td>
</tr>
<tr>
<td>Phosphate</td>
<td>70</td>
</tr>
<tr>
<td>Creatinine</td>
<td>113</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>1355</td>
</tr>
<tr>
<td>β2-microglobulin</td>
<td>11800</td>
</tr>
<tr>
<td>Albumin</td>
<td>66000</td>
</tr>
</tbody>
</table>

* Molecular weight expressed in daltons (Da)

- Membrane properties such as surface area and thickness are important for clearance. The larger the surface and the thinner the membrane the more effective the filters. A design that allows for maximum contact between blood and dialysate provides a higher clearance. Some molecules, such as proteins, may adhere to the walls of the membrane without coming into contact with the dialysate. This process is termed adsorption and is dependent on the membrane surface and materials. Membrane pore size, the diameter of the small hole in the membrane capillaries that enables diffusion, is important for efficiency. Previously, standard filters, so-called low-flux filters, had smaller pore size than high-flux filters that have greater permeability for large molecules. For example, β2-microglobulin can be removed with the help of these high-flux filters. Patients with renal failure without remaining kidney function are at high risk of β2-microglobulin accumulating in body tissues. It is known that patients treated for many years with HD suffer from amyloidosis. β2-microglobulin storage can lead to amyloidosis building. This in turn can lead to carpal tunnel syndrome, and joint, muscle and skeletal problems. In an autopsy study, where the presence of amyloids in the joints was examined, it was found that all patients undergoing HD treatment for more than 13 years had amyloidosis. A randomized, controlled trial (RCT) (HEMO-study) demonstrated a significant reduction in blood serum level of β2-microglobulin, but no survival advantage, with the use of high permeable filters in HD instead of low permeable filters. The so-called MPO (Membrane Permeability Outcome) study, an RCT design, used low serum albumin (less
than or 40 g/l) as an indicator for HD patients with increased mortality. Low s-albumin is associated with malnutrition, inflammation, atherosclerosis (fatty deposits in the artery), but also increased morbidity and mortality. Patients with low s-albumin had significantly better survival if they received treatment with high-flux filters compared with those treated with low-flux filter\textsuperscript{35}.

The effectiveness of a dialysis filter for cleaning the blood can be described by a constant: the mass transfer area coefficient, \( K_{oA} \). The constant specifies a filter’s maximum possible clearance of a substance when both blood and dialysate flow is the highest possible. In practice, blood and dialysate flow is not so high that maximum clearance can be achieved. The higher \( K_{oA} \) the greater the effect was by increasing blood flow. \( K_{oA} \) is the product of permeability constant (Ko) for a substance and effective membrane area (A). The constant does not change with blood flow, but increases slightly at higher dialysate flows. This is because higher flows better penetrate the membrane’s capillaries and provide a larger effective membrane area.

- The duration of dialysis treatment is important for the total volume of blood that is cleansed. In the beginning of treatment, the concentration difference of toxins from the blood and dialysate are greatest. In the rate that substances are dialyzed away concentration equalization occurs. Therefore, diffusion is most effective during the first hours. From this viewpoint, short, but more frequent, dialyses gives a better cleansing of the blood compared with longer and fewer dialyses. This effect is greater the smaller molecular size, and vice-versa\textsuperscript{4}.

**Embolism**

A particle circulating in the blood that can reduce blood flow to other areas than where it was formed is called an embolus. Emboli may consist of a thrombus (clot of coagulated blood) detached from the vessel wall. This is called thromboembolism. Emboli may also consist of other biological material such as cell aggregates, fat particles, bone or other tissue pieces that can occur after surgery (e.g. fracture or cancer surgery). Gas bubbles or foreign material (e.g. fragments of plastic tubes) can cause embolism\textsuperscript{36}.
Arterial embolism

Arterial emboli are emboli that occur in the pulmonary veins or the systemic circulation. These can lead to tissue ischemia because blood circulation is affected by emboli that adhere to the vessels. The result can be myocardial infarction, brain damage or death. Affected body parts become pale, cold and painful. Other symptoms are confusion, paralysis, convulsions, headache or coma\(^{37}\).

Venous embolism

Emboli in blood flowing to the heart, i.e. venous emboli, pass to the lungs via the pulmonary artery. If repeated over time this can lead to increased pulmonary pressure and lung damage. Larger emboli can cause blockage in the outflow from the right ventricle, which can lead to right ventricular enlargement and heart failure\(^{38}\). Shortness of breath, tachycardia, chest pain, cough, loss of consciousness and shock are symptoms that can be caused by venous emboli\(^{37}\).

Paradoxical embolism

The dividing wall between the right and left heart atrium has an opening in the foetus – the foramen ovale. This is to allow the oxygen rich blood from the placenta to pass directly from the inferior vena cava to the left atrium of the foetus without going to the lungs. At birth and during the early period after birth the opening closes, but in 25-30% of the population it remains open; this is called a persistent foramen ovale (PFO)\(^{39}\).

This has no impact haemodynamically, but provides the conditions for paradoxical embolism, i.e. that venous clots go out into the systemic circulation in the aorta instead of to the lungs\(^{40} \ 41\). Since the pressure in the left atrium is high, there is a flow of oxygen rich blood to the right atrium. If the pressure can be altered, there is reverse flow. This occurs, for example, with right ventricular infarction, cough or Valsalva manoeuvre (forceful exhaling against a closed glottis).

Other causes of paradoxical air embolism are abnormalities in the cardiovascular system or the lungs that cause the blood not to pass through the alveoli. The non-oxygenated venous blood from the right heart passes directly to the left heart and is mixed with oxygenated blood. This is called right-left shunt. A congenital ventricular defect resulting in blood flowing from the left to right ventricle, due to
pressure conditions, is called a left-to-right shunt. A simultaneous narrowing of the pulmonary artery (pulmonary stenosis) can change the pressure ratio so that the shunt reverses to a right-to-left shunt\cite{42}.

**The physiological right-to-left shunt**

Approximately 2\% of the blood in the arterial circulation never passes the lung capillaries, and therefore has not been oxygenated. There is a physiological right-left shunt due so that blood through the bronchial circulation that supplies lung tissue does not pass the lung capillaries, but instead passes through the pulmonary veins and mixes with the oxygenated blood. Likewise blood is emptied from the vessels that supply the left ventricle with oxygen, the coronary arteries, directly into the left atrium\cite{28}. If portions of the lung are not filled with air and ventilated, this means that a part of the circulation will not be oxygenated. This venous blood mixes with other well-ventilated lung parts to form an intrapulmonary shunt.

Atelectasis is collapsed non-ventilated lung areas, and in this condition shunting occurs according to the above description of non-oxygenated blood mixing with oxygenated blood. Microatelectasis is encountered during normal breathing at normal pressure conditions.

The venous blood in the pulmonary capillaries drains into the arterial circulation and may cause embolism with emboli of the size according to capillary diameter of 5-8 microns (red blood cells are about 7-8 microns\cite{28}).

Differences in ventilation/perfusion ratio due to alveoli without capillary networks or to blood flow through parts of the lung that are not ventilated occur in, for example, emphysema\cite{42}.

In animal models, air emboli can pass the lung barrier. If a sufficiently large air volume or a continuous amount of air is supplied to the venous system, the air may pass over to the arterial circulation. There are varying reports in literature regarding the quantity of air required for it to pass from the venous to the arterial side\cite{43 37}. More than 0.03 mL kg body weight per minute for continuous infusion, or 0.1 ml kg body weight as a single dose (bolus) is a suggested amount. This is determined from literature studies based on animal models and theoretical arguments\cite{39}.
Macro- and microembolism

Embolism can be divided into micro- and macroembolism. Microembolism is defined as emboli with a diameter smaller than 200 μm. These may occlude small vessels and arterioles and capillaries, while macroemboli occludes larger vessels and can lead to stroke and death as described above. Microemboli often produce no noticeable acute effects, except in sensitive tissues like the retina or in the subcortical regions of the brain.

Detection of emboli

At autopsy emboli can be identified, and the cause of disease and death can also be investigated. Using animal models, the effects of induced emboli such as air can be studied. Damage due to presumed emboli can be studied indirectly with the help of magnetic resonance imaging (MRI) or computed tomography (CT). The ultrasonic Doppler technique has made it possible to detect the presence of emboli in real time in humans. Technology to detect large solid and gas emboli was used as early as in the 1970s. The method was refined and a pulsed Doppler technique allowed the registration of microembolic signals (MES) as a marker of microembolic activity. The detected signal is referred to in the literature as MES because the source of the signal can be artefact (disturbance, motion triggered, apparatus or software, etc.) and the composition of emboli has not been possible to determine with certainty. New Doppler techniques with multi-frequency systems have made it possible for distinction between solid and gaseous emboli. The technique is based on the fact that solid microemboli reflect more ultrasound at higher frequencies compared with lower; this is contrary to that of gas emboli. Russell et al. described in 2002 how patients with carotid stenosis or mechanical heart valves were investigated with multi-frequency transcranial Doppler. Of the microemboli detected in patients with carotid stenosis, 94% were classified as solid; the identification of the other emboli was uncertain. In patients with mechanical heart valves 84% of the detected emboli were classified as gaseous. With Transcranial Doppler (TCD) placed over the arteia cerebri media (ACM), in connection with cardiac surgery when the heart-lung machine is used, the majority of emboli were found to be gaseous. This new technology has not been used widespread in research. In the future issues about the composition of emboli may have better outcomes.
Detecting MES is described in many contexts, amongst others in association with vascular imaging tests (angiography) and heart examinations such as with cardiac catheterization. Procedures that contribute to large amounts of or a continuous supply of emboli, such as during surgery when the heart-lung machine is used, mechanical heart valves and particularly during HD are discussed below. Even carotid and intracranial vascular stenosis and the presence of MES have also been studied.

**Microembolism with arterial origin**

Spencer et al. described MES detected by TCD for the first time in 1990. The prevalence of MES in ACM was measured during carotid endarterectomy surgery (CEA)\(^\text{49}\). Since then, the technology has been used in many studies with various issues. Despite the presence of large amounts of MES, patients may be symptom-free, but many studies have shown a correlation between MES and future clinical situations. In a systematic literature review from 2007 it was postulated that there is an increased risk of future strokes and transient neurological symptoms – transient ischemic attack (TIA) for patients with carotid stenosis who already had a stroke/TIA and the presence of MES. Patients with MES had an approximately seven-fold increased risk compared with those with no MES recorded. However, results should be interpreted with caution because the studies included were different in respect to the time MES was recorded, and that all studies were observational studies\(^\text{50}\). A prospective study monitored 114 patients with acute ischemic stroke and ACM stenosis for the presence of MES. In 22% of the patients MES was recorded. At follow-up at 13.6 months 12 of these patients had had a new stroke/TIA compared with 6 events in the group without MES detected; this gave a significant difference\(^\text{51}\). In a prospective observational study 482 asymptomatic patients with at least 70% carotid stenosis were examined with TCD over ACM. At follow-up significantly more patients with detected MES suffered ischemic cerebrovascular disease (stroke/TIA) or stroke compared with those with no MES detected. TCD was proposed as a method to identify asymptomatic patients with carotid stenosis, which would probably benefit from CEA\(^\text{52}\). There are also treatment studies involving MES. A randomized, double-blind, multicenter study showed differences in the incidence of MES depending on the therapy. Patients with recently symptomatic carotid stenosis were examined by TCD over ACM. The patients detected with MES were randomized to treatment with clopidogrel and acetylsalicylic acid (ASA) or ASA alone. The
patients who were treated with both clopidogrel and ASA had significantly fewer MES compared with the other group. Patients with recent symptomatic carotid stenosis were investigated in the same way as the above study. Patients were randomized to treatment with clopidogrel and ASA or dipyridamole and ASA. At follow-up the number of MES was reduced by approximately 75% in both groups; there was not difference between treatments. In both studies, all medications inhibited the aggregation of blood platelets, which have been shown to be effective against diseases caused by atherosclerosis such as myocardial infarction, stroke and TIA. ASA has, amongst other things, indication of secondary prophylaxis after myocardial infarction, and stroke/TIA; this is also the case for clopidogrel. Dipyridamole is used to prevent re-ischemic cerebrovascular disease. This suggests that the detected MES in both studies, which decreased after treatment, included emboli from atherosclerotic stenosis.

Silent brain infarcts are detected by CT or MRI examinations; they are without symptoms such as those seen with stroke/TIA. The presence of silent brain infarcts is associated with increased risk of developing dementia, and rapid deterioration of dementia caused by either Alzheimer's disease or vascular dementia. Vascular dementia primarily affects patients with cardiovascular risk factors such as hypertension, diabetes mellitus, hyperlipidaemia, smoking, etc. Even those with Alzheimer's disease have an increased prevalence of these risk factors. Changes in white matter of the brain are strongly associated with cognitive impairment and dementia. Microemboli are suspected to cause these changes. In a prospective study, 57 patients with single-sided (unilateral) symptomatic carotid stenosis were examined with MRI for changes in the white matter. This was done prior to CEA, and the excised atherosclerotic plaque was categorized histologically as stable or unstable regarding the risk of embolization. The instable plaque was associated with more than twice as many brain changes ipsilaterally compared with stable plaques.

Cerebral MES is common in both CEA and carotid angioplasty with stent placement (CAS). In CAS the vessel is widened where the constriction is. This is aided by a catheter in the artery femoralis that is threaded up to the stenosis, and widening occurs by inflating a balloon at the tip of the catheter. Then, a stent (metal cylinder) is put in place to widen the constriction and to help prevent a re-stenosis. Some articles show that the number of MES during CAS is associated
with neurological symptoms\textsuperscript{62}, but there are also articles that do not confirm this\textsuperscript{63}.

**Gas/air embolism**

The risks of air emboli have been known for a long time. As early as 1667 Redi described an experiment where he injected air into animals that later died. The first described observation of death in a human is in 1818\textsuperscript{39}.

**Formation of air embolism**

All invasive procedures can produce air emboli but since the atmospheric pressure is usually lower than venous and arterial pressures, vessel injury seldom leads to air embolism. During diving air emboli can arise from rapid lowering of pressure surrounding the body during rapid ascent to the surface\textsuperscript{64}.

**Decompression sickness**

Gases are always dissolved in body fluids and tissues\textsuperscript{65}. Tissues take up more dissolved gas when atmospheric pressure rises. When diving, the pressure rises and nitrogen accumulates even more in tissues. During surfacing the pressure reduces. Gas then diffuses into venous blood and is ventilated out in the lungs. A rapid drop in pressure because of ascending too fast prevents from being offloaded safely through the lungs; instead there is a risk that nitrogen gas changes into free form gas bubbles that build up in the body's tissues. If the diver, after having been submerged so long that a lot of nitrogen has accumulated, surfaces so quickly that the ambient pressure drops rapidly to about half, decompression sickness can occur. Gas bubbles can occur in many places in the body and can cause different reactions on adjacent tissues, joints, nerves and blood vessels. The bubbles in the capillaries can cause itching/tingling known as "diver's itch". These are the mildest of the symptoms and do not always require treatment but they can be a precursor to more serious symptoms like unbearable pain in large joints such as knees, elbows and shoulders. Shortness of breath, chest pain and coughing may occur. Neurological symptoms such as headaches, abnormal sensations, paralysis, dizziness, cramps, and unconsciousness are other serious symptoms; these are caused by gas bubbles on the arterial side that stick peripherally causing ischemia. Symptoms usually occur within an hour, but they can occur up to twelve hours
after the dive\textsuperscript{65}. Decompression sickness, caused by excess of inert gas, i.e. (not chemically reactive with their environment) dissolved in the body, can affect anyone exposed to pressure drops, for example, aviators, astronauts and caisson workers (construction and related trades under water).

Another problem that may exist in connection with diving is that pressure drops in the ascent, and if one has closed airways this can lead to lung rupture. This is caused by expanding gas volume in the lungs. Gas emboli can then pass to the arterial side. The neurological symptoms of this cannot be distinguished from decompression sickness\textsuperscript{66}.

A third cause of arterial gas emboli can be a foramen ovale that remains open. During ascent, nitrogen (instead of being blown out of the lungs) causes paradoxical embolism and this gives similar symptoms to decompression sickness. For divers with patent foramen ovale (PFO), the risk of severe decompression sickness is five times greater as compared with other divers\textsuperscript{67,68}. The absolute risk, however, is small, and the majority of those who suffer regain completely neurological function after treatment. Thus, PFO screening is not a recommendation for divers according to the European Divers Guidelines. In contrast, divers with known PFO and decompression sickness should stop diving\textsuperscript{69}.

Hyperbaric oxygen therapy is provided to compress the gas bubbles for the more severe cases of decompression sickness. In a pressurized chamber the diver is exposed to a high pressure and may breathe in 100\% oxygen via a mask. Excretion of the inert nitrogen gas and oxygenation in damaged tissues is accelerated. Treatment is continued until symptoms are alleviated. Recurrence is common, and this requires repeated hyperbaric oxygen therapy\textsuperscript{70}.

**Mechanical heart valves and air emboli**

In the area around mechanical heart valves MES were shown to occur and the initial suspicion was artefact. With improved technology MES could be perceived as microbubbles that are caused by cavitation phenomena due to the high pressure gradient of the valves\textsuperscript{71}. Cavitation phenomena can be described as follows: If the flow rate is sufficiently high, the pressure can be lower than the vapour pressure of the blood. The blood ‘boils’ locally and is released as gas bubbles (cavities). When these come in areas where the pressure is higher
than the vapour pressure, they can no longer exist without collapsing (implode)\textsuperscript{72}. However, it was not shown that emboli were mostly gaseous until Kap et al. succeeded in reducing MES, detected transcranially, by allowing patients with mechanical heart valves (aorta-/mitral valves) to breathe via a mask 100\% oxygen. Since diffusion equilibrium is normal between gas and blood in the alveoli, inhaling 100\% oxygen leads to increased diffusion of oxygen and the concentration in the blood increases while the nitrogen concentration decreases in blood\textsuperscript{28}. The ability of oxygen to be dissolved in blood is 4.8 times higher than for nitrogen. The oxygen bubbles formed by cavitation processes dissolves in the blood much better, and the time bubbles are in the blood is reduced\textsuperscript{73}. In a study of a similar design but with more patients (20 patients, all treated with anti-coagulant tablets) similar results were shown. Droste et al. suggested that an explanation could also be that oxygen inhibits cavitation processes in addition to decreasing the dissolution of gas bubbles in the blood\textsuperscript{74}. The same study also examined patients with known stenosis of the a.carotis or in intracranial vessels. The amount of detected MES did not decrease during oxygen therapy and these emboli were proposed to be solid, e.g. platelet aggregation or stenosis material\textsuperscript{74}. Support that microemboli were mostly made up of gas was obtained from a study where the characteristics of MES were compared between patients with mechanical valves and those with PFO that were injected intravenously with saline containing air bubbles. TCD was used measuring in ACM. No difference was seen between intensity, duration or the relative velocity of microemboli between the two groups. The authors concluded that the microemboli generated by mechanical heart valves were mostly made up of gas\textsuperscript{75}. By using TCD MES in arteries in the brain was detected after mechanical heart valve operation. Neurological effects may be a consequence of prolonged emboli effects in these patients. One study found significantly poorer working memory in those with mechanical heart valve who had MES in comparison with patients with biological heart valves and without MES\textsuperscript{76}.

**Heart-lung machine and air emboli**

Air emboli are a well-known problem in connection with the use of heart-lung machines during heart surgery\textsuperscript{77} \textsuperscript{78} \textsuperscript{79}. To be able to operate inside the heart, the heart must be empty of blood and should be still. This means that during a period of the operation heart and lung function must be replaced by a heart-lung machine. The blood is oxygenated and returned to the arterial circulation. Emboli of, for
example, gas, blood clots, fat and other biological material that occurs during the process, can affect ischemia-sensitive organs such as the heart and brain, and lead to neurological deficit or coma. Stroke, for example, may affect up to 9% of those over 75 years of age that undergo coronary artery-bypass surgery. Mortality and morbidity have decreased markedly because larger emboli are now quite rare. This is due in part to technical improvements such as blood filters after oxygenation. Oxygenators nowadays include a membrane that allows for diffusion of oxygen into the blood instead of supplying only oxygen bubbles as was previously done. Despite this, a large number of microemboli pass over to the patient's arterial circulation. Long-term effects are controversial. There are several reports of a relationship between the total number of brain emboli, as detected by TCD during surgery or by MRI after surgery, and reduced cognitive ability as measured by neuropsychological tests. A recent systematic review, however, could neither confirm nor refute such a link. It was therefore proposed to further develop measures to reduce emboli, according to the precautionary principle.

**Haemodialysis and air emboli**

During the earliest years of HD treatment the problem of acute symptomatic air emboli was a major challenge. Air embolism was the most sudden and deadly complication during HD treatment before air-sensitive detectors were developed. The risk of fatal air gas emboli still remains even today. In 1993 a fatal accident occurred in Sweden. A HD patient died due to air infusion after the alarm was overridden twice. As late as 2000 several sudden deaths during HD treatments have been reported. Manufacturing errors of dialysis filters led to development of gasemboli in the blood. Fifty-three people died as a result of this.

It is considered that it is uncommon with symptomatic air emboli associated with HD. However, the symptoms that could be caused by air emboli, such as shortness of breath, tachycardia, sweating, chest pain, coughing, headache and seizures occur during HD sessions. Moreover, it is not uncommon for dialysis nurses to report about observing air bubbles/foam in dialysis tubing, occasionally even after passing the air trap before the blood passes into the patient.
Hazardous air volume?

A critical factor for injury due to air emboli, aside from access to the arterial versus the venous circulation, is the amount of air supplied both as an instantaneous dose (bolus) and as a continuous infusion. Body location is a factor. Organs located in the upper half of the body, than standing up, are at greatest risk due to the lower density of air compared with blood. Also the morbidity of the individual plays a role on the extent of injury.

Still lacking is sufficient knowledge of the dose of air that could lead to death, or the amount injected into the bloodstream over a long term that can cause complications. The International Electrotechnical Commission (IEC) standards for infusion pumps and dialysis machines tolerate some air leakage. Less than 1 ml/15 min and bubbles less than 50 μl are accepted. Less than 0.03 mL kg body weight per minute for continuous infusion or 0.1 ml kg body weight as a single dose bolus is considered tolerable. This is based on a thorough study of the literature where animal models and theoretical arguments were included. This has been used as a safety limit for venous air infusion from medical equipment.

However, it is considered reasonable that air in the arterial system is dangerous and requires much less volume to cause damage than air in the venous system toward pulmonary circulation.

Haemodialysis and microembolic signals

In 2000 Woltman et al. used ultrasound duplex to examine two patients over their AV accesses during HD. MES was detected in both cases when blood flows were over 400 ml min. The origin of the microemboli was considered to be bubbles caused by cavitation due to pressure differences between the venous needle and the blood pump. Air from the dialysis machine and clots from the access were also considered potential sources for the emboli. In the same year a study was published with 25 patients that were examined with Doppler ultrasound (DU) before and during HD. MES were monitored in vena subclavia downstream of the AV fistula. In all patients, MES was detected during dialysis, but not prior to treatment. Blood flow was held constant at 300 mL min. Both low- and high-permeability filters were used. For measurements at the end of treatment, significantly more MES was detected in patients with high-flux filters. At no time did the air detector alert.
The intensity (measured as the energy of the reflected sound wave, i.e. echo, during Doppler examination) was not different between the MES. The relative intensity was high, which contradicts biological materials such as platelet aggregation and clots caused by complement activation or coagulation during the dialysis membrane-blood contact. Gas bubbles or synthetic material from, for example, tubes damaged by the dialysis pump were suggested as the cause of the MES.

A similar study described how 24 chronic patients treated with either HD or on-line HDF, were examined with DU of vena subclavia several times during the same treatment. All patients breathed in 100% oxygen at a subsequent time point that was the same as when measurements were done transcranially in patients with mechanical heart valves as previously described.

All patients were detected with MES and for those with on-line HDF the number was significantly more. For no detection did the air detector alert. However, the number of MES did not decrease during O2 delivery. This was thought to indicate that the detected were mainly solid microemboli or larger gasemboli that lasted longer before dissolving in the blood. An objection to this is that the measurements were made in the venous blood stream after the fistula, but before concentration equalization and ventilation in the lungs. The dialysis system can be a source of emboli, in some cases causing a continuous air emboli supply (with tube leakage).

In blood vessels after the passage of blood through the lungs, one can suspect that gas bubbles are decreased after being caught in the lung capillaries, but the number increases again when blood passes the dialysis system, the source of emboli. The study mentioned above was comparable in intensity to a study by Rolle et al. This suggests similar compositions in both studies and speaks against solid endogenous material like clots from access, etc. In these studies as well as in a review article by Barak et al. possible clinical implications of chronic microemboli associated with HD are also discussed. Since microemboli are detected in the pulmonary circulation, changes in the lungs of chronic HD patients have been discussed. It is well known that chronic HD patients have a high incidence of lung morbidity. In one study, 46 chronic HD patients who had died were examined for the presence of pathological abnormalities in the lungs. Over 80% of these patients had chronic lung diseases and the most common was interstitial fibrosis. A CT study of 117 chronic HD
patients demonstrated pulmonary fibrosis in over 30%\textsuperscript{99}. Pulmonary arterial hypertension (PAH) is common in chronic HD patients (29% of 51 patients examined with DU\textsuperscript{100}). PAH is defined as a mean pulmonary artery pressure at rest of 25 mmHg as measured with cardiac catheterization. Ultrasound of the heart (echocardiography) is used as a screening tool\textsuperscript{101}. The key to this is an increase in cardiac output – the amount of blood the heart pumps out per minute, due to AV access. This leads to pulmonary vasoconstriction, pulmonary hypertension and eventually to pulmonary fibrosis. Volume load, due to the absence of urine production and anaemia which are common in HD patients, further contributes to hypertension\textsuperscript{102}.

Another explanation may be chronic embolization. It is well known that the HD process causes the activation of coagulation, complementation, and platelets\textsuperscript{103 30 104}. The blood’s contact with the dialysis membrane initiates these processes, as well as the peristaltic pump of the haemodialysis apparatus that drives the blood forward contributing to microbubbles\textsuperscript{105}. Platelet aggregates and aggregates of white blood cells have been detected in connection with HD\textsuperscript{106}. Their effects on the microcirculation are unclear.

Changes caused by pulmonary hypertension are difficult to distinguish from changes caused by repeated thromboembolism\textsuperscript{36}. Additionally, repeated air emboli in animal models were shown to provide pulmonary hypertension\textsuperscript{107 108}. HD patients have a higher prevalence of pulmonary hypertension compared with patients with peritoneal dialysis or pre-dialysis patients\textsuperscript{109}.

Larger emboli on the venous side get trapped in the pulmonary circulation, except in the presence of PFO or abnormality in the cardiovascular system that changes pressure conditions in the heart. It should be noted, however, that the prevalence of PFO is as high as about 30%\textsuperscript{39}. Stroke disease without concomitant peripheral vascular disease, atrial fibrillation, small vessel disease (constriction of the small blood vessels in the brain’s white matter.), heart valve defects or recent myocardial infarct (within 6 weeks) is defined as cryptogenic stroke\textsuperscript{110}. The prevalence of PFO in cryptogenic stroke is 50%, and in non-cryptogenic stroke the prevalence of PFO is 10-15\textsuperscript{111}. Closure of the PFO is done in selected cases of cryptogenic stroke. Case-control studies suggest a risk reduction with PFO-closure\textsuperscript{112 113}. Since all the studies were without randomization they are considered of low quality, and therefore no guidelines for when to take action is given. Randomized, prospective, and controlled studies that can be the basis
for guidelines are currently in progress. Additionally, embolism arises due to physiological right-left shunting. Venous blood drains from the capillaries into the arterial circulation and allows emboli in the order of 5-8 μm to pass into the circulation. In addition to this, there are differences in the ventilation-perfusion ratio, such as in emphysema, which also enables paradoxical embolization (refer to the section ‘Paradoxical embolism’ in the Introduction’). Patient cases with fatal outcomes have been described\(^\text{114}\).

Silent brain infarcts are defined as infarct changes that are detected by CT or MRI but without symptoms of stroke/TIA\(^\text{56}\).

HD patients have a high prevalence of silent brain infarcts. One-hundred twenty three chronic HD patients were examined with MRI and nearly 50% showed ischemic change; by comparison in a healthy control group only 10% had brain infarcts\(^\text{115}\). Other morphological changes like brain atrophy are also common in HD patients\(^\text{116, 117}\). Cognitive functions as measured by neuropsychological tests are significantly reduced in HD patients compared with healthy subjects\(^\text{118, 119}\). Many factors such as uraemia, anaemia, hyperparathyroidism, hypertension and other risk factors for cardiovascular disease contribute to this deterioration\(^\text{120-123}\).

When comparing HD patients with peritoneal dialysis patients, the latter performed better on tests\(^\text{124, 125, 126}\). These results may be due to dialysis modality, but also as a result of selection bias since patients were not randomized to a type of dialysis treatment. In a later study patients with clinical signs of dementia or cognitive impairment were excluded and no significant difference in test results between HD and peritoneal dialysis were seen\(^\text{127}\).

**Microbubbles**

Microbubbles are microemboli that contain gas, often composed of air or oxygen but other gases such as carbon dioxide, nitrogen and nitrous gases may occur\(^\text{128, 129}\). Barak \textit{et al} describes air microbubble changes and the effects on the circulation based on experiments with animal models\(^\text{130}\). The bubbles are in a dynamic process. In terms of size, smaller bubbles coalesce with larger bubbles that break down into smaller bubbles continuously under the influence of factors such as pressure and temperature. The length of time microbubbles act as emboli before they dissolve in the blood has been a topic of discussion\(^\text{130}\). According to the Epstein-Plesset equation, used to
calculate the dissolution time of spherical bubbles in water, it takes 1-6 seconds for an air bubble with a diameter of 20 μm to be taken up in the bloodstream. This is because surface tension creates an over pressure in the bubble that collapses. Thus, it is so that microbubbles can change shape to become more cylindrical making it easier to follow the blood stream; this is according to in vivo experiment\textsuperscript{131}. The extension can be up to 5 times compared with the diameter of the original bubble and "survival time" increases by 50%. In addition, a layer of protein is formed between air and the blood around the microbubble, which extends the dissolution time\textsuperscript{132}. By the same token the time when the microbubble affects the environment is likely longer than previously estimated\textsuperscript{130}.

**Microbubbles’ effects on tissues**

Microbubbles follow the blood stream to the point that the vessel becomes so small that they get stuck and prevent blood flow; this leads to lack of oxygen (ischemia). Endothelial cell damage, inflammation, and coagulation are initiated due to mechanical damage\textsuperscript{81}. Gas presents as a foreign substances to the blood and the microbubbles initiate local inflammation\textsuperscript{133}. Neutrophil cells aggregate around the gas bubble and complement activation occurs. C3a and C4a, components of complement system activation, affect polymorphonuclear cells (PMN) and stimulate mast cells to release histamine. This leads to increased vascular permeability and interstitial edema\textsuperscript{134}. PMN cells are involved in the release of cell-killing agents, which damages the endothelial membrane\textsuperscript{135, 136}. Furthermore microbubbles activate coagulation and induce platelet aggregation thereby leading to thrombosis\textsuperscript{81}. In animal experiments, where air was injected into the carotid artery (a.carotis int.) neurological symptoms were produced. Thrombin is an important mediator in both the thrombotic and inflammatory response in ischemia, reperfusion and vascular injury. Thrombin also converts fibrinogen to fibrin, which clogs the vascular bed and, in particular, leads to endothelial damage\textsuperscript{137}. Since heparin inhibits fibrin formation and thrombin binding to endothelium heparin was proposed to protect against the result of the effects of air emboli. When heparin was administered prior to air injection significantly less neurological symptoms occurred. This could indicate that some of the endothelial damage caused by air emboli was caused only by inflammation and thrombus formation. In animal models it was shown that repeated air emboli gave rise to pulmonary hypertension and further pathological
changes that included destroyed micro arteries, as seen in arterial pulmonary hypertension\textsuperscript{108,138}.

**Microbubbles in-vitro**

In the studies where MES was detected with DU in patients' venous system in association with HD, the composition of microemboli was not determined. It has been discussed that the intensity of MES may provide clues about whether the emboli are solid or gaseous. However, both small gas bubble and a larger solid embolus can produce the same strong signal\textsuperscript{97}. Cavitation bubbles can form and may be an emboli source in HD. In a previous study, HD patients breathed in 100% oxygen during dialysis\textsuperscript{97}. This was to inhibit cavitation and to increase the concentration of oxygen, which is more soluble than nitrogen, in the blood and thereby to reduce the survival of bubbles in the blood. However, the number of MES in the vena subclavia was not diminished with increased oxygen delivery compared with without. It was considered that detection were mainly of solid microemboli or larger gasemboli that had survived longer before dissolving in the blood\textsuperscript{97}. Objections to this conclusion are that the measurements were made before the lungs where gas exchange takes place and after the emboli source in the dialysis system, and that the survival of the gas bubbles was extended due to a protein layer around the bubble as described previously\textsuperscript{130}.

There are no published studies using multi-frequency Doppler, which could shed light on the composition of microemboli in connection with HD.

Jonsson et al.\textsuperscript{91} conducted several in-vitro experiments with the main aim to determine if the air in HD could pass the venous chamber without the air alarm going off. Instead of blood, a dextran albumin solution, with similar viscosity and rheological properties of blood was used. The fluid was recycled in various HD systems. The flow rate was increased from 100 ml min to 500 ml min and fluid level in the air chamber (equivalent to the venous chamber for HD treatments) was varied from a lower to a higher level. A DU device, CMD-10 (Hatteland, Røyken, Norway), was used to detect the number of MES/min. A large number of MES was recorded without the air detector being alerted. When the liquid flow rate was 300-500 ml min air bubbles were visible without the alarm being activated. Not even when air was injected into the corresponding venous chamber, where
the sensor detects the air, did the alarm sound. When 10 ml, but not 5 ml, was injected the alarm sounded.

When fluid circulated in the system, and the number of emboli does not increase over time, this indicated that the emboli were not mainly composed of plastic or other foreign materials derived from the dialysis machine system or the filter. With the absence of blood and a dialysis patient in the system, emboli from platelet aggregates, clots or ruptured atherosclerotic plaque can be excluded. This, along with the fact that air bubbles could be seen with the naked eye suggests that the MES detected during HD consists of microbubbles.

Because the distance to the middle of the flow (tube radius) was known, the size of the microbubbles could be determined. Most were in the order of 5-15 μm. The higher the pump flow the more microbubbles (up to about 700 bubbles / min!), but these could be reduced if the fluid level was high in the venous chamber.

Additional in-vitro studies confirm these results. Stegmayr et al. examined various models of venous chambers with the aim of measuring their impact on the presence of microbubbles140. A dialysis system, similar to that in the previous study, used a dextran albumin solution which had a similar viscosity to blood. The same DU apparatus, CMD-10 (Hatteland Instrumentation Røyken, Norway) was used to detect microbubbles and to determine their size. A CMD-10 probe was installed 10 cm downstream of the venous chamber. The number of microbubbles was measured at fluid flows varied between 50-600 ml min. Two different fluid levels were used in the venous chamber. The high level was defined as a level just below the fluid inflow to the venous chamber where the air detector is placed. The low level was the level that was as low as possible without the air detector being alerted, i.e. approximately 50% of the venous chamber volume was filled with fluid. All air detectors allowed microbubbles to pass through without the alarm going off. Generally, the number of detections with increasing fluid flow and the high fluid level venous chamber significantly reduced the number of MES.

In the absence of dialyzer, that source of emboli is omitted. In the absence of blood or patient clots and other biological materials are omitted as MES sources. That the number of MES was affected by the venous chamber level strongly indicates that the emboli did not consist of plastic material as a result of wear and tear. In such cases, the number of the MES would not be affected by the venous chamber
level. As the distance from the fluid inflow to the fluid level in the venous chamber increases, the low fluid level can lead to more turbulence when the fluid jet reaches the level of liquid. Air from the venous chamber was sucked down with the fluid, which created turbulence. Air bubbles were formed that could follow the fluid flow past the air alarm. If the force of the fluid flow is higher than the bubble buoyancy, which is dependent on the bubble size, passage would be allowed. Higher fluid flows also increase turbulence by the fluid jet hitting the surface of the liquid in the venous chamber with greater force. Small air bubbles have lower buoyancy due to lower volume, according to Archimedes' principle, and this explains why it was the smallest air bubbles that appeared most. If the design of the venous chamber enabled fluid to flow along the wall, the result would be less turbulence and a lower number of air bubbles. Overall this speaks for the presence of gasemboli as the main reason for, the detection. Since there were no needles bubbles caused by cavitation was considered negligible. Conditions were not present for large pressure differences, which could cause cavitation. The peristaltic pump used at the fluid flows mentioned was not considered sufficient to cause large pressure gradients.

In addition to the effect that air bubbles, in-vivo, have on inflammation and coagulation, as previously described, survival in the bloodstream may be more than 40 minutes since air is composed of 80% nitrogen. Therefore there is a risk that micro-infarctions will occur in-vivo.

The same authors also investigated, in-vitro, whether different types of filters showed differences in the occurrence of air emboli. The dialyzers were a low-flux F8HPS and a high-flux FX-80, both manufactured by Fresenius Medical Care (Bad Homburg, Germany). Both filters were steam-sterilized and stored without a fluid content, i.e. a dry filter. The third filter was a gamma-sterilized filter, APS-18u (Asahi Kasei Medical, Tokyo, Japan), which was stored with a fluid-base. The filters were compared at the same dialysis loop as in the previous study, and again the dextran-albumin solution that mimics blood characteristics was used. A constant fluid flow of 500 ml min was used and each filter was examined with the help of Doppler device, CMD-10 (Hatteland Instrumentation Røyken, Norway), for the presence of air bubbles. Measurements were made before the filter, directly after the filter and after the venous chamber – where the air detector is mounted. Regardless of the filter used air bubbles passed the air detector without it being alerted. The reason for this is
that the air detector is calibrated to alarm for bigger air bubbles since it is at this point the bubbles are considered to be a safety issue (refer to the section above ‘Hazardous air volume?’). Why do air bubbles pass the venous chamber instead of rising? According to previous descriptions excess fluid flow forces small air bubbles to rise. Air bubbles smaller than 400 μm are passed down if the fluid flow is 500 ml/min. This is confirmed by the fact that no differences existed in the number of air bubbles before and after the air trap. For measurements before and after the two dry-stored filters there were significantly more air bubbles after the filters; this indicates that there is air left in filters despite the priming (by rinsing with fluid the parts of the dialysis systems to remove air, including filters, hoses, etc.). No difference was found between high- and low-flux filters, which indicate that the membrane pore size does not matter when it comes to the presence of microbubbles. However, there were significantly fewer air bubbles after the wet filter, indicating that the air remained in the filter instead of being released. Moreover, it was noted that the more fluid used in the priming process, the fewer number of air bubbles were detected.

The occurrence of microbubbles during HD has been also recognized by other researchers, and preventive measures have been proposed. An in vitro dialysis system with a mixture of serum and glycerine to replace blood was used. An ultrasound transducer with frequency 5 MHz generated sufficiently strong irradiation power to change the path of movement of air bubbles of the size 5-200 μm so that they were collected in a storage chamber. DU apparatus with a probe that had 2 MHz resonance frequency (Multidop X4, DWL, Sipplinghen, Germany) was used to detect air bubbles. These were placed before and after the "bubble trap" and in total 70% of the microbubbles were intercepted. The emboli that remained in circulation were deemed fragmented air bubbles that were much smaller than the original bubbles. Other experimental studies have shown positive effect of surfactant on bubble elimination. Surfactant seems to change bubble surface tension and promotes bubble absorption.

**Ultrasound device**

Ultrasound is sound waves with frequencies above 20 kHz, i.e. frequencies above the audible sound. The ultrasonic device has a sensor/transducer that generates sound waves of a known frequency. The transducer contains piezoelectric crystals that produce ultrasonic pulses and converts electrical energy into a mechanical pressure
wave- sound wave. With increasing frequency the energy of the ultrasound increases. Continuous irradiation of the tissue leads to energy accumulation and damage that includes to the cell's genetic material (DNA). To avoid this, the transducer generates very short pulses of ultrasound followed by a pause. The sound wave propagates through the body, and some are reflected (echo) back to the transmitter during the pause. The echo is formed when the sound wave reaches an interface between two tissues with different acoustic impedance. Acoustic impedance is a measure of the ease that a substance/tissue transmits ultrasonic waves. The product of the propagation velocity and the density of the tissue correspond to this value.

The body's tissues have an average propagation velocity of 1540 m/s, with the exception of bone and gas that have different values. The reflection of the ultrasound occurs when an ultrasonic wave suddenly hits on a change in tissue acoustic properties. The amount that is reflected depends on the difference in acoustic impedance in the two tissues and on the ultrasonic beam incident angle. The part of the ultrasonic wave that is not reflected continues further into the tissue, and when it encounters the next boundary the next reflection occurs. If the differences between the tissue densities are quite high a lot of ultrasonic wave energy is reflected. In practice this means that tissue lying under air filled-organs or a gas bubble cannot be studied. The energy of the reflected sound wave is converted into an electrical signal, which is then analysed by the ultrasonic device and presented in the form of a graph or image on a display screen.

In M-mode an ultrasonic beam is sent and the reflection points are marked along a time axis. The method is well suited for studying fast events such as motion, for example heart valvular movement.

In 2D echo, a sweeping ultrasonic beam is used where the reflection points for each beam are seen on the ultrasound screen, and these points combine to build up a tow-dimensional image of the investigated structure. A high frequency transmitter provides a good resolution but penetrates much shorter. If the ultrasound hits a material with different acoustic impedance and the interface is perpendicular to the ultrasound propagation direction, the reflected energy back toward the probe is stronger than if the ultrasound presents an oblique angle.
J. C. Doppler (1803-1853), an Austrian physicist, described the physical phenomenon that the Doppler technique is based on (the reason that Doppler is written with a capital ‘D’)\textsuperscript{42}. A sound has a higher frequency if the sound source is moving toward the observer and a lower frequency if the source is moving away from the observer. The change in frequency is called the Doppler shift. With the Doppler moving objects can be studied, such as red blood cells. The blood cells moving toward the transducer will reflect ultrasound at a higher frequency compared with the transmitted sound wave. The opposite occurs when the cells move from the transmitter, i.e. the reflected sound has a lower frequency than the transmitted ultrasound signal. The frequency difference, which represents the Doppler shift, is processed by the DU device (using the Doppler equation) and presented as velocity curves and audible sounds. This means that only the emitted ultrasonic encountered on a motion toward or away from the transmitter are registered. The technology has been around for a long time, but it was not until the 1980s that the Doppler became useful as a clinical instrument.

In pulsed wave Doppler (PW Doppler) the transducer sends a short pulse ultrasound and then waits until the reflected sound returns before sending a new pulse. With this technique Doppler shifts from specific areas can be registered. The area in which the rates are analysed is called sample volume (SV), and is placed in the desired location by the investigator. The disadvantage of pulsed Doppler is that it is not capable of recording high-frequency shifts. When the flow rate exceeds a certain limit, the frequency change is so great that it is perceived as speed in the opposite direction, i.e. aliasing.

Penn Doppler is an ultrasonic sensor that gives only a Doppler registration but no image. It is primarily used for examining vessels. Carotid arteries are relatively superficial and transducers with frequencies from 3 to 7.5 MHz are common for studies these, compared with other cardiac studies where frequencies of 2-5 MHz are used. This enables even a pulsed Doppler to have good penetration and yet accurately measure high speeds without the occurrence of aliasing.

Duplex means using equipment that provides both a 2D and Doppler echo image. Duplex is used in examining blood vessels, usually in the neck and legs\textsuperscript{42, 144}.  


Presence of microemboli during haemodialysis?

In the field of microemboli associated with HD there are no prospective, randomized, controlled studies. Neither are there studies of microemboli during HD and clinical endpoints. Patients who depend on HD for survival suffer from numerous symptoms between dialysis sessions as well as during a session. The causes are multifactorial with uraemia and cardiovascular disease being prominent. Survival is significantly reduced compared with healthy people of the same age. If HD, because of prolonged repeated exposure of microemboli may contribute to morbidity, efforts should be made for further research.

Using a DU device was shown to be a reliable method in detecting MES in vitro. This inspired us to move forward with clinical studies. Safety standards for air emboli associated with HD are based on animal models and theoretical arguments. Microbubbles of air less than 40 μm are considered to shrink rapidly and collapse in the circulation mainly due to surface tension forces. The standards are also based on the assumption that any air emboli associated with HD affect the venous side. The microbubbles that may remain in the blood are expected to lodge in the lungs and to be ventilated out. The microbubbles are believed not to affect the patients.
AIMS

The general aims of this thesis were to study in vivo the presence of microemboli during haemodialysis using Doppler ultrasound and examine methods that might reduce the exposure to microemboli.

The specific aims of the papers were:

I) To explore if microemboli can be detected in the venous blood line after the air trap.

II) To explore if microemboli can be detected in the patient’s vascular access and in the carotid artery.

III) To investigate if the amount of microemboli can be decreased by changing the blood level in the venous chamber.

IV) To investigate if the exposure to microemboli can be reduced by using different types of filters in combination with altering the blood level in the venous chamber.

V) To investigate if microbubbles can be detected in vivo by investigation of an autopsy of a patient on chronic haemodialysis.
MATERIALS AND METHODS

Paper I

Study population and design

The design was an explorative study. In a total of 46 HD sessions forty chronic patients (32 men, 8 women) were investigated regarding the presence of MES in the venous blood stream after the blood passed the air trap/venous chamber. The mean age was 59.2±14.6 years (range 23–89). The primary diagnosis leading to end stage renal disease can be seen in Table 4. Three patients’ data are missing.

Table 4. Primary diagnosis causing end-stage renal function

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic renal failure; aetiology uncertain</td>
<td>10</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>10</td>
</tr>
<tr>
<td>Diabetic nephropathy Type I</td>
<td>5</td>
</tr>
<tr>
<td>Renal vascular disease, hypertension</td>
<td>4</td>
</tr>
<tr>
<td>Medullary cystic disease</td>
<td>2</td>
</tr>
<tr>
<td>Diabetic nephropathy Type II</td>
<td>1</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>1</td>
</tr>
<tr>
<td>Myelomatosis</td>
<td>1</td>
</tr>
<tr>
<td>Polycystic kidney disease, adult type</td>
<td>1</td>
</tr>
<tr>
<td>Renal vascular disease, polyarteritis</td>
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</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>1</td>
</tr>
<tr>
<td>Data missing</td>
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</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
</tbody>
</table>

The patients attended the dialysis unit at Norrlands University Hospital. During the time of the study there were 53 chronic HD patients. All patients received oral and written information and gave their consent to participate. The only exclusion criteria were if the patient was demented or too ill in general to assimilate the information and give consent. The number of people excluded or who declined examination was not registered. A total of 231 measurements were performed during different sessions and several times during the sessions. The measurements were done within 30 minutes after
start of dialysis in all 40 patients, and in 25 of these just before the end of the same dialysis session.

Analyses were also performed in nine sessions with online haemodiafiltration, four of them first with conventional high-flux HD and then when they were switched to online treatment.

In six of the patients, isolated ultrafiltration was performed to remove fluid. Those measurements were made while on ultrafiltration mode, but also later when they changed for their standard dialysis.

To detect micrometer-sized microemboli during HD, a pulsed ultrasound Doppler probe (CMD-10, Hatteland, Røyken, Norway) was mounted in a vertical position on the outlet line 10 cm downstream of the venous air trap. The signal measured was from reflection of the ultrasound waves. The frequency of 1.5 MHz ensured that the ultrasound detector could recognize MES through the tubing.\(^{149}\). (Figure 4).

**Figure 4.** Haemodialysis circuit. The probe of CMD-10 (Hatteland, Røyken, Norway) placed below air trap/venous chamber
The ultrasound device had the ability both to count the number of MES and also to classify the size of each microemboli, divided into nine different size ranges. The mean for each detection level was for Range 1: 5µm(2.5-7.5); Range 2: 10µm(7.5-12.5); Range 3: 15µm(12.5-17.5) ; Range 4: 20µm(17.5-22.5); Range 5: 25µm (22.5-27.5); Range 6: 30µm (27.5-32.5); Range 7:35µm (32.5-37.5); Range 8:40µm (37.5-42.5). Range 9 represented detection level for microemboli above 42.5µm (Table 9).91

Material

Dialysis was performed using either Fresenius 4008 devices (Fresenius Medical Care, Bad Homburg, Germany) including online devices in 39 patients or a Baxter device (Baxter, Deerfield, IL, USA) in one patient. Dialysis tubing sets used were Fresenius FA404C/FV404B; these were steam sterilized. Dialysis filters used were either HPS8 (Fresenius Medical Care), or in online line sessions F60. The Doppler ultrasound equipment, CMD-10 (Hatteland Instrumentering, Røyken, Norway) described below, was used. The corresponding computer software Bubmon.exe was used to present and save the computed data.

CMD-10: Cardiovascular Microbubble Detector (Hatteland, Røyken, Norway)

CMD 10 is designed to detect microbubbles in a fluid, e.g. of blood that is oxygenated by an oxygenator, and for monitoring gas emboli in blood vessels through the skin (transcutaneous)150 151. The instrument is based on a pulsed Doppler technique that measures only bubbles in motion with velocities that exceed a certain threshold. This is to prevent echoes that can be misinterpreted as moving microbubbles. Microemboli consisting of other than gas can also be detected, but the apparatus is sensitive to differences in acoustic impedance (sound velocity multiplied by density). For example, plastic and rubber particles have acoustic impedance that is comparable with most fluids while gas bubbles have high impedance, which CMD-10 is more sensitive to.

When an ultrasonic pulse hits a bubble a large portion of the ultrasound wave is reflected, which is not the case with emboli of plastic/rubber. But despite the fact that a significant proportion of the ultrasound wave is reflected when it encounters a gas bubble, the reflected ultrasound wave distributes in different directions. A small
part of the ultrasonic wave hits the probe crystal. If the gas bubble moves towards or away from the probe a Doppler shift occurs, i.e. the reflected sound has a different frequency than the transmitted ultrasonic wave.

The ultrasonic frequency is 1.5 MHz or 3 MHz (transcutaneous measurements). Doppler shifts are presented as a spike, whose amplitude is proportional to the Doppler amplitude and the microbubble diameter. When the tube diameter is known, and therefore the distance to the bubbles known, the sizes can be determined. The spike width is inversely proportional to the microbubble velocity.

We monitored microemboli in the dialysis apparatus blood-filled tubes and used a probe designed for this (1.5 MHz). In order to process echoes and perform statistics a PC using the software BUBMON.exe was connected to CMD-10.

Calibration was performed according to the manual for CMD-10. Glass beads with a diameter of 200 μm were used. These give an echo that mimics air bubbles with a size of 50 μm\textsuperscript{150} \textsuperscript{151}. The glass beads were in a recirculation system with dialysis fluid, produced by the dearing system of a Fresenius 4008 device (Fresenius Medical Care, Bad Homburg, Germany). The system was contaminated with air in conjunction with an addition of glass beads, and ventilation was done with the help of the ventilation chamber. For a more detailed description of the calibration procedure\textsuperscript{91}.

The time that microbubbles are in the ultrasonic field (i.e. transient time) is short, about 5-100 ms, depending on the blood flow velocity and the tube size. This places demands on the equipment and to allow a good registration a signal is created that indicates the greatest Doppler amplitude during 100 ms intervals. In this way the largest Doppler amplitude for each 100 ms interval is recorded. If a small emboli is shadowed by a larger, the small one will not be recorded and the number of smaller microbubbles may be underestimated.

Another weakness with CMD-10 is that if two small emboli in the blood flow are moving in parallel, the interpretation will be the presence of one big microembolus. Inhomogeneity due to air bubbles in the plastic probe can also interfere with the measurements.
We have compared the CMD-10 with the newer dual channel bubble detector, Gampt BCC200 (GAMPT mbH, Leipzig, Germany). The test performed was that the probes were mounted in series on the same tube, and a fluid with a lot of bubbles was circulated. Both probes of the BCC200 detected more MES than the CMD-10. There was a good coherence in regarding the numbers between the both probes of the BCC200. The probe we used was, however, modified compared with CMD-10 original probe. The CMD-10 probe consists of a thick plastic cover with a high resistance. By removing some plastic the distance from the ultrasonic transducer to the center of the tube was decreased by 4 mm. Because of this the sensitivity to detect echoes was increased from the size of and emboli of 50 μm to about 2.5 μm. This was a significant improvement in sensitivity. A strength of the CMD-10 with the modified probe is the high reproducibility. We did experiments where we let the glass beads circulate in looped tubing containing ventilated water. The CMD-10 measured the number of detections. Each measurement period was one minute and the measurement was continued for twelve minutes. The fluid flow rate was 365 ml/min. For each minute the number of detections was compiled. The number in each measurement period averaged 1235 ± 23.1 (range 1198-1271)! After the glass beads were collected, there were no detections. This suggests that emboli from artificial materials, like plastic from the tubes, are not so common in dialysis tubing.

**Paper II**

**Study population and design**

The design was an explorative study. Fifty-five chronic HD patients from the dialysis units at Skellefteå County Hospital and Norrlands University Hospital were included. One patient was excluded before the measurements were completed since he suffered from muscular fibrillation that made reliable measurements impossible. All patients received both oral and written information and gave consent to participate. All 21 patients at the dialysis units in Skellefteå County Hospital accepted to participate and 34 patients out of a total of 49 dialysis patients at Norrlands University Hospital. The only exclusion criteria was if the patient was demented or not able to understand the information. Two patients met the exclusion criteria. The mean age was 63 ± 17.2 years (range 24-86 years of age). There were 24 females
and 30 males. The primary diagnosis causing end-stage renal function is presented in Table 5.

**Table 5. Primary diagnosis causing end-stage renal function**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic nephropathy Type I and Type II</td>
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</tr>
<tr>
<td>Chronic renal failure; aetiology uncertain</td>
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</tr>
<tr>
<td>Glomerulonephritis</td>
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<tr>
<td>Polycystic kidney disease</td>
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<tr>
<td>Renal vascular disease</td>
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<tr>
<td>Pyelonephritis</td>
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<td>Tubular necrosis</td>
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<td>Interstitial nephritis</td>
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<td>Alport Syndrome</td>
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<td>Congenital disease</td>
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<td>Goodpasture’s Syndrome</td>
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<td>Medullary cystic disease</td>
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<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>54</strong></td>
</tr>
</tbody>
</table>

During the HD sessions, an endovascular microemboli detector was used (EMEX-25, Hatteland Instrumentering, Røyken, Norway). The corresponding EmmonW software was used and it reduced the number of possible false-positive detections caused by unintentional probe movement. A handheld probe was used to investigate the presence of MES at the AV fistula/graft and carotid artery (either at the left or right side of the neck). If a CDC was used for access, the measurement was conducted only at the carotid artery. A recording was taken once before the patient was connected to the HD device and again any time after the first 10 minutes after onset of HD. This time was not registered since our previous in vivo study showed no difference in the presence of MES after the start compared with before the end of dialysis\(^{39}\). Since we expected most microbubbles to end up in the lungs, but not the smaller (refer to the section ‘The physiological right-to-left shunt’ in the Introduction), the time of measurement was prolonged up to 5 min at the carotid artery versus 2 min at the AV fistula.
The site of measurement on the AV fistula was within 10 cm proximal from the venous needle, at a site where a strong signal could be measured.

A control group that was healthy, and did not suffer from cardiovascular and kidney disease, was examined with Doppler ultrasound (EMEX-25, Hatteland Instrumentation, Røyken, Norway) for the presence of MES in a.carotis communis. The measurement lasted for 5 minutes. Sixteen participants, that included 10 females and 6 males, took part. The mean age was 44.1 ± 8.7 years (range 33–63).

**Material**

A standard treatment was carried out, and dialysis conducted using Gambro Lund, Sweden AK 200/200S devices in 27 sessions. The tubing sets used were Gambro BL207B that were steam sterilized. Fresenius Bad Homburg, Germany 4008H/S devices were used in 23 sessions, including online devices in two patients. The tubing set used was Fresenius FA404C that was steam sterilized. In one session, a Fresenius 5008 was used. In three cases, the dialysis session protocol did not include the information about what kind of dialysis device was used, although this is routinely requested. The dialysers used were Polyflux 17L, 140H, 170H, 210H, FX80 and F8HPS; all steam sterilized. During the HD sessions, the Doppler ultrasound equipment EMEX-25 (Hatteland Instrumentering, Røyken, Norway) described below was used.

Another part of the study investigated whether microemboli were able to pass an infusion pump with an air detector. A setup was used comprising an infusion of either 0.9% NaCl or Ringer's with 4% albumin as a replacement fluid (for infusion during, say, aphaeresis) or StructoKabiven Perifer (Fresenius Kabi, Uppsala, Sweden) plus an infusion pump Braun/Infusomat FMS (B. Braun, Melsungen, Germany). The fluid speed was set at 1000 ml/h for the NaCl and Ringer's with albumin infusion, while the speed for the StructoKabiven nutritional fluid was 200 mL/h, corresponding to the maximum recommended speed for a patient weighing 70 kg. The probe, used for measurement at the dialysis device (CMD 10 Hatteland, Røyken, Norway) was placed below the infusion pump after the air detector.
**EMEX-25: Endovascular Microemboli Detector** (Hatteland, Røyken, Norway)

EMEX-25 is designed primarily to detect microemboli in major peripheral arteries and veins. The ultrasonic frequency is 3.0 MHz. It is based on the same principles as CMD-10, i.e. pulsed Doppler technique where only emboli in motion with a speed and echo amplitude above a threshold value are recorded\(^{152}\). This is to minimize registration errors of echo from substances other than microemboli that follow in the blood stream. To process echoes and perform statistics a PC using the software EmmonW.exe was connected to EMEX-25. The program has an algorithm for removing artefacts, which reduces the number of potential false positive detections as caused by unintentional movement of the probe or electromagnetic interference with other devices. Larger probes or tissue motion should be avoided as these generate false detections\(^{152}\).

EMEX-25 is used transcutaneously and the probe directed at 45 degrees to the investigated vessel to perceive emboli movement. The instrument is sensitive to any emboli in the vessel. The degree of sensitivity is based on differences in acoustic impedance between the blood and the microemboli. The higher the impedance, the greater the sensitivity, which means that the instrument is more sensitive to microemboli consisting of gas compared with plastic or blood particles. Most of the echoes from air emboli are reflected, which is not the case with emboli from blood or plastic particles. There is a near linear relationship between the energy in the reflected sound wave from the microbubble and its cross sectional area, because the echo is nearly completely reflected\(^{152}\).

As with the CMD-10, Doppler shifts are presented as spikes, whose amplitudes are proportional to Doppler amplitude. Spike amplitude is closely proportional to the diameter of the microemboli, and the length is inversely proportional to the microbubble velocity\(^{152} 387\). Since the exact distance to the carotid artery was unknown (about 2-3 cm from the skin) only the emboli relative size (to each other) could be determined.

Validation of EMEX-25 was performed in a ventilated tube system with a dextran solution that had similar viscosity and rheological properties of blood and in different flow conditions. \(Q=300-350\) ml/min. The CMD-10, which we used in previous studies\(^{91} 140 141\) and showed good reproducibility, measured on a vertical part of the tube
to avoid the risk of larger microbubbles ascending such as to shadow the microbubbles that follow the main flow. The tubing was immersed in a container with water. The EMEX-25-probe was directed against the tubing in the water container and bubbles were detected. The result of the number of bubbles per minute in different size ranges for each DU device was compared. The devices had a high correlation in the case of number bubbles/min, $r^2=0.96$, $p<0.0001$ (Figure 5).

![Figure 5](image)

**Figure 5.** Correlation between the number of bubbles/min (all sizes) detected by CMD-10 (y-axis) and EMEX-25 (x-axis)

The EMEX-25 was significantly more sensitive for detecting small bubbles in the order of $\leq 10\mu$m. The number of bubbles/min for the size range 1 of the CMD-10 correlated to the number of bubbles/min for the size range 13 of EMEX-25 ($r^2=0.93$, $p<0.0001$). Hence the conclusion was that EMEX-25 is more sensitive in the measuring environment (water bath). The important point, however, was that when a lot of bubbles were detected with the CMD-10 a lot of bubbles were also detected with the EMEX-25.
Paper III

Study population and design

The design was a randomized, double blind, cross-over, intervention study. In vitro studies have shown that if the fluid level in the venous chamber is high the amount of MES is reduced\textsuperscript{140}. In line with these results, in more than 80% of the measurement occasions a reduction of MES were expected. Twenty patients were calculated to be sufficient to detect any difference between the study groups with respect to the number of detected MES and the selected level of significance. The patients served as their own controls by the use of a cross-over protocol. Twenty chronic HD patients participated in the study. Nine women and eleven men were included. The mean age was 67.2 ± 14.6 years (range 31-88 years). The primary diagnosis leading to end stage renal disease is presented in Table 6.

Table 6. Primary diagnosis leading to end stage renal disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycystic kidney disease, adult type</td>
<td>5</td>
</tr>
<tr>
<td>Chronic renal failure; aetiology uncertain</td>
<td>3</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>3</td>
</tr>
<tr>
<td>Diabetic nephropathy Type I</td>
<td>2</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>2</td>
</tr>
<tr>
<td>Renal vascular disease</td>
<td>2</td>
</tr>
<tr>
<td>Hemolytic uremic syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>1</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>20</strong></td>
</tr>
</tbody>
</table>

All 20 patients from the dialysis unit at Skellefteå County Hospital were invited to participate in the study. The patients were informed both orally and in writing (see Appendix 1). Fifteen patients accepted to participate in the study. To get a sufficient number of patients, according to the power calculation, patients from the dialysis unit at Norrlands University Hospital, Umeå, were also asked to take part. The first five patients asked agreed to participate in the study. In total there were 39 chronic HD patients at the dialysis unit at Norrlands University Hospital. During the study dialysis session, the patients
were randomly assigned to standard HD treatment with either a high blood level or low blood level setting in the air trap (Figure 6). If the level was defined as high, the blood level was right below the inlet into the air trap (at approximately 90% of total volume). If the level was denoted as low, the blood level was as low as possible without setting off any alarms (at approximately 50% of total volume) (Figure 6). The randomization process was performed as follows: The dialysis nurse in charge chose one envelope out of twenty. In ten envelopes there was a note with the text “HIGH” (high level) and in the other ten envelopes was a note with the text “LOW” (low level). The nurse set the air trap and neither the investigator nor the patient was aware of the setting as the air trap was completely covered. During the HD sessions, EMEX-25 (Hatteland Instrumentering, Røyken, Norway) was used. A handheld probe was used to determine the presence of MES at the arteriovenous fistula/graft. Corresponding EmmonW software was used. After approximately thirty minutes of HD a measurement was taken for two minutes.
During the recording the patient was in a sitting position and the access arm was held in a vertical position. This position was used in order to prevent microbubbles from sticking onto the vessel wall near the access site. This could potentially interrupt the continuity of flow of air microbubbles and lead to an unevenly distributed stream, thereby impairing the detection quality. By lowering the arm a more consistent flow of microbubbles is achieved. After completing this measurement the blood level was changed to the opposite setting. After another thirty minutes a new recording was carried out in an identical fashion.

Figure 6. Study design of paper III
Material

Standard HD was carried out using Gambro (Lund, Sweden) AK 200S devices in 19 of 20 sessions. The tubing sets used were Gambro BL207B that were steam sterilized. In one instance a Fresenius (Bad Homburg, Germany) 4008H system was used. The tubing set used was Fresenius FA404C that was steam sterilized. High-flux dialyzers Polyflux 210H, Xenium 170, 190 and FX80 were used after steam sterilisation. None was reused. In two sessions a low-flux filter FX10 was used. The blood flow was kept constant during the entire haemodialysis session for each individual patient, but could vary among the patients (range 260 to 400 ml/min). The Doppler ultrasound equipment EMEX-25 (Hatteland Instrumentering, Røyken, Norway), described above was used.

Paper IV

Study population and design

The design was a randomized, cross-over, intervention study. The patients served as their own controls. In vitro studies have shown that when a wet filter was used the amount of MES is reduced\textsuperscript{141}. In line with these results, in more than 80% of the measurement occasions a reduction of MES were expected. Twenty patients were calculated to be sufficient to detect any difference between the study groups with respect to the number of detected MES and the selected level of significance < 0.05. At baseline there were 39 chronic HD patients at Norrlands University Hospital. Of these, 21 patients were invited to participate and received both oral and written information and all gave their consent. One patient was excluded because she was transplanted. The mean age was 59.2±14.6 years (range 23–89). There were eight females and twelve males. The primary diagnosis resulting in end-stage renal disease is shown in Table 7.
Table 7. Primary diagnosis leading to end stage renal disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic renal failure; aetiology uncertain</td>
<td>7</td>
</tr>
<tr>
<td>Polycystic kidney disease, adult type</td>
<td>3</td>
</tr>
<tr>
<td>Diabetic nephropathy Type II</td>
<td>2</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>2</td>
</tr>
<tr>
<td>Renal vascular disease</td>
<td>2</td>
</tr>
<tr>
<td>Diabetic nephropathy Type I</td>
<td>1</td>
</tr>
<tr>
<td>Polyarteritis</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
</tbody>
</table>

Each patient was studied during standard HD in a randomized order with either:

a) a dry-stored dialyzer (F8HPS, Fresenius Medical Care, steam sterilized) with the blood level in the venous chamber set as “low” (DL). (The blood level was as low as possible without inducing any alarms on the dialysis device).

b) the same dialyzer as in above, but with the blood level in the venous chamber set as “high” (DH). (The blood level in the venous air trap was set right below the inlet into the air trap).

c) a wet-stored dialyzer (Rexeed, Asahi Kasei Medical, gamma sterilized) with the blood level in the venous chamber set as “high” as in series b above (WH). (Figure 7).

The randomization process was performed as follows: The dialysis nurse in charge chose one envelope out of three. In the envelopes there was a note. One note with the text DL and in the second envelope was a note with the text DH. In the third envelope was a note with the text WH.
Each patient underwent a total of three treatments according to settings described above and a total of 60 dialysis sessions were performed (Figure 7). The conditions were the same for each patient throughout the series except one case where the Fresenius 4008 device was used for the settings DL and DH, but the Fresenius 5008 device was used by mistake during the WH setting. The total number of MES was measured continuously during the first three hours of treatment. The measurements were conducted with an ultrasound
detector (CMD-10 Hatteland, Røyken, Norway) connected to the venous tubing and placed below the air detector, as described in study I.

**Material**

Standard HD was carried out using Fresenius (Bad Homburg, Germany) 4008/4008H/S devices in 56 sessions, and a 5008 in one session. The tubing sets used were Fresenius FA404C/FA404B that were steam sterilized. Gambro Lund AK 200S was used in three sessions. The tubing sets used were Gambro BL207B that were steam sterilized. The wet-stored filter Rexeed (Asahi Kasei Medical) was gamma sterilized; it was a low-flux filter and for single use. F8HPS (Fresenius Medical Care) was also a low-flux dialyzer and for single use; it was dry-stored and steam sterilized (Table 8). The Doppler ultrasound equipment CMD-10 (Hatteland Instrumentering, Røyken, Norway) described above was used.

**Table 8. Specifications for F8HPS and Rexeed-18L**

<table>
<thead>
<tr>
<th>Specifications</th>
<th>F8 HPS</th>
<th>Rexeed-18L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (ml/min)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>252</td>
<td>270</td>
</tr>
<tr>
<td>Creatinine</td>
<td>224</td>
<td>256</td>
</tr>
<tr>
<td>Phosphate</td>
<td>193</td>
<td>199</td>
</tr>
<tr>
<td>Vitamin B</td>
<td>118</td>
<td>117</td>
</tr>
<tr>
<td>UF-coefficient²</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Priming volume (ml)</td>
<td>113</td>
<td>103</td>
</tr>
<tr>
<td>Effective surface area (m²)</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Sterilization</td>
<td>steam</td>
<td>γ-ray</td>
</tr>
</tbody>
</table>

¹ Q_d=500mL/min,UF=0mL/min ² ml/h*mmHg

**Paper V**

The study was designed as a case report. We studied a 61 year-old man who suffered from diabetes mellitus from 30 years of age. Eighteen years later, he suffered from several thromboses. Thrombophilic investigation after his second thrombotic episode revealed no activated protein C resistance, no deficiencies of the coagulation inhibitors, antithrombin, protein C, or free protein S, but there were signs of hypofibrinolysis. After this, he was on lifelong
treatment with dicumarol. Due to diabetic nephropathy, HD was initiated at 56 years of age. Chronic HD was performed and as access a central dialysis catheter was used. Six years later, he died from cardiac arrest, 10 minutes after initiation of acute ultrafiltration due to pulmonary edema. He had been on a regular HD 2 days earlier. Autopsy was performed after informed consent was obtained from relatives (approved by the Umeå Ethical Committee). Tissue was collected at autopsy from the patient and was immersion-fixed in 4% paraformaldehyde in 0.1 M Na phosphate, pH 7.4, and paraffin embedded. The tissue was stained with hematoxylin/eosin and immunohistochemistry, using antibodies against C3, immunoglobulin G (IgG), IgM, and fibrinogen.

**Statistical analysis**

Two-tailed tests were performed and P-values below 0.05 were considered as statistically significant.

**Paper I**

Non-parametric tests, Mann–Whitney or Wilcoxon’s paired statistics, were used for non-normal distributed data, and the parametric Students t-test for normal distributed data. Pearson’s correlation test was used for correlation analysis. SPSS 16.0 software was used to calculate the statistics.

**Paper II**

A normal distribution could not be assumed. Besides descriptive statistics, a Wilcoxon non-parametric paired signed-rank test was used for comparison of the presence of MES before and during the HD sessions. Spearman's rank correlation test was used. Group comparisons were carried out using the Mann–Whitney U-test. A chi-square calculation was carried out to compare the presence of MES in this study with a hypothetical outcome that would be obtained if microemboli were to pass through an open foramen ovale in up to 30% of these patients (16 out of 54). SPSS 16.0 software was used to calculate the statistics.

**Paper III**

Normal distribution could not be assumed and the number of patients was limited, therefore Wilcoxon non-parametric paired signed-rank test was
used for comparison of the presence of MES measured with a high air trap level, and low air trap level, respectively. Spearman’s rank correlation test was used. Group comparisons were carried out using the Mann–Whitney $U$-test. The software used for calculating the statistics was SPSS 16.0.

**Paper IV**

Normal distribution could not be assumed and the number of patients was limited, therefore Wilcoxon non-parametric paired signed rank test was used for comparison of the number of MES measured within the first 30 minutes of HD and during the subsequent period. Group comparisons were carried out using Mann-Whitney $U$-test. Pearson’s test for correlation was used. The software used for calculating the statistics was SPSS 17.0. A multiple Poisson regression was used to test the effect of the type of dialyzer setting (DL, DH, and WH) on the total number of MES in the blood. A multi-level approach was used in which patients were considered to have repeated observations. An exchangeable correlation structure was assumed and the Poisson regression parameters were estimated by generalized estimation equations (GEE). These statistical analyses were performed with statistical software R ver.2.12.2.

**Ethical considerations**

All studies were approved by the Research Ethics Committee of Umeå University. Patients could discontinue participation without giving any explanation and without effect on continued treatment at any time. All patients gave written informed consent. In studies 1, 2 and 3 the examiner and physician was the same person, which may have encouraged the respondents to participate to a greater extent than would have otherwise been the case. All patients received both oral and written information and gave their consent to the staff who was not examiners. The data were linked to patients with a code. Only those responsible for studies and the research nurse had access to the patient code. There are no known side effects of Doppler ultrasound examinations. The settings of the air alarms in studies 3 and 4 are approved in accordance with applicable safety regulations. In study 5, family members were provided information they gave permission for the study. All studies complied with the Declaration of Helsinki.
RESULTS

Paper I

Measurements revealed the presence of MES in all of the series and in 90% of the measurements. The sensor in the air trap did not alarm during any of the measurements. The mean extent of microemboli (sum of all sizes) was at start $128 \pm 190$ (range 0–769) microemboli passing the detector per minute (Table 9).

**Table 9.** Distribution of microemboli related to size (grouped 1–9, and the sum of all) in 46 sessions during the first 30 min of dialysis (mean ± SD). Comparison of extent of microemboli between the smaller to the next larger size is given.

<table>
<thead>
<tr>
<th>Range of the size of microemboli (μm)</th>
<th>Number of microemboli/min mean values ± SD</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range 1 (2.5-7.5)</td>
<td>60 ± 118</td>
<td></td>
</tr>
<tr>
<td>Range 2 (7.5-12.5)</td>
<td>15 ± 20</td>
<td>0.009</td>
</tr>
<tr>
<td>Range 3 (12.5-17.5)</td>
<td>11 ± 18</td>
<td>0.000</td>
</tr>
<tr>
<td>Range 4 (17.5-22.5)</td>
<td>11 ± 23</td>
<td>n.s.</td>
</tr>
<tr>
<td>Range 5 (22.5-27.5)</td>
<td>8 ± 20</td>
<td>0.000</td>
</tr>
<tr>
<td>Range 6 (27.5-32.5)</td>
<td>7 ± 17</td>
<td>n.s.</td>
</tr>
<tr>
<td>Range 7 (32.5-37.5)</td>
<td>5 ± 14</td>
<td>0.023</td>
</tr>
<tr>
<td>Range 8 (37.5-42.5)</td>
<td>4 ± 12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Range 9 (&gt;42.5)</td>
<td>8 ± 29</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sum of all</td>
<td>128 ± 190</td>
<td></td>
</tr>
</tbody>
</table>

n.s., not significant.

Blood flow in the patients varied between 180 and 400 mL/min (mean during the start period was 346 ± 57, and at the end was 354 ± 59). There was no significant change between measures after start versus during the end of the HD session. Dialysate flow was 500 mL/min during all dialysis sessions. There was no difference between the values after start versus that before the end of dialysis regarding any of the microemboli sizes (Figure 8).
Figure 8. Extent of the sum of microemboli (counts per minute) during the start of dialysis versus during the end of dialysis (n=25). Lines connect the same patient. There were no significant changes.

There was significantly less extent of larger microemboli when comparing Range 2 to 1 also at the end of dialysis (Figure 9).
There was a weak but significant correlation between the blood flow and the number of microemboli for Range 1 \((r = 0.30, P = 0.021)\), Range 2 \((r = 0.37, P = 0.006)\), Range 3 \((r = 0.29, P = 0.026)\), and the sum of all microemboli \((r = 0.34, P = 0.01)\) (Figure 10).
The isolated ultrafiltration was performed in six runs. Those were compared with the standard dialysis sessions of the same patient. More microemboli were present within Range 1 ($P = 0.001$), Range 2 ($P = 0.015$), Range 5 ($P = 0.042$), and the total sum ($P = 0.003$) compared with the standard dialysis. Significantly more microemboli (Range 1, $P = 0.0001$; Range 2, $P = 0.003$; total sum, $P = 0.0001$) were present when comparing online high-flux dialysis ($n = 9$) with standard HD ($n = 41$ inclusive 4 with both online high-flux dialysis and HD). When measuring one meter downstream of the venous line from the air trap, instead of within 10 cm, there were fewer of the smallest microemboli ($P = 0.013$, $n = 21$ pairs), but no difference between the numbers of other sizes.
**Paper II**

There was a significant increase in MES during HD at the carotid artery in both the AV fistula and carotid artery of the patients (Table 10).

**Table 10.** Microembolic signals prior to haemodialysis (no-HD) and during (d-HD) at the AV fistula/graft and carotid artery

<table>
<thead>
<tr>
<th>AV access no-HD (n = 38)</th>
<th>AV access d-HD</th>
<th>P</th>
<th>Carotid artery no-HD (n = 54)</th>
<th>Carotid artery d-HD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (0-3)</td>
<td>4 (0-85)</td>
<td>0.000</td>
<td>1 (0-14)</td>
<td>2 (0-36)</td>
<td>0.008</td>
</tr>
<tr>
<td>0.2 ± 0.5</td>
<td>13.5 ± 20</td>
<td>1.7 ± 2.9</td>
<td>3.5 ± 5.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as median (range) (first row) and means ± SD (second row)

The mean blood flow was 309 ± 52 mL/min at the start and 304 ± 51 mL/min at the end. Blood flow varied between the patients from 200 to 450 mL/min. The air detector of the HD device did not give an alarm for any air passage during the dialysis procedures. There was no significant difference regarding the presence of MES during HD between those patients using high- and low-permeability filters, nor was the number of emboli affected by blood flow, age or access type. This applied to both the AV access site and carotid artery.

The control group (participants free of cardiovascular and renal illness) had a significantly lower number of MES compared with the HD patients before the start of HD as detected in arteria carotis communis (Table 11). There was a total of four MES in the control group. All four detections emerged when the investigator moved the probe or the participant moved the head.

**Table 11.** Microembolic signals prior to haemodialysis (no-HD) compared with a control group at carotid artery

<table>
<thead>
<tr>
<th>Carotid artery no-HD (n=54)</th>
<th>Control group (n=16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0-14)</td>
<td>0 (0-1)</td>
<td>0.016</td>
</tr>
<tr>
<td>1.7 ± 2.9</td>
<td>0.2 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as median (range) (first row) and means ± SD (second row)
Although measurement was for only 5 min, this study showed an increased number of MES at carotid artery in significantly more patients (31 out of 54, $P = 0.001$) than would be expected if the only explanation for the passage of microemboli was an open foramen ovale.

Peripheral vein infusion system

No air alarm was triggered during the infusion experiments. An infusion of sodium chloride (1000 ml/h) resulted in one count during a 55-min measurement. The median amount of MES during 11 runs was 206 counts per minute (range 34–443) when the infusion was conducted with a Ringer's–albumin solution. During the infusion of StructoKabiven Perifer (at 200 mL/h), a median of 0 counts per 5 min ($n = 17$ runs, 13 runs with 0 and 4 runs with one count per 5 min) was measured.
Comparing high blood level with low blood level settings in the venous chamber revealed significantly more MES at the site of AV access with low blood level. Median (range) was 2.5 (0-80) compared with 17.5 (0-77), respectively, (P = 0.044) (Figure 11).

**Figure 11.** Box plot for the number of microembolic signals measured in 2 minutes at the AV access. The median for each data set is indicated by the black center line, and the first and third quartiles are the edges of the area, i.e. the inter-quartile range (IQR). The extreme values (within 1.5 times the IQR from the upper or lower quartile) are the ends of the lines extending from the IQR. Points at a greater distance from the median than 1.5 times the IQR are plotted individually as circles. These circles represent potential outliers.

Mean blood flow during HD sessions was 324±30 ml/min. In each patient the blood flow was kept constant. Inter-individually the blood flow varied among the patients with a range of 260 to 400 ml/min. There was no correlation between blood flow and the number of MES when the whole group was studied. There was no significant
difference in the presence of MES during HD regarding gender or age, nor was the number of MES affected by type of access (fistula vs. graft) or dialyzer.

**Paper IV**

More MES/180 min were detected during dialysis with the setting dry-stored dialyzer with a low blood level (DL), versus wet-stored dialyzer with high blood level (WH) (OR 4.07, 95% CI 4.03-4.11, p<0.001) and dry-stored dialyzer with a high blood level (DH) versus WH (OR 1.18, 95% CI 1.17-1.19, p<0.001) and less for DH versus DL (OR 0.290, 95% CI 0.288-0.293, p<0.001). This implies that microemboli exposure was least when using WH, greater with DH, and most with DL (Table 12 and Figure 12).

**Table 12.** Effect of different dialyzer settings in relation to measured MES (counts/180 minutes). dry-stored dialyzer with a low blood level (DL), high blood level (DH) and the wet-stored dialyzer with high blood level (WH)

<table>
<thead>
<tr>
<th>Settings</th>
<th>OR</th>
<th>P</th>
<th>95% CI of odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL</td>
<td>4.07</td>
<td>&lt;0.001</td>
<td>4.03-4.11</td>
</tr>
<tr>
<td>DH</td>
<td>1.18</td>
<td>&lt;0.001</td>
<td>1.17-1.19</td>
</tr>
<tr>
<td>WH</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The number of MES (mean value) during the first three hours of treatment was 3263 counts/180 minutes (SD ±4998). The mean number of MES detected during 180 minutes of HD were 6373 counts/180 minutes (SD ±7511) for DL, 1848 (±1948) for DH, and 1564 (±1466) for WH (Fig 12).

**Figure 12.** The number of microembolic signals (mean) during 180 minutes of haemodialysis with different treatment settings: dry-stored dialyzer with a low blood level (DL), high blood level (DH) and the wet-stored dialyzer with high blood level (WH)
There was significantly greater exposure to microemboli during the first 30 minutes of HD compared with the median values for each 30 minutes increment of the remaining 150 minutes of HD for settings DL (p=0.001) and DH (p=0.004), but not for WH. This phenomenon was most pronounced for the DL, while DH had less, and WH had least microemboli (Figure 13).

**Figure 13.** The number of MES (median) during the first 30 minutes (black) versus the median values of the latter 30 minutes periods (white) with different treatment settings: dry-stored dialyzer with a low blood level (DL), high blood level (DH) and the wet-stored dialyzer with high blood level (WH)
MES were detected in all sessions (Table 13). One session revealed an extreme outlier with 174710 MES during the DH series. That series (Patient 16, see Table 13) was excluded when the statistical calculations were carried out. It was considered to be due to an inappropriately tightened connection between the AV-fistula cannula and arterial portion of dialysis tubes since the same extent of MES was noted during the entire dialysis session.

Table 13. Patient demographics, diagnosis, devices and numbers of microembolic signals (counts/180 minutes) using various dialysis modes: dry-stored dialyzer with a low blood level (DL), high blood level (DH) and the wet-stored dialyzer with high blood level (WH)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Devices</th>
<th>DL</th>
<th>DH</th>
<th>WH</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
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<td>Glomerulonephritis</td>
<td>F4008</td>
<td>16713</td>
<td>1985</td>
<td>2199</td>
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<td>2</td>
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<td>male</td>
<td>Polyarteritis</td>
<td>F4008</td>
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<tr>
<td>3</td>
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<td>PKD</td>
<td>F4008/5008</td>
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<td>2396</td>
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<tr>
<td>4</td>
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<td>1188</td>
<td>4</td>
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<tr>
<td>5</td>
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<td>754</td>
<td>69</td>
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<tr>
<td>7</td>
<td>77</td>
<td>male</td>
<td>Diabetes, type II</td>
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<td>819</td>
<td>569</td>
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<tr>
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<td>1778</td>
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<td>13309</td>
<td>485</td>
<td>3403</td>
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</table>

CRF= chronic renal failure (etiology uncertain), Hypertension= renovascular disease due to hypertension, PKD=polycystic kidney disease, adult type, Polyarteritis= renovascular disease due to polyarteritis, Glomerulonephritis= crescentic glomerulonephritis, F4008= Fresenius 4008, F5008=Fresenius 5008, G200=Gambro AK 200
The mean blood flow was 312±42 mL/min and varied from 200 to 400 mL/min between patients. The number of MES/180 minutes for the three settings correlated to the blood flow (see Table 14). The higher the blood flow the greater exposure to microemboli/180 minutes of HD. Table 15 shows, that with the setting of dry-stored dialyzer with low blood level (DL), that a blood flow below 300 ml/min resulted in less extent of MES.

**Table 14.** Correlation between the number of microembolic signals and blood flow during the first three hours of treatment with the three settings: dry-stored dialyzer with a low blood level (DL), high blood level (DH) and the wet-stored dialyzer with high blood level (WH)

<table>
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<tr>
<th>Settings</th>
<th>Pearson's correlation</th>
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<tr>
<td>DL</td>
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</tr>
<tr>
<td>DH</td>
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</tr>
<tr>
<td>WH</td>
<td>0.47</td>
<td>0.041</td>
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</table>

**Table 15.** Comparison of the effect of blood flow, either below 300 ml/min (n=11) or above 300 ml/min (n=8), on number of microembolic signals with the setting dry-stored dialyzer with a low blood level (DL). Data are given for the first 30 minutes after start (30s) or the mean of the following 30 minutes (30f) and for the whole 180 minutes period (180)

<table>
<thead>
<tr>
<th>Blood flow below 300 ml/min</th>
<th>Blood flow above 300 ml/min</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Median</td>
<td>Std. Dev.</td>
</tr>
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<tr>
<td>DL-30f</td>
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<td>132</td>
</tr>
<tr>
<td>DL-180</td>
<td>2675</td>
<td>942</td>
</tr>
</tbody>
</table>

There was no significant difference in the number of MES during HD related to gender or age, nor was the number of MES affected by type of access (central dialysis catheter or fistula and graft). The numbers of MES were not related to arterial line pressure. More than 40% of the microemboli were of the size of approximately 5 μ.
Autopsy revealed a closed foramen ovale and pulmonary edema. Microscopic evaluation verified the presence of bubbles of gas in the vessels of the lungs (Figure 14), the brain (Figure 15), and the heart. Anti-C3 and anti-IgG staining was negative around the bubbles while staining for antifibrinogen was positive (Figure 16).

**Figure 14.** Microscopic finding of microbubbles of gas (at arrow) in pulmonary capillary. In addition increased fibrosis of tissue (red areas)
Figure 15. Microscopic finding of microbubbles of gas in the brain (at arrow)
Figure 16. Microscopic finding of microbubbles of air in the brain after staining with antifibrinogen. Bubbles are surrounded by a brighter zone indicating clotting. The bright field, illuminated, marks aggregation of fibrin. IMH, immunohistochemistry
DISCUSSION

External validity

External validity refers to whether the results of the studies can be generalized to other HD patients. The results may appear in a certain way due to the individual subjects. A representative selection increases the external validity. To see if the patients included in the studies differed significantly from other HD patients, the study patients were compared with patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS) (Table 16, 17). DOPPS is a prospective, observational study intended to gain an understanding of which treatment patterns (e.g. type of access, treatment, unit size, etc.) during HD are associated with the best outcomes. This is a cohort study where data have been collected longitudinally. The study began in 1996. The data are a random sample of dialysis units from 19 countries from around the world, including Sweden. The hypothesis is that differences in treatment patterns correlate to differences in performance, and that knowledge of these can lead to improved care and reduced mortality and morbidity. DOPPS annual reports include, aside from demographic (patient descriptive) data, even comorbidity of patients included in the study. The type of morbidity reported is described, for example, in an article by Pisoni et al.

Table 16. Descriptive statistics for demographics for patients from DOPPS countries and from patients included in papers 1, 2, 3, 4 (P1-4). N=number of patients. Female=percent of patients with female sex. Age, Time=time on dialysis, values are presented as years and means ± SEM

<table>
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<tr>
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<th>Italy</th>
<th>Japan</th>
<th>Spain</th>
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<td>0.5</td>
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<td>18.9</td>
<td>46.3</td>
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Table 17. Co-morbidity for patients from DOPPS countries and from patients included in papers 1, 2, 3, 4 (P1-4). Presented in percent of all patients

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<th>Japan</th>
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<th>P2</th>
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<td>8.1</td>
<td>9.3</td>
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</table>

CHD=Coronary heart disease, Ca=Cancer other than skin, CaVD=Other cardiovascular disease, CeVD=Cerebrovascular disease, CHF=Congestive heart failure, DM=Diabetes Mellitus, GIB=Gastrointestinal bleeding, HT=Hypertension, LD=Lung disease, ND=Neurologic disease, PD=Psychiatric disorder, PVD=Peripheral vascular disease, RC/G=Recurrent cellulitis, gangrene

The demographic data and co-morbidity for the patients included in paper 1, 2, 3, 4 are as expected, except that there was a smaller number of women in paper 1 and the patients in our studies have been on dialysis a shorter time.
General discussion

The present studies of this thesis have resulted in increased knowledge regarding development and distribution of MES in the dialysis circuit and body of the patient. However, the presence of MES in HD was reported as early as 1975\textsuperscript{155}. \textit{In-vitro} as well as \textit{in-vivo} studies using DU during HD sessions have revealed MES in the veins\textsuperscript{95-97} \textsuperscript{156}. The authors suggested these MES to be composed of fibrinogen, artificial material or air bubbles. In one study, the authors suggested that the composition of the emboli were either synthetic particles or microbubbles due to the intensity of the MES\textsuperscript{96}. They suggested that the roller pump might induce the formation of signals from tube damage or by cavitation.

Although we cannot determine the composition of the detected MES with our DU devices, indirectly there are several facts that point in the same direction. In our previous \textit{in-vitro} studies, we observed:

- that MES were detected when we used dextran, instead of blood, which rules out emboli from platelet aggregates, cell aggregates, clots or ruptured atherosclerotic plaques as causes for the MES\textsuperscript{91 140 141}.

- calibration of CMD-10 meant that the fluid was deaerated and only the glass marbles remained. The number of MES did not increase with time as would be the case if it was an issue of plastic emboli from plastic tubing\textsuperscript{91}.

- the number of MES did not vary according to the level of the fluid in the air chamber if the emboli were of artificial material\textsuperscript{91 140}.

- artificial MES due to measurement errors, the method used, or to artefacts caused by unintentional movement of the probe or electromagnetic interference from other devices can occur. However, the problem is the same for all measurements and paired statistics were used, which minimizes the risk that false detections would significantly affect the results.

Additional:
- air bubbles could be seen with the naked eye during the experiments\textsuperscript{91}. 

• the DU device, CMD 10, is much more sensitive for microemboli that consist of gas (because of its high acoustic impedance) compared with plastics material or blood particles\textsuperscript{150}.

These data suggest that larger amounts of detected MES consist of air bubbles.

In our clinical studies:

• blood levels of the venous chamber affect the number of MES\textsuperscript{157, 158}.

• wet filters generate fewer MES, which can be explained by less air in the wet-stored filter compared with a dry-stored filter\textsuperscript{158}.

• DU devices, based on the same technology as \textit{in-vitro} studies were used\textsuperscript{139, 157-159}. They are more sensitive to microemboli that consist of gas.

• the autopsy study of a chronic HD patient showed small cavities, which were not stained by hematoxylin/eosin or influenced by immunohistochemistry. These cavities were probably caused by micro-bubbles of gas (personal communication T.Brännström). The gas bubbles were detected in the capillaries of the brain, lungs and heart. The gas bubbles were surrounded by fibrin, proving existence before death occurred. These findings indicate that gas bubbles formed before death occur in both the venous and the arterial circulation\textsuperscript{160}.

Altogether, the results indicate that microbubbles were responsible for most of the detected MES in the studies. But where does the air enter the dialysis circuit? Where do the bubbles come from? Theoretical air sources have been discussed in the literature\textsuperscript{39 94} (Figure 17).
Figure 17. Below are shown five hypothetical sources of air contamination in the extracorporeal system.

1. Air remaining after priming. For example in the tubing system, air trap/venous chamber and dialyzer (header and pores). Microbubbles are generated by turbulence in the venous chamber. Air is taken up by the blood jet entering the venous chamber.\textsuperscript{94, 140, 161}

2. The presence of air leakage in the HD circuit due to negative pressure. For example, infusion- or drug injection ports, luer-lock connection between the tubing set and
catheter/fistula, arterial side injection port, infusion port of anticoagulants, sampling, arterial pressure gauges\textsuperscript{94}.

3. The dialysate is degassed in the dialysis system. Only if malfunction in the degassing system occurs, may gas diffuse from the dialysis fluid to blood in the dialyzer (transmembrane diffusion). Back filtration: in locally low TMP dialysate may flow to the blood through the membrane\textsuperscript{94}.

4. Bubbles created by the cavitation phenomenon. Cavitation occurs in blood when the local blood pressure drops below the vapor pressure so that blood is vaporized and bubbles (cavities) are formed. Blood pressure can be theoretically reduced locally by high flow velocities and acceleration. In the dialysis system, a pressure decrease occurs in the dialysis needle on the artery side, but only by 40 mmHg (Blood Flow: QB 400 ml / min, needle size 1.6 mm). This pressure decrease is too small for cavitation to occur. According to some researchers bubbles are not formed from air cavitation in the dialysis system\textsuperscript{161}.

5. During priming procedure or during infusion saline is used. Air bubbles can be formed from over saturated saline\textsuperscript{161}.

Paper I

This study showed that patients are exposed to microemboli during HD treatment. MES was detected in over 90\% of the measurements. The air alarm alerted at no time during the dialysis treatments. The reason for this is that air alarms are not designed to detect microbubbles since they are not considered to be a safety risk for the patients (see section on Hazardous air volume? in the Introduction). Safety standards for air emboli associated with HD are founded on literature studies based on animal models and theoretical arguments\textsuperscript{94}. Microbubbles less than 40 μm are believed to shrink rapidly and collapse in the circulation mainly due to surface tension\textsuperscript{39, 147, 148}. Standards are also based on the assumption that air emboli associated with HD affect the venous side and are therefore harmless in smaller amounts\textsuperscript{92, 93}. The microbubbles that remain in the blood
are expected to lodge in the lungs and then be ventilated\textsuperscript{39}. Thus, they are considered not to affect the patients to a great extent. Most MES detected in this study were small (range 1 corresponding diameter around 5\textmu m) and there was a weak correlation between blood flow and the number of MES.

**Paper II**

After the start of HD significantly more MES were detected in the AV access compared with before starting dialysis. The results confirmed earlier studies on MES in the venous circulation of HD patients as reported by other research groups\textsuperscript{96} \textsuperscript{95} \textsuperscript{97}. These results argue against that the fact that resolution time of microbubbles in the blood circulation is only a few seconds and thus is the time when the microbubble affects the environment is probably longer than previously calculated\textsuperscript{130}.

Significantly more MES were detected during HD compared with before HD at carotid artery, which suggests that the HD treatment generates microemboli not only in the venous circulation but also in the arterial circulation. One possible explanation for this result is that microbubbles activate the complement system\textsuperscript{162} \textsuperscript{163}, which leads to thromboembolism in venous and arterial circulation.

Another explanation could be that microbubbles pass the lung barrier and are recorded as MES in the carotid artery. Most of the microemboli detected in previous \textit{in-vitro} experiments\textsuperscript{91} \textsuperscript{140} \textsuperscript{141}, as well as in paper I, were on the order of 5 \textmu m. These could theoretically pass the pulmonary capillaries and lead to embolization (refer to the section ‘The physiological right-to-left shunt’ in the Introduction). MES detected in carotid artery may be related to paradoxical embolism in the presence of PFO connection with a pressure change, Valsava manoeuvre, or embolization passing the pulmonary capillaries. The high prevalence of MES in the carotid artery found in our study can not be explained only by paradoxical embolization in the presence of PFO.

Whatever the explanation is for the MES in carotid artery, they may present unfavourable risks to patients. Results from a study in 2009 on patients who underwent CEA or CAS suggested that both solid and gaseous emboli can be harmful to the brain\textsuperscript{164}. The study showed a correlation between microemboli and ischemic stroke or MRI verified infarcts ipsilateral to the CEA or CAS. Eighty-five consecutive patients
with >70% carotid stenosis with different additional indications, e.g. neurologic symptoms ipsilateral or prophylactically before cardiac surgery, were included. All patients underwent clinical neurological examination before and after the intervention. The patients who had neurological symptoms during or after the intervention underwent MRI. All patients underwent examination with multi-frequency TCD with the ability to distinguish between solid or gas emboli ipsilateral during operation. The results showed that both the solid and gaseous microemboli were independently associated with procedure-related ipsilateral cerebral infarcts and/or changes in MRI.  

Emboli on the artery side can occlude the capillaries and cause ischemic lesions. In a recent observational study on well dialyzed HD patients with absence of TIA/stroke disease and optimized Hb (115 ± 11 g / l) were compared with a control group regarding cognitive function as measured by neuropsychological tests. The control group did not have kidney disease but were matched for age and co-morbidity. HD patients had significantly poorer performance on tests. The difference between groups was independent of risk factors for dementia. The prevalence of cognitive impairment is high in the HD patient population. The cause is probably multifactorial. The authors suggest that, in addition to the critical research on factors specific to kidney disease, the contribution that the HD process has on deterioration should be clarified. Can microemboli contribute to the high prevalence of cognitive impairment in HD patients?  

To firmly establish a correlation between microemboli during HD and morbidity, other types of studies are needed.  

If microemboli can enter the blood during HD treatment, it is likely that this can also occur in patients receiving fluid infusions. In-vitro experiments with fluid infusions suggest that microemboli associated with intravenous infusion may occur.  

MES in both carotid artery and AV access were detected prior to initiation of HD. This can be explained: a) partly by the fact that there are enough small movements of the patient or the probe to risk the occurrence of MES; b) partly by the fact that the presence of atherosclerosis and vascular injuries are common in HD patients and there is a risk of thromboembolism.  

The risks are the same during HD and patients were their own controls, but the study design was not blind and systematic errors
may occur. The healthy control group had significantly fewer MES. Movement of either the investigator or the person examined was observed for all four detections. Keeping the probe stationary for 5 min was exhausting, and many patients found it difficult to lie still. Another possible explanation for detections before the start of HD is that there may have been air bubbles from newly administered fluid infusions. Alternatively, small bubbles may recirculate several days from a previous dialysis session.

In a recently published study, 51 HD patients examined with transthoracic echocardiograph for the presence of PFO. There was 21.3% with PFO. The number of MES in the AV fistula was measured with DU (ST3 Digital Transcranial Doppler System, Spencer Technologies, Seattle, WA, USA) before and after the start of HD in eight patients. Significantly more MES was detected during HD than before starting HD.

Forty of these HD patients were examined during HD with DU over ACM. No MES were detected, although Valsava manoeuvre was done in patients with PFO. This contradicts the results in the paper II. However, different DU apparatus was used and measurements were done at different places in the two studies. No direct comparison is made between apparatus and there are no data on the difference in detection sensitivity. Studies with simultaneous measurement of microemboli in carotid artery and ACM during HD and with the same equipment are desirable.

**Paper III**

This study measured the number of MES in the AV access of HD patients with the same DU device as in study 2, i.e. using a handheld probe. Patients were their own controls. The patients as well as the investigator were blinded to the intervention to reduce the risk for bias. The number of MES was assessed during the 2-min survey at two different times on the same patient during the same dialysis session. The level of blood in the venous chamber differed between the measurements. The number of microemboli was significantly reduced when the blood level was high. The results confirmed data from a previous *in-vitro* study. The reason why the level in the venous chamber had an impact on the number of microemboli is discussed in the above mentioned article. As the distance from the inflow of blood to the blood level in venous chamber is increased, at low blood levels, this leads to more turbulence as the blood stream
reaches the blood level. Air from the venous chamber can be pulled down with the blood. Air bubbles can be formed that can follow the blood flow past the air trap. If the momentum of the blood flow exceeds the bubble buoyancy, which is dependent on the bubble size, such passage is possible.

No correlation between the degree of blood flow and the number of MES were present in this study as well as in paper II. This is in contrast to earlier in-vitro experiments that demonstrated a strong association between fluid flow and the number of microemboli. An explanation for this difference may be that the in-vitro flux studies varied in the set blood flow from 50 to 600 ml/min. The large increase in the number of MES occurred at higher blood flow rates with about 300 ml/min as a breakpoint. In the clinic blood flows around and below 300 ml/min are common. In paper II the mean blood flow was just over 300 ml/min and in paper III it was 324 ml/min. In paper I a weak correlation existed between blood flow and the number of MES; the mean blood flow was approximately 350 ml/min. One explanation for the correlation may be that a higher blood flow also increases the turbulence by the blood stream striking the surface of the blood venous chamber with greater force. Turbulence results in the air above the blood level in venous chamber being sucked into the blood and forming air bubbles.

HD patients have a higher prevalence of pulmonary hypertension compared with patients with peritoneal dialysis or pre-dialysis patients. This is supported by a recently published retrospective observational study that compared chronic HD and peritoneal dialysis patients undergoing transthoracic cardiac echo examination. 42% percent of HD patients and 19% of the PD patients had pulmonary hypertension. Various reasons for these significant differences were discussed. Nitric oxide, produced by endothelial cells, decreases vascular tone and has been proposed as a factor in the occurrence of PAH in HD patients. HD patients with AV access have a lower level of nitric oxide compared with the control group without HD treatment. AV access leads to increased cardiac output, which is believed to reduce nitric oxide production and causes an increase in pulmonary vascular tonus. This does not explain the difference because even PD patients have a decreased production of nitric oxide. Microbubbles from the tubing set or filter are suggested to be a potential etiological explanation. The microbubbles can clog pulmonary capillaries, causing ischemia, inflammatory response, and complement activation. This can lead to permanent vascular changes
and pulmonary hypertension. Randomized, prospective treatment studies are needed to clarify if presence of microbubbles, which arise during HD, increases the risk of lung damage.

In summary, exposure of potentially harmful microemboli is reduced by an easy machine adjustment, i.e. by raising the level of blood in the venous chamber, without additional cost to the health service.

**Paper IV**

During the first three hours of HD treatment the number of MES was measured with a DU device (CMD-10, Hatteland, Røyken, Norway). Measurements were made on the venous side of the tube after the venous chamber. Patients were randomized to three different treatment options, and the results confirmed the previous results from both the *in-vitro* and *in-vivo* studies. If a wet filter and a high blood level of the venous chamber were used, the minimum numbers of MES were detected. If instead a dry stored filter in combination with a low blood level of the venous chamber were used, more MES were detected than if the same filter was used with a high blood level.

That a greater number of microemboli were noted when the dry-stored filter was used instead of the wet-stored filter can be explained by the fact that more air remains in the dry filter in spite of the priming process. That the number of microemboli was significantly more during the 30 first min compared with subsequent 30-min intervals for the dry filters indicate that the filters would be less air containing when the blood flows through the membrane capillaries. Another explanation may be blood-membrane interactions that can lead to emboli. It is well known that the HD process causes the activation of coagulation, complement, and platelets. Blood contact with the dialysis membrane can initiate these processes. Maybe gamma sterilization of wet filter or the wet storage itself affect biocompatibility by inhibiting coagulations and complement activation?

Platelet aggregates and aggregates of white blood cells have been detected in connection with HD. Its effect on the microcirculation is unclear.

In one of our patients, 174,710 MES were measured during a three-hour HD session. Since the number of MES/30 min was constant
during the measurement time there was a continuous supply of emboli. We assumed that the cause was air leakage on the arterial side due to, for example, insufficiently tight coupling.

In this study there was a correlation between the number MES and the degree of blood flow.

In papers I-IV the detected MES that appeared in both the venous and arterial circulation may consist of:

- Microbubbles that pass through the foramen ovale (25-30% of the population\textsuperscript{39}) to the arterial side with a temporary increase in pressure on the venous side (Valsava manoeuvre) or large number of micro air bubbles that are of the same size or smaller than red blood cells passing through the lung capillaries or physiological shunts to the systemic circulation (refer to the section ‘The physiological right-to-left shunt’ in the Introduction).

- Thromboemboli that may develop besides air bubbles due to the blood dialysis membrane interaction and the dialysis process itself that initiates coagulation\textsuperscript{104}. Thromboemboli may also develop due to the presence of air bubbles that themselves activate haemostasis\textsuperscript{163}. Such emboli may also enter the venous circulation and lungs. Eventually they may pass through the pulmonary barrier or physiological shunt to the arterial circulation (refer to the section ‘The physiological right-to-left shunt’ in the Introduction).

**Paper V**

In a deceased chronic HD patient small cavities were revealed, which were not stained by hematoxylin/eosin or influenced by immunohistochemistry. These cavities were probably caused by microbubbles of gas (personal communication T.Brännström). The gas bubbles were detected in the capillaries of the brain, lungs and heart. The gas bubbles were surrounded by fibrin. These findings indicate that the gas bubbles were formed before death and occurred not only in the lungs but also in the systemic circulation.

Additional autopsy data from HD patients were reported by us in a poster presentation\textsuperscript{160}. Five chronic HD patients who died between 10 to 3333 min after HD were included in that study. As a control group
seven patients who died due to amyotrophic lateral sclerosis (ALS) were included. Lung tissue was examined microscopically after staining with fluorescent antibodies against fibrinogen as a marker for the presence of microbubbles before death. Ten fields were viewed microscopically. In all five HD patients microbubbles in lung tissue were found, while only two of seven patients with ALS had such findings (Table 18).

Table 18. Findings (MB) and absence (no-MB) of microbubbles in the lungs of HD-patients and ALS-patients. (Fischers test p=0.0278, RR=3.5, CI 1.08-11.3)

<table>
<thead>
<tr>
<th></th>
<th>MB</th>
<th>no-MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD-patients</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>ALS-patients</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

HD patients had more microbubbles in the lung tissue compared with the ALS patients (Student t-test, p <0.05).

In all five HD patients fibrosis in lung tissue was found, whereas none of the seven patients with ALS had such findings (Table 19).

Table 19. Findings (Fibrosis) and absence (no-Fibrosis) of pulmonary fibrosis in HD-patients and ALS-patients

<table>
<thead>
<tr>
<th></th>
<th>Fibrosis</th>
<th>no-Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD-patients</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>ALS-patients</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

All HD patients showed moderate to extensive pulmonary fibrosis, but none of ALS patients showed such findings. The microbubbles were surrounded by fibrin, supporting the existence of these when the patients were still alive.

This extended study confirmed the presence of gas bubbles in the lung tissue before death in HD patients. There were significantly more microbubbles detected in HD patients compared with ALS patients. However, two ALS patients did have microbubbles in the
lung tissue. Perhaps these patients had had fluid infusions before death, and thus air bubbles entered the blood circulation\textsuperscript{459}.
Clinical perspectives

Patients who needed HD and have no remaining renal function are recommended to undergo at least three treatments four hours a week for adequate cleaning of the blood of uraemic toxins and excess fluid\textsuperscript{170}. This means that patients are connected to a machine in time equivalent to 26 days per year. Just the suspicion that this essential cleaning process can contribute to the multi-morbidity patients suffer justifies efforts to minimize potential risks to patients, according to the precautionary principle; however, further studies of HD treatment effects on the patient are needed.

The proposed measures below can be taken to reduce patients' exposure to microbubbles in association with HD:

- Close connectors carefully to prevent air leakage at the connections sites of the tubes on the arterial side. Choose caps and plastic clamps for heparin lines that are airtight\textsuperscript{91}.

- Do not tap on or turn the dialyzer during dialysis to avoid increasing the number of microbubbles downstream the dialysis circuit\textsuperscript{141}.

- A larger than recommended amount of priming volume is needed, to reduce the amount of microbubbles\textsuperscript{91,141}. The amount of priming volume needed to reduce microbubbles during HD has to be further investigated \textit{in-vivo}.

- A lower blood pump speed, $Q_b < 300$ ml/min, may reduce the numbers of microbubbles in the blood\textsuperscript{91,139,140,158}.

- Using a wet-stored dialyzer will further reduce the amount of microbubbles\textsuperscript{141,158}.

- A high blood level in the venous chamber may help to reduce exposure to microbubbles\textsuperscript{91,140,157,158}. 

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CONCLUSIONS

*In-vivo* studies in haemodialysis patients have shown that microemboli:

1) pass the air trap and are detected on the venous side where the blood is pumped to the patient

2) increase during haemodialysis treatment
   a) at the aterio-venous access
   b) at the carotid artery

3) decrease in number if blood level in the venous chamber is high

4) decrease in number if a wet-stored dialyzer is used
   and that

5) gas bubbles are present at autopsy in both the venous and arterial circulation in a patient treated with haemodialysis
ACKNOWLEDGEMENTS

I would like to express my appreciation and sincere gratitude to all of you who in different ways have contributed to making this thesis possible. I especially wish to express my thanks to:

Umeå university, the Rector of the University, Lena Gustavsson, the Dean of the Medical Faculty, Anders Berg, the Prefect of the Department of Public Health and Clinical Medicine Lars Weinehall, and the head of section of Clinical Medicine, Bo Carlberg for academic support.

My supervisor, Professor Bernd Stegmayr, for your encouragement and for sharing your knowledge. Without your enthusiasm and unlimited support this thesis would not be finished.

My co-supervisor, Per Jonsson, for your patient teaching on scientific and technical matters. For you always have the time to help and give positive guidance.

All the patients who participated in the investigations.

My co-authors for valuable comments and for sharing your expertise: Christofer Stegmayr, Thomas Brännström, Johan Hultdin, Fredrik Jonsson, Kristina Nilsson Ekdahl and Bo Nilsson.

Research nurses Maria Grubbström, Heidi Lindmark and Malin Skagerlind for skilful assistance and for taking good care of the patients.

Professor Kurt Boman och Professor Jan-Håkan Jansson, who taught me as a young doctor the value of science.

All my colleagues at the Department of Medicine and Geriatrics, Skellefteå County Hospital.

All the personnel at the dialysis unit in Skellefteå County Hospital.

Lars Johansson, Jonas Andersson and Marcus Lind for your valuable comments and for sharing your expertise.
Philip Cohen and Thomas Suh, for your skilful proofreading.

All my colleagues at the unit for Nephrology, Department of Internal Medicine and the personnel at the dialysis unit in Norrland University Hospital.

Susanne, my beloved wife, for your selfless support and encouragement.

My children Sara, Samuel and Sanna, who makes me happy.

My brother, sister and relatives for your support.

My parents, who always encouraged me and taught me to love books.

This study was supported by generous grants from the County Council of Västerbotten, Njurföreningen i Västerbotten and the Foundation for Medical Research in Skellefteå.
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APPENDIX

PATIENT INFORMATION

Vid svår njursjukdom klarar inte njurarna av sina uppgifter bla att rena blodet från slaggprodukter. Patienten blir då beroende av regelbunden dialysbehandling.

I samband med hemodialys (bloddialys) används olika utrustningar/materiel (bla dialysfilter, slangar, tillförselvätskor mm.) för att så långt som möjligt individualisera behandlingen för varje enskild patient.

Det saknas dock forskningsdata som tydligt talar om för oss hur vi ska göra detta på allra bästa sätt. Man vet att dialysbehandlingen påverkar kroppen på olika vis med bl.a. trötthet efter dialysen hos vissa patienter.

Alla dialysapparater är konstruerade på ett sådant sätt att luft förhindras att komma in i patienten (luftvakt i slangsystemet).

Dock är det känt att mikroskopiskt små mängder luft kan passera in i patientens blod under dialysbehandling oavsett vilken dialysapparat som används. Det är inte klarlagt om dessa mikroskopiska fynd utgör någon risk för patienten på lång sikt.

Vi önskar undersöka om det går att minska luftmängden som kan upptäckas i dialysfistel/graften genom att göra mindre dialysapparatjusteringar (som är godkända enligt gällande säkerhetsföreskrifter).

Undersökningens utformning:

I samband med sedanlig dialys kommer luftvaktnivån ställas på antingen låg eller hög nivå vid start. Antalet luftbubblor i dialysfistel/graft mäts mha en ultraljudsapparat under ca 2 min. Sedan ställs luftvaktnivån om och nya mätningar görs.

Det finns inga kända biverkningar av ultraljudsundersökningen.

De data som samlas in från denna undersökning förvaras i ett dataregister. I detta register finns patienten enbart noterad med kod (tex Per Eriksson som...
P01). Endast ansvarig läkare samt medarbetare kan sammankoppla koden med patient.

Deltagande i undersökningen är helt frivillig.

Patienten kan när som helst avbryta deltagandet utan att behöva ge någon förklaring.

Beslutet kommer inte heller att påverka fortsatt behandling.

Vid frågor hänvisas till ansvarig läkare Ulf Forsberg tel :0910-771000.

Medarbetare: Bernd Stegmayr (läkare, Umeå), Maria Grubbström (forskningssjuksköterska, Umeå), Per Jonsson (tekniker, Umeå).