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## Lipid Micro Emboli in Cardiac Surgery

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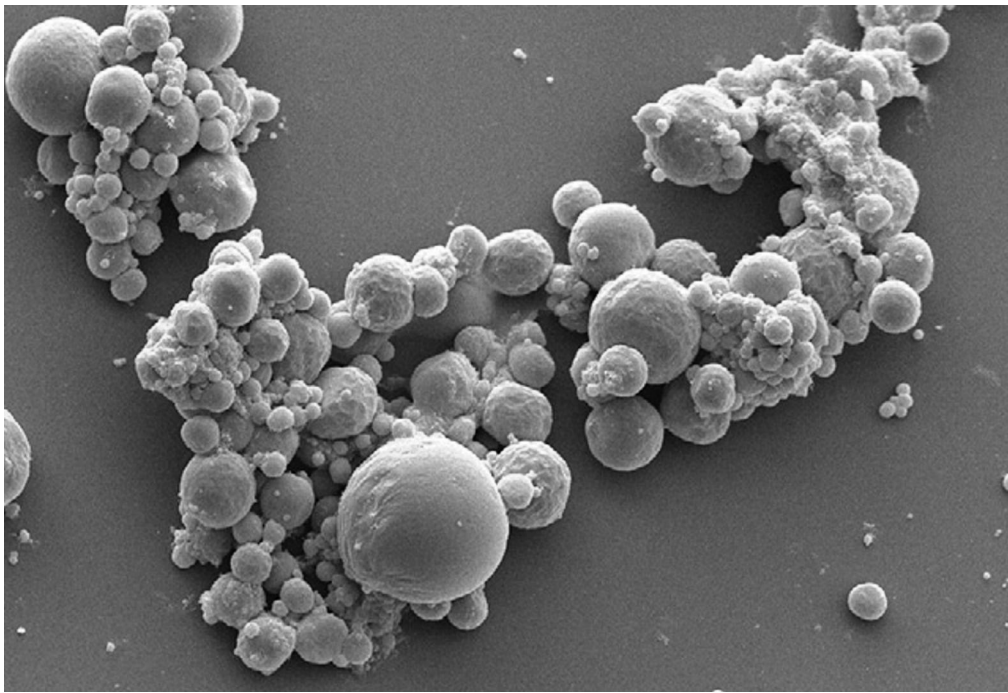
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# Lipid Micro Emboli in Cardiac Surgery

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*Dedicated to Lára*



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# Summary

This thesis is based on five studies. It includes the characterization of lipid micro emboli in shed mediastinal blood during cardiac surgery. The reuse of blood directly by cardiotomy suction is a common practice in order to reduce the need of blood transfusions. In shed mediastinal blood we showed that there is an abundance of lipid particles in the size range of 10-60 $\mu$ m. These lipid particles are also recirculated by the cardiotomy suction, through filters and the plastic tubing in the cardiopulmonary bypass (CPB) circuit. The lipid particles are quite probably in an emulsified state which renders them hydrophilic and less prone to stick to the plastic surfaces of tubing and filters.

With a series of samples from the mediastinal blood and from the arterial circulation we found that the lipid particles are formed during the operation. Probable sites of origin are the bone marrow after sternotomy and the mediastinal fat tissue manipulated during the surgery in order to expose the heart for cannulation. When the blood was collected in a separate transfusion bag and transfused over a short period of time the increase of lipid micro emboli could be detected in the arterial circulation of the CPB circuit. This was measured by using a Coulter counter to analyze and count emboli in a series of blood samples. The Coulter counter was shown in the first study to be a valuable technique to evaluate embolic loads of lipid micro emboli in blood.

The lungs act as physiological filters for the circulation. Embolic material is filtered effectively by the lungs. Using this filtering property of the lungs could therefore be an option in retransfusing the lipid laden mediastinal blood gathered during cardiac surgery. Using a pig model and a shed blood phantom laden with radioactive triolein as embolic material, we registered the hemodynamic results of this during CPB. Furthermore we registered the embolic load in the circulation by measuring beta radioactivity in a series of blood samples. We found that the lungs do act to trap a great deal of these lipid particles but these are then to a certain extent recirculated again. This was especially evident when the cardiac output and pressure were increased by a bolus of volume and vasoconstrictors.

Lipid emboli find their way into the circulation during cardiac surgery, where they can be detected and counted by the Coulter counter. When simultaneously registering micro embolic signals from a transcranial Doppler and counting lipid micro emboli in blood samples taken from the arterial line, we found a strong correlation between the two phenomena. Lipid material was also tagged by beta radioactivity and found to end up in different organs. By comparing two shed blood phantoms, one with non-emulsified lipids and the other with emulsified lipids we found that the lipid embolic matter increases in the circulation and is more easily deposited in the tissue.

The overall purpose was to show that lipid micro emboli are released into the shed mediastinal blood during cardiac surgery. When this blood is retransfused, it is the source of a bombardment of lipid micro emboli in the circulation. These emboli are lodged in various organs. Lipid emboli in the emulsified state are more likely to pass into the circulation during retransfusion of shed mediastinal blood. These emboli can be detected by a transcranial Doppler in the arterial circulation of patients undergoing cardiac surgery.

# Populärvetenskaplig sammanfattning

Alltmer komplicerade hjärtoperationer kan göras med hjälp av hjärt-lungmaskin. Under tiden hjärtat behöver stå stilla kan maskinen syresätta och cirkulera patientens blod genom kroppen. Det är vanligt att återanvända sårblodet som samlas runt hjärtat under operationen genom att det sugts upp och förs tillbaka till hjärt-lungmaskinen genom enkla filter. Detta kan också vara en livräddande åtgärd om större tillfälliga blödningar uppstår under operationen.

På senare tid har det uppmärksammas att sårblodet innehåller små partiklar av fett som kan ta sig genom filtersystemet och hamna i patientens blodomlopp. Detta är en av möjliga orsaker till skador som kan uppstå under en hjärtoperation.

Med närmare analys av blodprov från det uppsamlade blodet innan det återförs till hjärt-lungmaskinen har vi funnit att det finns en enorm mängd fett partiklar. Dessa är av små storlekar men tillräckligt stora för att kunna fastna i kroppens minsta kärl och eventuellt orsaka skada i flera olika organ.

Med analys av blodprov från patienternas cirkulation strax efter att det uppsamlade sårblodet återförs, kan vi åter hitta dessa partiklar och se hur de finns nu i blodomloppet. Genom djurförsök har vi återfört blod med fettpartiklar till lungkretsloppet för att eventuellt lungorna skulle kunna fånga upp partiklarna och på en naturlig väg göra sig av med dessa innan de cirkulerade vidare till övriga kroppen. Detta visar sig kunna ha drastiska konsekvenser, med bl.a. nedsättning av hjärtats funktion. Dessutom hittar partiklarna sin väg genom lungkärlen och dyker upp i övriga blodomloppet och kan där hamna i övriga organ med möjliga skadliga följder.

När väl sårblod som samlas under en operation tillförs genom filter, finner vi fettpartiklar i blodomloppet med analys av blodprover, men dessutom kan vi upptäcka dessa med transcraniell Doppler, en ultraljudsteknik som

riktar sig till att upptäcka embolier i hjärnans kärl. Det finns alltså goda grunder att anta att det som finns i blodomloppet också hittar vägen till hjärnan. När vi tillför fettpartiklar i blodblandning som skall likna sårblodet kan vi se att genom att göra fettpartiklarna mer vattenlösliga hittar dessa partiklar ännu lättare in i blodomloppet och kan hamna i en större utsträckning i många olika organ i kroppen. Möjligheten till skada ökar med större mängd fettpartiklar som hamnar i cirkulationen.

# Original Papers

## Study I

Eyolfsson A, Scicluna S, Johnsson P, Petersson F, Jönsson H. Characterization of lipid particles in shed mediastinal blood. *Ann Thorac Surg.* 2008 Mar;85(3):978-81

## Study II

Jönsson H, Eyolfsson A, Scicluna S, Paulsson P, Johnsson P. Circulating particles during cardiac surgery, *Interact Cardiovasc Thorac Surg.* 2009 May;8(5):538-42

## Study III

Eyolfsson A, Plaza I, Brondén B, Johnsson P, Dencker M, Bjursten H. Cardiorespiratory effects of venous lipid micro embolization in an experimental model of mediastinal shed blood reinfusion. *J Cardiothorac Surg* 2009;4:48

## Study IV

Eyolfsson A, Al-Rashidi F, Dencker M, Scicluna S, Brondén F, Koul B, Bjursten H. Comparison between transcranial Doppler and Coulter counter for detection of lipid micro embolization from mediastinal shed blood reinfusion during cardiac surgery. *Perfusion* published online early 15 August 2011, DOI: 10.1177/0267659111419033

## Study V



Eyjolfsson A, Dencker M, Brondén B, Scicluna S, Johnsson P, Bjursten H.  
Lipid emboli distribution in cardiac surgery is dependent on the state of  
emulsification. Submitted for publication.

# Abbreviations

AUC	area under the curve
BMI	body mass index
CABG	coronary artery bypass grafting
CBC	complete blood count
CCS	Canadian Cardiovascular Society
CI	confidence interval
CPB	cardiopulmonary bypass
DPM	disintegrations per minute
F	French (1F =0.33mm)
LIMA	left internal mammary artery
MCA	middle cerebral artery
mCi	milliCurie
MES	micro embolic signal
NYHA	New York Heart Association
OPCAB	off pump coronary artery bypass
OsO <sub>4</sub>	osmium tetroxide
PVR	pulmonary vascular resistance
SCAD	small capillary arteriolar dilatations
SD	standard deviation
SEM	scanning electron microscopy
SEM	standard error of the mean
SVR	systemic vascular resistance
TCD	transcranial Doppler

$V_d/V_t$  ratio of dead space to tidal volume

# Introduction:

In cardiac surgery with cardiopulmonary bypass (CPB), retransfusion of shed mediastinal blood is common practice to minimize blood loss(1) .The rationale for intraoperative salvage of blood is to reduce the need for allogenic blood transfusions (2). In addition to being costly (3), allogenic blood transfusions have been shown to increase the risk of infection(4) and to have a negative effect on the long term survival after cardiac surgery (5-8). The reuse of salvaged blood from the mediastinum by cardiotomy suction has however been questioned because of its potentially severe negative effects. Not only is there a systemic inflammatory response to the reuse of cardiotomy suction blood (9-11), but also it is contaminated with embolic material (12, 13). It has been shown that this blood is contaminated with lipids, which may act as emboli (14). Furthermore, these particles pass through the CPB circuit and find their way into various organs (15). Even with the use of various cell saving devices, there are high levels of inflammatory markers and lipid micro emboli in the processed mediastinal blood(16-18).

In earlier studies the focus has largely been on the potential threat of lipid micro emboli on the brain and the neurological outcome (12, 14, 19-23). Recent studies however have shown that lipid emboli travel to various organ systems of the body and can potentially inflict damage or disturb their function (24). The main purpose of this thesis is to characterize these micro emboli, to establish their sizes and number and to take a closer look at the route in the circulation. It is also to evaluate the effect of transfusion of lipid laden blood to the venous side of the circulation. In addition, we wanted to see if these emboli could be detected noninvasively. In all, the focus is to build a case for eliminating the potential threat of contaminated mediastinal blood during cardiac surgery.

## Use of cardiotomy suction blood

The reuse of mediastinal blood with cardiotomy suction is more or less mandatory in open cardiac surgery (1). Not only does this enable the

surgeon to visualize the field but saves the shed blood from being discarded and thereby avoiding costly and potentially dangerous blood transfusions of donor blood (25, 26). In the case of profuse bleeding the use of cardiomy suction can also be lifesaving whereas the blood can be immediately retransfused through the CPB circuit (27).

Filters in the cardiomy suction are used to decrease the risk of solid emboli making their way into the circulation . More recently, the issue of embolisation of lipid particles has come into focus, and these filters can only partially remove the lipid micro emboli (28-30). Several authors have shown that microembolisation is a potential threat during cardiac surgery, and will influence the outcome (31-33).

Previous studies in orthopedic trauma have shown that lipid embolisation is a known cause of tissue damage, where both neurological, renal and pulmonary damage can cause fatal outcomes (34-36).

Opening of the mediastinum during cardiac surgery involves trauma. The sternum is opened with an oscillating saw and thereby exposing the marrow. Bone marrow is relatively rich in fat and consists of 40% fat in the adult (37). With electorcautery the subcutaneous and mediastinal fat is exposed. Both bone marrow and adipocytes in the wound are a probable source of lipid which can be seen in the shed mediastinal blood at surgery. Small and large fat droplets can be observed in the pool of blood collecting in the surgical field (15). It is this contaminated blood that is common practice to collect and reuse during surgery by means of cardiomy suction.



**Figure 1. Surface of shed blood from the surgical field where lipid droplets are easily seen.**

## The lungs acting as filters

Fat embolism syndrome is a dire complication of long bone trauma (34). It is usually associated with neurological, hematological and respiratory involvement and can be fatal. The mechanism in which these emboli travel into the systemic circulation is usually considered to be by direct contact with circulating arterial blood. However pulmonary contusion and lung damage is also an important mechanism for fat emboli to pass through the lungs (38). The lungs can serve as a filter for debris and emboli up to a certain extent, but will eventually release smaller emboli through to the systemic circulation (39).

In cardiac surgery with cardiotomy suction it is known that lipid micro emboli find their way into the capillaries of the brain. The evidence of this is found in the brain of patients post mortem (40). This has been corroborated in animal studies where lipid laden blood transfusions result in cerebral emboli as well as embolisation of other organs (15).

Little is known about the size, nature and number of the potentially dangerous lipid micro emboli in cardiac surgery. The filters used in the CPB circuit are not effective in eliminating these micro lipid particles in the size ranges of 10 - 60  $\mu\text{m}$  (30, 41). This results in a bombardment of tissue by these emboli. It is therefore highly doubtful that the lungs will effectively serve as filters of lipid micro emboli without the risk of systemic embolisation and direct damage to the lungs themselves (42).

## Profiling lipid micro emboli

Early studies have been able to photograph emboli in the retinal vessels after CPB (12, 43). Later studies showed lipid emboli in the microcirculation of the brain. These were defined as small capillary and arteriolar dilatations (SCADs) found in the brain of subjects that had earlier undergone cardiac surgery with CPB (21, 40).

Characterization of these emboli is an important factor in further reducing the risk of complications for patients undergoing cardiac surgery. The size and number of lipid particles being circulated and their physical properties are key factors in developing methods and strategies aiming to minimize the embolic load in cardiac surgery (28, 44).

## Coulter Counter

The Coulter Counter is a vital tool in both clinical and research laboratories. Its primary function is the quick and accurate analysis of complete blood counts (CBC). The CBC is used to determine the number or proportion of white and red blood cells in the body. The Coulter counter is also routinely employed for quality control in laboratory medicine and industry (45, 46). The use of the Coulter Counter analysis of blood during cardiac surgery is a novel method. The method is well established in the accurate determination of particles such as microspheres, blood cells, bacteria and cells from cell cultures. It is able to count tens of thousands of particle in each sample quickly and accurately. Moreover the device determines the volume of each counted particle (45, 46).

## Transcranial Doppler (TCD)

To non-invasively monitor intracranial circulation by TCD is not new. Aaslid and co-workers first described intracranial detection and measurement of blood flow and flow velocity in 1982 (47). This technique has then been used for a variety of different purposes as for example monitoring of intracranial vessel recanalization during stroke (48), monitoring of intracranial cerebral hemodynamics after subarachnoid hemorrhage (49), in patients with increased intracranial pressure (50) or for evaluation of brain death (51). TCD has also been used to detect right-to-left shunts in the presence of patent foramen ovale in stroke patients (52), and to detect micro emboli (53-57). The micro emboli detection has, however, up until now mainly been restricted to detection of air or atherosclerotic plaque debris embolus.

## Radiation detection by Scintillation

The principle for liquid scintillation used in the studies in this thesis is a standard protocol, where a radioactive material is mixed with a solvent and scintillation fluid.

Radioactive tissue and blood samples were dissolved and decolorized. The radioactive substance bound to the lipid disintegrates and can be detected by the beta particles being emitted and colliding with the solvent used.

This in turn will be transferred to the scintillator molecules in the solution to be recorded as light photons. The light photon is then detected by a photo multiplication device. This device converts the light from every photon to a detectable electrical signal, which is also amplified for detection and quantification. Different radiation signals for different samples can thus be detected (58).

## State of emulsification of lipids

The state of emulsification of lipids is of paramount importance in how the lipids interact with other materials and surfaces. Emulsified lipids are hydrophilic and easily soluble in water, whereas non-emulsified lipids will not solve in water because of their hydrophobic/lipophilic properties.

Cardiotomy suction blood collected from the surgical field during cardiac surgery with CPB has been shown to be contaminated by lipid material. This lipid material can form emboli in several organs. Lipids normally appear in the emulsified state in the circulation (59). This is best exemplified in the transfer of micelles from the intestines into the blood via lymphatic vessels. Even though micelles are of size ranges far smaller than those representing lipid emboli in cardiac surgery suction blood, the same physical properties render these particles emulsified. Electron microscopy of shed mediastinal blood shows typical spherical forms of lipids in state of emulsion. There is an abundance of agents normally found in blood which can act as emulsifiers, and render these lipid particles hydrophilic.





# Aims of the studies

I

To characterize the lipid particles in shed mediastinal blood, by determining the size and number of particles found in shed mediastinal blood during cardiac surgery. In addition, to determine the microscopical appearance and the triglyceride content

II

To determine the occurrence of lipid micro embolic material at several sampling locations and events during cardiac surgery, and to follow the lipids in the circulation

III

To study the effect of retransfusion of blood containing lipid micro emboli on the venous side of the circulation with a special focus on hemodynamic and respiratory effects together with the embolic removal capacity of the lungs.

IV

To evaluate if transcranial Doppler has the capacity to noninvasively detect lipid micro emboli during cardiac surgery.

V

To evaluate differences in the kinetics and deposition between emulsified and non-emulsified lipid embolisation during cardiopulmonary bypass.



# Material and Methods

## Study design.

### Study I

After approval by the local ethics committee and written information, 24 patients undergoing routine elective cardiac surgery with CPB were included in the study. After anesthesia and median sternotomy all patients were cannulated with a standard 22 F aortic cannula in the ascending aorta and a two stage venous cannula in the right atrium. In each case, 10 ml of shed mediastinal blood was collected from the pericardium after the administration of heparin and after aortic cannulation, but prior to the initiation of CPB. At the same time, 10 ml of the patient's arterial blood was drawn from a peripheral arterial line. An additional 5 ml of shed mediastinal blood and a sample of mediastinal adipose tissue were taken from the first nine patients for comparative analysis by gas chromatography. Coulter counter analysis was performed immediately, while the samples taken for gas chromatography were frozen for later analysis. Complete Coulter counter data was achieved in 16 patients in both centrifuged and non-centrifuged series. The non centrifuged blood was used in a subtraction analysis by arterial blood to collaborate the embolic load in the collected mediastinal blood. Supernatant of the mediastinal blood was also studied with Scanning Electron Microscopy to further enhance the character of the lipid material found.

### Study II

After approval of the local ethics committee and informed consent, 44 patients undergoing routine elective coronary artery bypass grafting (CABG) with CPB were included in the study. After anesthesia and median sternotomy, and after heparin was administered a background sample of arterial blood was drawn from the peripheral arterial line. Surgery was performed in a standardized manner, where the left internal mammary

artery (LIMA) is routinely harvested before the pericardium is opened. Heparin was then administered and the LIMA divided. All cardiotomy suction blood from the mediastinum was collected in a transfusion bag separate from the CPB circuit. Blood samples were collected at different times during the operation from the surgical field. A sample of pericardial fluid was collected immediately after the pericardium was opened. Samples of blood from the pericardium were then collected after cannulation of the aorta and right atrium, and then after removal of the aortic cross clamp. A sample was gathered after removal of the venous and arterial cannula but prior to administration of protamine for reversal of the heparin effect. In addition, a sample of the blood that had gathered in the pleural space was collected.

At the time the aortic cross clamp was released the collected blood was measured and retransfused into the cardiotomy reservoir of the CPB circuit as quickly as possible, using a quick-prime set. Blood samples were collected before the infusion from the CPB circuit and then at 30, 60, 120, 150, 180, 300 and 600 sec. All samples were analyzed with a Coulter Counter immediately after the final sample was drawn.

### Study III

After approval of the regional animal study ethics committee, 7 adult pigs were studied. The animals, which weighed roughly 70kg, were anesthetized and mechanically ventilated. All animals were hemodynamically monitored and fitted with catheters in the internal jugular vein, the left and the right atrium, the pulmonary artery and the femoral and carotid arteries. The animals were heparinized and a median sternotomy performed. During the experiment, arterial and pulmonary blood pressures, central venous and left atrial pressures, pulse, nasopharyngeal temperature, ventilator settings and puls-oximetry were monitored continuously. Cardiac output was measured by a flow probe on the pulmonary artery.

A resting period of 10 minutes was observed after the animals were hemodynamically stable.

After this period, a shed blood surrogate containing radioactive lipid micro emboli was infused on the venous side. Blood sampling continued for 3 hours from a separate arterial line for the detection of radioactivity representing the micro emboli infused. After this the animals were given a bolus dose of Ringers lactate and epinephrine to increase pressures and

cardiac output. Blood sampling and monitoring was then continued for one more hour before the animals were sacrificed.

The blood samples were all measured for radioactivity. The samples were decolorized and the level of beta radioactivity determined by liquid scintillation.

#### Study IV

After approval of the local ethics committee and written informed consent, 15 patients undergoing CABG with CPB were included in the study. After heparin was administered all blood from the operating field was collected in a transfusion bag separate from the CPB circuit. The collected blood was then retransfused into the reservoir of the heart lung machine as quickly as possible. Blood samples were taken from the peripheral arterial line starting at the time of retransfusion. Samples were collected at 0, 30, 60, 90, 120, 150, 180, 240 and 300 seconds after retransfusion. Blood samples were analyzed by a Coulter Counter.

Transcranial Doppler (TCD) was fitted transtemporally by a head brace to continuously monitor the cerebral artery bilaterally. The detection level was for micro embolic signals (MESs). Signals above 20dB were not counted to avoid counting gaseous emboli (60). Embolic counts were performed off-line after surgery using the continuous data gathered two minutes after the start of retransfusion.

#### Study V

After approval from the regional animal study ethics committee, twelve adult pigs were included in the study. Six pigs were first exposed to the nonemulsified lipid emboli and later the second series of six pigs were exposed to the emulsified lipid emboli.

After premedication and anesthesia the pigs were supported on a ventilator. Heparin was given in a full dose before cannulation of the right atrium and ascending aorta. All animals underwent a standardized perfusion for 40 minutes with approximately 2.0 l/m<sup>2</sup>/min and hemodynamic stability.

Two different types of lipid laden solutions were given to the two groups of animals. The first six animals received a non emulsified shed blood phantom. This solution was prepared with 5ml of radioactive triolein

gently mixed with 200ml of the animals own blood and 200ml of saline. The second group of animals received an emulsified shed blood phantom which was prepared the same way. In this latter solution only, we added an emulsifier to the 5ml of radioactive triolein and mixing the two in a mixer for one minute. After having produced this emulsified triolein, a mixture of 200ml of blood and 200 ml of saline were added and gently agitated. Both shed blood phantoms were infused at room temperature into the cardiotomy reservoir of the heart lung machine after 20 minutes of CPB.

Blood samples were gathered in both groups. Each sample was 0.2ml of blood drawn from the internal jugular vein catheter. The first sample was drawn at the start of CPB, and then at 0,20,40,60,80,100,120 seconds, 3,4,5,6,7,8,9,10,15 and 20 minutes after the start of infusion of the shed blood phantom. After the last blood sample was drawn, the animal was sacrificed. The blood samples were then gathered for later analysis for radioactivity with liquid scintillation.

After sacrificing the animals, tissue samples were taken from the white matter of the cerebrum, gray matter of the cerebrum, brainstem, hippocampus, cerebellum, heart, left lung, liver, cortex of one kidney, spleen small intestine and skeletal muscle. Four different tissue samples of 100-200 mg were dissected from each investigated organ.

The sample preparation for all samples was done by dissolving the tissue cells and then decolorize. Liquid scintillation was done to determine the amount of radiation in each sample. Two separate measurements were performed for every sample, and the mean value was used in the calculations.

## Patients in the clinical studies (study I, II and IV)

All patients included in the studies (I,II and IV) were recruited from the Dept. of Cardiothoracic Surgery at Lund University hospital. All patients underwent routine cardiac surgery with CPB.

In study I, 24 patients undergoing routine cardiac surgery were included.

In study II, 44 patients undergoing only routine elective coronary artery bypass graft surgery with CPB were included.

In study IV, 15 patients undergoing routine elective CABG surgery with CPB were included, and in this study, two patients had to be excluded because of the inability to obtain adequate TCD data.

## Animals in the laboratory studies (study III and V)

All animal laboratory studies were performed at the Igelösa Life Science laboratories outside of Lund.

In study III, 7 pigs were included in the study. One animal was excluded because of incomplete data when the animal died early in the experimental process.

In Study V, 12 adult pigs were included and complete data was collected from all of the subjects.

## Anesthesia in the clinical studies

All patients underwent routine cardiac surgery. Anesthesia was induced by fentanyl (Fentanyl; B. Braun Melzungen AG, Melzungen, Germany), midazolam (Dormicum; F. Hoffmann-La Roche Ltd., Basel, Switzerland), propofol (Propofol-Lipuro; B. Braun Melzungen AG) and suxamethonium (Celocurin; Ipex Medical AB, Solna, Sweden). Anesthesia was maintained by propofol, fentanyl and vecuronium bromide (Norcuron; Schering-Plough AB, Stockholm, Sweden). In a few cases, isoflurane (Foren; Abbott Laboratories, Chicago, IL) was added. All this was done according to the standard protocol for anesthesia of cardiac surgery patients. Inotropic drugs were used at the discretion of the anesthesiologist in charge. The primary inotropic drug used was: Dobutamine (Dobutamine; Hospira Inc., Lake Forest, IL) and norepinephrine (Noradrenalin APL; Apoteket AB Stockholm, Sweden) was used for vasoconstriction.



## Anesthesia in the laboratory studies

Premedication was performed with an intramuscular injection of 15 mg/kg ketamine chloride (Ketalar®, Pfizer Inc., New York, NY) and 0.2 mg/kg xylazine (Rompun®, Bayer, Göteborg, Sweden). Induction of anesthesia was performed with an intravenous injection of thiopental sodium (Pentothal®, Abbot, North Chicago, IL) 10 mg/kg and atropine (Atropin®, Kabi Pharmacia, Uppsala, Sweden) 0.02 mg/kg. Maintenance of anesthesia was performed using an infusion of 0.15 mg/kg/min ketamine chloride and 0.01 mg/kg/min pancuroniumbromide (Pavulon®, N.V. Organon, Oss, the Netherlands), or an infusion of  $0.1 \pm 0.2$  mg/kg/min propofol (Diprivan®, Astra-Zeneca, Södertälje, Sweden) together with intermittent intravenous injections of fentanyl (Leptanal®, Lilly, France) 0.02 mg/kg and atracriumbesylate (Tracrium®, Glaxo, Täby, Sweden)  $0.2 \pm 0.5$  mg/kg. The animals underwent tracheotomy, and an endotracheal tube was inserted after the animals had received an intravenous injection of succinylcholine (Celocurin®, Ipex, Solna, Sweden) to obtain muscle relaxation.

## Cardiac surgery in the studies

The patients in study I,II and IV underwent routine cardiac surgery with CPB. After anesthesia median sternotomy was done. When the internal mammary artery was harvested, it was done before the pericardium was opened. Heparin was administered and the patients were cannulated with a cannula in the ascending aorta for arterial return of blood, and in the right atrium to the vena cava inferior for draining blood into the CPB circuit.

CABG surgery was done with CPB and aortic cross clamping. Distal anastomoses were performed with the heart arrested by cardioplegia normally administered into the aortic root. Cardioplegia was either hyperkalemic crystalloid cold cardioplegia or cold blood cardioplegia. The cross clamp was removed before the proximal anastomosis were completed with the help of a tangential occluder on the ascending aorta. When needed the patients rhythm was then converted to a sinus rhythm with an electric shock of 10 joule directly on the myocardium. The patients were then weaned from CPB, and supported with inotropic or vasoconstrictive drugs for stable hemodynamic conditions if needed.

# Preparation and administration of radioactive shed blood phantom

In the laboratory studies (III and V) radioactive shed blood phantoms were used.

## Study III

Radioactive triolein (Amersham BioSciences, Little Chalfont, UK) was mixed with 65% non-radioactive triolein solution (Carl Roth GmbH., Karlsruhe, Germany). The proportions used were such that 5 ml of the final solution contained 1 mCi of radioactivity. A Shed blood surrogate was then produced by mixing 200ml of the arterial blood with 200ml saline and 5ml of the 1 mCi radioactive triolein solution. This yielded approximately 1% lipid content. The surrogate was then gently agitated for five minutes and then retransfused from a pressurized transfusion bag into the central venous line.

## Study V

A solution of radioactive triolein was achieved by mixing a 65% non-radioactive triolein solution (Carl Roth GmbH., Karlsruhe, Germany) with radioactive tritium-labeled triolein (Amersham BioSciences, Buckinghamshire, UK). The proportions used were such that 5 ml of the final solution should contain 1 mCi of radioactivity. In order to mimic shed blood in the surgical field, which is normally diluted, shed blood phantoms were constructed. A non-emulsified shed blood phantom was constructed by mixing 200 ml blood from the cardiotomy reservoir with 200 ml saline and 5 ml radioactive triolein solution, and was then gently agitated for approximately one minute. The emulsified shed blood phantom was prepared in the same way except an emulsifier (0.2g Pluronic F127, Sigma-Aldrich, USA) was added to the triolein solution before the blood was added. Emulsification was obtained by firstly mixing the triolein and emulsifier solution in a blender for one minute (61). The ratio between emulsifier and lipids was controlled to obtain lipid micro particles in the size range between 1-60 microns to emulate the lipid particles found in shed blood (62). The second phantom was constructed by gently agitating 200 ml blood from the cardiotomy reservoir with 200 ml saline and the emulsified lipids for one minute. Both shed blood phantoms were infused

at room temperature into the cardiotomy reservoir of the heart-lung machine after 20 minutes of bypass. It was allowed to flow into the reservoir as quickly as possible. In all animals the shed blood phantom reached the reservoir to the full extent within one minute.

## Use of triolein as lipid micro emboli

To mimic mediastinal shed blood in the laboratory studies (III and V), triolein was used to represent the lipid content that has shown to become lipid micro emboli in cardiac surgery. Triolein has been extensively used to study lipid embolization of the lung and brain (63-67). Triolein consists of three chains of oleic acid, and almost 50% of human adipose tissue is composed of oleic acid (68).

## Coulter counter analysis in the clinical studies(I, II and IV)

A Multisizer™ 3, Beckman Coulter Counter® with a 100 µm aperture probe (Beckman Coulter Inc., Fullerton, CA, USA) was used for particle size determination (45). The narrow aperture between the electrodes constitutes the sensing zone through which suspended particles pass. In the sensing zone each particle displaces its own volume of electrolyte. The volume displaced is recorded as a voltage pulse; the height of each pulse being proportional to the volume of the particle. The device was programmed to count and determine the size of all particles with diameters between 2 and 60 µm, at 0.2 µm intervals.

In study I, all blood samples were divided into two separate aliquots of 7 and 3 ml. The 7 ml aliquot was centrifuged at 4200 rpm for 45 minutes to separate lipid material from blood cells. Twenty µl of the supernatant was diluted with 100 ml saline, and was used for analysis in the Coulter counter. In the protocol used, particles were counted for 100 seconds. From the 3 ml aliquot of non-centrifuged blood, 1 µl of blood was diluted with 100 ml saline. A fixed volume of 10 ml was then analyzed in the Coulter counter.

In study II, all blood samples and pericardial fluid were centrifuged at 4200 rpm for 45 min to separate blood cells and lighter particles (e.g. lipid particles). The supernatant was collected and stirred, and 400 µl of this plasma was diluted with 100 ml saline for analysis in the Coulter counter. A Multisizer 3, Beckman Coulter Counter with a 100-mm aperture probe (Beckman Coulter Inc, Fullerton, CA, USA) was used for particle size determination. In the protocol used, 2 ml of the diluted specimen was analyzed at room temperature with a setting for counting particles between 2 and 60 µm, at 0.2 µm intervals.

In Study IV the blood samples were centrifuged at 4,200 rpm for 15 minutes to separate lipid material from blood cells. Four hundred µl from the supernatant was diluted with 100 ml of saline solution, and was used for analysis in the Coulter counter. In the protocol used, 2 ml of the final solution was counted.

## Scanning Electron Microscopy (SEM) in study I

Supernatant from shed blood was mixed with a 1% solution of osmium tetroxide ( $\text{OsO}_4$ ), (Link Nordic, Stockholm, Sweden), which was solved in 0.1 M Sörensen buffer at 7.2 pH. It was placed on a SuperFrost® glass slide (Menzel-Gläser, Braunschweig, Germany) to allow for sedimentation. The samples were then dried and transferred to buttons for SEM. The samples were covered with 15 nm layer of gold in a Polaron E5150 SEM coating unit (Quorum Technologies Ltd, East Sussex, UK). SEM was performed with a Philips 515 scanning electron microscope (Philips, Amsterdam, the Netherlands).

## Gas chromatography study (I)

Gas chromatography was performed on mediastinal adipose tissue and the supernatant of shed blood from the first nine patients. The samples were prepared in a three-step process including extraction, lipid fractioning, and trans-esterification (69, 70). Neutral lipids and phospholipids were then analyzed separately using a Hewlett-Packard HP-5 column in a Hewlett-

Packard 6890 gas chromatograph (both supplied by Agilent Technologies Sweden AB, Kista Sweden).

## Analysis by Liquid scintillation in the laboratory studies (III and V)

In study III and V each blood sample was 0.2ml. Sample preparation was done by adding to each sample of blood, 2 ml Solutene-350® (Packard Bioscience, Groningen, the Netherlands) to dissolve the cells. The sample was left in an air heater at 37°C overnight. To decolorize the samples, 0.2 ml hydrogen peroxide was added twice, with overnight incubation at 37°C between. One ml 95% ethanol was added, followed by 15 ml scintillation fluid (Hionic Fluor, Packard Bioscience, Groningen, the Netherlands) (71). The samples were then left to rest for 4-6 days in order for the chemoluminescence to decrease.

In study V, the samples were blood samples and tissue samples. The same procedure was used for all samples. To each sample, 2 ml Solutene 350 (Packard Bioscience, Groningen, Netherlands) was added to dissolve either tissue cells or blood. The samples were placed in an air heater at 37°C for 24 hours. When the tissue samples had dissolved, 0.2 ml hydrogen peroxide was added to decolorize the samples, and they were then placed in the air heater at 60°C for 30 minutes. An additional 0.2 ml hydrogen peroxide was added. Fifteen milliliters of scintillation fluid (Hionic Fluor; Packard Bioscience) was added, and the samples were then left to stand for 4 to 6 days to allow the chemoluminescence to decrease (71).

In both studies the beta radiation from the tritium-labeled triolein was used as a marker for triolein content. The level of radioactivity (beta radiation) was measured by scintillation counting, using a liquid scintillation counter (14814 Win Spectral Guardian; Wallac Oy, Turku, Finland). The specific activity of tritium was calculated for each sample. Two separate measurements were performed for every sample, and the mean value was used. For each tissue the mean value for the four samples was reported. Radioactivity is reported as the number of disintegrations per minute per gram sample (DPM/g) for tissue, and (DPM/ml) for blood.

## Analysis by Transcranial Doppler (IV)

Transcranial Doppler was performed and the bilateral cerebral artery was monitored continuously using multifrequency TCD scanning (Doppler box; DWL, Singen, Germany). The probe was fixed transtemporally by a head brace and all hits were counted on-line automatically. The detection level for micro-embolic signal was defined as an increase in power of more than 10 dB above background level and an embolus/blood ratio that lasted 4 ms or longer simultaneously in both 2.0 and 2.25 MHz frequency channels. The insonation and reference gate depths were between 50 and 60 mm, ample volume was 10mm, filter setting was 150Hz, power was 180mW and gain was 10. The multifrequency Doppler has a sensitivity of 98.6% and a specificity of 97.2% for detection of emboli versus artifacts (72). In this study, micro emboli signals above 20dB were not counted to avoid counting any gaseous emboli (60). Emboli counts were performed after surgery using the software program included in the system. Background noise was measured two minutes before the infusion of the collected blood.

## Statistical analysis

### Study I

This is a descriptive analysis focusing on the characterization of lipid micro emboli. Triglyceride profile of mediastinal adipose tissue were normalized to Palmitic acid to yield relative concentrations for easier comparison.

### Study II

All data were presented as the mean  $\pm$  one standard deviation (SD) or as a  $\pm$  95% confidence interval (CI) was used. A Student's t-test with homoscedastic variance was used for group comparison. Univariate analysis of the difference between the number of particles at the different location points during surgery and the response measured as the AUC.

### Study III

All values are expressed as the mean  $\pm$  one SD. Comparisons between groups were made with a two-tailed Students t-test. To quantify the physiological response in terms of a decrease or increase in blood pressure, the area under the curve (AUC) was calculated from the period of blood pressure change. The end of the change was defined as the point of time when the blood pressure had returned to the pre event value.

### Study IV

Data are presented as means  $\pm$  one SD. Spearman rank order correlation was used to evaluate the relationship between micro embolic signals detected by TCD and particle characterization by the Coulter counter, and a paired t-test to compare MES before and after retransfusion of gathered blood. A P-value less than 0.05 was considered significant.

### Study V

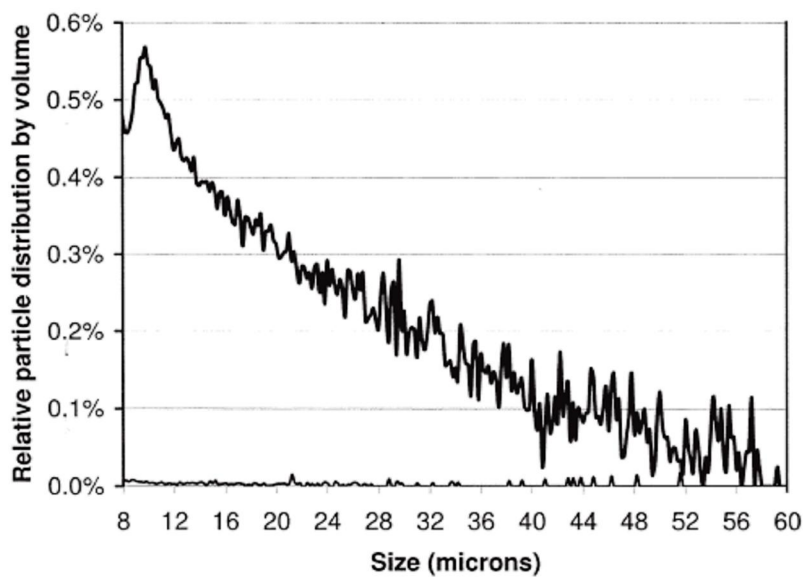
Data are presented as means  $\pm$  one SD. In both groups, the area under curve (AUC) was calculated from the start of infusion to 20 minutes after the start of infusion. Background radiation (determined as the mean of the pre-infusion sample and the sample taken when infusion was started) was subtracted from the levels used for the calculation of the AUC. Non-parametric test (Mann-Whitney U Test) was used to compare differences between the two groups in radioactive deposition in blood and different tissues. A p-value less than 0.05 was considered significant.

All statistical analyses were carried out using Statistica ver. 7.1 to 9 (StatSoft Inc, Tulsa, OK, USA).

# Results

## Study I

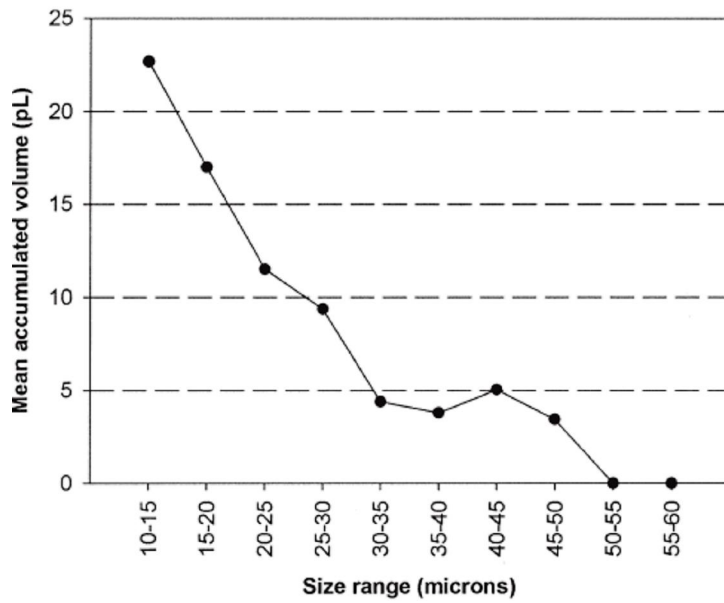
Coulter counter analysis of centrifuged shed mediastinal blood showed much higher levels of particles in the range of 10 to 60  $\mu\text{m}$  than in centrifuged arterial line blood (Figure 2). The highest number of particles had sizes around 10  $\mu\text{m}$  and the amount decreased steadily to 60  $\mu\text{m}$ .



**Figure 2. Relative size distribution of particles found in the supernatant (after centrifugation) of arterial (lower curve) and shed mediastinal blood (upper curve).**

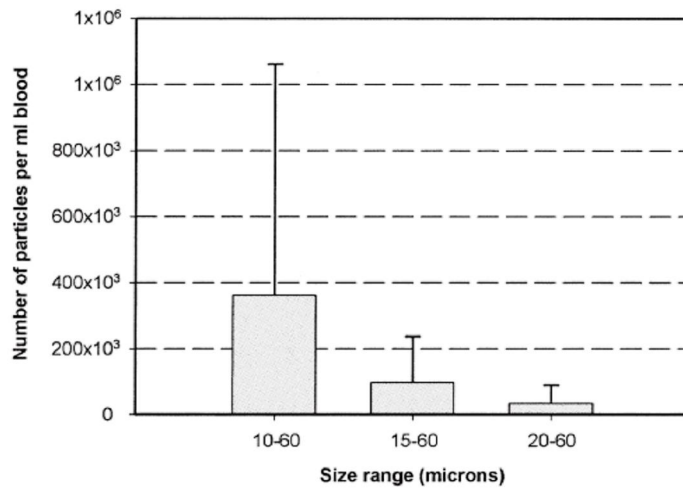
A similar pattern regarding size and distribution was observed in non-centrifuged blood after subtraction analysis (Figure 3).





**Figure 3. Size distribution of particles in whole shed mediastinal blood. Size distribution was calculated by subtracting the number of particles in the arterial line blood from the number of particles in shed mediastinal blood.**

The subtraction analysis also provided the number of particles per ml shed blood:  $361,468 \pm 699,678$  in the range 10–60  $\mu\text{m}$ ,  $96,963 \pm 140,317$  in the range 15–60  $\mu\text{m}$ , and  $33,137 \pm 55,808$  in the range 20–60  $\mu\text{m}$  (Figure 4).



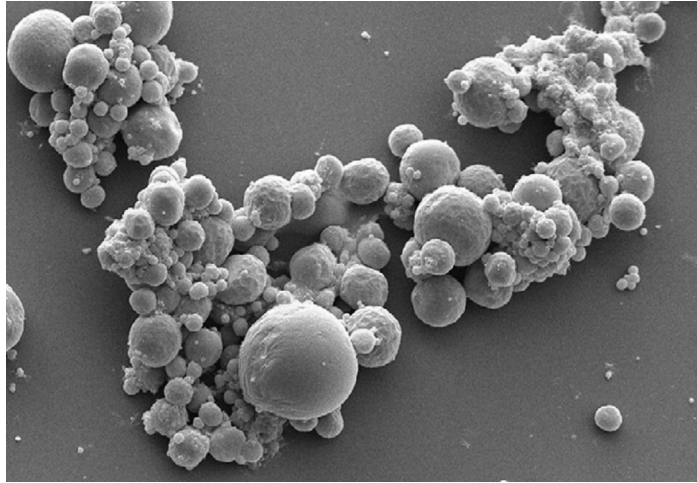
**Figure 4. Number of particles found in each milliliter of shed blood grouped in different size ranges.**

Gas chromatography revealed a pattern of triglycerides in the supernatant of shed blood similar to that found in mediastinal adipose tissue (Table 1).

Free fatty acid	C:Double bonds	Mediastinal adipose tissue (%)	Shed blood (%)
Myristic	14:0	16.5	8.7
Palmitoleic	16:1	15.9	11.7
Palmitic	16:0	100.0	100.0
Linolic	18:2	36.3	65.2
Oleic	18:1	171.9	183.1
Stearic	18:0	25.2	19.5

**Table 1. triglyceride Profile. Triglyceride profiles of mediastinal adipose tissue and shed mediastinal blood. The values are normalized to 16:0 (palmitic acid) to yield relative concentrations, for easier comparison.**

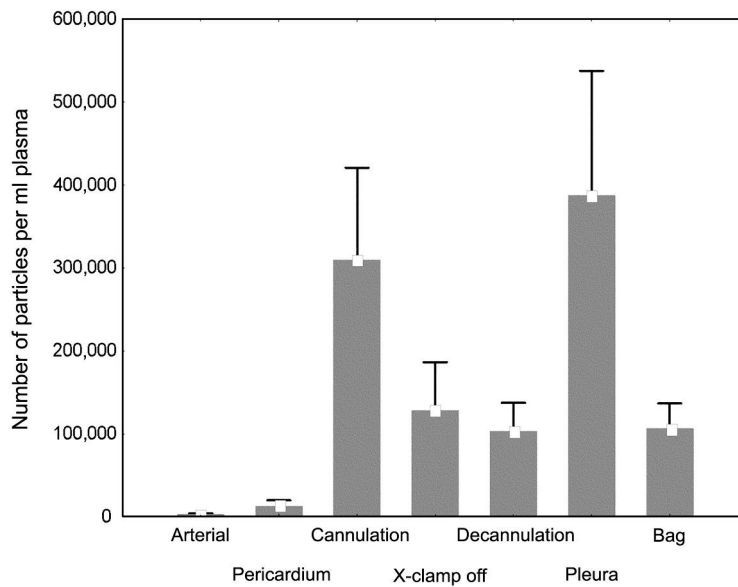
Scanning electron microscopy showed a variation in size and a spherical appearance of the lipid emboli in centrifuged shed blood (Figure 5).



**Figure 5. Scanning Electron Micrograph of lipid particles found in shed mediastinal blood.**

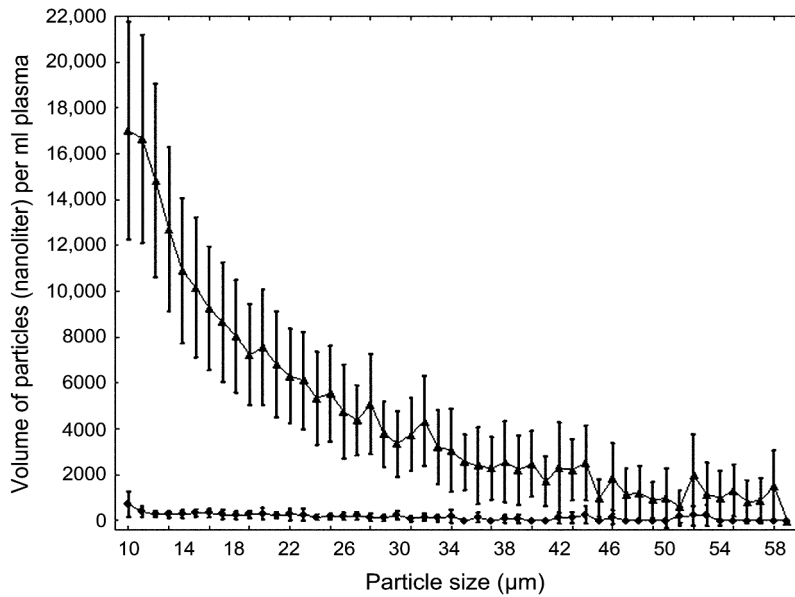
## Study II

The highest number of particles larger than 10  $\mu\text{m}$  was found in blood collected from the surgical field and from the transfusion bags (Figure 6), and was significantly higher than the number of particles found in arterial blood before cannulation and in pericardial fluid after pericardectomy ( $P < 0.0001$ ).



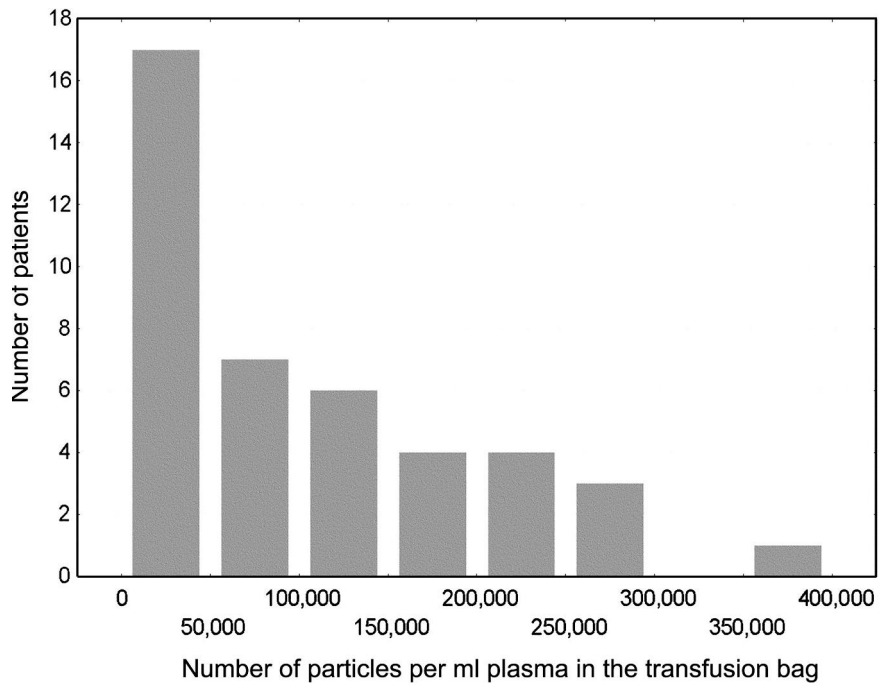
**Figure. 6. Mean ( $\pm 95\%$  CI) number of particles  $\geq 10 \mu\text{m}$  at different sampling locations (arterial blood, pericardial fluid, pleural space and transfusion bag) and from the pericardium at different times during surgery (cannulation, removal of the cross-clamp and decannulation)**

The blood in the transfusion bags showed an abundance of particles in the range  $10\text{-}60 \mu\text{m}$ , compared to that found in arterial blood before cannulation (Figure 7).



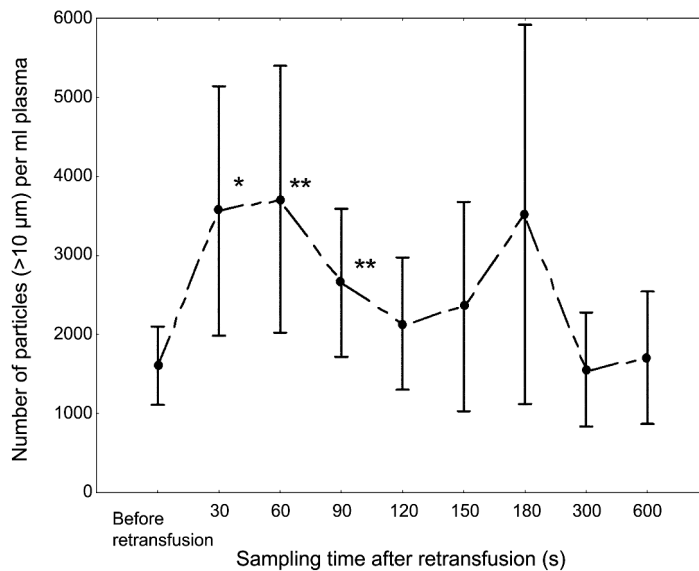
**Figure 7. Size distribution (mean±1 SD) of particles found in the supernatant (after centrifugation) of arterial blood (○) and blood from transfusion bags (●) grouped in 1 µm intervals.**

In addition, there was a significant difference between levels of particles in blood collected from the surgical field at different location points and that in the transfusion bag ( $P < 0.0001$ , one-way ANOVA). A large interindividual variation was found in particle concentration in the transfusion bag blood (Figure 8).



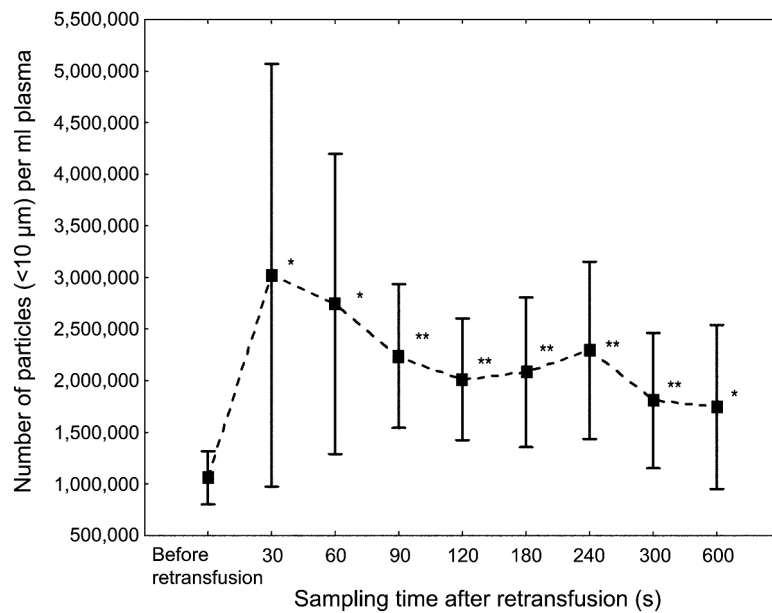
**Figure 8. Histogram showing the distribution of particle concentration found in blood from the transfusion bags for different patients.**

After retransfusion of the blood from the transfusion bag, the concentration of particles in the arterial line in the CPB circuit showed a biphasic response (Figure 9).



**Figure 9. Number of particles ( $\geq 10 \mu\text{m}$ ) in the perfusion circuit at different points in time after retransfusion of cardiotomy suction blood (mean $\pm$ 95% CI). The number of particles is significantly different from that at baseline (0 s sample); \* $P < 0.05$  and \*\* $P < 0.005$ .**

The levels of particles  $\geq 10 \mu\text{m}$  at 30, 60 and 90 s after retransfusion were significantly higher than before retransfusion (3562 $\pm$ 5063, 3711 $\pm$ 5416 and 2654 $\pm$ 3004 vs. 1604 $\pm$ 1593 particles/ml plasma, respectively, all  $P < 0.001$ ). However, no significant difference was found 120, 150, 180, 300 and 600 s after transfusion when compared with levels before retransfusion (2137 $\pm$ 2650, 2369 $\pm$ 2650, 3517 $\pm$ 7701, 1556 $\pm$ 2314 and 1705 $\pm$ 2688 particles/ml, respectively). The levels of particles  $< 10 \mu\text{m}$  were significantly increased at all times after retransfusion compared to that before transfusion ( $P < 0.05$  at 30, 60 and 600 s vs. 0 s,  $P < 0.005$  at 90, 120, 150, 180 and 300 s vs. 0 s, Figure 10).



**Figure 10. Number of particles (>10 μm) in the perfusion circuit after retransfusion of cardiotomy suction blood (mean±95% CI). The number of particles is significantly different from that at baseline (0 s sample); \*P<0.05 and \*\*P<0.005.**

Univariate analysis of the difference between the number of particles at the different location points during surgery and the response measured as the AUC only showed a significant correlation between the number of particles after cannulation and the AUC ( $r=0.41$ ,  $P<0.05$ ).

When testing perioperative variables (Table 2) and the number of particles in the transfusion bag using univariate analysis, only gender was found to be correlated with the number of particles. Higher numbers of particles ( $\geq 10 \mu\text{m}$ ) were found in the transfusion bag blood collected from men than from women ( $122,287 \pm 98,537$  vs.  $27,250 \pm 24,912$  particles/ml plasma,  $P<0.005$ ). No significant gender effect was seen on particle numbers at any of the other location points. No correlation was found between the concentration particles in the bag and the volume of the blood in the bag.



Variable (*n*=42)

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Age (years)	67.9±10.2
Sex (M/F)	33/9
BMI (kg/m <sup>2</sup> )	27.7±4.0
Hypercholesterolemia	95%
Diabetes mellitus	
Insulin	14%
Oral antidiabetics	17%
Hypertension	50%
EuroSCORE	4.4±3.1
NYHA class	
I	29
II	6
III	4
IV	3
CCS class	
I	1
II	14
III	22
IV	5
Perfusion time (min)	81.4±21.5
Cross-clamp time (min)	48.2±14.6
Distal anastomoses	3.7±0.9
Amount blood collected (ml)	339.5±213.5

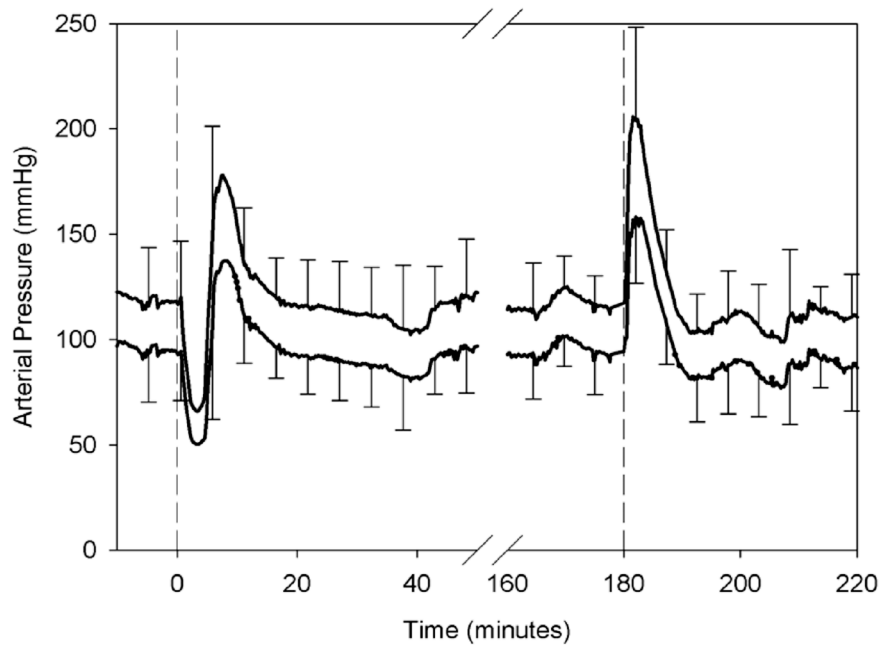
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**Table 2 Pre- and intra-operative variables. Hypercholesterolemia defined as treated with statins before surgery. Hypertension defined as diagnosed and treated with antihypertensive drugs**

### Study III

Infusion of the shed blood surrogate resulted in an almost immediate increase in the pulmonary pressure. In one animal, the infusion led to total circulatory collapse within 10 minutes due to acute right heart failure, despite attempts to reverse the condition with epinephrine, the animal could not be saved. This animal was excluded from the analysis. Thus, the results from 6 animals are presented.

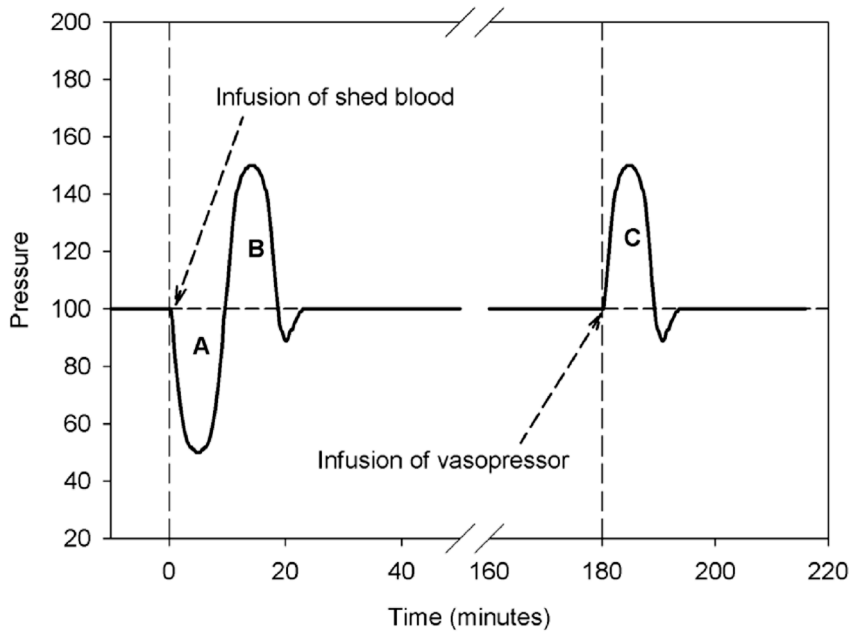
The response of the arterial blood pressure after infusion of shed blood was biphasic (Figure 11). Five of the 6 animals exhibited an initial decline in arterial blood pressure, followed by an increase in pressure. This initial decrease in systolic blood pressure was  $53 \pm 39$  mmHg from baseline, and was recorded after a mean of  $158 \pm 51$  seconds. The subsequent increase in systolic blood pressure from pre-infusion baseline was  $70 \pm 69$  mmHg.



**Figure 11.** Systolic and diastolic arterial blood pressure. Mean values  $\pm$  1 SD during the experiment. The first dashed line indicates the infusion of the

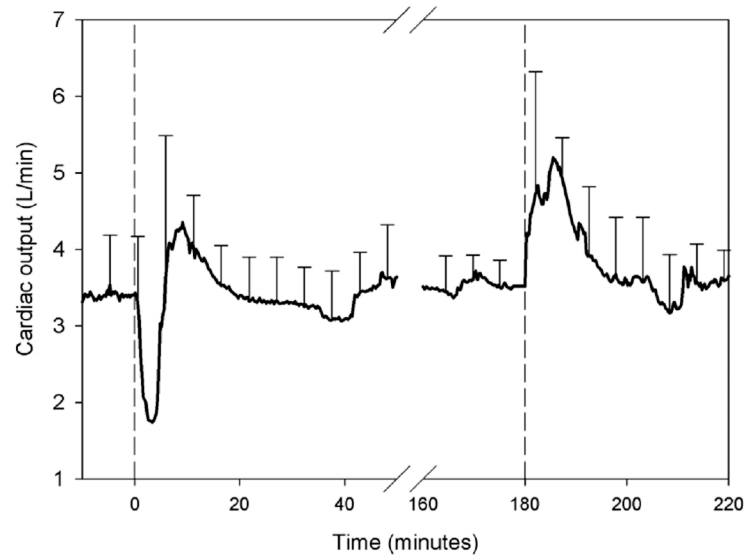
shed blood surrogate. The second dashed line indicates the infusion of epinephrine and Ringer's lactate.

The physiological response measured as the AUC for the decreasing period (Figure 12A) was significantly different from no response ( $p < 0.05$ ). The response expressed as the AUC for the increase in arterial pressure did not reach significance ( $p < 0.10$ ) (Figure 12B).



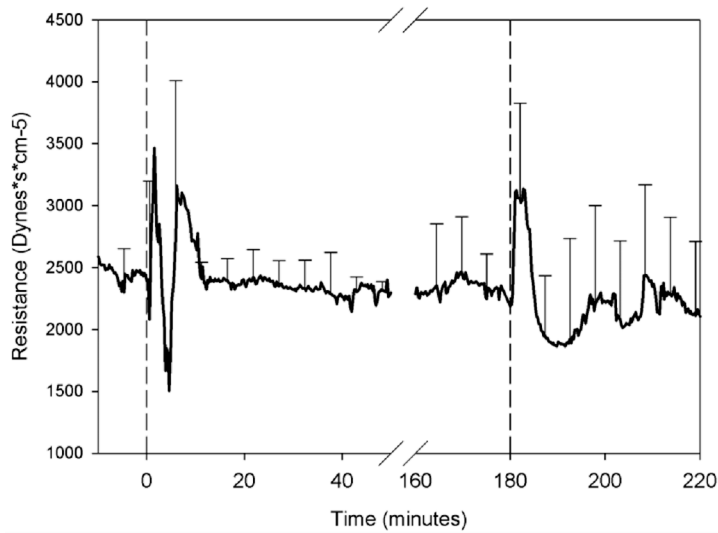
**Figure 12. The protocol used for determining physiological response. The vertical dashed lines indicate the infusion of the shed blood surrogate, and the infusion of epinephrine and Ringer's lactate. A denotes the Area Under the Curve (AUC) as a measure of decline in blood pressure over time. B denotes the AUC indicating blood pressure increase. C denotes the increase in blood pressure after the period of increased blood pressure and flow induced with epinephrine and Ringer's lactate.**

Cardiac output declined concomitantly with the decrease in arterial pressure (Figure 13), from  $3.39 \pm 0.68$  to  $1.59 \pm 1.95$  L/minute ( $p < 0,001$ ) and was on average 53% from base-line.



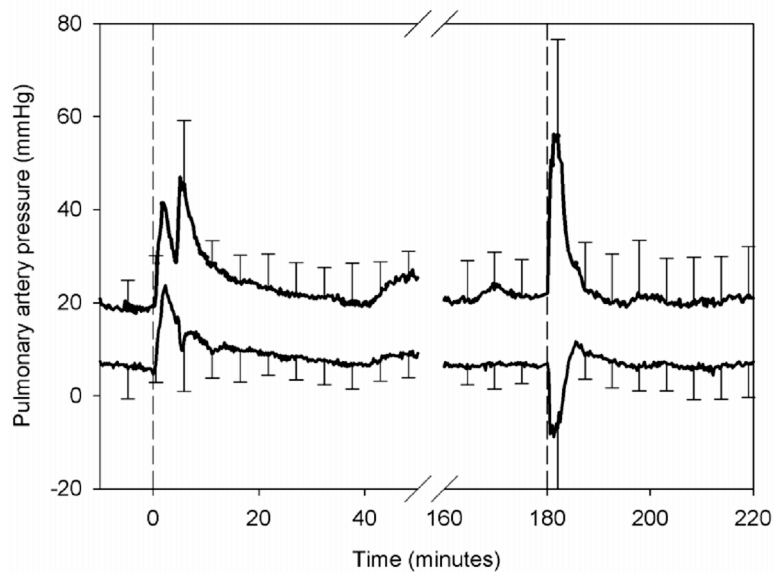
**Figure 13. Cardiac output. Mean values  $\pm$  1 SD during the experiment. The first dashed line indicates the infusion of shed blood surrogate. The second dashed line indicates the infusion of epinephrine and Ringer's lactate.**

The changes in systemic vascular resistance (SVR) varied from animal to animal. In 2 animals there was almost no response. In the other animals there were both rapid increases and decreases in SVR during the initial period of hemodynamic instability, but no pattern could be discerned (Figure 14).



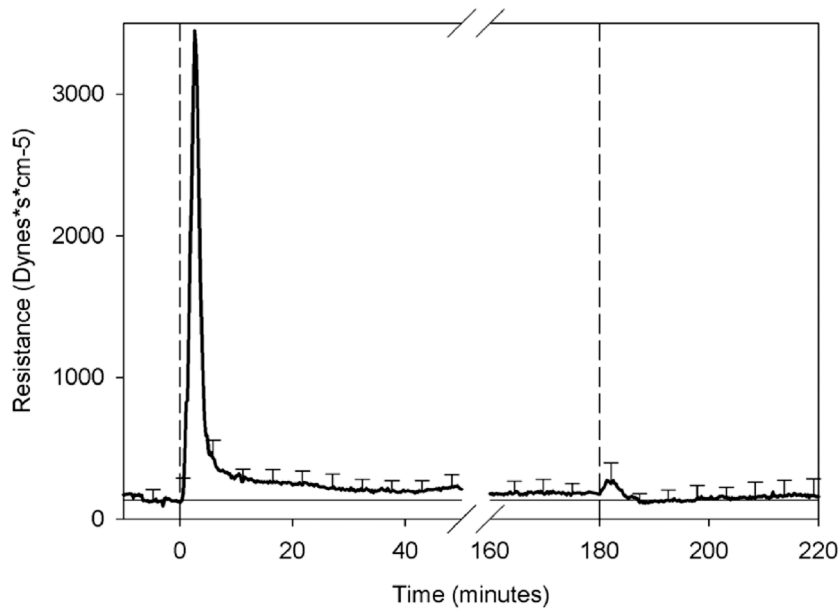
**Figure 14. Peripheral vascular resistance. Mean peripheral vascular resistance as determined from arterial blood pressure and cardiac output. The first dashed line indicates the infusion of shed blood surrogate. The second dashed line indicates the infusion of epinephrine and Ringer's lactate.**

The response of the pulmonary pressure after infusion of the shed blood was biphasic (Figure 15). All animals showed an initial increase in pulmonary pressure, followed by a short decrease before a second rapid increase. The initial increase in systolic pulmonary pressure from baseline was  $36 \pm 10$  mmHg ( $p < 0.05$ ), which represents a 156% increase in pulmonary systolic pressure. The secondary increase in pulmonary pressure was  $47 \pm 17$  mmHg ( $p < 0.05$ ) above baseline.



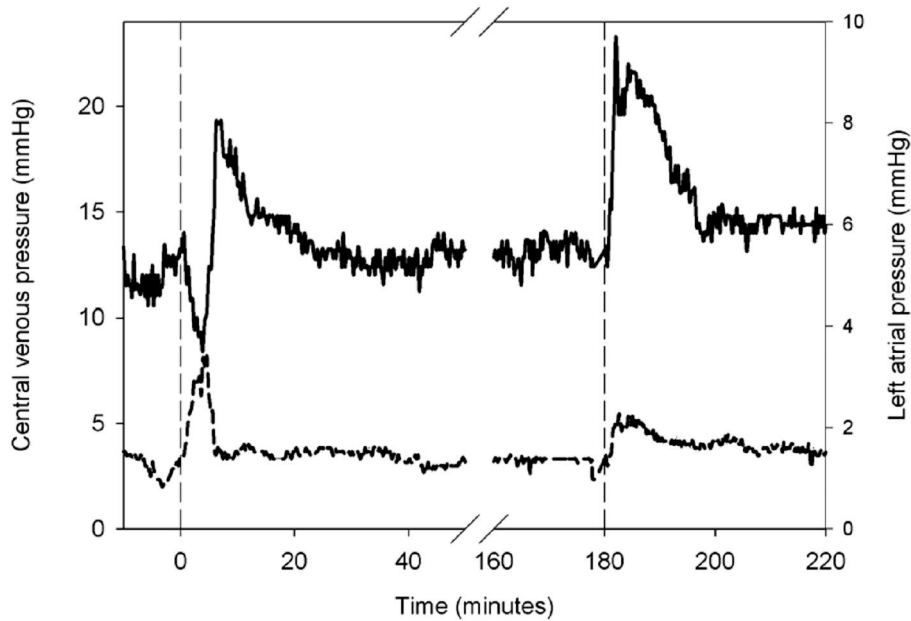
**Figure 15. Systolic and diastolic pulmonary blood pressure. Mean systolic and diastolic pulmonary blood pressure  $\pm$  1 SD during the experiment. The first dashed line indicates the infusion of shed blood surrogate. The second dashed line indicates the infusion of epinephrine and Ringer's lactate.**

Pulmonary vascular resistance (PVR) increased significantly in all animals (Figure 16). The mean increase in PVR from  $116 \pm 67$  dynes  $\cdot$  s  $\cdot$  cm<sup>-5</sup> before infusion to  $3446 \pm 3676$  dynes  $\cdot$  s  $\cdot$  cm<sup>-5</sup> at the maximum PVR, and did not return completely to baseline until after the infusion of epinephrine and Ringer's lactate (Figure 16).



**Figure 16. Pulmonary vascular resistance. Mean pulmonary vascular resistance as determined from arterial blood pressure and cardiac output. The first dashed line indicates the infusion of shed blood surrogate. The second dashed line indicates the infusion of epinephrine and Ringer's lactate. The horizontal line denotes the baseline value calculated from the PVR during the 10-minute resting period (prior to the shed blood surrogate infusion).**

The central venous pressure increased and the left atrial pressure decreased, in response to the infusion of the shed blood surrogate (Figure 17).

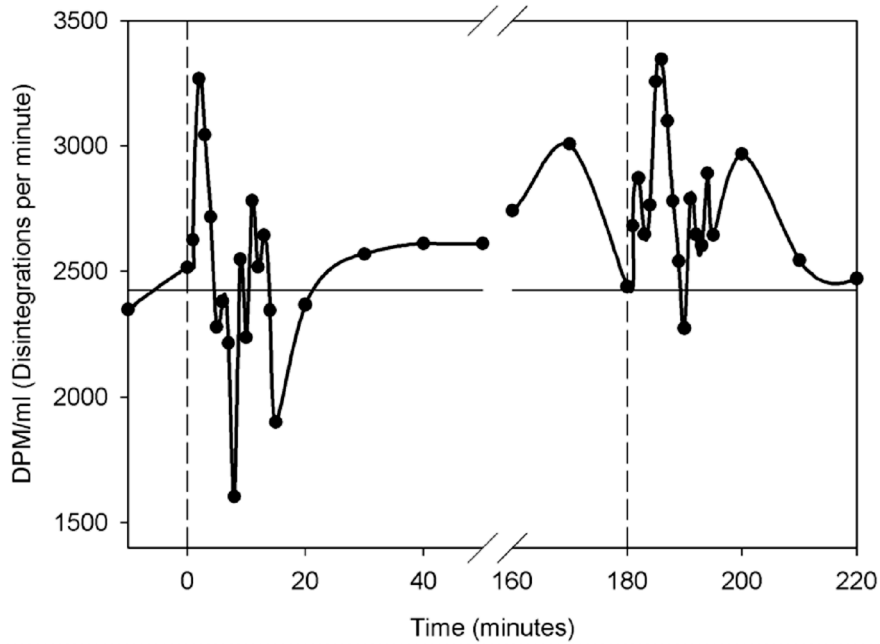


**Figure 17. Central venous pressure and left atrial pressure. Mean central venous pressure (dashed line) and left atrial pressure (solid line). The first dashed vertical line indicates the infusion of shed blood surrogate. The second dashed vertical line indicates the infusion of epinephrine and Ringer's lactate.**

After infusion of epinephrine and Ringer's lactate an increase in arterial blood pressure ensued (Figure 11 and 12C), as shown by a significant increase in the AUC of the blood pressure ( $p < 0.001$ ). In addition, there was an increase in cardiac output and SVR (Figure 13 and 14). Pulmonary artery pressure and PVR increased transiently after the infusion of epinephrine and Ringer's lactate.

The radioactivity levels in arterial blood increased after the infusion of the shed blood surrogate (Figure 18). From the baseline level of  $2369 \pm 1164$  DPM/ml levels increased to a peak of  $3953 \pm 1532$  DPM/ml ( $p < 0.05$ ), at a mean time of  $100 \pm 50$  seconds after infusion.

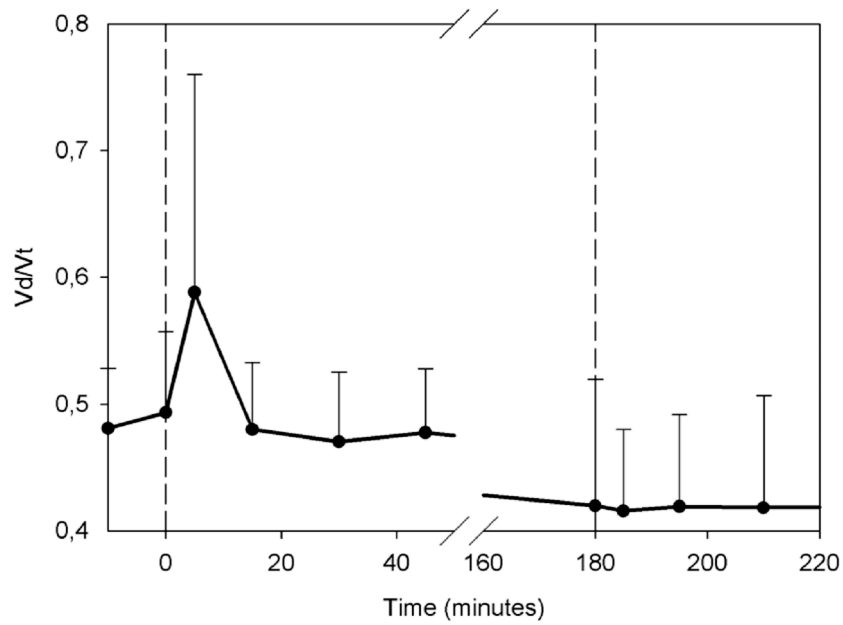




**Figure 18. Radioactivity.** Mean amount of radioactivity in the carotid artery at each sampling time, as a measure of the amount of emboli passing through the pulmonary circulation. The first dashed line indicates the infusion of shed blood surrogate. The second dashed line indicates the infusion of epinephrine and Ringer's lactate. The horizontal line denotes the baseline value calculated from the two samples taken before infusion.

After the period of increased pulmonary blood flow and pressure, the peak level was  $4080 \pm 981$  DPM/ml at a mean time of  $390 \pm 112$  seconds, and was significantly higher than the baseline value of  $2369 \pm 1164$  DPM/ml ( $p < 0.05$ ).

The deadspace ( $V_d/V_t$ ) increased after infusion of the shed blood surrogate (Figure 19), and reached its maximal levels after 5, 15 or 30 minutes in 5 of the 6 animals. In one animal, no change in deadspace was observed. The mean level of  $V_d/V_t$  before infusion of the shed blood was  $0.49 \pm 0.06$ , compared to the highest levels after infusion  $0.61 \pm 0.15$  ( $p < 0.06$ ).



**Figure 19. Ventilatory deadspace. Mean ventilatory deadspace estimated from blood gas analysis and capnography. The first dashed line indicates the infusion of shed blood surrogate. The second dashed line indicates the infusion of epinephrine and Ringer's lactate.**

#### Study IV

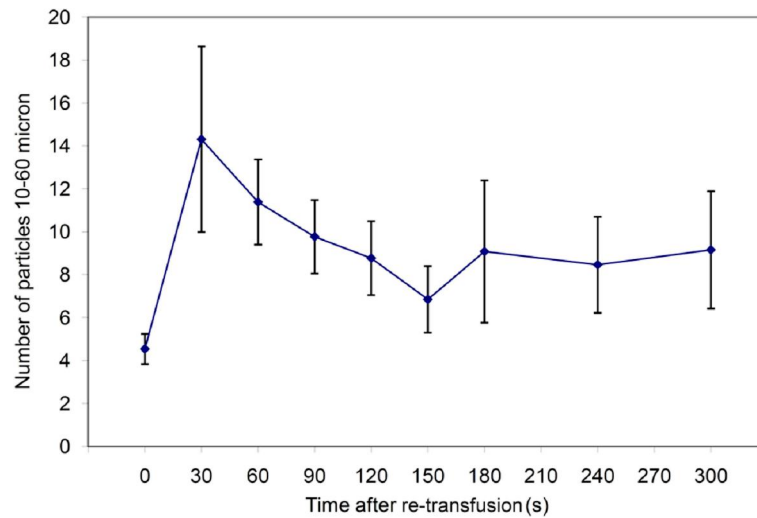
A total of 15 patients were included in the study. Two patients had to be excluded because of the inability to obtain adequate TCD measurements. Satisfactory TCD and Coulter counter measurements were obtained in all other patients, thus the final study population consisted of 13 patients (11 male and 2 female).

**Table 3. Descriptive statistics of the study population (n=13), values are mean  $\pm$  1 SD.**

Variable	Mean $\pm$ SD
Age (y)	66 $\pm$ 6
Sex (male/female)	11/2
Weight (kg)	87 $\pm$ 16
Height (m)	173 $\pm$ 7
BMI (kg/m <sup>2</sup> )	29 $\pm$ 5
NYHA (I-IV)	2.0 $\pm$ 1.2
CCS (I-IV)	3.0 $\pm$ 0.9
Euroscore points	3.3 $\pm$ 2.2
Retransfusion volume (ml)	269 $\pm$ 116
Perfusion time (min)	100 $\pm$ 48
Aortic x-clamp time (min)	55 $\pm$ 21

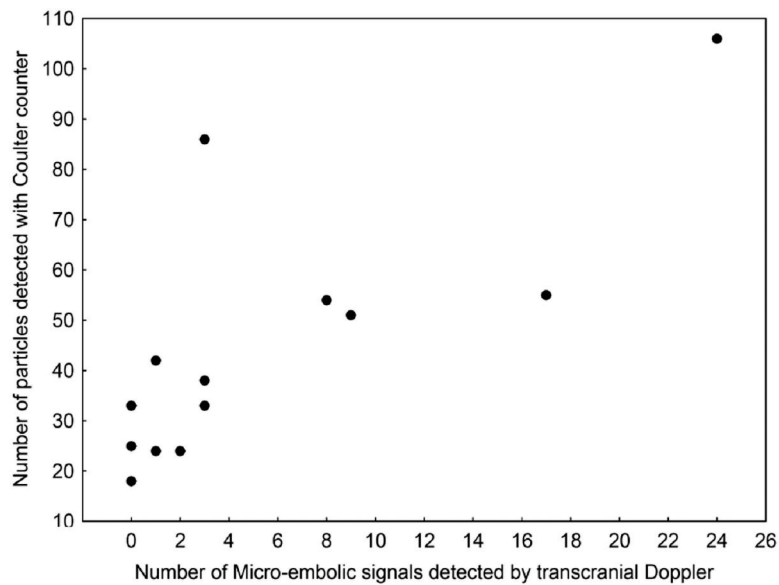
All patients were in sinus rhythm. One patient had stable angina and the remaining 12 patients had previous symptoms of unstable angina and had been stabilized before surgery. Descriptive statistics for the study population are displayed in Table 3.

In this study the identification of particles in arterial blood was analyzed from the moment of retransfusion of collected mediastinal blood. The distribution of particles in the size range of 10 – 60  $\mu$ m during the first 300 seconds after retransfusion of shed blood were counted by the Coulter counter. There was a typical biphasic pattern (Figure 20). However, the majority of the embolic load is found in the first 120 seconds after retransfusion.



**Figure 20. Number of micro-emboli (size 10-60  $\mu\text{m}$ ) detected by Coulter counter over the first 300 seconds (s) after re-transfusion of shed blood (mean and standard error (SE)).**

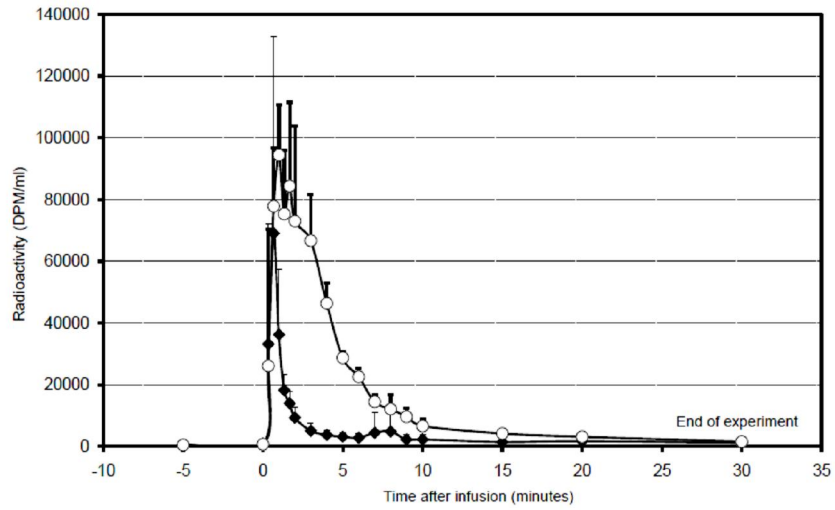
The graph shows a biphasic pattern, where the embolic load mainly appears during the first two minutes after re-transfusion. This was therefore the time frame chosen for comparison to the TCD registration. The number of micro embolic signals detected by the TCD during the two minute pre transfusion period was  $0.15 \pm 0.37$  and for the first 120 seconds after retransfusion,  $5.5 \pm 7.4$  ( $P < 0.05$ ). There was a strong correlation between aggregated embolic loads, as measured by the Coulter counter and the TCD during the first 120 seconds ( $r = 0.79$ ,  $P < 0.005$ ). Figure 21 shows the relationship between micro embolic signals detected by TCD versus emboli from simultaneously sampled blood analyzed for micro emboli in the Coulter counter.



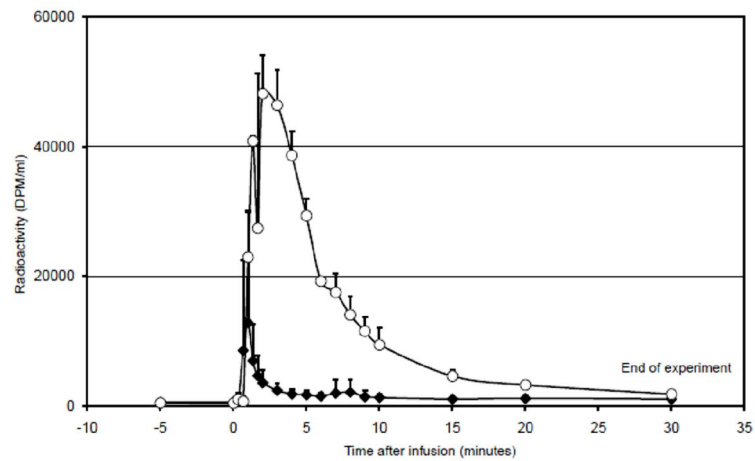
**Figure 21. Relationship between micro-embolic signals detected by transcranial Doppler and emboli in simultaneously sampled blood characterised by Coulter counter during the first two minutes after re-transfusion of shed blood. Correlation with Spearman rank order ( $r=0.79$ ,  $P<0.005$ ).**

#### Study V

In this animal study the animals that received the emulsified lipids had higher levels of radioactivity in the blood. AUC for the arterial samples between the group exposed to non-emulsified versus emulsified lipid micro emboli were  $104\ 758 \pm 69\ 146$  DPM/ml vs.  $553\ 590 \pm 140\ 862$  DPM/ml,  $p<0.01$ . The corresponding values for venous samples were  $32\ 550 \pm 29\ 287$  DPM/ml vs.  $398\ 491 \pm 98\ 536$  DPM/ml,  $p<0.01$ . Thus emulsified lipid micro emboli generated a 5.3-fold higher embolic load in the arterial and a 12.2-fold higher in the venous circulation, compared to non-emulsified lipid micro emboli. Figure 22 displays the kinetics in the arterial circulation after introduction of the two different shed blood phantoms, and Figure 23 represents the corresponding graph for the venous circulation.



**Figure 22.** The kinetics in the arterial circulation after introduction of the two different shed blood phantoms. Open circles represents emulsified and filled squares represents non-emulsified lipid micro emboli. Levels expressed as mean±SEM.



**Figure 23.** The kinetics in the venous circulation after introduction of the two different shed blood phantoms. Open circles represents emulsified and filled squares represents non-emulsified lipid micro emboli. Levels expressed as mean±SEM.

Moreover, emulsified lipid micro emboli resulted in a 2-15-fold higher tissue deposition in investigated organs compared to non-emulsified lipid micro emboli (Table 4). The only organs where no statistically significant differences could be detected were the hippocampus (borderline significant), the brain stem (also borderline significant), and the lung, although the deposition was higher in the group that had received emulsified lipid micro emboli. The biggest differences in uptake were found in the heart, liver, spleen, muscle and brain (white matter).

**Table 4. Specific activity of tritium for each tissue sample. Radioactivity of tissue samples is reported as the number of disintegrations per minute per gram sample (DPM/g). Values are mean  $\pm$  1 SD. Ratio refers to the ratio between emulsified and non-emulsified embolic load.**

Variable	Non-emulsified lipid micro emboli (n=6)	Emulsified lipid-micro-emboli (n=6)	p-value	Ratio
Brain mean	9 948 $\pm$ 11 384	29 238 $\pm$ 12 338	0.04	2.94
Brain - White Matter	7 935 $\pm$ 9 332	30 941 $\pm$ 12 060	0.01	3.90
Brain - Grey Matter	15 095 $\pm$ 15 652	38 930 $\pm$ 17 611	0.02	2.58
Brain - Brainstem	6 386 $\pm$ 7 183	14 142 $\pm$ 6 365	0.08 NS	2.21
Brain - Hippocampus	9 347 $\pm$ 14 602	26 670 $\pm$ 11 036	0.05 NS	2.85
Brain - Cerebellum	10 977 $\pm$ 11 287	35 505 $\pm$ 18 322	0.02	3.23
Heart	7 795 $\pm$ 11385	119 665 $\pm$ 69 640	0.004	15.35
Lung	22 077 $\pm$ 27 243	38 934 $\pm$ 28 732	0.20 NS	1.76
Liver	25 104 $\pm$ 21 644	194 261 $\pm$ 116 385	0.006	7.74
Kidney	186 959 $\pm$ 126 730	508 177 $\pm$ 145 054	0.006	2.23
Spleen	166 485 $\pm$ 151 141	821 500 $\pm$ 823 961	0.02	4.93
Small Intestine	14 678 $\pm$ 8 817	39 131 $\pm$ 11 533	0.006	2.67
Muscle	2 146 $\pm$ 2 187	9 979 $\pm$ 7 905	0.02	4.65

# Discussion

The reuse of blood with cardiotomy suction during CPB is standard procedure. The salvaging of blood and recirculating it during surgery is a way to reduce the need for blood transfusions and a safety measure in case of major bleeding. This thesis has tried to describe the phenomena of lipid micro emboli found in cardiotomy suction blood, and study the route of these emboli in the circulation.

## Characterization of lipid micro emboli

In the first study in this thesis, the focus is primarily on characterizing lipid micro emboli in cardiac surgery. The primary finding was that there are considerable numbers of particles larger than 10  $\mu\text{m}$  in shed mediastinal blood. These particles are in the size range that can pass through standard filters in a CPB circuit, but are larger than the diameter of capillary vessels. In the past, focus has been on the possible deleterious effects of lipid micro emboli originating in cardiotomy suction blood (14, 17, 40, 64). Attention has been focused on the possible complications on the brain, and this has earlier been the main cause of investigation. Recently however, other organ systems have also come into focus (15).

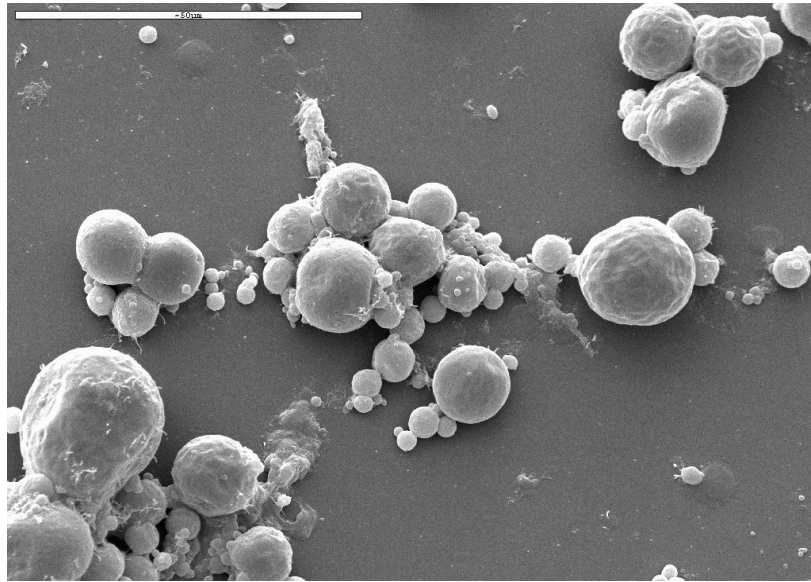
Studying the adipose tissue and the supernatant of shed mediastinal blood containing lipid material with gas chromatography we found striking similarities. The cardiac operation by median sternotomy involves a great deal of exposure of mediastinal fat. The wound is subjected to electrocautery and manipulation which can easily be the origin of lipid particles found in the mediastinal blood during surgery. The bone marrow of the sternum is another possible source of lipid material as it is split and the marrow exposed to the mediastinum. Since the bone marrow contains 40 % fat in the adult patient(37), this also is a potential source of lipid emboli gathering in shed mediastinal blood. It can therefore be assumed that mediastinal adipose tissue and bone marrow are the primary source for lipid material in the shed blood. The results of the gas chromatography seem to strengthen this assumption (62).



Using the Coulter Counter, we could count particles and determine the volume of each particle in shed mediastinal blood. This analysis revealed that the particles are in the size range of 10-60  $\mu\text{m}$ , with over 300.000 per ml of shed blood. This number of particles that potentially can lodge in the arteriolar system when circulated during cardiac surgery supports further research on the topic of micro embolisation as a source of organ dysfunction.

## Lipid micro emboli in the circulation

The second study in this thesis showed that lipid material gathered in the mediastinum during the operation, and also provided a connection between the reinfusion of shed mediastinal blood and the detection of lipid micro emboli in the circulation. When blood is aspirated from the surgical field during cardiac surgery, it contains substantially higher amounts of particles larger than 10  $\mu\text{m}$  than circulating arterial line blood. This was also shown in the first study where we characterized lipid micro emboli, showing hundreds of thousands of particles between 10-60 $\mu\text{m}$  in size, in each ml of blood. When this shed blood was retransfused to the CPB circuit an increase in the number of particles was observed in the arterial line of the CPB circuit. The Coulter counter principle was used in our earlier characterization study. In that study, the supernatant containing particles was studied with gas chromatography, and a pattern of lipid distribution similar to that in mediastinal adipose tissue was found. Moreover, since the blood was centrifuged and the supernatant used for analysis in the Coulter counter, only embolic material with the same or a lower density than plasma was analyzed. In addition, the results of this study, in terms of lipid particle size distribution are also consistent with the results of a study by Kaza et al., in which a lipophilic dye was used to detect lipid emboli (28). It is therefore likely that the particles detected by the Coulter counter represent, at least to a majority, lipid particulate matter.



**Figure 24. Lipid micro emboli in shed mediastinal blood as seen by Scanning Electronmicroscopy. White bar 100µm.**

## The formation of lipid particles during cardiac surgery

We found that there was a large variation in the number of particles in the blood aspirated from the surgical field at different sampling locations and times, suggesting that the release of lipid particles may be related to the mechanical manipulation of the surgical field. This is also supported by the finding that there were minimal numbers of particles in the closed space of the pericardium at the time it was opened to expose the heart (Fig. 6). Surprisingly, there was a considerable interpatient variation in particle concentration in the transfusion bags, as can be seen in Fig. 8. The study offered no explanation for these differences.

In study V, we used two different types of lipid laden shed blood phantoms infused into the circulation of animals. One of the phantoms with emulsified lipids and the other non-emulsified lipids. These were tagged with radioactive tritium to be able to trace the lipid emboli in the circulation. Also to further establish the embolic properties of lipids in the emulsified state, as this is most likely the state of lipids during cardiac

surgery. Emulsified lipid micro emboli gave a higher embolic load in the arterial and venous circulation, and a higher tissue deposition in all analyzed organs despite the fact that we added the same amount of lipids and radioactivity in both groups (Table 4.). There are different plausible explanations for these findings. Non-emulsified lipid emboli, because of their hydrophobic state, adhere to such surfaces as silicone tubing in the CPB circuit and to polyethylene in the cannulation tubes or the polypropylene in the micro porous surfaces of the filters and oxygenator in the heart-lung machine (62, 73, 74).

## Detection of circulating lipid emboli

When we registered the MES by TCD during the infusion of shed mediastinal blood in study IV, we found a sound correlation between the embolic load in the median cerebral artery (MCA) as measured by Doppler and in samples of arterial blood analyzed by Coulter counter. This clearly shows the pathway of lipid micro emboli from the mediastinal blood into the circulation and finally into the arterial system of the brain. The evidence is overwhelming. It shows that patients undergoing cardiac surgery with CPB, and the reuse of cardiotomy suction blood, are subjected to a degree of lipid micro embolism that can potentially be harmful (75).

## Avoiding lipid micro emboli

The lungs can act as filters for lipid material (76, 77). Using the venous side in order to reduce the risk of arterial embolism is not an option, as we showed in our experimental study III. Normally an infusion of volume would yield an increased right ventricular filling and subsequent increase in cardiac output (78). In this case we found that the circulatory reactions were different than expected. There were signs of strain on the right heart, possibly by the mechanical obstruction of lipids in the capillaries of the lungs. This strain on the right heart could put a patient at risk for hemodynamic complications during surgery. Furthermore the lipid emboli were recirculated from the lungs when the circulation is stimulated with vasoconstrictors and volume. Possibly the embolic material is pushed

through the capillaries of the lungs by increased pressure and flow. The lungs themselves are affected by lipid emboli and toxic effects of fatty acids can facilitate this (67).

Using the lungs as a filter for lipid micro emboli does not seem to be an acceptable option to decrease the risk of lipid micro embolism on the arterial side. We found substantial evidence of radioactive lipid emboli on the arterial side.

## Clinical implications

Indeed, recent attempts to reduce lipid micro emboli during surgery have been shown to reduce the embolic load (33), and result in reduced cognitive dysfunction postoperatively (79). Some surgeons even discard the shed mediastinal blood completely to eliminate the potential embolic load altogether (9, 80). This can be done when the surgical technique is adapted to minimize blood loss. Processing the shed mediastinal blood is standard procedure with off pump coronary artery bypass surgery (OPCAB). This greatly reduces the risk of lipid micro-emboli but does not eliminate it.

In addition, there are a number of new techniques in use or emerging in order to eliminate lipid emboli in shed mediastinal blood. Closed-circuit CPB (81) and various cell saving devices are already in use (18, 79, 82). Particle separation by ultrasound (PARSUS) and sedimentation based separation are promising new techniques under development (74, 83, 84).



# Conclusions

## Conclusions of the studies

### Study I

This study effectively characterized the lipid particles found in shed mediastinal blood during cardiac surgery. It showed the size and number of particles that can pass through the filters and lines of the CPB circuit. With Electron microscopy these particles were clearly shown to take the spherical forms expected of emulsified lipid particles. The lipids are of a very similar fatty acid composition as the mediastinal fat in the surgical patient.

### Study II

This study shows that the lipid particles found in shed mediastinal blood gather during the operation. They are found in the collected shed mediastinal blood and can be traced to the arterial line of the CPB circuit after retransfusion.

### Study III

This experimental study in an animal model showed that the retransfusion of lipid laden blood can have severe hemodynamic effects on the circulatory stability during CPB. It further showed that using the filtering capacity of the lungs is not an efficient way to reduce lipid micro emboli load.

### Study IV

This study shows that the lipid micro emboli from retransfused shed mediastinal are traced as micro embolic signals in the middle cerebral artery. This correlates with an increase in the embolic load in the arterial circulation.

#### Study V

In this study we showed that lipid micro emboli are lodged in various parts of the body. With the lipid material in an emulsified state this effect is much greater. It is thus highly debatable whether retransfusion of shed mediastinal blood laden with lipid emboli should be used without methods of reducing the embolic load.

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