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MEASURES TO PREVENT MICROEMBOLIZATION IN CARDIAC SURGERY AND DURING ANGIOGRAPHY WITH SPECIAL REFERENCE TO CARBON DIOXIDE

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MEASURES TO PREVENT MICROEMBOLIZATION IN CARDIAC SURGERY AND DURING ANGIOGRAPHY WITH SPECIAL REFERENCE TO CARBON DIOXIDE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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“All the knowledge I possess everyone else can acquire, but my heart is all my own”

– *Johann Wolfgang von Goethe*

ABSTRACT

Background: Cardiovascular interventions including open heart surgery using cardiopulmonary bypass (CPB) and coronary angiography may introduce air and particulate arterial embolism that may lead to tissue lesions of the brain and other organs.

Aims: To assess: if CO_2-insufflation of an empty CPB circuit decreases number of gaseous emboli in the prime compared with a conventional CPB circuit, which holds air before fluid priming (**Study I**); the efficacy of a new mini-diffuser device for CO_2 de-airing in a minimally invasive open cardiothoracic wound cavity model and in patients undergoing minimally invasive open aortic valve surgery (**Study II**); if an extra separate venous reservoir abolishes CO_2-insufflation induced hypercapnia and retains sweep gas flow of the oxygenator constant during open heart surgery (**Study III**); if there is a difference in the incidence of cerebral microemboli when using either the femoral or the radial approach during coronary angiography (**Study IV**).

Methods: Study I-II were experimental. **Study I**: Number of gaseous microemboli in the arterial line were counted after randomization to insufflation with CO_2-gas or not prior to priming in 20 CPB-circuits. **Study II**: Air displacement efficacy of a mini-diffuser and of an open-ended tube was measured during CO_2-insufflation in a minimally invasive open cardiothoracic wound cavity model and in patients undergoing minimally invasive open aortic valve surgery. **Study III**: A separate reservoir was used during CPB in addition to a standard venous reservoir. The separate reservoir received drained blood and CO_2-gas continuously via a suction drain (1 L/min) and handheld suction devices from the surgical wound. CO_2-gas was insufflated via a gas-diffuser in the open wound at 10 L/min. In a cross-over design for each patient, gas and blood were either uninterruptedly drained from the separate to the standard venous reservoir or not. $PaCO_2$ was determined after tuning of sweep-gas flow as necessary and following steady state of $PaCO_2$ with an online monitor. **Study IV:** We randomized 51 patients to either right femoral or right radial arterial approach and documented with transcranial Doppler the number of particulate microemboli circulating through the middle cerebral arteries.

Results: Study I. Throughout the experiment, the median microembolic count per minute in the CO_2 group stayed lower than in the control group ($p \leq 0.004$). **Study II.** The air content was <1% and 10-75% in the open wound model during CO_2 inflow of 2–10 L/min with the mini diffuser and the open-ended tube, respectively. In 6 patients air content in the open surgical wound stayed <1% during CO_2 flows of 5 and 8 L/min via the mini-diffuser. **Study III.** Median $PaCO_2$ did not vary between setups (5.41; 5.29-5.57, interquartile range [IQR] vs. 5.41; 5.24-5.58, p=0.92), while sweep-gas flow (L/min) was lower (2.58; 2.50-3.16 vs. 4.42; 4.00-5.40, p=0.002) when CO_2-gas was not drained from the separate to the standard reservoir. **Study IV.** The median (range) number of particulate emboli was significantly higher with radial 10 (1–120) than with femoral 6 (1–19) approach. Also, with the radial approach more particulate microemboli circulated through the right sided middle cerebral artery compared with the femoral approach.

Conclusions: CO_2-insufflation of an empty CPB circuit reduces the number of gaseous emboli in the prime compared with conventional CPB circuit priming (**Study I**); CO_2 de-airing with the mini-diffuser was effective in a minimally invasive open cardiothoracic wound cavity model and in patients (**Study II**); A separate venous reservoir, with a clamped connecting tube to the standard venous reservoir for evacuation of gas and blood from the open surgical wound, prevents CO_2-insufflation induced hypercapnia in open heart surgery, keeping $PaCO_2$ and sweep gas flow constant (**Study III**); When performing coronary angiography, the radial access produces a higher number of particulate cerebral microemboli than the femoral alternative (**Study IV**). This approach is hence advocated in this aspect.

LIST OF SCIENTIFIC PAPERS

I. **Nyman J**, Rundby C, Svenarud P, van der Linden J. Does CO2 flushing of the empty CPB circuit decrease the number of gaseous emboli in the prime? *Perfusion* 2009;24(4) 249–255.

II. **Nyman J**, Svenarud P, van der Linden J. Carbon dioxide de-airing in minimal invasive cardiac surgery, a new effective device.
Journal of Cardiothoracic Surgery 2019;14(1):12

III. **Nyman J**, Holm M, Fux T, Sesartic V, Fredby M, Svenarud P, van der Linden J. Elimination of CO_2-insufflation induced hypercapnia in open heart surgery using an additional venous reservoir for suction of blood from the open surgical wound.
(Submitted)

IV. Jurga J*, **Nyman J***, Tornvall P, Nastase Mannila M, Svenarud P, MD, van der Linden J, Sarkar N. Cerebral Microembolism During Coronary Angiography. A Randomized Comparison Between Femoral and Radial Arterial Access.
Stroke 2011;42:1475-1477.
*J.J. and J.N. contributed equally to this work

CONTENTS

LIST OF ABBREVIATIONS

AVR	Aortic valve replacement
CABG	Coronary artery bypass grafting
CPB	Cardiopulmonary bypass
CO_2	Carbon dioxide
dEBR	Delta embolus blood ratio
EGFR	Estimated glomerular filtration rate
HITS	High-intensity transient signals
IQR	Interquartile range
MCA	Middle cerebral artery
MRI	Magnetic resonance imaging
MACE	Major adverse cardiac events
MES	Microembolic signals
MI	Myocardial infarction
MVR	Mitral valve replacement
N_2	Nitrogen
O_2	Oxygen
$PaCO_2$	Partial pressure of carbon dioxide in arterial blood
PCI	Percutaneous coronary intervention
PMD	Power M-mode Doppler
PVC	Polyvinyl chloride
RCT	Randomized clinical trial
SD	Standard deviation
TCD	Transcranial Doppler
TEE	Transesophageal echocardiography
TIA	Transient ischemic attack

1 INTRODUCTION AND BACKGROUND

1.1 CLINICAL MANIFESTATION OF MACRO- VERSUS MICROEMBOLISM

1.1.1 Macroembolism in cardiac surgery and during angiography

In open heart valvular surgery there is a high risk of perioperative stroke, mostly considered to be due to macro-particulate and air embolization.[1] Bucerius et al. reported in a prospective study involving approximately 16,000 cardiac surgery patients, with a mean follow-up time of 11.7 days, that the risk of stroke was 4.8%, 8.8%, and 9.7 after isolated aortic valve replacement (AVR), mitral valve replacement (MVR), and multivalve surgery, respectively.[1] In contrast to cardiac surgery, the incidence of procedure-related stroke or TIA after coronary angiography and percutaneous coronary intervention (PCI) is very low, < 0.3%.[2]

1.1.2 Microembolism in cardiac surgery and during coronary angiography

Cerebral microemboli may be detected with the transcranial Doppler (TCD) technique, both during open cardiac surgery [3] and during catheter-based cardiac procedures.[4-6] The great risks of cerebral microemboli in open heart surgery imply, as highlighted by a Cochrane review, that the number cerebral microemboli correlates with the quantity of postoperative neuropsychological disturbance.[7]

The content in cerebral microemboli varies and may consist of air or particulate material including fat emboli, atheromatous debris or blood clots.[8] Microemboli have been associated with ischemic lesions detected with cerebral magnet resonance imaging (MRI).[4, 9] Silent cerebral infarctions without overt clinical signs of stroke may give subtle neurological symptoms including signs of depression, minor visual defects and frailty.[10] Though the clinical impact of microembolism is debated, silent ischemic lesions have been associated with neuropsychological impairment and augmented risk of dementia. [4, 10]

1.2 AIR EMBOLISM

Air consists mainly of nitrogen (N_2, 78.1%),[11] and does not dissolve easily in blood. This is the reason why arterial air embolism may cause perfusion defects.[12] Air microemboli in blood create foreign surfaces, which start the formation of microthrombi,[13] activation of platelets and leukocytes, as well as changing the erythrocyte count. [13-15] These gas-liquid interfaces of air microemboli in blood will also initiate adsorption and denaturation of proteins in plasma.[13, 14, 16] The first protein that is layered on an air bubble surface is fibrinogen, which has non-polar hydrophilic parts that may act as binding sites for various blood-borne elements, including fatty acids [16] and lipid particles. These particles may stimulate platelet adhesion and aggregation.[17] Platelets that are activated via air microemboli get a similar structural change as when activated by collagen or thrombin.[18] Air microemboli instantly affect the vascular endothelium, causing functional changes, and also promote aggregation as well as activation of platelets and leukocytes.[19-24] The subsequent changes in microvascular permeability are the result of the release of inflammatory factors from activated cells, involving prostaglandins, leukotrienes, and thromboxanes.[25, 26] Arterioles having an inner diameter < 30 - 60 µm usually get obstructed by air microemboli. Even an air micro-embolus with a diameter of 25 µm that obstructs a cerebral arteriole for < 30 seconds will disturb brain function.[27] In a clinical study by Kirmani et al., evaluating number of arterial air microemboli, postoperative cognitive impairment has directly been linked with the number of perfusionists´ interventions during cardiopulmonary bypass (CPB).[7] Moreover, arterial air embolism during open heart procedures may produce various postoperative complications in different organs, e.g. cerebral injury, arrhythmias, and myocardial dysfunction.[28-34]

1.2.1 Air micro-embolism in cardiac surgery

In cardiac surgery air embolism may cause cerebral and peripheral organ dysfunction. When the heart or the large vessels are opened during cardiac surgery, air will enter the heart and the large vessels. Also, air emboli may already enter the arterial bed at the initiation of CPB. During CPB, microemboli have been causally related to several perioperative neurological complications. In this setting, one should not forget that the brain is particularly susceptible to ischemia caused by emboli. Postoperative neuropsychological impairment is seen in 30-80% of patients undergoing cardiac surgery using CPB and these pathological deficiencies may even advance and persist.[35]

Even when conventional de-airing techniques are used in open heart surgery, many arterial air microemboli will nevertheless appear, particularly when the patient is weaned from CPB.[3, 36]

1.2.2 Prevention of air micro-embolism in cardiac surgery

Microembolic events occur when using CPB, despite use of new technologies, including microporous membrane oxygenators, better-quality arterial line filters, and CPB surface coatings, which all have been documented to reduce the amount of microemboli.[3]

1.2.2.1 Insufflation of CO_2-gas in the open surgical wound

Since the 1950s, many cardiac centers routinely insufflate the open cardiothoracic wound cavity with CO_2-gas during surgery to prevent air from getting into the circulation. As the density of CO_2-gas is 50% higher than air, CO_2-gas will fill and displace air by gravity from the open wound cavity. Specifically, the density of CO_2-gas is heavier than air as the molecular mass CO_2 is 44 ($12 + 2 \times 16 = 44$), which should be compared with that of the average molecular mass of air, which is 29 (80% N_2 + 20% O_2 = $0.8 \times 28 + 0.2 \times 32 = 29$). Thus, the relation in molecular mass is $44/29 = 1.5$ i.e. CO_2 has a mass that is approximately 50% higher. As CO_2-gas is > 25 times more soluble in blood than air at 37 – 38 °C,[37, 38] arteriole obstruction or flow disturbance of the brain and other organs is believed to be reduced if blocked by CO_2-gas.[39] Arterial CO_2-emboli are much quicker resolved in blood and much better tolerated than air emboli.[29-32, 40, 41] As indicated in earlier studies, CO_2-gas cannot due to turbulence effectively de-air an open wound cavity if insufflated via an open tube or other devises unless a high flow-low velocity device is applied, i.e. a gas-diffuser.[42-44]

De-airing with CO_2-gas insufflation in minimally invasive open heart surgery is extra critical compared with conventional open heart surgery via a full sternotomy, as the former procedure does not allow ordinary de-airing maneuvers. Besides, the much smaller wound cavity in minimally invasive open heart surgery, necessitates a considerably tinier device for CO_2-gas insufflation. Until now, no device has been assessed for this purpose.

CO_2-gas evacuated from the open surgical wound cavity with a coronary suction device into the venous reservoir of the CPB circuit affects $PaCO_2$ of the patient due to the large interface between blood and gas, where CO_2-gas will dissolve in the continuous venous blood stream. Ventilation of the patient via the oxygenator in the CPB circuit must then be increased to maintain an approximately normal $PaCO_2$ of 5.3 kPa, normal range 4.6 – 6 kPa (40 mmHg, normal range 35-45mmHg). If CO_2-gas from the wound cavity may be separated from the standard venous reservoir, there should be no impact of the CO_2-gas on the patient´s $PaCO_2$. This would avoid CO_2 induced hyperperfusion of the brain and thus decrease the risk of cerebral embolization during CO_2-insufflation for de-airing in open heart surgery.

1.2.2.2 Insufflation of CO_2-gas in the CPB circuit

Previous reports have identified when cerebral emboli appear during cardiac surgery, e.g. when the aortic cross-clamp is removed, and especially during weaning from CPB,[3] and how these may be prevented. However, only a limited number of studies have focused on microembolism when CPB is initiated. A prerequisite to decrease these microemboli is the understanding of how the CPB circuit is built and primed. The components of the standard CPB circuit comprise an oxygenator (with or without an integrated venous and/or cardiotomy reservoir), a cardioplegia delivery set and an arterial filter, **Figure 1**.

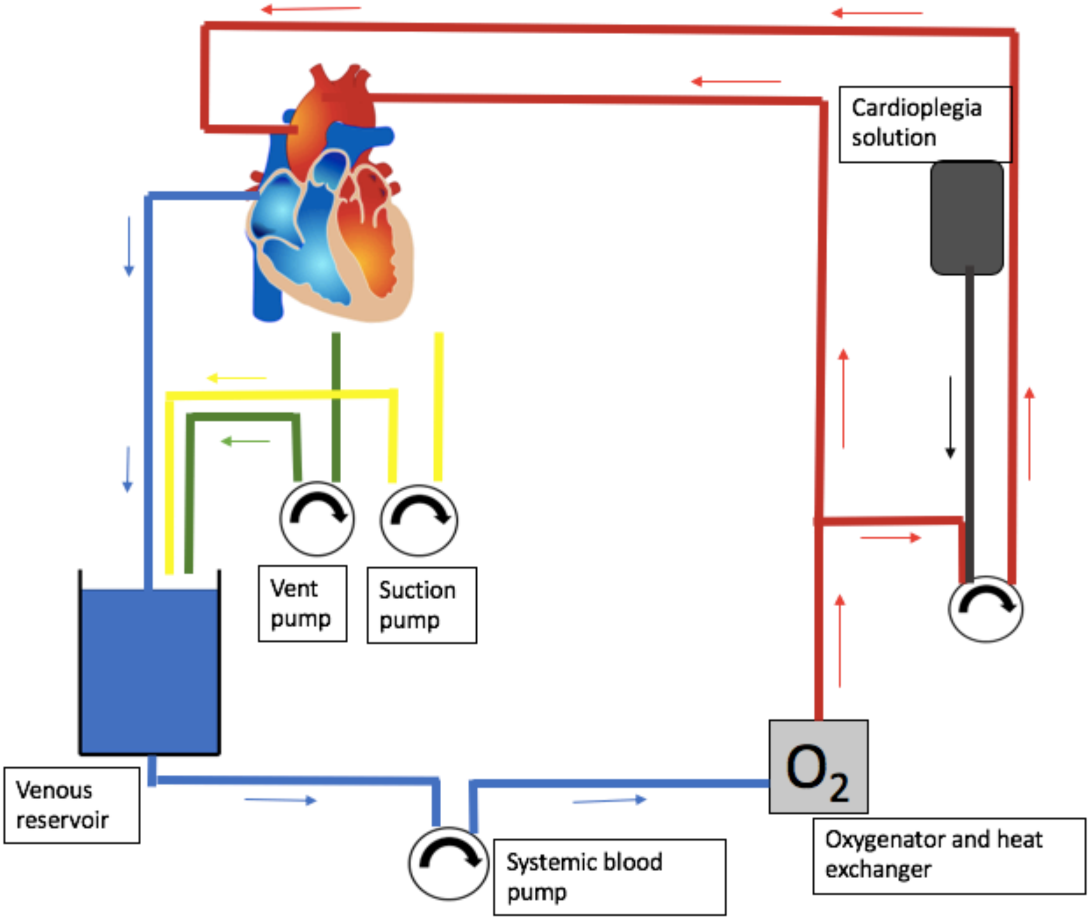

FIGURE 1. This figure illustrates the conventional parts of the extra-corporeal circuit. The color blue represents venous desaturated and hypercapnic blood. The color red illustrates arterial blood fully saturated with oxygen. The green and yellow colors represent blood from the aortic root and/or left ventricle and/or the lowest part of the open surgical wound cavity, which is emptied via suction devices to the venous cardiotomy reservoir" (re-printed with permission from Gunilla Kjellberg).

A venous cannula is conventionally used to passively drain venous blood from the right atrium to a cardiotomy reservoir. Alternatively, a venous cannula is positioned in the superior and inferior caval vein, respectively, and joined to one tube via a Y-connector. The venous blood is then transported from the cardiotomy reservoir via a roller or a centrifugal pump past an oxygenator. When passing the oxygenator, CO_2 is removed, and the venous blood gets saturated with oxygen. The oxygenator's sweep gas flow rate and the oxygen concentration regulate the oxygen tension and saturation and CO_2 pressure in the outgoing arterial blood. A heater/cooler system connected to the oxygenator controls the temperature of passing blood. The outgoing oxygenated arterial blood reenters the patient by an arterial cannula, which usually is positioned in the distal part of the ascending aorta. Blood passing the CPB circuit will have contact with tubes and the main pump, as well as with additional pumps and tubes that drain blood from the surgical wound cavity to the venous reservoir. Totally, blood gets in contact with a foreign surface area of approximately 2 - 3 m^2. The pump flow rate is usually 2.4 L/m^2/minute but will be adjusted to several factors including the patient´s body surface area, hematocrit and temperature.

1.2.2.3 Traditional de-airing maneuvers of the CPB circuit

The CPB circuit contains sterile air prior to priming with fluids. After all parts of the CPB circuit have been filled with prime fluid, the circuit will be re-circulated with the prime for several minutes. Additionally, to release trapped air, the perfusionist usually taps on the CPB circuit with a rubber hammer or a metal clamp. The knocking is typically sustained until the perfusionist confirms that the prime of the CPB circuit is well de-aired using a bubble sensor. This sensor is an integrated part of most CPB circuits and will usually detect single bubbles with a diameter of ≥ 300 µm (Stöckert S3, Sorin Group, Munich, Germany). To further efficiently evacuate air from the CPB circuit, perfusionists may insufflate the CPB circuit with CO_2-gas prior to prime filling. However, studies evaluating this potentially important measure are missing.

1.2.3 Air and particulate microembolism in coronary angiography

The frequency of silent brain infarctions may be substantially higher than overt strokes during and early after coronary angiography[28] and microemboli have been observed passing the middle cerebral arteries (MCAs) during coronary angiography.[29] Current studies with diffusion weighted magnetic resonance imaging (MRI) indicate that microemboli may be the source of asymptomatic ischemic cerebral defects, but their clinical relevance is debatable.[30-32]

1.2.3.1 Femoral versus radial access for coronary angiography

There are several advantages of using the radial artery access such as the superficial anatomic position that allows easy compression, no important close structures, shorter duration of immobilization, and less periprocedural distress. All these factors may explain why this approach is preferred by patients.[45, 46] Women have smaller radial arteries than men, which may clarify why radial arteries in women are more likely to spasm. The spasm complication is a major reason of failure to perform coronary angiography via the radial access.[47, 48]. Also, the small artery size in women may restrict the use of larger interventional devices.[49, 50]

Various analyses comparing the radial and femoral access for cardiac catheterizations in terms of clinical outcome has been made. The first meta-analysis[51] found considerably fewer complications related to access site when comparing the radial with the femoral approach, but the hazard of major adverse cardiac events (MACE) did not differ. A following larger meta-analysis showed that the radial approach led to fewer major bleedings, although not effecting the combined endpoint of death, myocardial infarction (MI) and stroke.[52] A later meta-analysis that combined randomized clinical trials (RCTs) and observational studies including more than 750,000 patients, also found fewer major bleedings, nonetheless indicated a reduced mortality rate using the radial access.[53]

The first large RCT comparing the two vascular approaches, the RIVAL trial, did not demonstrate a significant difference in the merged endpoint (death, stroke, MI, major bleeding). However, a subgroup analysis discovered that the radial access was beneficial for STEMI patients.[54] Two later RCTs, the RIFLE-STEACS and the STEMI-RADIAL, uncovered benefits for patients with ST-segment elevation in acute coronary syndrome using the radial approach regarding the composite outcome of cardiac death, MI, stroke, revascularization of the culprit lesion, and bleeding.[55, 56] A separate analysis of the composites of the combined endpoint showed that bleeding rates and cardiac mortality were significantly lower in the radial group of the RIFLE-STEACS trial only, and not in the STEMI-RADIAL trial.[55, 56] In the following MATRIX trial, the radial approach was linked with a lower rate of the merged endpoint (death, stroke, MI, and bleeding in acute coronary syndrome patients).[57]

No significant differences in frequency of stroke was presented in the above mentioned RCTs, though they had a considerably higher incidence of stroke compared with register-based incidences (0.3-0.7%).[54-57] However, when considering the low incidence of stroke or transient ischemic attack (TIA) related to the intervention, a type II error may be anticipated, as the RCTs were not adequately powered for this purpose. Besides, register studies may lack validity regarding stroke and TIA.

In contrast to the low incidence rate of stroke and TIA, microemboli occur during most angiography procedures. This allows studies to be performed using a very limited number of patients. In a non-randomized study by Spencer et al., cerebral microemboli appeared more frequently during coronary angiography using the radial than with the femoral approach.[32] Therefore, we designed the randomized **Study IV** to examine if the number of cerebral microemboli may differ between the radial and femoral approach during coronary angiography.

2 AIMS

The specific aims were to:

- evaluate if CO_2-insufflation of an empty CPB circuit reduces number of gaseous emboli in the prime compared with a conventional CPB circuit, which contains air before fluid priming.

- evaluate 1) a method to warrant a high content of CO_2-gas (> 99%) in the empty CPB circuit, 2) if tapping on the oxygenator releases gaseous emboli, 3) if the duration of re-circulating the circuit with fluid prime has an impact on the number of gaseous emboli.

- assess efficiency of a mini-diffuser device for CO_2 de-airing in aminimally invasive open cardiothoracic wound cavity model with and without intermittent rough suction as well as in patients undergoingminimally invasive open aortic valve surgery.

- evaluate if an additional separate venous reservoir eliminates CO_2-insufflation induced hypercapnia and keeps sweep gas flow of the oxygenator constant during open heart surgery.

- test if there is a difference in incidence of cerebral microemboli between the femoral and the radial access site during coronary angiography.

3 METHODS

3.1 STUDY DESIGN AND PATIENT SELECTION

The experimental randomized **Study I** was divided into two sections. In the first part, we developed a technique to confirm that the empty CPB circuit was totally filled with CO_2-gas (> 99%). In the second part, we measured the amount of gaseous microemboli in the prime circulating in the CPB system. Prior to the beginning of each test, we randomized the CPB circuit to an interventional or a control group using unmarked envelopes.

Study II consisted of laboratory tests followed by confirmation of the de-airing efficacy of the used device by point measurements of air content in an open minimally invasive surgical wound cavity and in six patients having AVR surgery.

Study III (ClinicalTrials.gov Identifier: NCT04202575) included clinical measurements during CPB in 10 patients undergoing AVR with either coronary artery bypass grafting (CABG) or not, where the patients served as their own controls using a cross-over design.

The clinical **Study IV** (ClinicalTrials.gov Identifier: NCT01428947) was a prospective, randomized, single-center trial. Three-hundred-forty patients were screened from February 2007 to June 2008, whereof 51 were included and randomized to coronary angiography via either radial (n = 28) or femoral (n = 23) approach. All patients had stable angina pectoris and were planned for diagnostic coronary angiography. Exclusion criteria included valvular heart disease, previous CABG, a pathological Allen´s test (indicating absence of collateral arterial blood flow to the hand)[58] of the right hand, severe kidney disease with an estimated glomerular filtration rate (eGFR) less than 30 mL/min calculated with the Cockcroft-Gault formula, and if not blood flow velocity was measurable in both MCAs with TCD.

Study I-IV were performed at the Karolinska University Hospital, Stockholm, Sweden.

3.2 CLINICAL PRACTICE

3.2.1 Cardiopulmonary bypass system

In **Study I** we used a Stöckert S3 heart-lung machine, which consisted of a roller pump and a Dideco pre-mounted tubing set with Compactflo EVO adult Phisio-coated membrane oxygenator (Dideco, Sorin Group, SpA, Mirandola, Italy). The volume of the CPB system was 5.5 L. We primed the CPB system with 1500 mL Ringer´s Acetate, 250 mL Mannitol 15% and 5,000 units of heparin. The temperature of the priming solutions was 20-22°C. We passively filled each CPB-circuit and attached the tubing of the pump house to the main pump.

In **Study III** we used a Stöckert S5 heart-lung machine and the CPB circuit set (Sorin Perfusion Tubing Systems, Sorin Group Italia S.r.l. Mirandola (MO), Italy) consisted of an oxygenator, a centrifugal pump and a standard venous reservoir was set up with an additional venous reservoir (Sorin Inspire HVR, Sorin Group Italia S.r.l. Mirandola (MO), Italy), to which the coronary suction device (suction rate of 0.25-1 L/min,) was connected as well as an extra cardiotomy suction device (suction rate 1 L/min), placed in the wound cavity 4 cm below the skin surface. The additional venous reservoir was positioned above and connected with a tube to the standard CPB cardiotomy reservoir. To enable blood sampling Luer lock connections were inserted in the CPB circuit; 1) at the venous return tubing before entrance to the standard venous reservoir, 2) between the standard venous reservoir and the oxygenator, and 3) on the arterial line of the circuit. An on-line device for blood gas analyses (CDI™ 500, Terumo Cardiovascular Systems Corporation, MI, USA), was connected to the arterial line between the oxygenator and the patient. The CPB system was primed with 1500 mL Ringer´s Acetate, 250 mL Mannitol 15% and 7,500 units of heparin.

3.2.2 Angiography

In **Study IV**, all patients were treated with aspirin prior to coronary angiography. During coronary angiography, the Seldinger technique was used by puncturing the vessel wall with a needle through which a flexible guidewire was pushed forward and left intravascularly as the needle was withdrawn, where after a sheath was advanced over the guidewire. The sheath was equipped with a valve and a side port for catheter placement and drug administration, respectively. For decades the femoral access has been the conventional approach, for percutaneous coronary interventions, allowing the use of large sized catheters.[59] The common femoral artery was punctured 1-2 cm distal of the inguinal ligament. After flushing the catheter, a guidewire was inserted and advanced to the thoracic aorta. Currently, two catheterization techniques are used: either the guidewire is withdrawn in the descending aorta or catheters are advanced through the aortic arch by a guidewire. The latter technique is thought to minimize the risk of dislodging arteriosclerotic debris from the aortic arch. Local traditions decide preference to either technique but hard evidence regarding ischemic neurological complications is rare. After catheterization, the femoral arterial sheath was removed and to achieve hemostasis, vascular puncture site was either compressed manually or by a mechanical device (FemoStop®, Radi Medical systems, Uppsala, Sweden). Alternatively, the arterial puncture site was closed by insertion of a resorbable plug *(AngioSeal®, St.Jude Medical),* which reduces the duration for hemostasis.[60]

3.3 SPECIFIC DEVICES AND METHODS

3.3.1 Measurement of air content

In **Study II**, we based the size of the minimally invasive cardiothoracic wound model on measurements (median) of minimally invasive cardiothoracic wound cavities of 3 patients undergoing minimally invasive open heart procedure using an incomplete J-shaped sternotomy. The orifice of the wound model had a radius and a depth of 35 mm and 44 mm, respectively. Additionally, an aortic model measuring 50 x 40 x 40 mm was placed at its base.

Air displacement in the experimental circuit, **Study I**, and in the experimental as well as patients´ minimally invasive wound cavities, **Study II**, was evaluated by measuring residual O_2-gas concentration. This was calculated to percentage of CO_2-content using the simple method: $\%CO_2 = [100 - (\%O_2 \text{ measured} \times 100 / \%O_2 \text{ initial})]$. The O_2-content was determined using an ceramic sensor (CheckMate 9900, PBI-Dansensor A/S, Ringsted, Denmark), with a gas sampling volume of < 2 mL, a response duration below 2 seconds (> 20.95% change in bidirectional O_2-concentration), a measurement range of 0.0001% - 100% O_2, and a 1% accuracy of the measured value. A 1.5 mm thick Teflon tube was used as a sampling probe. The O_2-concentration is approx. 1/5 of air, to be precise, 20.95% close to sea level.[11] Although air is a mixture of gases, including O_2, air performs as one single gas at normal pressure and temperature. According to Avogadro’s law, “At constant pressure and temperature equal volumes of different gases contain equal amounts of gas molecules”. Consequently, for every 5 CO_2-molecules insufflated to each measuring point, 5 air molecules will be moved and one of these 5 molecules will be an O_2-molecule. Hence, by measuring the O_2-content, the concentration of air and CO_2-gas is given concomitantly. The O_2-measuring device has a heated ceramic sensor that measures displacement of air more accurately than ordinarily used CO_2-sensors that are based on an infrared sensor technique to measure CO_2-concentrations up to 100%. The ceramic O_2-sensor has in comparison a higher accuracy, requires less gas, and is quicker than standard CO_2-sensors.

3.3.2 Single channel microbubble Doppler detector

In **Study I** we used a single channel microbubble Doppler detector (CMD-10, Hatteland Instrumentering, Royken, Norway), which was attached to the arterial line. The velocity range of the bubble detector was 2.5 - 5 m/s and measured bubbles sizing from around 10 - 300 µm. The maximum counting rate was around 2000 microbubbles/min. A thirteen mm (3/8” I.D. tubing) conventional probe was connected to the arterial line. The CMD-10 was linked to a personal computer using the BubmonWv1.3 software to record number of microemboli. The Hatteland CMD10 single-channel microbubble detector does not measure bubble sizes accurately.

3.3.3 Multifrequency transcranial Doppler

Study IV: The noninvasive TCD technique measures blood flow velocity in cerebral arteries using the Doppler effect. It is used clinically to discover and evaluate intracranial vasospasm in patients with subarachnoid hemorrhage, diagnose cerebrovascular ischemia (steno-occlusive disease, acute ischemic stroke), assess intracranial arterio-venous malformations, cerebral microemboli, and cerebral perfusion after circulatory arrest.[61]

An ultrasound probe positioned over the thinnest part of the temporal bone, the temporal window, emits waves that are transmitted through the skull and the frequency change of the reflected ultrasound waves are also registered by the probe and correspond to the velocity of the moving red blood cells within the insonated cerebral vessel (the Doppler shift frequency). Also, the direction of the blood flow can be detected. A 2 MHz frequency ultrasound probe was used for the TCD measurements, (Embodop®, DWL, Singen, Germany) including the identification of the intracranial carotid artery bifurcation, where after the MCA can be identified by having a blood flow direction towards the probe.[61, 62]

Microemboli passing the insonated vessel deflect ultrasound waves, which return and are registered as high-intensity transient signals (HITS), **Figure 2**. The multifrequency technique allows automatic differentiation of gaseous and solid microemboli due to different backscatter profiles. Solid emboli reflect more intense ultrasound signals at the higher frequency, whereas gaseous microemboli reflect more intense signals at the lower frequency.[63-65] We used the power M-mode Doppler (PMD) that provides simultaneous flow signals by overlapping sample volumes that facilitates the “tracking” of the emboli.[66] Particulate as well as gaseous microemboli are registered as microembolic signals (MES). The cut-off level for detection of microemboli was set as a ≥ 7 dB increase above the background level (delta embolus blood ratio [dEBR]) lasting ≥ 4 ms concurrently in the 2.0- and the 2.5-MHz frequency channel, respectively. The patients´ MCAs were detected at a depth of 40 - 55 mm from the skin level at the temporal window. We used a sample volume of 13 mm, no filter, a power of 200 mW, and an 80 cm/s scale. To reject artifacts online a reference gate was set 15 mm superficially to the MCA insonation gate.

TCD monitoring was continuously performed from the beginning of the sheath insertion and ended when the final catheter had been withdrawn and corresponded to predefined procedural steps during coronary angiography.[61, 63-65]

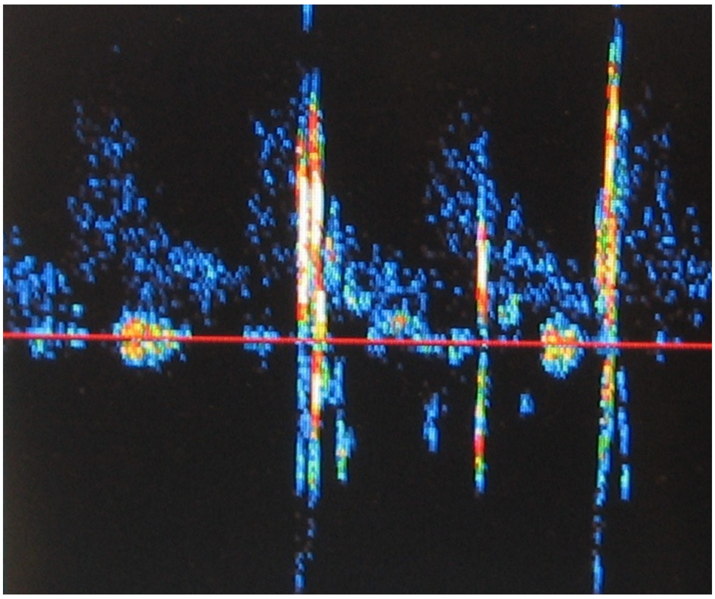

FIGURE 2. Pulsating transcranial Doppler blood flow velocity signal including several high-intensity transient signals from the insonated middle cerebral artery.

3.3.4 Devices for insufflation of CO_2-gas

3.3.4.1 Devices for insufflation of CO_2-gas in minimally invasive open-heart surgery

The CO_2-diffuser (CarbonMini® Cardia Innovation AB, Stockholm, Sweden), a single-use insufflation device, consisting of three parts. A two meter long polyvinyl chloride (PVC) tube that has an inner diameter of ¼ inch (6.35 mm), a bacterial filter (ORO1H from Pall Medical, Portsmouth, U.K.), a 0.20 m PVC tube that has an inner diameter of 1 mm inside which a metal thread runs, and a cylindrical diffuser consisting of soft polyurethane, with a diameter of 7 mm and a length of 17 mm, and corresponding to a surface area of approximately 4 cm^2, **Figure 3**. As a control device, we used a plastic tube with an inner diameter of 1 mm, that is frequently used for CO_2-insufflation during minimally invasive open heart surgery.

FIGURE 3. The mini CO_2-diffuser (CarbonMini® Cardia Innovation AB, Stockholm, Sweden), is a single-use insufflation device that consists of three parts, a two m long PVC tube, a bacterial filter, a 0.20 m PVC tube that has an inner diameter of 1 mm inside which a metal thread runs, and a cylindrical diffuser consisting of soft polyurethane (approx. 4 cm^2).

3.3.4.2 Device for insufflation of CO_2-gas in full sternotomy open heart surgery

In **Study III**, de-airing of the open cardio-thoracic surgical wound cavity was performed before cannulation and continued using CO_2-gas insufflation at 10 L/min via a CO_2-diffusor (CarbonAid® Cardia Innovation AB, Stockholm, Sweden), **Figure 4**, until closure of the heart and aorta. We positioned the CO_2-diffuser in the open surgical wound cavity at a depth of > 4 cm from the edge of the wound surface.

FIGURE 4. The standard CO_2-diffuser (CarbonAid® Cardia Innovation AB, Stockholm, Sweden), a single-use insufflation device, which includes three parts, a two m long PVC tube, a bacterial filter, a 0.20 m PVC tube that has an inner diameter of 1 mm inside which a metal thread runs, and a cylindrical diffuser consisting of soft polyurethane (approx. 10 cm^2).

3.3.5 Suction rate measurements

Using a back-pressure compensated O_2-flowmeter, the CO_2-gas flow was determined, as CO_2-flowmeters are seldom used clinically. The O_2-reading scale was accustomed for CO_2 with a universal flowmeter (ABB/Fisher & Porter, Göttingen, Germany), due to the higher density of CO_2-gas. The universal flowmeter comprised of a measuring tube (FP ¼ -16 G-5/81) including a spherical stainless-steel float (SS-14). Nevertheless, we did not apply the universal flowmeter for direct quantifications in **Study II**, because of absence of compensation for back-pressure. During the calibration this disadvantage was circumvented by determining the CO_2-outflow distal to the insufflation devices. The reading scale of the universal flowmeter was calibrated for medical CO_2-gas (AGA Gas AB, Stockholm, Sweden) at 20 ºC and at 1013 mbar using a software (FlowSelect version 2.0, ABB/Fisher & Porter, Göttingen, Germany). We avoided the use of a coronary suction device during measurements. The suction rate of the rough suction device was 5, 10, and 15 L/min, respectively, determined with the help of a flowmeter.

3.4 STUDY INTERVENTIONS

Study I, an in vitro study, consisted of two parts. First, we developed a procedure to guarantee absolute filling of the empty CPB-system with CO_2-gas (> 99%). Secondly, we measured the amount of microemboli passing through the circuit in the prime fluid. Ten CPB circuits were used in the first study part, with the aim to ensure that more than 99% of the inner volume of the CPB system contained CO_2-gas. **Figure 5** depicts the used CPB system, which was filled with CO_2-gas from the highest part of the venous reservoir (A).

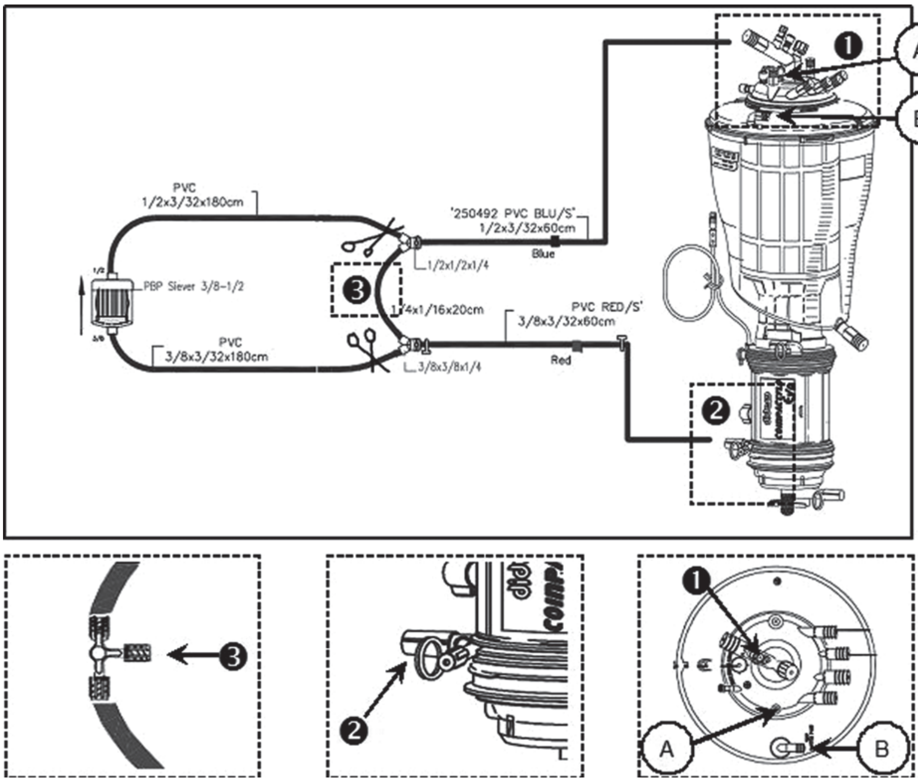

FIGURE 5. The pictures summarize how **Study I** was setup. (A) corresponds to the inlet for CO_2-gas and (B) represents the overpressure valve. The measuring points are situated at highest part of the venous reservoir (1), at the outflow of the oxygenator (2), and at the 3-way Luer lock where the samples from the shunt were taken (3).

The CO_2-gas was insufflated via a 0.2 µm bacterial filter at a flow rate of 10 L/min and the CO_2-concentration was determined at 3 sites; The measuring points are situated at highest part of the venous reservoir (1), at the outflow of the oxygenator (2), and at the 3-way Luer lock where the samples from the shunt, between the arterial and venous tube, were taken (3). In order to evacuate surplus gas, we detached the overpressure peg-valve (B) situated on the highest part of the venous reservoir. The measurements at the above described 3 sites were performed during a period of ten minutes with one-minute intervals. We determined the CO_2-concentration at 3 locations, each site denoting an important part of a standard CPB circuit, which was not performed in the single previous report on this topic.[67] However, in that research paper the CPB system lacked a venous reservoir and the CO_2 measurements were sampled in the same arterio-venous line, distal to the CO_2-gas inlet. There was only a 40 µm arterial filter between those two measuring sites, and the total circuit volume was just 575 ml, around 10% of the volume applied in our study, when measured from the CO_2-gas inlet point of the sampling port.

In the following segment of **Study I** we used 20 CPB circuits. CPB-circuits were randomized to an intervention (n = 10) or a control group (n = 10), immediately before the start of each experiment using unmarked envelopes. In the intervention group, we insufflated each CPB system with CO_2-gas at the highest point of the venous reservoir for 5 minutes, see above.

Thereafter, the CPB-system was filled slowly with priming fluid during continuous CO_2-gas insufflation. In the control group we abstained from filling the CPB systems with CO_2-gas, whereby the CPB-circuits only held air. After stopping CO_2-gas insufflation in the intervention group, a perfusionist blinded to group assignment set the flow rate of the main pump to 5 L/min and the 100% O_2-sweep gas flow of the oxygenator to 3 L/min, respectively. Subsequently, during the first minute, the perfusionist knocked on the oxygenator 20 times with an arterial line cross-clamp. Next, the whole CPB system was running without any intervention for 12 minutes. During the 14th minute the perfusionist once more tapped 20 times on the oxygenator. Number of microemboli were counted till the 15th minute had passed.

Study II: We placed the distal end of the mini-diffuser pointing to the base of the experimental surgical wound cavity at a depth of 25 mm, measured from the edge of the wound opening. CO_2-gas was insufflated in the wound cavity via the mini-diffuser at 2, 3, 4, 5, and 10 L/min, respectively. The air concentration of the experimental wound cavity was determined during steady state in each quadrant, 15 mm below the edge of the wound opening and at the bottom of the wound cavity. The de-airing efficacy of the mini-diffuser and the control 1 mm open-ended tube, respectively, was measured during fixed settings without involvement of suction devices. Thereafter, the efficacy of the mini-diffuser was additionally tested with different suction rates, using a suction device (Kendall Argyle Yankauer suction tube (fine), Tyco Healthcare, 4.0 mm, Ireland) during and after a 2 second suction period, or during uninterrupted suction at 15 mm beneath the edge of the wound orifice. The O_2-concentration was determined 40 mm below the wound area opening at steady state, i.e. 60 seconds of stable values, prior to start of intermittent (2 s) or uninterrupted suction, with ten measurements under each condition. Following initiation of intermittent suction, the O_2-concentration was determined every 5 seconds for 25 seconds. Following initiation of uninterrupted suction, the O_2- concentration was determined every ten seconds for ninety seconds. Residual CO_2-gas in the cavity was evacuated via a rough suction device prior to each alteration in CO_2-flow rate, insufflation device, or measuring site. In 6 patients undergoing minimally invasive AVR via a six cm J-shaped partial sternotomy, from the cranial part of the manubrium to the 3rd intercostal space, air concentration was determined four cm beneath the edge of the wound opening, when insufflating the open surgical wound cavity at a CO_2-flow of 5 and 8 L/min, respectively, with a mini-diffuser, positioned contra-lateral to the site of measurement.

In **Study III**, a CPB circuit set (Sorin Perfusion Tubing Systems, Sorin Group Italia S.r.l. Mirandola (MO), Italy) consisting of an oxygenator, a centrifugal pump and a standard venous reservoir was set up with an additional venous reservoir (Sorin Inspire HVR, Sorin Group Italia S.r.l. Mirandola (MO), Italy), to which the coronary suction device (suction rate of 0.25-1 L/min,) was connected as well as an extra cardiotomy suction device (suction rate 1 L/min), placed in the wound cavity 4 cm below the skin surface. The additional venous reservoir was positioned above and connected with a tube to the standard CPB cardiotomy reservoir. To enable blood sampling Luer lock connections were inserted in the CPB circuit; 1) at the venous return tubing before entrance to the standard venous reservoir, 2) between the standard venous reservoir and the oxygenator, and 3) on the arterial line of the circuit. An on-line device for blood gas analyses (CDI™ 500, Terumo Cardiovascular Systems Corporation, MI, USA), was attached to the arterial line. During CPB with standard configuration, the **conventional setup**, blood as well as gas from both the coronary and cardiotomy suction devices were without interruption drained through the separate reservoir to the conventional reservoir. During the **intervention setup** the line connecting the separate and conventional venous reservoir was closed with a clamp. Accordingly, drained blood and gas from the coronary and cardiotomy suction devices stayed in the separate venous reservoir. If the blood volume in the separate venous reservoir exceeded 800 ml it was drained to the conventional reservoir, leaving at least 100 ml of blood in the separate venous reservoir to avoid spillover of CO_2-gas.

The patients were treated according to routine before clamping of the aorta, including venous and arterial cannulation of the right atrium and the ascending aorta, respectively. De-airing of the open surgical wound was initiated before cannulation and continued using CO_2-gas insufflation of 10 L/min via CO_2-diffusor (CarbonAid® Cardia Innovation AB, Stockholm, Sweden) until closure of the heart and aorta. A recent study by independent authors found the device used in our study, the CarbonAid® diffuser, to be superior to all other tested devices, delivering 100% CO_2 concentration in the tested surgical cavity with CO_2-gas insufflation rates of 7 - 10 L/min.[44] We positioned the CO_2-diffuser in the open surgical wound cavity more than 4 cm below the edge of the wound. The perfusionist adjusted the sweep gas flow of the oxygenator aiming to keep a $PaCO_2$ of approximately 5.3 kPa by following the on-line arterial gas data (CDI™ 500). Five minutes after arterial blood gas steady state was reached in either setup, blood gas samples were drawn from the three sample points (see above) and measured with a conventional blood gas analyzer (ABL 825 Flex, Radiometer Medical ApS, Brønshøj, Denmark). Using a crossover design, the setup was switched as many times as possible, ranging between 2 and 6 times (median 4) for each patient between the **interventional** and the **conventional setup** and the mean values from the three sampling points for each setup for each patient were used for comparison between patients.

TABLE 1. Predefined parts of the coronary angiography

Stages	Practical implementation
1. Arterial access	Insertion and flushing of introducer
2. Insertion of first catheter	Insertion of guidewire* Insertion of catheter Removal of guidewire Withdrawal of blood and flushing of catheter
3. Coronary angiography	Engagement of coronary ostium Contrast injection
4. Exchange of catheters	Disengagement of catheter Introduction of guidewire Removal of first catheter Insertion of new catheter Removal of guidewire Withdrawal of blood and flushing of catheter
5. Coronary angiography	Engagement of coronary ostium Contrast injection
6. Removal of guidewire and catheter	Disengagement of catheter Introduction of guidewire Removal of guidewire and the catheter

(*) with the femoral access technique, guidewire and catheter were inserted together

Study IV. Following randomization to radial or femoral approach, the coronary angiography process was separated into six predefined parts reflecting catheter swap and engagement of coronary ostia, **Table 1**. Coronary angiography was performed by two interventional cardiologists using selective catheters for the right and the left coronary artery, respectively. When using the radial access, a pharmacological mix consisting of 250 µg glycerylnitrate, 5000 units of Heparin, and 2.5 mg verapamil hydrochloridium in 15 ml of 0.9% saline, was injected into the 6 French Flexor Radial sheath (Cook, Denmark). Directly after its insertion, the tip of the 6 French JL 3.5 and JR 4 catheter (Boston Scientific), respectively, was positioned in the Valsalva sinus and substituted over a 0.035” 250 cm guide wire (Cordis). Ultimum 6 French sheaths (St. Jude Medical) were used to access the femoral artery, and 6 French JL and JR 4 catheters (Boston Scientific) and 0.035” 150 cm guide wires (Boston Scientific) were inserted into the ascending aorta. All catheters and guide wires were heparinized before use. Every patient received the standard contrast medium, Visipaque 320 mg/mL (GE Healthcare). Duration of fluoroscopy and volume of injected contrast were recorded. Simultaneously, we recorded the amount of passing cerebral microemboli the MCAs bilaterally with a multifrequency TCD system (Embodop, DWL). Using a synchronized clock, TCD measurements were correlated with the predefined parts of the coronary angiography procedure.

3.5 ETHICS

The research papers in the thesis follow the principles of the Helsinki Declaration and the Regional Ethical Review Board in Stockholm endorsed the studies (**Study II**: 2015/323–32; **Study III**: 2018/1091–31; **Study IV**: 2006/1077-31/2). All included patients agreed to participate in the studies, **Study II-IV**.

3.6 STATISTICS

Study I-IV. Data were analyzed with SPSS® statistical program (http://www.spss.com). We considered a two-sided probability level of less than 0.05 as significant.

Study I. The Kolmogorov-Smirnov analysis was applied to test for normality distribution of data. Otherwise, conventional non-parametric tests were used. We expressed results as median, with interquartile range (IQR).

Study II. Data were described as mean ± standard deviation (SD). Due to the small numbers, we used the non-parametric Kruskal-Wallis and Mann-Whitney U-tests, which were used when appropriate.

Study III. No power calculation was performed as there are no available data with this setup. We applied the paired Mann-Whitney U-test for comparison between groups, as there were few patients.

Study IV. We used the Mann–Whitney U-test for continuous variables, and X^2-test and Fisher's exact test for categorical data. Based on data from Lund et al.[4] reporting a 40% decrease in number of particulate microemboli when using the femoral (n = 10) compared with the radial (n = 37) artery access, approximately 22 patients were needed to find a significant difference between the two approaches with a power of 80%.

4 RESULTS

4.1 STUDY I

According to **Figure 6**, CO_2-gas insufflation into the CPB system caused a quick rise in CO_2-concentration at the 3 measuring sites reaching more than 99.00% within 4 minutes. Then, the mean values stabilized and barely augmented with centesimal of percentages for every additional minute. The measured data at the oxygenator (Site 2) were significantly lower compared with measured data at the venous reservoir and at the shunt (Site 1 and 3) for each time point.

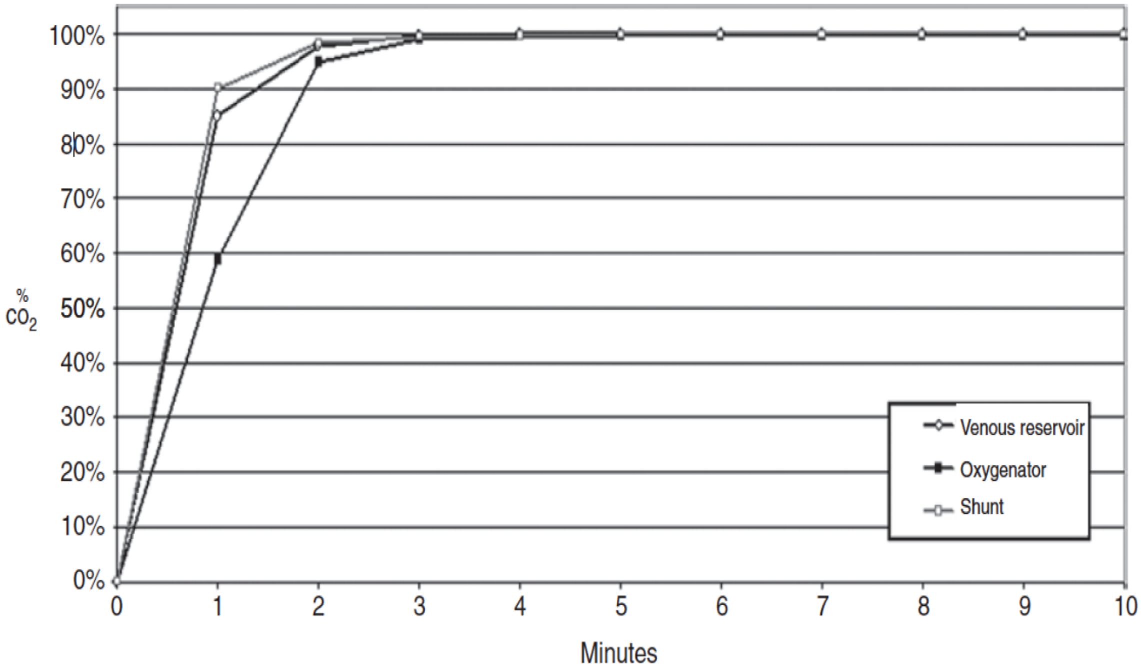

FIGURE 6. Mean CO_2 (%) at 3 measuring sites during CO_2-insufflation into the CPB circuit.

Figure 7 shows median amount of microemboli for the groups for each minute throughout the experiment, which lasted 15 minutes. During the first minute, the median number of microemboli peaked in both the control and the intervention group, with 380.5 (IQR 288.75/422.25) and 264.5 (IQR 171.75/422.25) counts, respectively ($p = 0.01$). The number of microemboli stayed higher ($p \leq 0.004$) in the intervention group with CO_2-gas, compared with the control group, when tested every minute during the experiment. In both groups, median values fell considerably between the first two minutes, with median values falling to 12% (44.5/380.5, $p < 0.01$) in the control compared with 4% (9.5/264.5, $p < 0.01$) in the intervention group. Thereafter, between minute 2 and 13 in both groups, the number of microemboli declined gradually, with the lowermost values at the 13th minute, with median counts of 7.5 in the control group versus 0 in the intervention group. The 14th minute showed the same pattern as the 1st, during which the oxygenator was once again tapped on 20 times. This resulted in a remarkable rise in number of microemboli versus the 13th minute ($p < 0.01$). The median values increased to 173 in the control group versus 87 in the intervention group. During the 15th minute the number of microemboli once again fell substantially in the two groups ($p < 0.01$), resulting in a median value of 15.5 in the control group versus 0.5 in the intervention group. These values corresponded to 9% in the control group and 0.5% in the intervention group of the values registered during the 14th minute. Those values corresponded to 35% (15.5/44.5) in the control group versus 5% (0.5/9.5) in the intervention group of the values during the 2nd minute.

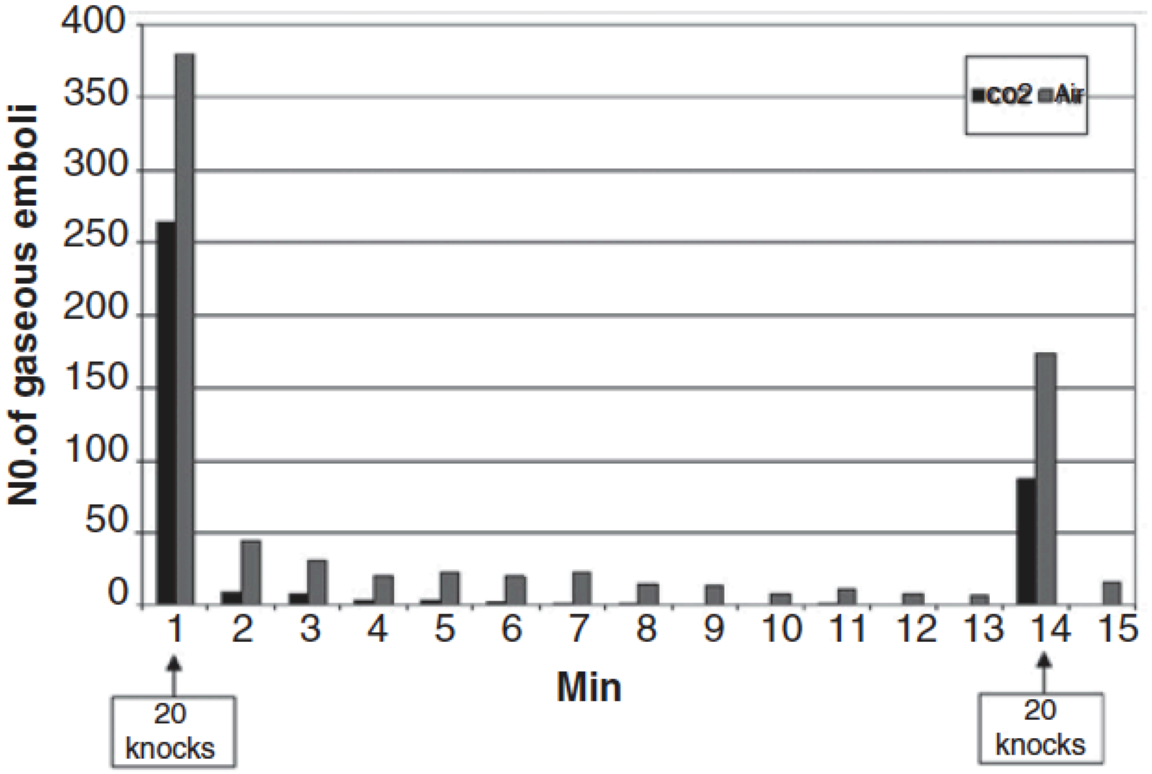

FIGURE 7. Median microembolic count for the intervention group (CO_2-gas) and the control group (air) during the 15 minutes of the study. The perfusionist tapped on the oxygenator twenty times during the 1st and 14th minute (arrows), which resulted in an increase in number of gaseous microembolic counts.

4.2 STUDY II

In the wound model using the mini-diffuser the content of air at a depth of 15 mm was 0.5 ± 0.2%, 0.5 ± 0.2%, 0.6 ± 0.3%, 0.6 ± 0.3%, and 1.5 ± 1.2% during continuous CO_2-insufflation of 2, 3, 4, 5, and 10 L/min, respectively. 40 mm below the edge of the wound cavity opening the related values were 0.8 ± 0.3%, 0.5 ± 0.2%, 0.4 ± 0.2%, 0.3 ± 0.2%, and 0.2 ± 0.1%, respectively, **Figure 8**.

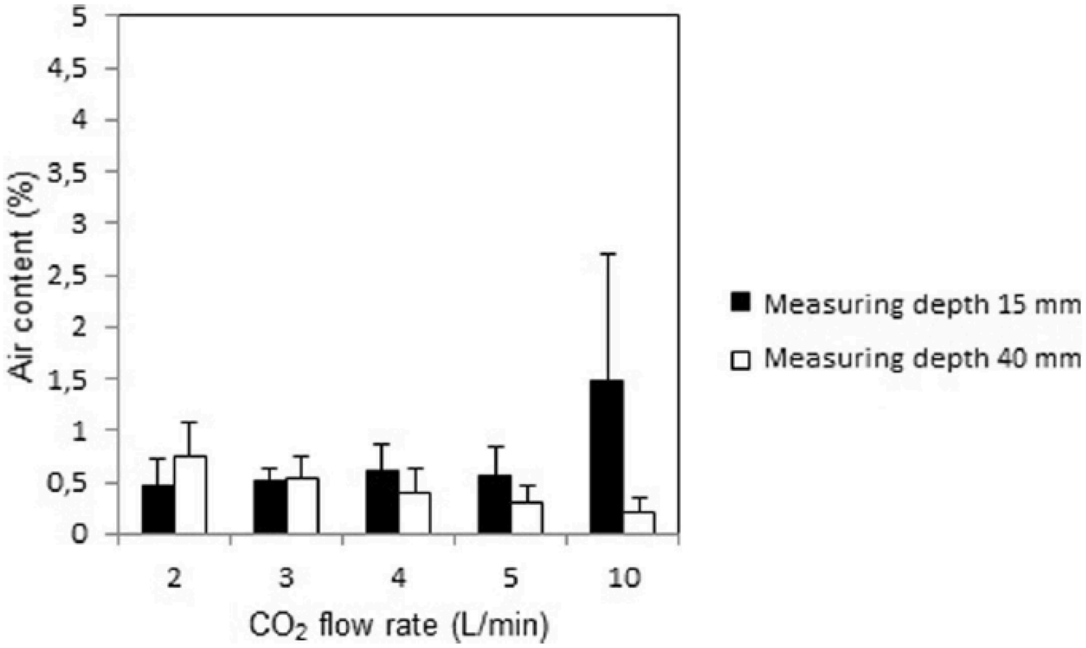

FIGURE 8. Measured content of air under stable conditions during inflow of CO_2-gas when applying various flows in an experimental minimally invasive surgical wound cavity with the new mini-diffuser. The air concentration was assessed 15 mm and 40 mm beneath the edge of the wound cavity opening.

Using the open-ended tube, the control device, to fill the experimental wound cavity with CO_2-gas, the mean concentration of air was 10% - 20% at 2 and 3 L/min of CO_2-gas, and the mean concentration of air augmented to 20% - 75% at CO_2-flows of 4, 5 and 10 L/min, respectively,

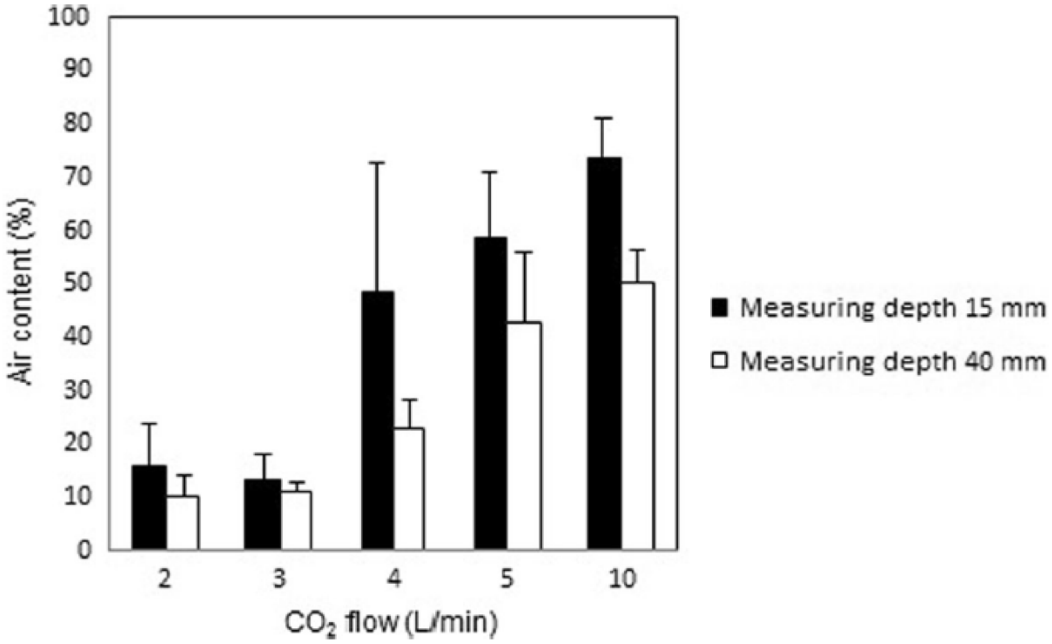

FIGURE 9. Measured content of air at steady state during CO_2-insufflating at different flow rates in an experimental minimally invasive surgical wound cavity using an open-ended tube (inner diameter of 1 mm). The air concentration was determined 15 mm and 40 mm beneath the opening edge of the cavity.

Figure 9. Consequently, when compared with the control, the mini-diffuser displaced air in the experimental wound cavity substantially more effectively at all flows ($p = 0.02$). The mini-diffuser de-aired the experimental minimally invasive surgical wound cavity extremely efficiently, < 1% residual air, in contrast with the open-ended tube, > 40% residual air, **Table 2**.

TABLE 2. Summarizing comparison between the experimental results of the new device to the control.

Insufflation device	Open-ended tube (1 mm inner diameter)		Mini-diffuser	
CO_2-gas flow	5 L/min	10 L/min	5 L/min	10 L/min
Mean air concentration in the experimental surgical wound cavity	43%	49%	< 1%	< 1%

Figure 10 illustrates the air concentration when applying a rough suction rate of 10 L/min for 2 seconds. A minor significant increase in concentration of air happened after 5 seconds at every flow rate ($p < 0.001$). Thereafter, the concentration of air returned to stationary levels for all flows after ten seconds. Hence, despite a period of 2 seconds with rough suction rate of 10 L/min the concentration of air stayed < 1% at every CO_2-flow, with the exception of the low flow rate of 2 L/min. This flow rate increased the content of air to circa 2.4% ($p < 0.001$). Figure 11 depicts air concentration after a 2 second period of rough suction of 15 L/min. When a suction force of 10 L/min was added, the mean concentration of air in the cavity increased significantly after five seconds at every CO_2-gas flow rate ($p < 0.001$). Conversely, the mean concentration of air merely rose over 1% at a CO_2-flow of 2 and 10 L/min. The concentration of air reverted to stable levels for every CO_2-flow in 10 seconds that was like when using a suction force of 10 L/min. The continuous use of a rough suction force of 5, 10, and 15 L/min, at a CO_2-flow of 2, 3, 4, 5, and 10 L/min, respectively, caused an unambiguous outline. For each rise in CO_2-gas flow rate the concentration of air diminished significantly ($p < 0.001$) for the 3 suction rates. The only exception was when we augmented the CO_2-gas flow from 5 to 10 L/min, the content of air did not change statistically when applying a rough suction force of 5 L/min ($p = 0.21$).

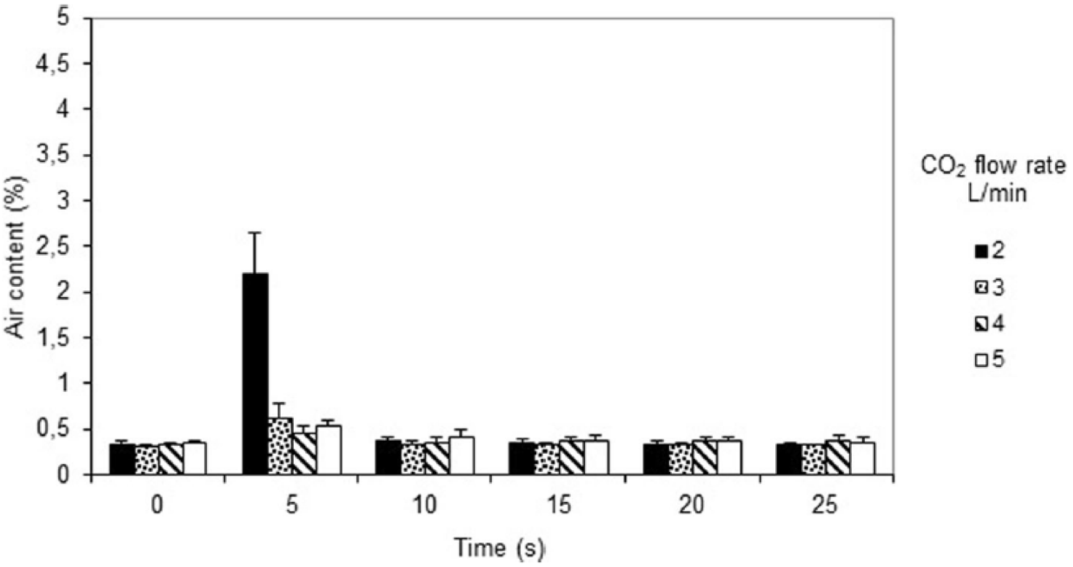

FIGURE 10. Content of air measured every 5 seconds during CO_2-gas insufflation with the new mini-diffuser and after applying a rough suction force of 10 L/min during the first two seconds. CO_2-gas flow rates of 2, 3, 4, and 5 L/min, respectively, were used in this setup.

The mean concentration of air was ≤ 10% at a CO_2-flow rate of 4, 5, and 10 L/min, respectively, with a uninterrupted rough suction force of 5 and 10 L/min, **Figure 12**. Using a CO_2-flow of 10 L/min the concentration of air stayed beneath 10% when applying uninterrupted suction forces of 5, 10, and 15 L/min. The mean concentration of air in the minimally invasive cardiothoracic wound cavity of six patients, who underwent AVR, was 0.4 ± 0.5% and 0.6 ± 0.7%, when delivering CO_2-gas at a rate of 5 and 8 L/min, respectively, via the mini-diffuser.

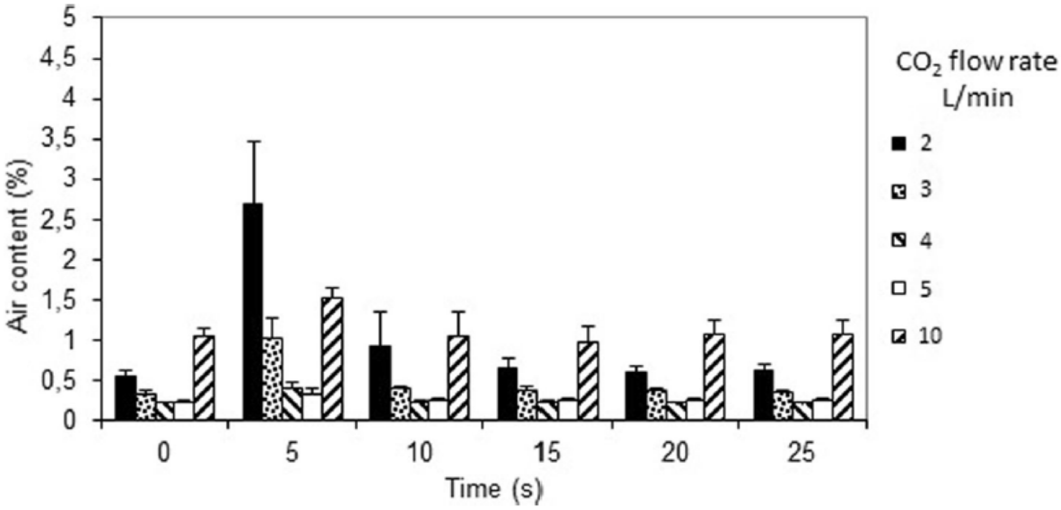

FIGURE 11. Content of air measured every 5 seconds during insufflation of CO_2-gas with the new mini-diffuser and after applying a rough suction force of 15 L/min during the first two seconds. CO_2-gas flow rates of 2, 3, 4, 5, and 10 L/min, respectively, were used in this setup.

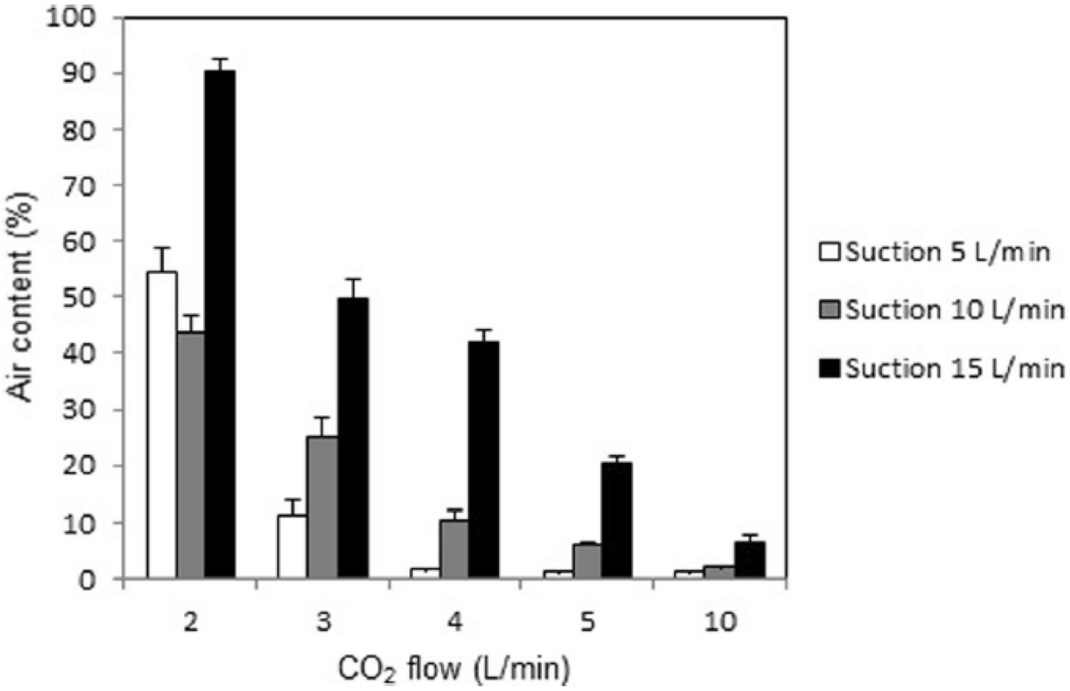

FIGURE 12. Air concentration at stable conditions when insufflating CO_2-gas with the new mini-diffuser and when concomitantly applying uninterrupted a rough suction rate of 5, 10, and 15 L/min. CO_2-gas flow rates of 2, 3, 4, 5, and 10 L/min, respectively, were used in this setup.

No patient had symptoms of postoperative cerebral or cardiac deterioration, **Table 3**.

TABLE 3. The essential results when applying the mini-diffuser in six adults undergoing minimally invasive aortic valve replacement.

	Mini-diffuser		**Postoperative stroke or cognitive dysfunction**	**Decrease in regional right or left ventricular function after CPB**	**Ventricular arrhythmias after CPB**
CO_2-flow rate	5 L/min	8 L/min	0/6	0/6	0/6
Mean air content in surgical cavity	<1%	<1%			

CPB = Cardio-pulmonary bypass

4.3 STUDY III

Table 4 depicts patient characteristics. Eighty percent of the patients were men and the median age was 62 years (IQR: 54.25 - 64.5).

TABLE 4. Patient characteristics

Characteristic	median (IQR)
Age, years	62 (54.25-64.5)
Female gender, no (%)	2 (20)
Height, cm	179 (175 – 185)
Weight, kg	80 (75 – 88)
Body mass index, kg/m^2	26.1 (22.8 – 29.3)
Creatinine µmol/L	92 (83 – 102)
eGFR, mL/min	66 (59 – 76)
EuroSCORE II	1.08 (0.67 – 2.73)
Diabetes Mellitus, no (%)	1 (10)
CPB duration, min	90 (80 – 108)
Aortic cross clamp, min	69 (65 – 90)
Pump flow, L/min	4.70 (4.58 – 5.03)

Abbreviations: CPB = Cardio-pulmonary bypass, eGFR = estimated glomerular filtration rate, was calculated using the Cockcroft-Gault formula.

Median $PaCO_2$ did not differ between the conventional and the interventional setups (5.41; IQR: 5.29 - 5.57, vs. 5.41; IQR: 5.24 - 5.58, p = 0.92), whereas median sweep gas flow (L/min) was significantly lower (2.58, IQR: 2.50 - 3.16 vs. 4.42, IQR: 4.00 - 5.40, p = 0.002) when CO_2-gas was not drained from the additional to the standard reservoir, the interventional setup. All blood gas results from the three sampling points and the sweep gas flows during the interventional and conventional setups are presented in **Table 5**.

TABLE 5. Results from separating collection of blood and gas from the open surgical wound during cardio-pulmonary bypass with an additional cardiotomy reservoir during de-airing with CO2-gas insufflation

Characteristic	Use of additional separate venous reservoir	Standard use of venous reservoir	*P*-value
$PaCO_2$, kPa	5.41 (5.29 – 5.57)	5.41 (5.24 – 5.58)	0.92
Arterial pH	7.35 (7.33 – 7.37)	7.36 (7.33 – 7.37)	0.39
$PvCO_2$, kPa	6.17 (5.92 – 6.44)	6.13 (6.00 – 6.55)	0.77
Venous pH	7.33 (7.31 – 7.34)	7.32 (7.31 – 7.34)	0.87
Pre-oxygenator PCO_2, kPa	6.16 (5.91 – 6.31)	6.70 (6.28 – 7.00)	0.004
Pre-oxygenator pH	7.33 (7.31 – 7.35)	7.30 (7.28 – 7.33)	0.008
Sweep gas flow, L/min	2.58 (2.50 – 3.16)	4.42 (4.00 – 5.43)	0.002

Variables are described as median (Interquartile range). Abbreviations: $PaCO_2$ = CO_2 pressure in arterial blood. $PvCO_2$ = CO_2 pressure in blood from the venous cannula draining blood from the vena cava. Pre-oxygenator PCO_2 = CO_2 pressure in blood from the venous blood that passed the venous reservoir just before entering the oxygenator.

4.4 STUDY IV

340 patients were screened from February 2007 to June 2008. 51 patients were randomized to either radial (n = 28) or femoral (n = 23) approach, respectively. **Table 6** describes baseline characteristics. Every patient was on aspirin and patients in the radial group were given 5,000 units of intravenous heparin at start of coronary angiography.

TABLE 6. Patient characteristics

Characteristic	Angiography via Right Femoral Artery (n = 23)	Angiography via Right Radial Artery (n = 20)	*P* value
Age, y, mean (SD)	66.3 (7.6)	61.6 (8.8)	0.08
Female sex (%)	4 (17)	2 (10)	0.67
Hypertension	16 (70)	12 (60)	0.51
Diabetes mellitus	12 (52)	2 (10)	0.004
Previous stroke/Transitory ischemic attack	4 (17)	2 (10)	0.68
Previous acute myocardial infarction	3 (13)	4 (20)	0.68
Previous percutaneous coronary intervention	3 (13)	4 (20)	0.69
Number of coronary stenoses			0.03
No stenosis	5 (22)	12 (60)	
1-vessel disease	12 (52)	3 (15)	
2-vessel disease	4 (17)	2 (10)	
3-vessel disease	2 (9)	3 (15)	
Contrast volume (mL)	75 (60 - 100)	82.5 (50 - 160)	0.31
Fluoroscopy time (min)	2.5 (1 - 5)	6 (2 - 12)	<0.0001
Number of gaseous microemboli	44 (7 - 131)	58 (19 - 469)	0.08
Number of particulate microemboli	6 (1 - 19)	10 (1 - 120)	0.02
Right middle cerebral artery	2 (0 - 9)	7 (1 - 65)	0.004
Left middle cerebral artery	3 (0 - 15)	3 (0 - 55)	0.57

Values are mean ± SD, numbers (%) or median (minimum–maximum range).

We did not include eight patients in the analysis because of conversion from radial to femoral access. More patients in the radial group did not present pathological coronary arteries and had longer fluoroscopy durations. We identified cerebral microemboli in every patient.

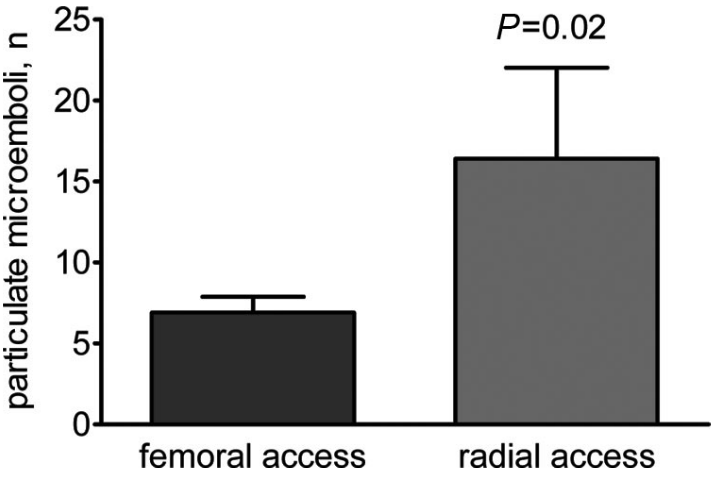

FIGURE 13. Number of particulate cerebral microemboli during coronary angiography. Mean ±SEM.

Most microemboli were gaseous, and predominately occurred during flushing of the catheters. However, number of gaseous microemboli differed insignificantly between the groups, **Table 6**. The median number of particulate microemboli was higher (67%) in the radial group (p = 0.02) compared with femoral group, **Table 6** and **Figure 13**.

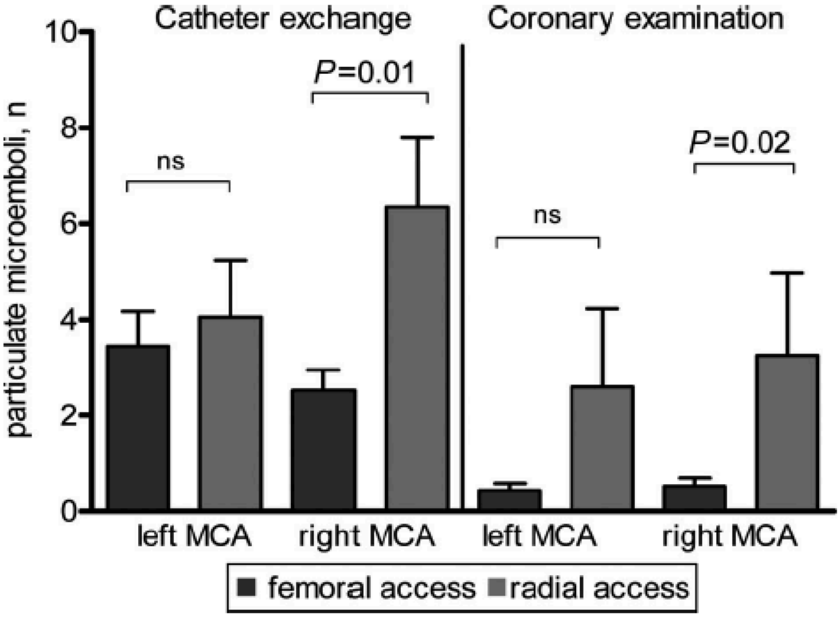

FIGURE 14. Number of particulate cerebral microemboli during coronary angiography when split into procedural phases. Mean ± SEM; ns = non-significant.

More particulate microemboli were registered during catheter exchanges than when the coronary arteries were examined in both groups (7 [range, 0 - 51] versus 1 [range, 0 - 67]; $p < 0.001$). When the left and right MCA were separately analyzed, significantly more particulate microemboli circulated through the right MCA with the radial approach, **Table 6**, mostly during catheter exchanges, **Figure 14**. We did not detect any strokes.

5 DISCUSSION

To minimize the occurrence of microemboli in cardiac surgery and coronary angiography several measures may be used. In the experimental **Study I**, it was shown that if an empty CPB circuit is insufflated with CO_2-gas from the highest part of the venous reservoir at a flow of 10 L/min, > 99% of all air is evacuated within 3 - 4 minutes. Moreover, in the experimental and clinical **Study II**, efficient CO_2 de-airing was accomplished with the mini-diffuser, reaching < 1% residual air in an experimental minimally invasive cardiothoracic surgical wound cavity, whether applying an intermittent rough suction force or not, and in patients undergoing minimally invasive AVR. In the clinical cross-over **Study III**, it was shown that the use of an additional venous reservoir for evacuation of blood from the open cardiothoracic surgical wound cavity prevents CO_2-insufflation induced hypercapnia, keeping $PaCO_2$ and sweep gas flow stable. This prevents CO_2 induced hyperperfusion of the brain and thus decreases the probability of cerebral embolization during CO_2-gas de-airing in open cardiac surgery. Finally, the RCT, **Study IV**, indicated that the radial approach for coronary angiography produced a significantly higher number of particulate cerebral microemboli than the alternative femoral approach, and thus consequently may have an impact on the incidence of silent cerebral lesions.

5.1 DE-AIRING OF A CPB CIRCUIT

The aim of **Study I** was to ensure a high CO_2-gas content in the CPB system as quickly as possible using conventional CPB setup. Subsequently, we applied a fixed high CO_2-gas flow of 10 L/min and we limited the time of CO_2-gas insufflation to 10 minutes. We detected that a sufficiently high CO_2-concentration, more than 99.5%, had been achieved at all 3 measuring sites of the CPB system within 4 minutes. Unexpectedly, the CO_2-gas concentration rose significantly slower in the oxygenator than at the highest point of the venous reservoir and at the shunt. One may speculate about the reason for these differences. It may be caused by an irregular allocation of the CO_2-gas in the hollow fibers of the oxygenator.

Not many studies have investigated the presence of microemboli during the initiation of CPB. To our knowledge, the study by Bucerius et al. is the only previous clinical study in this field. Those authors reported a higher number of air emboli at the start of CPB.[3] Furthermore, an experimental study by Martens et al. showed that arterial CO_2 microemboli disappeared significantly earlier than air bubbles, when entering a de-aired CPB system.[39] In **Study I**, when CPB-circuits containing air were recirculated for 15 minutes with prime fluid, involving 2 minutes when the perfusionist tapped with a clamp on the oxygenator, proficient de-airing of the CPB circuit was not accomplished. A prolonged duration of re-circulation, and/or with an extended time of knocking may possibly have accomplished this, but that is still uncertain. Even if these measures were sufficient, it would still be more time consuming and more laborious. In contrast, the CPB-circuit was efficiently de-aired when insufflated with CO_2-gas.

5.2 CLINICAL CONSEQUENCES OF AIR EMBOLI

One may debate whether rigorous de-airing of the primed CPB system is clinically relevant when starting CPB. However, previous studies have demonstrated that CO_2-gas microemboli are considerably better abided compared with air microemboli.[29-32, 40, 41] Injection of air, 0.5 mL/lb body weight, in the pulmonary vein in cats filled the cats' coronary arteries with air and was fatal to all animals.[32] Conversely, the same volume of CO_2-gas injected in the same manner was well tolerated in all cats. Even when a large volume of CO_2-gas, 6 mL/lb body weight, which was 12 times the fatal volume of air, was injected, none of the cats died, though a temporary total occlusion of the coronaries with CO_2-gas, was observed. These results clearly indicate that there is a remarkable difference in potential clinical outcomes between arterial air and CO_2 emboli. Likewise, Borger et al. found that postoperative neurocognitive deficiency correlated directly with number of perfusionist interventions that caused arterial air microemboli.[34]

5.3 DE-AIRING IN OPEN HEART SURGERY

To prevent air embolism in standard open cardiac surgery, CO_2-gas has for many years been insufflated in the open surgical wound cavity. However, almost two decades ago, conventional CO_2-insufflation with an open-ended tube was found to be ineffective because it produced turbulence and thus poor de-airing of the open surgical wound due to the outgoing high velocity jet.[43] This study by Persson et al. demonstrated that effective de-airing of an open surgical wound cavity could only be accomplished with a gas-diffuser that allows a high CO_2-gas outflow with a low velocity.[43] Additionally, to achieve efficient de-airing of the open surgical wound cavity the CO_2-diffuser has to be positioned inside and not at the orifice or outside the open surgical wound cavity.[43] The gas-diffuser is nowadays applied in open heart surgery in most western countries according to its manufacturer (Cardia Innovation AB, Stockholm, Sweden).

5.3.1 De-airing in minimally invasive open heart surgery

There is a need for a new small CO_2-diffuser that does not interfere in minimally invasive open heart surgery. However, the new CO_2-diffuser needs to be large enough to accomplish effective de-airing when used in a small open surgical wound cavity. The tested small gas-diffuser was devised to fulfill those prerequisites, and in **Study II** we verified the mini-diffusers effectiveness and also compared it with an alternative device, an open-ended tube, which may sometimes be applied in minimally invasive open heart surgery. We used an experimental setup to ensure that several variables known to have an impact on de-airing efficiency during cardiac surgery in an open wound could be altered, including wound cavity geometry, CO_2-flow rates, and suction forces in the wound cavity when using a suction device.[68] We measured the wound size in adults undergoing minimally invasive AVR via a partial sternotomy wound to create an experimental wound cavity model.

5.3.1.1 Influence of CO_2-insufflation rate

Continuous CO_2-insufflation is a prerequisite to avert air from getting into the open heart and vessels, as else air confined in these cavities cannot easily be displaced. The CO_2-insufflation flow was between 2 and 10 L/min. The lower flow rate should neutralize the impact of diffusion from ambient air, and the high flow rate is the advocated CO_2-insufflation rate of the large diffuser when used in adult patients undergoing open heart procedures via a full sternotomy. Likewise, we determined the air concentration both at the bottom of the experimental cavity as well as near its orifice. This corresponds to the anterior part of the ascending aorta, where the force of diffusion from ambient air will be strongest.

5.3.1.2 Influence of rough suction rate

The intermittent application of a usually handheld suction device is an additional factor, which may have an impact on the efficacy of CO_2 de-airing in open heart surgery. For this reason, we placed a rough suction device in the experimental minimally invasive wound cavity and utilized a suction force of either 5, 10, or 15 L/min where a bleeding from the artificial ascending aorta would most likely occur. De-airing using the open-ended tube caused an air content $> 10\%$ at a CO_2-insufflation rate of 2 L/min and augmented markedly with every increase in CO_2-gas insufflation rate. The resulting high concentrations of air were most likely due to turbulence produced by the high outflow velocity of the CO_2-gas exiting the tiny opening of the open-ended tube. Conversely, with the new mini diffuser, de-airing of the experimental minimally invasive wound cavity produced an air concentration that stayed $< 1\%$ at CO_2-insufflation flows between 2 and 5 L/min, and $< 2\%$ at a CO_2-insufflation flow of 10 L/min. Using the mini-diffuser, CO_2-insufflation rates between 3 and 5 L/min minimizes the remaining air content, while the CO_2-insufflation flow of 10 L/min minimizes the varying impact of the surgeon´s hand movements and different kinds of suction devices on de-airing efficiency.

In cardiac surgery two different suction devices are usually applied. A device with a low suction rate, 0.25-1 L/min, is frequently used at the bottom of the cardiotomy for constant removal of blood. This suction device does not impede de-airing unless the suction force is approximately similar to or above the used CO_2-gas flow.

A high suction rate is intermittently applied with a rough suction device to evacuate blood from the operation field and to facilitate optimal surgical exposure without affecting CO_2 de-airing in standard cardiac surgery via a complete sternotomy.[68] When intermittently applying a rough suction rate of 10 L/min, the air concentration was $< 1\%$ at CO_2-gas flows between 3 and 5 L/min in the experimental minimally invasive surgical wound cavity, while the concentration of air exceeded 2.5% at a CO_2-gas flow of 2 L/min. An augmentation of the discontinuous suction rate to 15 L/min would have required an increased CO_2-gas flow. Undeniably, using a discontinuous suction force of 15 L/min, the air concentration stayed $< 1\%$ with CO_2-insufflation flows of 4 to 5 L/min, and close to 1% at a CO_2-gas flow of 10 L/min. When applying a continuous rough suction force of 15 L/min in the experimental minimally invasive surgical wound cavity at CO_2-gas flows of 2 to 5 L/min, the air concentration remained over 20%. With a CO_2-insufflation flow of 10 L/min, the air concentration dropped below 7%. Consequently, when applying a continuous rough suction rate of 10 L/min in the experimental minimally invasive surgical wound cavity, the air concentration remained $> 20\%$ with a CO_2-insufflation flow of 2 and 3 L/min, while with a CO_2-insufflation flow of 4, 5, and 10 L/min the air concentration was below 10%. Thus, to prevent air confinement uninterrupted suction should be circumvented, particularly when the heart and large vessels are opened. The coronary suction devise will usually be applied with a substantially lower suction force for continuous suction of blood if profuse bleeding occurs, and will hence not influence de-airing. The mini-diffuser was also examined in patients undergoing minimally invasive AVR and the mini-diffuser de-aired the small open wound cavity effectively, with an air content $< 1\%$, at an uninterrupted CO_2-insufflation flow of both 5 and 8 L/min. Accordingly, the high CO_2-insufflation rate of 8 L/min should be applied when the surgical team anticipates to apply a rough suction device very often or for longer periods.

5.3.1.3 Does CO_2 de-airing affect cerebral microembolization?

A previous RCT in adults having conventional valve surgery via a complete sternotomy[12] and either receiving CO_2-insufflation in the open surgical wound cavity via a conventional gas-diffuser, or not, number of microemboli in the left ventricle, left atrium and in the ascending aorta were estimated using intraoperative transesophageal echocardiography (TEE). Period of measurement began at the time-point of X-clamp release till 20 minutes after terminating CPB. The cardiac surgeon executed conventional de-airing movements while blinded to the TEE results. Later, a blinded evaluator compiled the maximal count of microemboli minute by minute. The median count of microemboli during the study was 161 and 723 in the CO_2-insufflation group and in the control group, respectively ($p < 0.001$). In the CO_2-insufflation group, the estimated median microemboli count dropped to null seven minutes after end of CPB in contrast to 19 minutes in the control group ($p < 0.001$). The studied microemboli followed a typical pattern. Initially, the number of microemboli peaked immediately after the aortic cross-clamp had been released. Thereafter, the majority of microemboli whirled in the left ventricle and atrium without being ejected, and only few microemboli emerged in the ascending aorta. Another top emerged when the beating heart commenced to eject blood throughout weaning from CPB. During this stage, the majority of microemboli came from the pulmonary veins. The microemboli emerged as floating chaplets at the anterior wall of the left atrium, and subsequently circulated into the left ventricle, after which they appeared in the ascending aorta. Notwithstanding elaborate surgical de-airing maneuvers, new microemboli persisted to materialize in the left atrium until the end of the study period, 20 minutes after termination of CPB. The second peak of microemboli coincides with the findings of a previous TCD report during open heart surgery, where the majority of cerebral microemboli appeared during and after termination of CPB.[3]. This is the precarious time point, since it is during termination of CPB that the heart resumes to emit microemboli to the cerebral circulation. Hence, it is this time point when the distinction in the count of microemboli between patients given CO_2-insufflation and controls becomes evident.[3] Likewise, one should take in consideration that in patients experiencing CO_2-insufflation, the number of microemboli were fewer and diverged in their composition from those in the control group.[12] They contained CO_2-gas and not air.

5.3.1.4 How to eliminate CO_2-insufflation induced hypercapnia in open heart surgery

In **Study III** we used an additional cardiotomy suction device at a constant suction rate of 1 L/min as the coronary suction device suction rate may vary between 0.25 - 1 L/min and as the latter device is sometimes held outside the wound cavity. In this way, CO_2-gas was continuously drained to the additional cardiotomy reservoir followed by drainage to the conventional cardiotomy reservoir, where CO_2-gas was quickly absorbed in the circulating venous blood. As a consequence, the impact of CO_2-gas on $PaCO_2$ in the conventional setup was manifest resulting in increased sweep gas flows in order to keep normocapnia. Importantly, the conventional setup functionally corresponds to standard practice using a setup without an additional venous reservoir.

In contrast, during the interventional setup, CO_2-gas could not be drained from the additional to the standard venous reservoir and could thus not be absorbed by circulating venous blood in the standard venous reservoir. This resulted in a mean sweep gas flow of 2.6 L/min compared with 4.4 L/min in the conventional group, which was approximately 40% higher. Extrapolating these data to clinical practice, the use of an additional venous reservoir, with a clamped connection to the standard venous reservoir, for suction of gas and blood from the surgical wound cavity during de-airing with CO_2-gas insufflation prevents fluctuation of $PaCO_2$ levels and the need of changing sweep gas flows according to the amount of CO_2-gas being evacuated to the venous reservoir. It may also in this setup be difficult to achieve normocapnia in very large patients where the capacity of the oxygenator to remove CO_2 is reaching its limitation when very high sweep gas flows are needed. Moreover, as the amount of CO_2-gas entering the standard cardiotomy reservoir during clinical practice may vary due to the used coronary suction rate and if the coronary suction device is held within the wound or not, $PaCO_2$ may still fluctuate if a fixed increased sweep gas flow rate is used. Only the interventional setup can prevent this from happening, avoiding changes in the arterial pressure of carbon dioxide ($PaCO_2$) dependent cerebral perfusion. Furthermore, use of on-line arterial blood gas monitoring may not prevent but only mitigate fluctuations in $PaCO_2$ during de-airing with CO_2-insufflation.

5.4 MINIMIZING CEREBRAL MICROEMBOLISM IN CORONARY ANGIOGRAPHY

Study IV, a RCT, demonstrated that cerebral microemboli emerged during coronary angiography in every patient with the femoral as well as the right radial access. However, the right radial access produced significantly more particulate microemboli than the femoral approach, and these findings are in agreement with a previous non-randomized study.[4] With the right radial approach, particulate microemboli occurred more frequently in the right than the left MCA, particularly when catheters were exchanged, in contrast to the femoral approach. Particulate microemboli are probably more harmful as one may expect them to permanently block small cerebral arteries and arterioles. When using the right radial access, the catheters used for angiography need to be pushed past the orifice of the right brachiocephalic artery and be distinctly curved into the ascending aorta. This protruding movement might dislocate atherosclerotic plaques from the arterial wall with the risk of ensuing embolization. Furthermore, due to the longer period of angiography using the radial approach, the angiography catheters may themselves compose an extra source of emboli. **Study IV** thus advocates the use of a single catheter. Moreover, in patients with vascular dementia diffusion-weighted MRI studies suggest that small clinically silent defects may promote to neuropsychological decline.[69] Prospective reports using diffusion-weighted MRI have revealed new silent ischemic cerebral defects following cardiac catheterization, with a rate of 5% to 22%[4-6, 70, 71] and cerebral particulate microemboli were confirmed using TCD in three of the studies.[4-6] Additionally, in one of the reports cognitive tests suggested a causal relationship between microemboli and neuropsychological deficiency,[4] indicating that the findings of **Study IV** are of clinical relevance.

5.5 CLINICAL IMPLICATIONS

Study I indicates that to minimize microemboli in the CPB prime the perfusionist should insufflate CO_2-gas into the empty CPB circuit from the highest point of the venous reservoir for 3 - 4 minutes and then recirculate the prime fluid for 15 minutes as well as to tap on the oxygenator for 2 minutes. Those precautions are easy to perform, will minimize the de-airing procedure, are cheap, and will protect the patients from air microembolization. However, if CO_2-insufflation is not used, the perfusionist's maneuvers, including 15 minutes of re-circulating the CPB system with prime fluid, including 2 minutes of tapping on the oxygenator, will fail to de-air the CPB system, and may increase the risk of adverse clinical events.

Study II demonstrates that the mini-diffuser can be used clinically for efficient CO_2 de-airing in minimally invasive open heart surgery also when normal types of suction devices are applied.

Study III shows that the simple use of an additional venous reservoir for evacuation of blood and gas from the open surgical wound eliminates possible hypercapnia induced by CO_2-insufflation in open heart surgery and thus keeps $PaCO_2$ and sweep gas flow constant. In this way, hyperperfusion of the brain induced by CO_2-insufflation for de-airing in open heart surgery is prevented and the risk of particulate cerebral microembolization is decreased.

Study IV advocates the use of a single angiography catheter to minimize cerebral microembolization. As the right radial approach produced more particulate microemboli to the brain than the femoral approach one may consider using the latter in patients with severe arteriosclerosis of the thoracic aorta.

5.6 LIMITATIONS

There are some limitations in **Study I**. First, a pre-bypass filter was not applied. Such a filter is sometimes omitted in clinical practice and, even if it had been used, it could not prevent the release of air microemboli from the oxygenator. The Hatteland bubble detector that was applied in **Study I** has the drawback that it cannot identify small bubbles with a diameter less than 10 μm. Nevertheless, as far as we know, such small air microemboli have never been identified to be of any physiological or clinical importance.

In **Study II** most measurements were performed in an experimental cavity preventing comprehensive testing of the mini-diffuser at various set-ups in patients. It would have been unethical to undertake all these different measurements in patients, as this would have considerably prolonged the duration on CPB. Moreover, in the few patients undergoing minimally invasive AVR where we actually performed single measurements, we did not evaluate the possible consequence on neuropsychological function using continuous CO_2 insufflation for prevention of air microembolism. Effective de-airing with CO_2-gas of the minimally invasive surgical wound cavity should inhibit air from getting into the open heart and great vessels, and consequently prevent air microemboli from entering the arterial circulation.

A limitation of **Study III** is that the number of patients was very limited, only ten patients. However, the patients were used as their own controls and the differences were highly significant both statistically and in numbers indicating that the number of patients was sufficient and that the results have a clinical impact. Moreover, we did not use a dual chamber cardiotomy reservoir that is commercially available (Sorin Inspire HVR Dual, Sorin Group Italia S.r.l. Mirandola (MO), Italy) because the wall separating the two chambers is open at its upper part allowing for gas exchange. Instead we used an additional venous reservoir connected to the standard venous reservoir via a tube enabling the possibility to separate the content of the reservoirs by clamping the connecting tube. It may also be argued that the occasional drainage of blood from the additional to the standard venous reservoir in the interventional group may have influenced outcome values. However, it can be presumed that the absorption of CO_2-gas into the gas-blood interface of blood in the additional venous reservoir will very quickly be reduced as the surface blood is stagnant and the volume is relatively small compared with the standard setup where CO_2-gas will interfere with a large blood surface area that is constantly exchanged with a blood flow of 4.6 L/min. Our data indicate that this presumption was correct.

There are several limitations in **Study IV**. There was a high crossover rate from radial to femoral access. Those patients were not included in the analysis. Also, the coronary angiographers, who were experienced with both approaches, preferred to alter access site rather than using additional catheters, as indicated by the low number of additional catheters used. Furthermore, most cerebral microemboli were detected during maneuvers, which were rather independent of the skill of the coronary angiographer, including catheter insertions and exchanges. The duration of fluoroscopy with the radial approach exceeded that of the femoral approach, indicating a potential confounding factor caused by longer duration of contact between the catheters and the arterial vessel walls. Even though the difference in fluoroscopy time between the access sites has dropped over the years, the radial access is linked to a longer exposure of radiation.[49, 50] The relation between the higher cerebral microembolic rate and the radial access is supported by the finding of fewer cerebral microemboli in the femoral group, notwithstanding the higher risk factors for stroke with the femoral access, including absence of additional heparin use, rate of diabetes mellitus and severe coronary artery disease.

6 CONCLUSIONS

The specific conclusions were:

- CO_2-insufflation of an empty CPB circuit reduces the number of gaseous emboli in the prime compared with a conventional CPB circuit, which contains air before fluid priming.

- More than 99% of air is displaced within 3 to 4 minutes of re-circulation with CO_2-gas at a flow rate of 10 L/min, if the empty CPB circuit is insufflated from the highest part of the venous reservoir. Knocking on the oxygenator releases gaseous emboli. Duration of re-circulating the CPB circuit with fluid prime reduces the number of microemboli, and 15 minutes of re-circulation is efficient.

- The mini-diffuser was effective for CO_2 de-airing, with $< 1\%$ remaining air, in a minimally invasive open cardiothoracic wound cavity model with and without intermittent rough suction and in patients undergoing minimally invasive aortic valve surgery.

- An additional venous reservoir, with a clamped connecting tube to the standard venous reservoir, for evacuation of gas and blood from the open surgical wound eliminates CO_2-insufflation induced hypercapnia in open heart surgery keeping $PaCO_2$ and sweep gas flow constant.

- For coronary angiography, the radial access site generates more particulate cerebral microemboli than the femoral access site.

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