BLOOD PRESSURE-DEPENDENT CHANGES IN PLASMA VOLUME, GLYCOCALYX AND PLATELET FUNCTION DURING ANAESTHESIA

Clinical and experimental studies

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Blood pressure-dependent changes in plasma volume,
glycocalyx and platelet function during anaesthesia

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“I need to write down my observations.
Even the tiniest ones; they´re
the most important?”

Tove Jansson

ABSTRACT

Background: Worldwide, more than 300 million surgeries are performed each year. General anaesthesia provides the surgical patient with a state of controlled loss of sensation and awareness. It is common that general anaesthesia causes hypotension. Anaesthesia-induced hypotension is associated with haemodilution and increased plasma volume (PV). The increased PV could potentially release atrial natriuretic peptide (ANP) that is suspected to degrade the endothelial glycocalyx (EG) layer.

Aims: To explore physiological and pathophysiological mechanisms resulting from anaesthesia-related hypotension we: 1) investigated the magnitude and dynamics of PV expansion secondary to anaesthesia induction; 2) assessed whether anaesthesia induction-related increase in PV could be attenuated by maintaining the mean arterial pressure (MAP) at pre-induction levels with norepinephrine (NE) infusion; 3) evaluated the consequences of anaesthesia induction-related PV expansion on the release of ANP and its effects on the EG. We also investigated whether exogenous administration of ANP caused degradation of the EG. Finally, we investigated the effect of NE infusion on platelet function and clot formation.

Methods: We conducted two prospective, randomised, single-centre studies on patients that underwent elective coronary artery bypass grafting (CABG). The patients were randomised to maintain pre-induction MAP (intervention group) or MAP 60 mm Hg (control group) by titration of NE. Baseline PV was measured by ^{125}I-albumin and the change in PV was calculated from the change in haematocrit (Hct). Changes in Mid Regional-pro Atrial Natriuretic Peptide (MR-proANP) and EG-components were measured.

In a prospective, randomised, blinded, experimental study, 20 pigs were randomised to receive an infusion of either ANP or NaCl. Changes in EG components, Hct, calculated PV and colloid osmotic pressure (COP) were measured.

Platelet aggregation was assessed with impedance aggregometry and clot formation with rotational thromboelastometry in study IV.

Results: Lower MAP, (60 mm Hg) secondary to anaesthesia induction increased the PV by 12%, while the PV increased by 2,6% in the intervention group with maintained pre-operative MAP. MR-proANP increased in the group with lower MAP but no degradation of the EG was detected. There was no increase in EG components secondary to an infusion of ANP, but the PV decreased. Intraoperative NE infusion improved platelet aggregation and clot formation.

Conclusions: Haematocrit decreased and plasma volume increased shortly after anaesthesia induction caused hypotension. The increase in plasma volume could be prevented by maintaining pre-induction blood-pressure levels with a norepinephrine infusion. No ANP-induced degradation of the EG was detected. Norepinephrine could contribute to a better perioperative haemostasis.

Keywords: Anaesthesia, blood pressure, hypotension, norepinephrine, haematocrit, plasma volume, atrial natriuretic peptide, glycocalyx, platelet aggregation.

SAMMANFATTNING PÅ SVENSKA

Varje år utförs fler än 300 miljoner kirurgiska ingrepp i världen. Narkos eller sövning ges för att patienten inte skall ha ont eller vara medveten under det kirurgiska ingreppet. Läkemedel ges för smärtlindring, sömn och muskelrelaxation. Det är vanligt att de läkemedel som ges vid narkosinduktion minskar hjärtats pumpförmåga och vidgar blodkärlen så att blodtrycket sjunker. Noradrenalin är ett kroppseget hormon som bland annat utsöndras vid stress och verkar främst genom att dra ihop blodkärlen och därmed höja blodtrycket. Noradrenalin, som läkemedel används ofta vid hjärtoperationer för att höja blodtrycket.

Hjärtats förmak kan, vid uttänjning t.ex. på grund av ökad blodvolym utsöndra ett hormon, förmakspeptid. Förmakshormonet utsöndras vid vätsketillförsel bl.a. hos patienter med blodförgiftning och misstänks kunna påverka och bryta ned blodkärlsväggarnas innersta skikt, glykokalyxlagret, vilket kan öka vätskeutträdet till omkringliggande vävnader.

Patienter som drabbas av akut kranskärlssjukdom behandlas idag ofta med ett kateterbundet ingrepp eller kranskärlskirurgi. Dessa patienter behandlas med trombocythämning för att minska risken för blodproppsbildning i hjärtats kranskärl. Om en patient med dubbel trombocythämning behöver akut kirurgi finns en stor risk för blödning.

Syftet med avhandlingen var att undersöka hur plasmavolymen förändras av blodtrycksänkningen vid narkosinduktion och om dessa förändringar kan undvikas om blodtrycket bibehålls med noradrenalin. Vidare undersöktes utsöndringen av hjärtats förmakshormon vid denna plasmavolymsförändring och om det fanns ett samband med nedbrytning av kärlväggens glykokalyxlager. Experimentellt undersöktes om kärlväggens glykokalyxlager och genomsläpplighet påverkades av att tillföra förmakshormon. Blodtrycksbevarande behandling med noradrenalin och dess effekt på blodplättarna och koagelbildningen undersöktes som en möjlig behandling vid blödning.

Avhandlingen omfattar fyra delarbeten. I delarbete 1 och 2 lottades sammanlagt 48 patienter till antingen en studiegrupp där utgångsblodtrycket bibehölls med hjälp av noradrenalininfusion eller till en kontrollgrupp där medelblodtrycket tilläts sjunka till 60 mm Hg. Förändringar av blodets hematokrit och plasmavolym samt utsöndring av förmakshormon och glykokalyxkomponenter mättes. I delarbete 3 lottades 20 grisar till att antingen få en infusion av förmakshormon (intervention) eller koksalt (kontroll) för att se om förmakshormon söndrar glykokalyx. Glykokalyxkomponenter mättes, liksom förändring av plasmavolym. I delarbete 4 mättes effekten av den givna blodtryckshöjande noradrenalinsdosen på blodplättarnas vidhäftningsförmåga och blodets koagelbildning.

Sammantaget undersöktes olika målblodtrycks inverkan på plasmavolymen, utsöndring av förmakshormon, kärlväggen samt koagulationen vid sövning under hjärtoperationer. Vi fann att plasmavolymen ökade med 12% i gruppen med lägre blodtryck. I denna grupp utsöndrades även förmakshormon, men det söndrade inte kärlväggens glykokalyx. Noradrenalininfusion förbättrade blodplättarnas förmåga att klumpas ihop samt blodets koagelstyrka.

LIST OF PAPERS

This thesis is based on the following appended papers, which are referred to in the text by their assigned Roman numerals:

I. Damén T, Reinsfelt B, Redfors B, Nygren A

Pressure-dependent changes in haematocrit and plasma volume during anaesthesia, a randomised clinical trial

Acta Anaesthesiol Scand. 2016:60(5):560-568

DOI: 10.1111/aas.12687

II. Damén T, Saadati S, Forssell-Aronsson E, Hesse C, Bentzer P, Ricksten SE, Nygren A

Effects of different mean arterial pressure targets on plasma volume, ANP and glycocalyx-A randomized trial

Acta Anaesthesiol Scand. 2021:65(2):220-227

DOI: 10.1111/aas.13710

III. Damén T, Kolsrud O, Dellgren G, Hesse C, Ricksten SE, Nygren A

Atrial natriuretic peptide does not degrade the endothelial glycocalyx: a secondary analysis of a randomized porcine model.

Accepted for publication, Acta Anaesthesiol Scand. 2021

IV. Singh S, Damén T, Dellborg M, Jeppsson A, Nygren A

Intraoperative infusion of noradrenaline improves platelet aggregation in patients undergoing coronary artery bypass grafting: a randomized controlled trial

J. Thromb. Haemost. 17: 657–665

DOI: 10.1111/jth.14408

CONTENTS

ABBREVIATIONS

ACS	acute coronary syndrome
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
ASA	acetylsalicylic acid
BSA	body surface area
CABG	coronary artery bypass grafting
cGMP	cyclic guanosine monophosphate
CI	cardiac index
CI	confidence interval
COP	colloid osmotic pressure
CPB	cardiopulmonary bypass
CVP	central venous pressure
DAPT	dual antiplatelet therapy
EG	endothelial glycocalyx
GAG	glycosaminoglycan
GC-A	guanylyl cyclase receptors of type A
Hb	haemoglobin
Hct	haematocrit
HR	heart rate
INVOS	brain tissue oxygen saturation
LSM	least square means
MAP	mean arterial pressure
MPAP	mean pulmonary artery pressure
MR-proANP	Mid Regional-pro Atrial Natriuretic Peptide
NE	norepinephrine
NSTEMI	non-ST-elevation myocardial infarction
PAC	pulmonary artery catheter
PCI	percutaneous coronary intervention
PI	perfusion index
PV	plasma volume
PVI	pleth variability index
PAWP	pulmonary artery wedge pressure
SD	standard deviation
SpHb	non-invasive continuous haemoglobin
SpO2	peripheral oxygen saturation
STEMI	ST-elevation myocardial infarction
SV	stroke volume
SVR	systemic vascular resistance
TER	transcapillary escape rate
vWF	von Willebrand factor

1. INTRODUCTION

1.1 Background

It is important to provide each cardiac surgery patient with proper anaesthesia an analgesia, and just as crucial to ensure each and every organ a good perfusion. Physiological and pathophysiological mechanisms resulting from anaesthesia-related hypotension need to be characterised in order to be able to fine-tune perioperative interventions to physiologically relevant endpoints.

1.1.1 Blood pressure

Arterial blood pressure is the derivative of cardiac output and systemic vascular resistance. Blood pressure is usually described by systolic blood pressure, mean arterial pressure (MAP) and diastolic blood pressure. MAP is defined as the average arterial pressure throughout one cardiac cycle, measured from the area under the pressure curve or calculated as: diastolic pressure + 1/3 of the pulse pressure.

The clinical reference blood pressure measurement method is a measurement of direct continuous intraarterial blood pressure using an arterial catheter. During cardiac surgery, direct continuous intraarterial blood pressure measurement is standard.

1.1.2 Anaesthesia and hypotension

Mean arterial pressure is affected by changes in either cardiac output and/or systemic vascular resistance. Anaesthesia induction affects both cardiac output and systemic vascular resistance by myocardial depression, direct vasodilatory effects and sympathetic inhibition, usually leading to reduced blood pressure.[1]

There is currently no universal definition of intraoperative hypotension. Hypotension is, however, usually defined as absolute or relative thresholds for either systolic blood pressure or MAP. An absolute MAP of less than 65 mmHg is frequently used to define intraoperative hypotension and is a common intervention threshold in clinical practice.[2] There is increasing evidence that intraoperative MAPs below 60-70 mmHg are associated with myocardial injury, acute kidney injury, and death.[2,3] Still, it remains unclear which perioperative blood pressures should be targeted. The general perioperative goal is to provide appropriate organ perfusion and therefore haemodynamic optimisation can make a difference with regard to complications.

1.1.3 Management of perioperative hypotension

Anaesthesia-related hypotension is perioperatively modifiable, with interventions improving the cardiac output and systemic vascular resistance. A common treatment strategy is to improve cardiac output by increasing preload with fluids and/or to improve cardiac contractility with inotropes. If vasodilatation is the main problem, systemic vascular resistance can be increased by using a vasopressor such as norepinephrine (NE) or phenylephrine.

1.2 Norepinephrine

1.2.1 History

Norepinephrine was identified as an adrenergic neurotransmitter in 1946. The Swedish physiologist Ulf von Euler studied the distribution and excretion of NE in his laboratory at the Karolinska Institute. In 1970 he was awarded the Nobel Prize in Physiology or Medicine.

Norepinephrine was primarily found to be stored and released as a neurotransmitter from neurons in the sympathetic nervous system. To a lesser extent NE was found to be

released as a hormone from the medulla of adrenal glands.

In 1950, NE was approved for medical use in the United States. According to the Surviving Sepsis Guidelines, NE is recommended as the first-line vasopressor for the treatment of sepsis-related vasodilatation and hypotension.[4]

1.2.2 Pharmacology

Norepinephrine functions as an endogenous catecholamine both as a neurotransmitter in the sympathetic nervous system and as a hormone and is together with epinephrine vital for the body´s flight-and fight response. This is fundamental in a threatening situation in order to increase the force in skeletal muscles as well as the force and the rate of the heart.

Norepinephrine binds to α- and β- adrenergic receptors. NE predominantly acts on α-receptors by producing vasoconstriction of resistance and capacitance vessels, thereby increasing the vascular resistance and the central blood volume. β_1-receptor stimulation causes a positive inotropic effect increasing the cardiac output and initially also a positive chronotropic effect increasing the heart rate. Because the blood pressure is a function of systemic vascular resistance and cardiac output NE increases the blood pressure by increasing the systemic vascular resistance and to some extent the cardiac output.

In clinical practice, NE is administered as intermittent intravenous injections but more commonly as an infusion, which is a routine practice in treating hypotension in cardiac surgery patients and intensive care patients.

1.3 Haemoglobin, haematocrit and plasma volume

In experimental studies a NE-infusion-related increase in arterial blood pressure has been shown to induce a loss of PV.[5,6] In a clinical study on mechanically ventilated intensive care patients with a vasodilatory shock, haemoglobin and haematocrit increased significantly with NE-induced increase in MAP.[7] Contrastingly, anaesthesia induction-related hypotension has been associated with haemodilution and increased PV.[8]

1.3.1 Haemoglobin

Haemoglobin (Hb) is the oxygen transport binding protein in red blood cells, reported as the concentration of haemoglobin in whole blood as grams per litre (g/l). The reference range is 117-153 g/l for women and 134-170 g/l for men.

1.3.2 Haematocrit

Haematocrit (Hct) is the percentage of blood volume that is occupied by red blood cells. The reference range is 0.350-0.458 for women and 0.393-0.501 for men.

1.3.3 Plasma, haemodilution and anaemia

Plasma forms the liquid base of blood. Around 55% of whole blood volume is plasma. Plasma contains up to 92% water, but also the protein albumin, coagulation factors and electrolytes.

Together with the blood cells, plasma maintains the blood volume and enables a normal venous return and preload for the heart in order to keep up the cardiac output and blood pressure. Plasma carries electrolytes, coagulation factors and immunoglobulins in order to maintain the cellular homeostasis, the vascular haemostasis and to defend the body against foreign viruses and bacteria. Albumin is the most important protein for maintaining the colloid osmotic pressure (COP).

The magnitude and dynamics of the anaesthesia-induction-associated decrease

in Hct and increase in PV and the possible effects of release of atrial natriuretic peptide and its effects on the endothelial glycocalyx layer of the capillary wall have not previously been studied.

Perioperative anaemia is common during open-heart surgery due to the use of cardiopulmonary bypass, the invasiveness of the surgery and fluid administration. Approximately every second patient going through open-heart surgery is transfused with red blood cells. Avoidance of unnecessary transfusions is important considering both undesirable side effects and also because of the health economy. Efforts to optimise perioperative Hb levels and reduce red blood cell transfusions during cardiac surgery are therefore warranted.

1.4 Starling principle and fluid exchange

In the 19th century, Ernest Starling showed that isotonic saline injected into connective tissue was absorbed into venous blood that became hemodiluted.[9] When serum was injected into connective tissue, no absorption and no venous haemodilution was observed.[9] Starling hypothesised that the capillary walls were semipermeable and that the fluid exchange was dependent on differences in transcapillary hydrostatic and osmotic pressures. Eugene Landis developed a technique for the measurement of capillary pressure and proposed that his findings of a positive correlation between capillary pressure and the transcapillary flow rate could be disclosed by a mathematical equation that later emerged as the classic Starling equation.

The classic Starling equation:

$Jv = L_pA[(P_c - P_i) - \sigma(\pi_p - \pi_i)]$ (Figure 1)

- J_v = net transendothelial fluid movement
- L_p = the hydraulic conductance of the membrane
- A = the surface area for filtration
- P_c = capillary hydrostatic pressure
- P_i = interstitial hydrostatic pressure
- σ = reflection coefficient for the membrane
- π_p = plasma colloid osmotic pressure
- π_i = interstitial colloid osmotic pressure

The Starling principle describes the movement of fluid across the capillary wall. According to the classic Starling principle, fluid is, at the arteriolar portion of the capillaries, filtered to the interstitium due to a dominant hydrostatic pressure gradient and reabsorbed at the venular end due to a dominant colloid osmotic pressure gradient.

To also understand the transfer of solutes and large molecules through the capillary wall, the two-pore theory for transcapillary fluid exchange was developed. According to the two-pore theory, flow of water and solutes mostly occurs through small pores, while macromolecular transport occurs through large pores.[10] Transport of macromolecules through the large pores is dependent on diffusion (the concentration difference of the macromolecules on each side of the capillary wall), convection (the solvent flow through the large pores that is dependent on the difference in transcapillary hydrostatic pressure) and the permeability to macromolecules.[6]

The discovery of the endothelial glycocalyx layer has challenged the classic Starling principle.

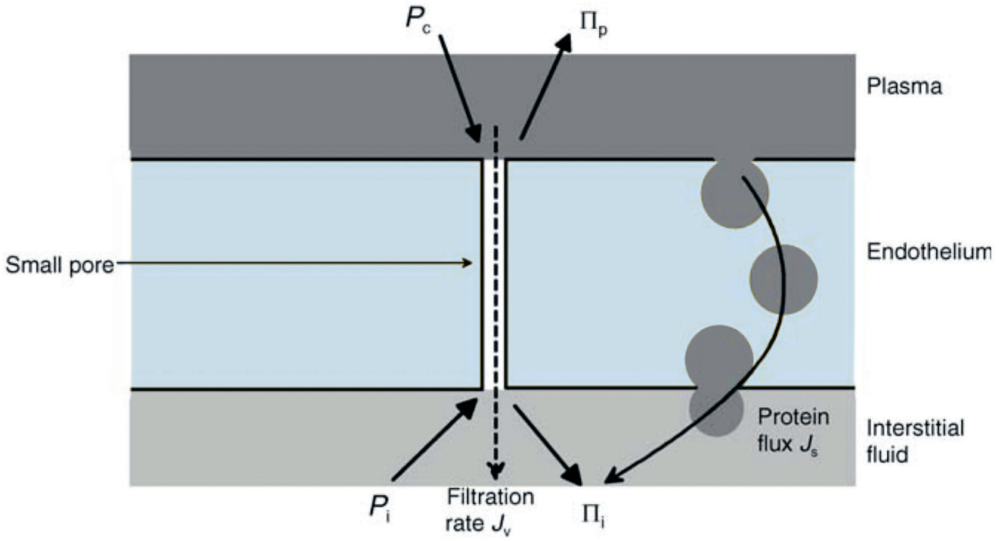

Figure 1. Schematic of the classic Starling principle. $Jv = L_pA[(P_c\text{-}P_i) - \sigma(\pi_p\text{-}\pi_i)]$.
Reprinted from Levick, Cardiovascular Research 2010 with permission

1.5 The endothelial glycocalyx (EG)

The endothelial glycocalyx layer is a sugar-based extracellular matrix lining the luminal surface of the endothelial cells. The underlying capillary wall comprises the endothelial cell monolayer, the basement membrane and the supporting cells. The endothelial glycocalyx layer is an integral part of the vascular endothelium and vascular integrity is maintained by both the endothelial glycocalyx layer and healthy endothelial cells. The word glycocalyx translates from the Greek for sugar coat, glykys meaning sweet and kalyx meaning husk.[11]

1.5.1 History

In the 1940s it was proposed that a thin endocapillary layer might cover the luminal endothelium.[12,13] In 1966 the fine structure of the capillary and the endocapillary layer was acknowledged.[14] In 2004 it was shown that the balance of Starling forces regulating transvascular fluid exchange was primarily regulated by the plasma protein concentration difference across the glycocalyx and not the whole capillary wall.[15]

1.5.2 Structure

The endothelial glycocalyx layer is composed of proteoglycans, glycoproteins, glycosaminoglycans (GAG), glycolipids and associated plasma proteins such as albumin and antithrombin (Figure 2).[16] The transmembrane syndecans and the membrane-bound glypicans are proteoglycans. Several GAGs, such as heparan sulphates and chondroitin sulphates, are covalently attached to proteoglycans. Heparan sulphate normally constitutes at least 50% of the GAGs. Hyaluronic acid is a GAG that does not bind to proteoglycans, but instead interacts with the cell-membrane glycoprotein CD-44.[17] The EG components form a fibrous network with a quasi-periodic inner matrix and a porous outer region.[18] The EG layer has a thickness of 0.2 μm up to 8 μm.

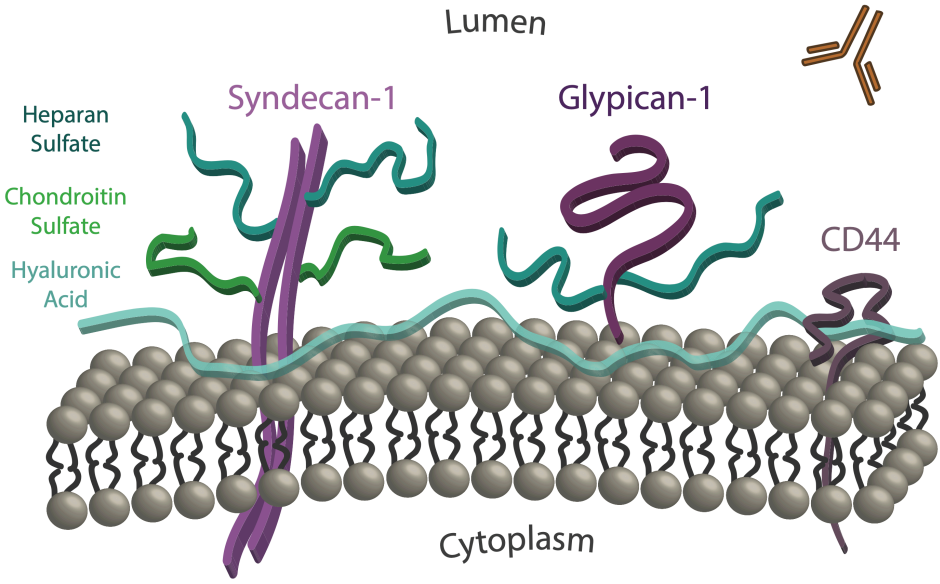

Figure 2. Schematic of the endothelial glycocalyx and its proteoglycan, glycoprotein and glycosaminoglycan components. Reprinted from Bartosch, Biophysical Journal 2017 with permission

1.5.3 Function

The EG forms a contact surface between blood and vascular endothelial cells. The EG has several functions necessary for vascular homeostasis.[19] The quasi-periodic inner matrix forms the permeability barrier and the porous outer layer determines red cell and white cell dynamics. The EG regulates vascular permeability and microvascular tone, inhibits microvascular thrombosis and helps to regulate leukocyte adhesion on the endothelium.[17,19]

1.6 The revised Starling principle

The discovery of the EG layer has led researchers to challenge the classic Starling principle. The classic Starling principle states that the rate of fluid movement across the capillary wall is proportional to the transendothelial hydrostatic pressure difference and the colloid osmotic pressure difference. Accordingly, fluid is filtered at the arterial and absorbed at the venular end of the capillary.[20]

The revised Starling principle, also called the Michel-Weinbaum model or the steady state Starling model, states that the EG layer establishes the osmotic pressure difference of the plasma proteins instead of the entire capillary wall (Figure 3). Thus the rate of fluid movement across the capillary wall is proportional to the transendothelial hydrostatic pressure difference and the trans glycocalyx layer colloid osmotic pressure difference.[20] The revised Starling principle also states that in a steady state there is a capillary filtration but no absorption at the venous end and that the filtrated fluid is returned to the blood circulation via the lymphatic circulation.[20]

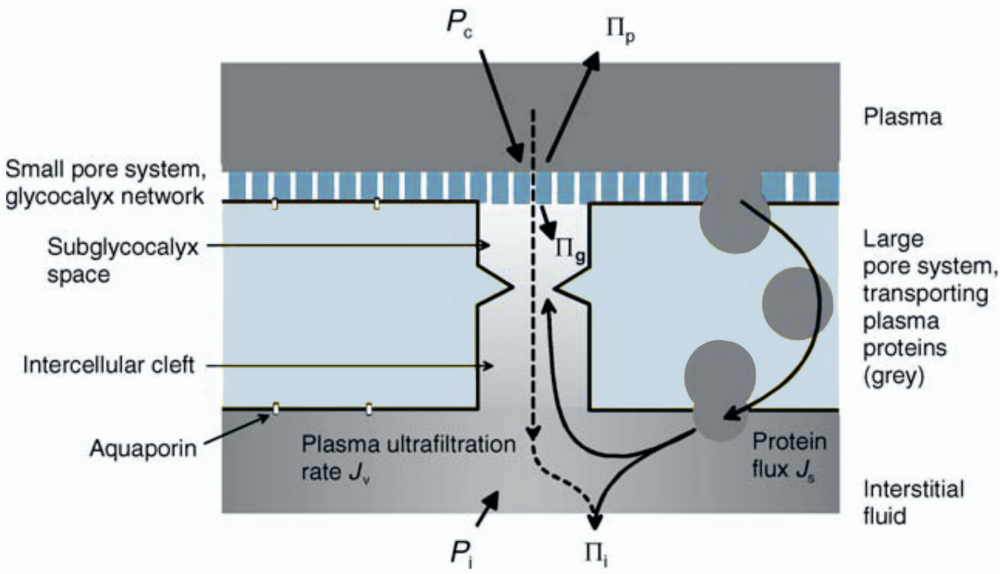

Figure 3. Schematic of the revised Starling principle. $Jv = L_pA[(P_c-P_i) - \sigma(\pi_p-\pi_g)]$.
Reprinted from Levick, Cardiovascular Research 2010 with permission

1.7 Atrial natriuretic peptide

1.7.1 History

Electron microscopic findings of storage granules in the cardiac atria raised the question of possible heart-hormonal effects. In 1981 it was discovered that intravenously injected cardiac atrial extract was causing a rapid increase of sodium, chloride and urine excretion.[21] This made it evident that the heart was an endocrine organ, in addition to its role as a functional blood pump. It was found that atria produced a polypeptide hormone that was named atrial natriuretic factor, nowadays known as atrial natriuretic peptide (ANP).[22]

1.7.2 Atrial natriuretic peptide

The active form of human circulating ANP was found to be a 28 amino acid peptide synthesised by and secreted from atrial myocytes in response to increased atrial wall stress. ANP reduces increased atrial pressure through an antihypervolaemic and antihypertensive effect by influencing several other organs that control cardiovascular function.[22] ANP binds to guanylyl cyclase receptors of type A (GC-A) generating the second messenger cyclic guanosine monophosphate (cGMP) that causes physiological actions as increased natriuresis and vasodilatation.[23,24]

1.7.3 ANP, vascular permeability and the endothelial glycocalyx

In an experiment, infusing healthy humans with a low dose of ANP, a shift of plasma volume, albumin and electrolytes from the circulation to the interstitium as well as a rise in haematocrit was noted.[25] Intravital microscopy studies in mice showed that ANP enhanced transendothelial caveolae-mediated albumin transport via its GC-A receptor, thus regulating the intravascular volume.[26] Endothelium-specific deletion (knockout) of GC-A has been shown to result in hypervolemic hypertension.[27] Thus, several studies

have shown the association between ANP and increased vascular permeability in regulating intravascular volume.

An intact EG is a part of the primary barrier to transendothelial transport of solvents, solutes and plasma proteins. In humans, the vascular permeability has been suspected to be affected by ANP-induced degradation of the EG.[28] However, contradictory results regarding ANP and glycocalyx degradation have been reported.[29]

1.8 Acute coronary syndrome, antiplatelet therapy and cardiac surgery

1.8.1 Atherosclerosis and atherothrombosis

A ruptured or eroded atherosclerotic plaque is usually the mechanism behind the activation and aggregation of platelets that, together with the coagulation cascade, lead to the formation of an atherothrombosis. If the atherothrombosis, i.e. the clot, is located in the coronary artery circulation it might partly or totally obstruct the arterial blood supply to the heart causing Acute Coronary Syndrome (ACS).

1.8.2 Acute Coronary Syndrome

Acute Coronary Syndrome is a condition of acute myocardial ischemia or infarction due to reduced or obstructed coronary artery blood flow. Unstable angina, Non-ST-elevation myocardial infarction (NSTEMI) and ST-elevation MI (STEMI) are the three types of ACS. Management of ACS includes the treatment of coronary artery occlusion by thrombolysis, percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). After initial management of ACS, the prevention of secondary thrombotic events is a fundamental part. One of the cornerstones in secondary prevention is the inhibition of platelet activation and aggregation by using a combination of acetylsalicylic acid (ASA) and a P2Y12 inhibitor, the so-called dual antiplatelet therapy (DAPT).

1.8.3 Dual antiplatelet therapy

According to the current guidelines for coronary artery disease, the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) recommend DAPT in all patients with ACS, independent of revascularisation strategy.[30]

A daily dose of 75-100 mg of ASA irreversibly inhibits the platelet cyclooxygenase-1 enzyme which is required for the production of thromboxane-A2. Thromboxane-A2 mediates vasoconstriction, platelet activation and stimulates platelet aggregation via activation of platelet GPIIb/IIIa-receptors.[31]

P2Y12 receptor inhibitors bind to the G-protein-coupled platelet receptor P2Y12 and inhibit ADP-induced platelet aggregation.[32]

Dual antiplatelet therapy combining ASA and a P2Y12 receptor inhibitor has been shown to reduce recurrent major adverse cardiovascular events in patients with ACS compared with ASA treatment alone, but at the expense of an increased risk of bleeding.[33]

1.8.4 Cardiac surgery, DAPT and bleeding

The current guidelines of the European Association for Cardio-Thoracic Surgery (EACTS) and the European Association of Cardiothoracic Anaesthesiology (EACTA), recommend ASA to be considered to be stopped 5 days preoperatively in patients refusing blood transfusion or at high risk of bleeding and undergoing non-coronary cardiac surgery; P2Y12 receptor inhibitors should be discontinued 3-7 days before non-emergent cardiac surgery.[34] However, patients treated with DAPT might need to undergo emergent cardiac surgery. For instan-

ce, Type A Aortic Dissection patients or ACS patients in need of emergent CABG surgery have to undergo cardiac surgery without being able to discontinue their DAPT. There is currently no antidote available to P2Y12 receptor inhibitors. In a Swedish nationwide retrospective, observational study the incidence of major bleeding complications was 38% and 31%, respectively, when ticagrelor/clopidogrel was discontinued <24 h before surgery.[35] These patients are, from a haemostatic point of view, challenging and in need of new alternative strategies to reduce perioperative bleeding complications.

1.8.5 Haemostasis

Haemostasis is a cascade that starts with a vascular injury and culminates in a formation of a clot with platelets and fibrin polymers that seal the lesion. The haemostasis cascade includes vasoconstriction of the wounded vessel, formation of a platelet plug (primary haemostasis) and coagulation (secondary haemostasis) that stabilises the formed platelet plug.

1.8.5.1 Primary haemostasis

The biconvex platelets derived from megakaryocytes are responsible for initiating the repair of an injured vascular endothelium. A break in the vascular endothelium exposes platelets to collagen fibrils and the von Willebrand factor (vWF) in the connective tissue matrix. The platelets interact with the collagen fibrils and vWF and thereby attach (adhere) to the injured vessel wall and become activated. Activated platelets change their shape and secrete, for example, ADP and thromboxane A2 from dense bodies and fibrinogen, vWF, factor V and XIII from alfa granules. Released ADP and thromboxane A2 activate circulating platelets which in turn secrete more ADP and thromboxane A2. The coagulation protein fibrinogen (released from activated platelets or derived from circulating blood) binds to platelet GPIIb/IIIa receptors and thereby initiates platelets to aggregate.

1.8.5.2 Secondary haemostasis

The secondary haemostasis, also named coagulation, is a cascade, a series of steps that ends with fibrin strands that stabilise the aggregated platelets. Tissue factor exposed at the site of vessel injury initiates the coagulation cascade by interacting with the circulating coagulation factor VII. The negatively charged surface on activated platelets is important for the activation and propagation of the coagulation cascade.

1.8.6 Norepinephrine as an alternative treatment

Due to the P2Y12 receptor inhibition, the ADP-induced platelet activation and aggregation are impaired, leading to a deteriorated primary haemostasis. For complete ADP-induced platelet activation and aggregation, a simultaneous activation of both the P2Y1- and P2Y12-mediated pathway is needed.[32] The P2Y12 receptor is a G- protein-coupled receptor that signals through Gi and inhibits adenylyl cyclase and activates phosphoinositide 3-kinase.[36] If P2Y12 is blocked, activation of another G-coupled receptor, the α_{2A}-adrenergic receptor, can cause intracellular signalling similar or identical to P2Y12 activation.[37] Activation of the platelet surface G protein- coupled receptor Gz results in both inhibition of adenylyl cyclase and activation of phosphoinositide 3-kinase, which is exactly the same as the P2Y12 receptor signalling through Gi. Norepinephrine could, by activating the Gz coupled α_{2A}-receptor, offer an alternative treatment for ADP-induced platelet activation and aggregation by coactivation of the P2Y1- and α_{2A}-mediated pathways and thereby relieve perioperative bleeding complications.

2. AIMS

I. To investigate the magnitude and dynamics of the plasma volume expansion during anaesthesia induction

II. To assess whether anaesthesia induction-related increase in plasma volume can be attenuated, by maintaining the blood pressure at pre-induction levels with norepinephrine infusion

III. To evaluate the consequence of anaesthesia induction-related plasma volume expansion on release of atrial natriuretic peptide and its effects on the endothelial glycocalyx

IV. To investigate whether exogenous administration of atrial natriuretic peptide causes degradation of the endothelial glycocalyx in an experimental porcine model

V. To investigate the effect of norepinephrine infusion on platelet aggregation and clot formation in elective cardiac surgery patients treated with ASA

3. PATIENTS AND METHODS

All four studies, as described in Papers I-IV, are prospective, randomised and controlled. Papers I, II and IV are human studies on adult cardiac surgery patients. Paper IV was a predefined sub-study to Paper II. Paper III is an experimental porcine study. Twenty-four patients were included in Paper I, 24 in Papers II and IV and 20 pigs were studied in Paper III.

All human studies were conducted in accordance with the current (2013) version of the Declaration of Helsinki and good clinical practice regarding international ethical and scientific quality standards.[38,39]

The pigs received care in accordance with the Swedish Board of Agriculture regulations and common advice concerning research animals (SJVFS 2015:38).

3.1 Paper I

3.1.1 Study design

The study was a prospective parallel-group randomised controlled single-centre investigation. The study protocol was reviewed and approved by the Regional Ethical Review Board in Gothenburg, Sweden (protocol number: 1052-13) and registered at http://www.ClinicalTrials.gov (id: NCT02412189).

3.1.2 Inclusion, exclusion criteria, randomisation

Patients who were scheduled for elective CABG surgery at Sahlgrenska University Hospital, Gothenburg were screened for eligibility. After providing oral and written consent from 26 patients, 24 were included in the study. Exclusion criteria were age less than 18 years, untreated hypertension, diabetes mellitus, a left-ventricular systolic ejection fraction of 45% or less and former stroke and/or a known carotid artery stenosis.

The 24 included patients were randomised into two groups using sealed envelopes. The randomisation was patient-blinded.

3.1.3 Experimental protocol

A schematic of the experimental protocol is presented in Figure 4.

In the intervention group, the MAP was maintained at pre-induction levels with norepinephrine infusion. In the control group, norepinephrine was administered only if MAP decreased below 60 mmHg. Blood gases were drawn and levels of INVOS (brain tissue oxygen saturation), SpHb (non-invasive continuous haemoglobin), SpO2 (peripheral oxygen saturation), PI (perfusion index) and PVI (pleth variability index) were collected before anaesthesia induction and during the experimental procedure; that is to say every 10 minutes during the first 70 minutes of the anaesthesia. The amount of urine passed was measured at the start of extracorporeal circulation and the mean urine flow (ml/min) was calculated.

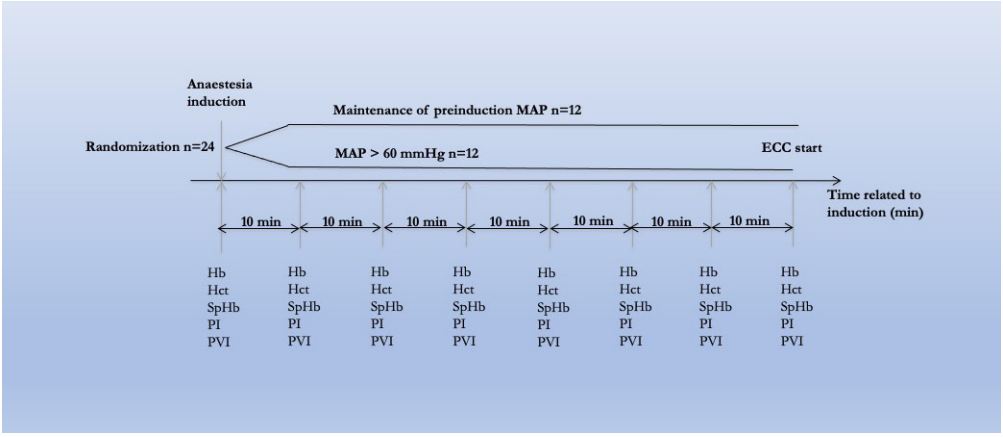

Figure 4. Flowchart of Study I. ECC: extracorporeal circulation, Hb: haemoglobin, Hct: haematocrit, MAP: mean arterial pressure, PI: perfusion index, PVI: pleth variability index, SpHb: continuous non-invasive haemoglobin

3.2 Papers II and IV

Papers II and IV are both concerned with the PV-GLY-ANP study and will thus be described together.

3.2.1 Study design

The studies were prospective parallel-group randomised controlled single-centre investigations. The common study protocol was reviewed and approved by the Regional Ethical Review Board in Gothenburg, Sweden (protocol number: 389-16), by the Sahlgrenska Radiation Safety Committee (protocol number: 16-20), by the Swedish Medical Products Agency (EduraCT number: 2016-004961-16) and registered at http://www.ClinicalTrials.gov (id: NCT02832596).

3.2.2 Inclusion, exclusion criteria, randomisation

Patients >40 years of age scheduled for elective CABG surgery at Sahlgrenska University Hospital, Gothenburg were screened for eligibility. After providing oral and written consent from 26 patients, 24 were included in the study. Exclusion criteria were pregnancy and breastfeeding, untreated hypertension, a left-ventricular ejection fraction of 45% or less, diabetes mellitus and a known carotid artery stenosis or a former stroke. After inclusion, 24 patients were randomised using block randomisation. Six blocks were labelled male and two blocks were labelled female according to the gender distribution of CABG patients. The randomisation was patient-blinded.

3.2.3 Experimental protocol

The experimental protocol considering anaesthesia and titration of norepinephrine to MAP at baseline level (intervention group) or 60 mm Hg (control group) is identical for Papers I, II and IV.

3.2.3.1 Experimental protocol Paper II

A schematic of the experimental protocol is presented in Figure 5.

Baseline PV was measured by ^{125}I-albumin and the change in PV was calculated from the change in Hct. Changes in haemodynamic parameters [MAP, central venous pressure (CVP), cardiac index (CI), mean pulmonary artery pressure (MPAP) and pulmonary artery wedge pressure (PAWP)], plasma ^{125}I-albumin, transcapillary escape rate (TER),

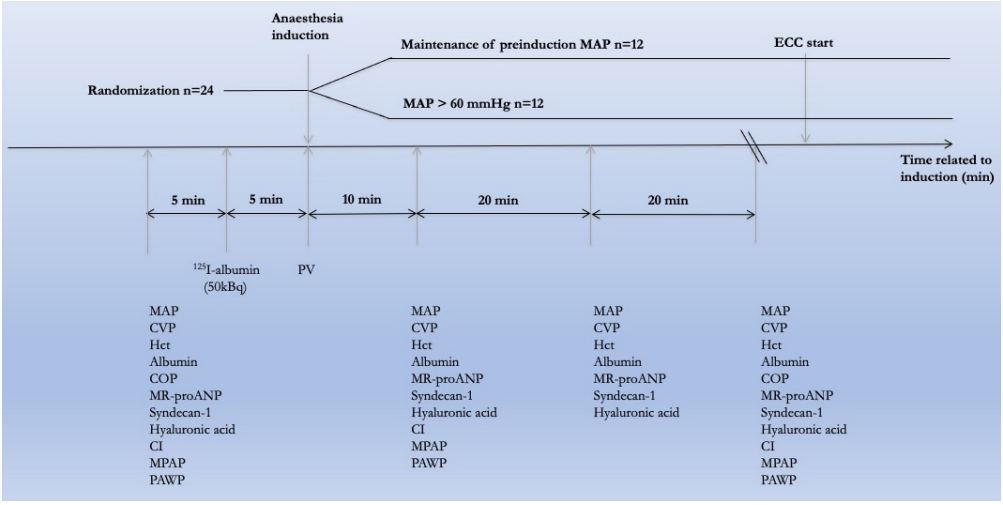

Figure 5. Flowchart of Study II. CI: cardiac index, COP: colloid osmotic pressure, CVP: central venous pressure, ECC: extracorporeal circulation, Hct: haematocrit, MAP: mean arterial pressure, MPAP: mean pulmonary arterial pressure, MR-proANP: Mid-Regional-pro-Atrial Natriuretic Peptide, PAWP: pulmonary artery wedge pressure

colloid osmotic pressure (COP), albumin, Mid Regional-pro Atrial Natriuretic Peptide (MR-proANP),the endothelial glycocalyx components hyaluronic acid and syndecan-1 and thrombomodulin were measured.

Measurements were performed at baseline and 10, 30 and 50 minutes after anaesthesia induction. CI, MPAP and PAWP were measured at baseline, 10 and 50 minutes and COP at baseline and 50 minutes. The amount of urine passed was measured at 50 minutes.

3.2.3.2 Experimental protocol Paper IV

A schematic of the experimental protocol is presented in Figure 6.

Platelet aggregation assessed with impedance aggregometry and clot formation assessed with rotational thromboelastometry were performed from blood samples collected 10 minutes before and 50 minutes after anaesthesia induction.

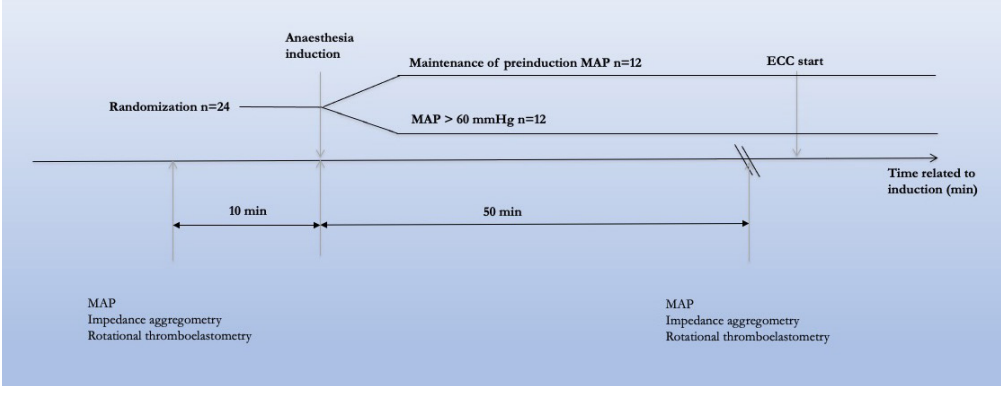

Figure 6. Flowchart of Study IV. ECC: extracorporeal circulation, MAP: mean arterial pressure.

3.3 Paper III

3.3.1 Study design

The study was an in-advance planned and predefined prospective, placebo-controlled, blinded sub-study of a randomised trial investigating the effects of ANP on renal function during cardiopulmonary bypass.[40] Ethical approval was provided by the Ethics Committee on Animal Experiments at the University of Gothenburg (no 107-2016).

3.3.2 Inclusion, exclusion criteria, randomisation

Twenty Swedish-bred (Vallrum farm, Ransta, Sweden), specific pathogen free female Yorkshire pigs were randomised into two groups using sealed envelopes. The randomisation was investigator-blinded.

3.3.3 Experimental protocol Paper III

A schematic of the experimental protocol is presented in Figure 7.

An infusion of either ANP (50 ng/kg/min) or saline (NaCl) was given during 60 minutes. EG components (porcine heparan sulphate proteoglycan, hyaluronic acid and syndecan-1), COP, Hct, calculated PV and urine output were measured from baseline to 60 minutes together with MAP and CVP.

3.4 Haemodynamic measurements

Arterial pressure, CVP, PAWP and MPAP were measured via the arterial, the central venous and the pulmonary artery catheters (PAC). The PAC was used only in study II and inserted together with the central line in local anaesthesia before anaesthesia induction.

Cardiac index was measured in triplicate using the thermodilution technique (mean of three 10 ml ice-cold saline injections in the PAC). Transducers were zeroed at the mid-axillary line.

3.5 Haemoglobin and haematocrit measurement

Haemoglobin and lactate were measured using an automated blood gas analyser (ABL 825 Flex in Paper I and RAPIDPoint 500 in Paper II). The blood gas analyser measures light absorbance by a spectrophotometer to calculate the Hb level and Hct is then calculated by the formula: Hb (g/l) x 0.2941.

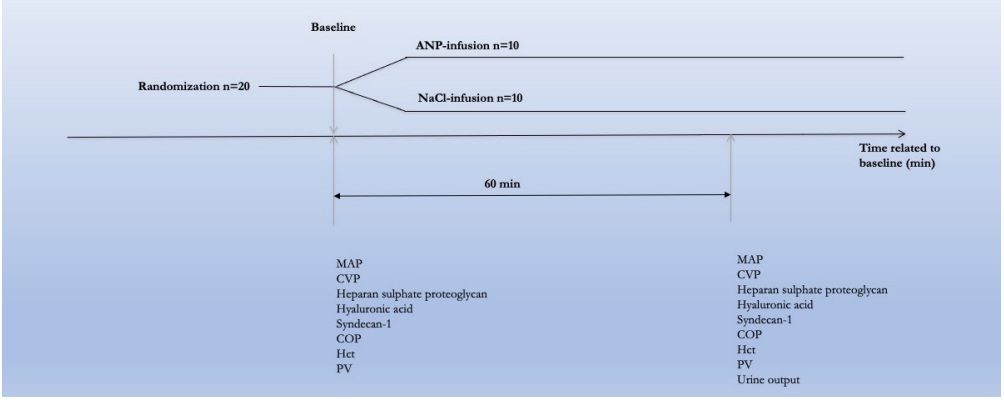

Figure 7. Flowchart of Study III. ANP: atrial natriuretic peptide, COP: colloid osmotic pressure, CVP: central venous pressure, Hct: haematocrit, PV: plasma volume

3.6 Plasma volume

In Paper II, the plasma volume before intervention and the TER were determined using ^{125}I-labelled human serum albumin (SERALB-125®, CIS bio international, Gif-Sur-Yvette Cedex, France). Each patient received an intravenous injection of 0.3 ml ^{125}I-albumin (50 kBq) in the central line 5 minutes before anaesthesia induction. Plasma samples were collected from the arterial line 10 minutes before anaesthesia induction and at 0 (just before anaesthesia induction), 10 and 30 minutes after induction. The administered ^{125}I activity, A_{inj}, was individually determined by weighing the syringe before (m_{before}) and after (m_{after}) injection, in combination with measurement of the activity concentration in a standard sample (with an activity, $A_{standard}$, of ca 20 kBq, and a mass, $m_{standard}$, of ca 0.1 g) collected from the same vial, $C_{standard} = A_{standard}/m_{standard}$. Possible ^{125}I activity adsorbed to the syringes was determined and found negligible. Thus:

$$A_{inj} = (m_{before} - m_{after}) \times C_{standard} \quad (1)$$

The plasma volume before intervention was determined as:

$$PV_0 = A_{inj}/C_{plasma,0}\ , \quad (2)$$

where A_{inj} is the activity injected at time -5, $C_{plasma,0}$ is the measured ^{125}I concentration in plasma at time 0, assuming total distribution in plasma and negligible (or similar) TER during these 5 minutes.

TER was determined as λ by fitting a monoexponential curve to the measured plasma concentrations of ^{125}I at times 0, 10 and 30 minutes versus time for each patient.

TER was calculated both without (uncorrected TER) and with correction (corrected TER) for plasma volume changes with time. Determination of corrected TER was made using the measured ^{125}I-albumin concentrations at 10 and 30 minutes post-injection multiplied by the relative change in plasma volume at the respective time point. The ^{125}I activity in plasma and standard samples was measured in a gamma counter (Wizard 1480; Wallac Oy). Corrections were performed for detector background signal and physical decay.

Plasma volume changes in Papers I and II were calculated with the formula 100 x (Hct_{pre}/Hct_{post} – 1) / (1-Hct_{pre}), where Hct is expressed as a fraction.[7]

3.7 Biomarker analyses

Plasma concentration of MR-proANP was determined by an automated immunofluorescent assay (Brahms).

Plasma concentrations of syndecan-1 (Human sCD138, Diaclone SAS and Pig Syndecan-1/CD138, SDC1, Cusabio technology LLC), hyaluronic acid (Echelon Biosciences Inc), porcine heparan sulphate proteoglycan (Amsbio), thrombomodulin (Human sCD141, Diaclone SAS) and albumin (Roche Diagnostics) were determined by immunologic assays according to the manufacturers' instructions.

The colloid osmotic pressure was measured by the OSMOMAT 050 (Colloid Osmometer, Gonotec GmbH).

3.8 Platelet aggregation and clot formation

3.8.1 Platelet aggregation

An impedance aggregometer (Multiplate®) was used to study platelet aggregation in Paper IV. In hirudin tubes (0.15 mg/l) the whole blood collected was allowed to incubate in the test cell for 3 minutes. Aggregation agonists were thereafter added and the impedance between two electrodes in the test cell was measured for 6 minutes. The AUC (U) was reported.

3.8.2 Thromboelastometry

The viscoelastic assay rotational thromboelastometry (ROTEM®) was used to assess clot formation in Paper IV. The INTEM, HEPTEM, EXTEM and FIBTEM tests were used. A total of 300 µl of blood collected in citrated tubes (0.19 M citrate) was added to the test cup together with an activator. The clotting time, clot formation time and maximum clot firmness were reported.

3.9 Statistical analysis

Statistical analysis was performed using the GraphPad Prism, version 8.3.1 (332) and 8.4.3 (471), SPSS Statistics, version 20, 22 and 25 (IBM) and SAS Software version, 9.4.

For Paper I, a power analysis based on pilot data revealed a total sample size of 10 patients for detecting a change in Hb by 50% with a significance level of 0.05 and a power of 0.80.[41]

The sample size in Paper II was based on the results of Paper I.[41] The power analysis revealed a total sample size of 12 patients for detecting a calculated difference in plasma volume change of 50% with a significance level of 0.05 and power of 0.80.[42]

Paper III was an analysis of a secondary outcome measure in a randomised blinded trial and therefore no sample size calculation was performed.[40]

For Paper IV, the sample size was the same as in Paper II.[42] A post-hoc analysis resulted in a power of 0.93, 0.59 and 0.40 given the observed values for differences in ADP-, AA- and TRAP-induced aggregation respectively.[43]

Categorical baseline data were compared using Fisher´s exact test. The Shapiro-Wilk test and histograms were used for assessment of a normal distribution. Normally distributed baseline data between two groups were compared using Student´s t-test. A paired t-test or repeated measures one-way ANOVA (analysis of variance) were used for assessment of intragroup changes and a two-way ANOVA for repeated measurements between groups. Non-parametric tests used for not normally distributed data were the Mann-Whitney test for continuous data compared between two groups and the Wilcoxon signed-rank test (matched pairs) for within-group changes. A flowchart for selecting used statistical tests is presented in Figure 8.

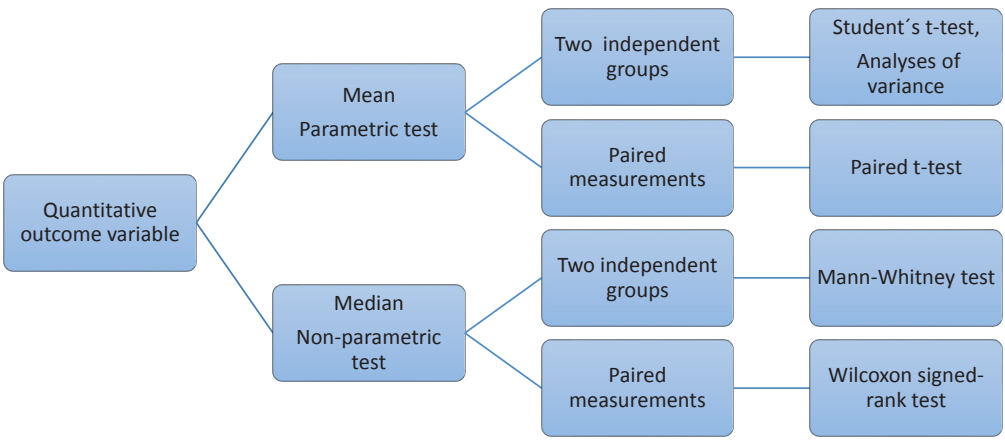

Figure 8. A flowchart for in Paper I-IV used statistical tests.

Data are presented as either mean ± standard deviation (SD) for the normally distributed data and median and 95% confidence intervals (CI) or 25th-75th percentiles for the nonparametric tests.

In Paper II a repeated measures correlation was used for the assessment of correlation between PV and MAP as well as PV and NE.

In Paper IV regression models with natural cubic splines were used to evaluate the effect of NE-infusion rate on the changes in platelet aggregation between baseline and 50 minutes after anaesthesia induction. Piecewise linear functions were used to simplify the splines. The difference in Least Square Means (ΔLSM) with 95% CI is presented for each 0.01 μg/kg/min increase in NE-infusion rate.

4. RESULTS

4.1 Effects of different mean arterial pressure targets on haematocrit and plasma volume (Papers I and II)

Patient demographics and baseline characteristics, MAP levels; norepinephrine dose, haematocrit and plasma volume are reported together – i.e. the 24 patients in Paper I and the 24 patients in Paper II. The remaining results are reported separately for Papers I, II, III and IV.

4.1.1 Patients (Papers I and II)

An overview of the clinical trial flowchart for **Papers I** and **II** is provided in Figure 9. Overall, 110 patients were assessed for eligibility and 49 patients were included. Of these, one patient was not analysed due to accidental intraoperative fluid administration, resulting in 48 patients in the final analyses.

The mean age of the participants was 65 ± 8.5 years and the female/male ratio was 10/38. All patients were scheduled for elective coronary artery bypass surgery. Patient demographics and baseline characteristics are detailed in Table 1.

	Control (n=24)	Intervention (n=24)
Age (years)	66 ± 7.3	64 ± 9.6
Sex (f/m)	7/17	3/21
Weight (kg)	82 ± 18	89 ± 18
MAP (mmHg)	97 ± 9.5	95 ± 8.5
Haematocrit (%)	42 ± 3.8	40 ± 3.8
Calculated PV (l)	3.4 ± 0.7	3.9 ± 0.8

Table 1. Patient demographics and baseline characteristics (Papers I and II). Values are mean ± standard deviation. MAP: mean arterial pressure, PV: plasma volume.

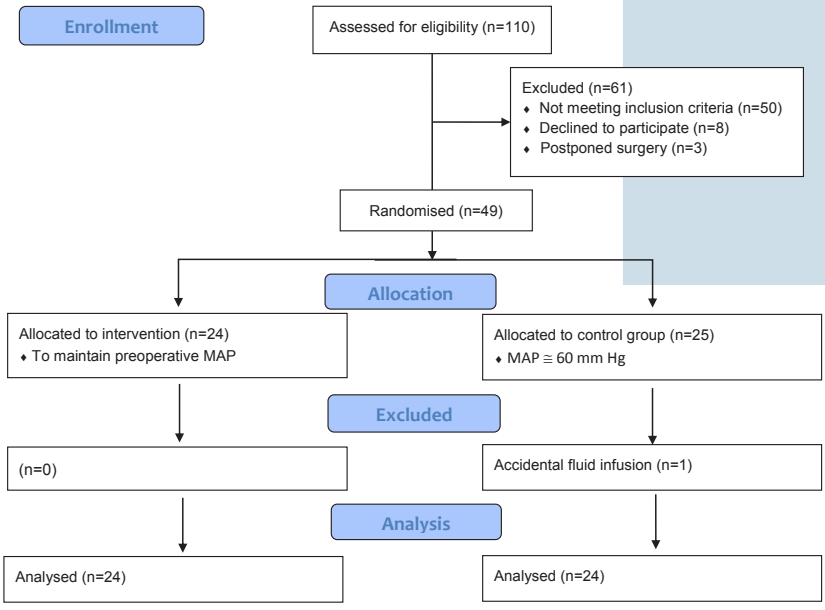

Figure 9. CONSORT flow diagram showing patient inclusion (Papers I and II).

4.1.2 Mean arterial pressure

Mean arterial pressure before anaesthesia induction was 97 ± 9.5 mmHg in the control groups and 95 ± 8.5 mmHg in the intervention groups (p = 0.495). After anaesthesia induction, MAP decreased significantly in the control groups and remained unchanged in the intervention groups (Figure 10). The MAP during the trial was 64 ± 8.7 mmHg in the control groups and 93 ± 9.5 mmHg in the intervention groups (p<0.0001).

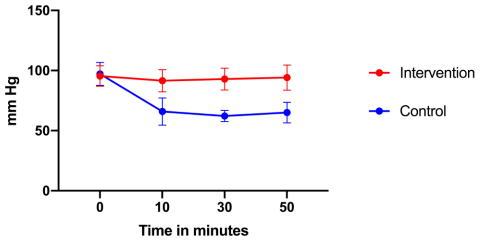

Figure 10. Change in mean arterial blood pressure. Data are presented as mean ± SD, p< 0.0001.

4.1.3 Norepinephrine

All patients in the intervention groups required NE at a dose of mean 0.12 ± 0.07 (range 0- 0.34) µg/kg/min. In the control groups, ten out of 24 patients needed NE (range 0.01-0.12 µg/kg/min) at some time point to maintain MAP above 60 mmHg. The results for changes in norepinephrine dose for both groups are shown in Figure 11.

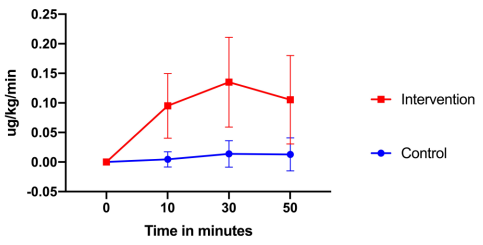

Figure 11. Change in norepinephrine dose. Data are presented as mean ± SD, p < 0.0001.

4.1.4 Haematocrit

Arterial blood haematocrit decreased significantly more in the control groups compared to the intervention groups (p < 0.0001). Ten minutes after anaesthesia induction a significant change in haematocrit was seen in the control groups (p < 0.0001). The reduction in haematocrit at 10 minutes was mean -2.1 ± 0.8 % units in the control groups and mean -0.3 ± 0.7 % units in the intervention groups (Figure 12).

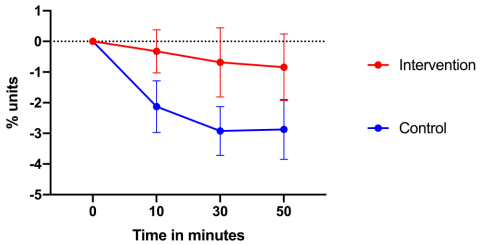

Figure 12. Change in haematocrit. Data are presented as mean ± SD, p < 0.0001.

4.1.5 Plasma volume

The increase in calculated plasma volume was mean 12 ± 4.4% in the control groups (p < 0.0001) and mean 2.6 ± 4.2% in the intervention groups (p = 0.0004). The increase in calculated plasma volume was significantly higher in the control groups compared to the intervention groups (p < 0.0001) (Figure 13).

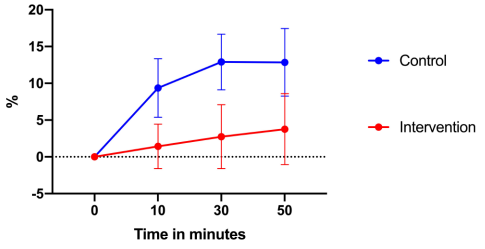

Figure 13. Change in calculated plasma volume. Data are presented as mean ± SD, p < 0.0001.

4.2 Effects of different mean arterial pressure targets on plasma volume, ANP and glycocalyx (Paper II)

Results concerning MAP and change in PV are partly presented in subchapter 4:1 together with the results from Paper I.

4.2.1 Haemodynamics

Changes in haemodynamic variables are presented in Table 2.

After anaesthesia induction, CVP increased in both the control group and the intervention group with no difference between the groups. MPAP increased in the intervention group with no change in the control group. CI and SV decreased significantly in both the control and intervention group with no difference between the groups. PAWP increased in the intervention group but did not change in the control group. SVR increased in the intervention group and decreased in the control group.

4.2.2 Changes in plasma volume, TER, albumin and colloid osmotic pressure

Baseline plasma volume assessed by ^{125}I-albumin was 3.0 ± 0.4 l and 3.2 ± 0.7 l in the control and intervention group respectively (p=0.293). Baseline plasma volume indexed for weight was 37 ± 6 ml/kg and 38 ± 4 ml/kg in the control and intervention group respectively (p=0.754).

After anaesthesia induction the calculated PV increased significantly more in the control group (range 104 – 881 ml) compared to the intervention group (range -205 – 294 ml) (p<0.001). A repeated measures correlation showed a significant correlation between the change in MAP and PV (p<0.01), whereas no significant correlation was noted between NE and the change in PV (p=0.537).

The mean value of uncorrected TER for ^{125}I-albumin was significantly different between the control group (22 ± 6%/h) and the intervention group (6.9 ± 5.9%/h) (p<0.001). There was a positive correlation

Variables	Group	Baseline	10 min	30 min	50 min	Within-group ANOVA, P-value	Between-group ANOVA, P-valube
MAP (mm Hg)	Control	94 ± 14	63 ± 6	62 ± 4	62 ± 5	<,001	<,001
	Intervention	92 ± 12	91 ± 9	91 ± 7	96 ± 12	,171	
CVP (mm Hg)	Control	6 ± 2	10 ± 4	11 ± 5	7 ± 3	,002	,251
	Intervention	6 ± 3	10 ± 5	11 ± 3	10 ± 5	,001	
MPAP (mm Hg)	Control	19 ± 5	19 ± 5	21 ± 5	18 ± 4	,045	,010
	Intervention	18 ± 4	22 ± 4	23 ± 5	22 ± 5	,006	
CI (L/min/m$^{2)}$	Control	2,6 ± 0,5	1,8 ± 0,5		2,0 ± 0,6	<,001	,306
	Intervention	2,6 ± 0,7	1,7 ± 0,4		2,2 ± 0,9	<,001	
SV (mL)	Control	75 ± 10	59 ± 11		53 ± 12	<,001	,414
	Intervention	79 ± 14	68 ± 15		65 ± 18	,016	
PAWP (mm Hg)	Control	13 ± 5	13 ± 5		12 ± 5	,626	,046
	Intervention	10 ± 3	13 ± 4		14 ± 4	,011	
SVR (dynscm^{-5})	Control	1539 ± 307	1353 ± 269		1260 ± 187	,014	<,001
	Intervention	1320 ± 219	1857 ± 421		1647 ± 508	,001	

Table 2. Haemodynamics. Abbreviations: ANOVA, analysis of variance; CI, cardiac index; CVP, central venous pressure; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; SV, stroke volume; SVR, systemic vascular resistance. Values are mean ± SD.
Source: Damén, T. et al. Acta Anaesthesiol Scand. 2020

between TER and the change of calculated PV at 30 minutes (r=0.838). Corrected TER, adjusted for the PV change, was -0.1 ± 5.4%/h and 5.7 ± 8.3%/h in the control and intervention groups respectively (p=0.055).

The baseline serum albumin was 38 ± 2.3 g/l in the control group and 38 ± 2.5 g/l in the intervention group (p>0.999). Serum albumin decreased in both the control group (p<0.001) and the intervention group (p=0.004) but to a greater extent in the control group compared to the intervention group (p=0.001).

The baseline COP was 25 ± 3.2 mm Hg in the control group and 24 ± 2.7 mmHg in the intervention group (p=0.400). COP decreased by -2.4 ± 1.6 mm Hg in the control group (p=0.0004) and -0.8 ± 1.2 mm Hg in the intervention group (p=0.043) with a significant difference between the groups (p=0.013).

4.2.3 Changes in MR-proANP and glycocalyx products

The changes in MR-proANP, hyaluronic acid and syndecan-1 are presented in Table 3. MR-proANP increased in the control group (p<0.001) with no change in the intervention group (p=0.401). There was no difference in the change in MR-proANP between the groups (p=0.114). In the control group there was no difference in the ANP response between patients receiving or not receiving NE (p=0.998). Changes in hyaluronic acid and syndecan-1 after anaesthesia induction did not differ between the groups (p=0.222 and 0.513 respectively).

4.3 Atrial natriuretic peptide and endothelial glycocalyx (Paper III)

4.3.1 Animals

Twenty-eight female Yorkshire pigs were enrolled. Three pigs established the model as pilots and five were sham-operated upon. The remaining 20 pigs were blindly randomised into either a control group (n=10) or an intervention group (n=10).

The baseline characteristics for the 20 pigs allocated to the control and intervention groups are provided in Table 4.

	Group	Baseline	Change 10 min	Change 30 min	Chan 50 min	Within-group ANOVA, P-value	Between-group ANOVA, P-valube
MR-proANP (pmol/L)	Control	103 ± 34	+7,9 ± 10	+21 ± 14	+23 ± 17	<,001	,114
MR-proANP (pmil/L)	Intervention	93 ± 57	+4,0 ± 26	+6,5 ± 21	+9,4 ± 25	,401	
HA (ng/mL)	Control	110 ± 20	-1,3 ± 16	-3,6 ± 19	-8,9 ± 22	,386	,222
HA (ng/mL)	Intervention	91 ± 14	+2,0 ± 13	+8,8 ± 17	+2,3 ± 12	,209	
Syndecan-1 (ng/mL)	Control	32 ± 38	-1,7 ± 5,7	-7,4 ± 7,9	+2,3 ± 16	,143	,513
Syndecan-1 (ng/mL)	Intervention	24 ± 27	-4,1 ± 6,2	-7,8 ± 11	-3,8 ± 6,2	,040	,

Table 3. Change in MR-proANP, hyaluronic acid and syndecan-1. Abbreviations: HA, hyaluronic acid; MR-proANP, Mid-Regional-pro-Atrial-Natriuretic-Peptide.
Source Damén, T. et al. Acta Anaesthesiol Scand. 2020

	NaCl (n=10)	ANP (n=10)
Body weight (kg)	57 ± 5.3	55 ± 7.1
MAP (mm Hg)	78 ± 9.3	74 ± 10.4
CVP (mm Hg)	8 ± 3.5	7 ± 2.4
Haemoglobin (g/l)	93 ± 9.2	91 ± 5.4
Haematocrit (%)	28 ± 3	27 ± 2
Heparan sulphate proteoglycan (ng/ml)	15 ± 2.9	15 ± 4.8
Hyaluronic acid (ng/ml)	230 ± 79	230 ± 36
Diuresis [(ml/min)/BSA]	0.93 ± 0.42	1.1 ± 0.45
Arterial lactate (mmol/l)	2.44 ± 0.49	2.15 ± 0.38
Arterial oxygen saturation	99.6 ± 0.27	99.5 ± 0.38

Table 4. Demographics and baseline characteristics of the pigs (Paper III). MAP: Mean Arterial Pressure. CVP: Central Venous Pressure. BSA: Body Surface Area. Values are mean ± SD.

4.3.2 Effect of ANP on circulating glycocalyx fragments

The heparan sulphate proteoglycan and hyaluronic acid corrected for the change in plasma volume did not significantly change in either group (p=0.221 and p=0.078 respectively for NaCl; p=0.066 and p=0.780 respectively for ANP). There was no significant difference between the groups for heparan sulphate proteoglycan and hyaluronic acid corrected for the change in plasma volume (p=0.333 and p=0.197, respectively) (Figure 14). All syndecan-1 samples were under the limit of detection.

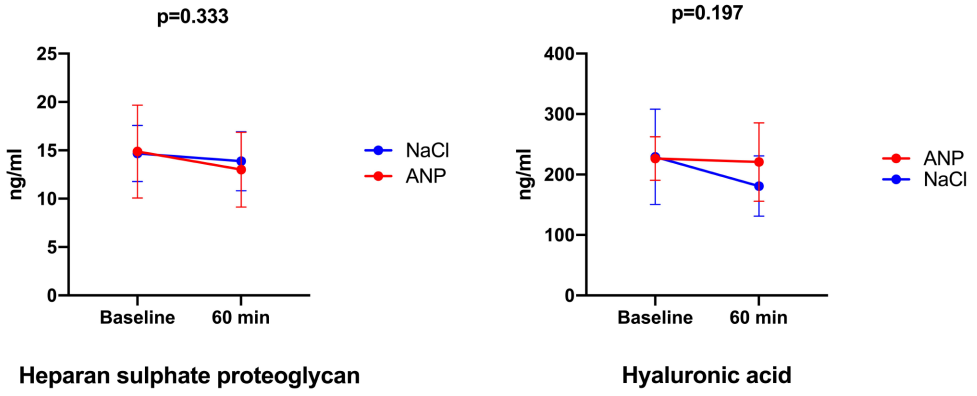

Figure 14. Change in circulating glycocalyx degradation products corrected for changes in plasma volume. Data are presented as mean ± SD.

4.3.3 Effect on haematocrit, plasma volume and colloid osmotic pressure

Arterial blood haematocrit increased with 1.8 ± 2.2% units in the ANP group (p=0.029) with no change (-0.5 ± 2.3% units) in the control group (p=0.504). The change in haematocrit significantly differed between the groups (p=0.034) (Figure 15.)

The calculated plasma volume decreased with -8.4 ± 10% in the ANP group (p=0.034) with no change (3.1 ± 12%) in the control group (p=0.427). The change in calculated plasma volume was significant between the groups (p=0.037) (Figure 15).

Colloid osmotic pressure changed in median 0.9 [95% CI, 0.00 to 1.58] mm Hg in the ANP group and median -0.39 [95% CI, -1.88 to 0.13] mm Hg in the control group, respectively. The change in colloid osmotic pressure was significant between the groups (p = 0.012) (Figure 16).

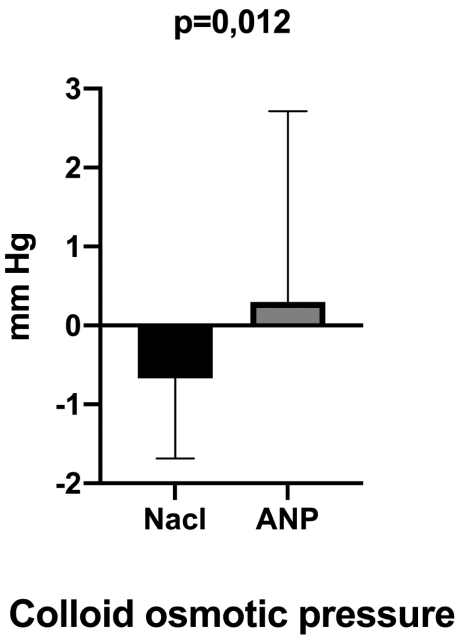

Figure 16. Change in colloid osmotic pressure. Data are presented as median ± 95% CI.

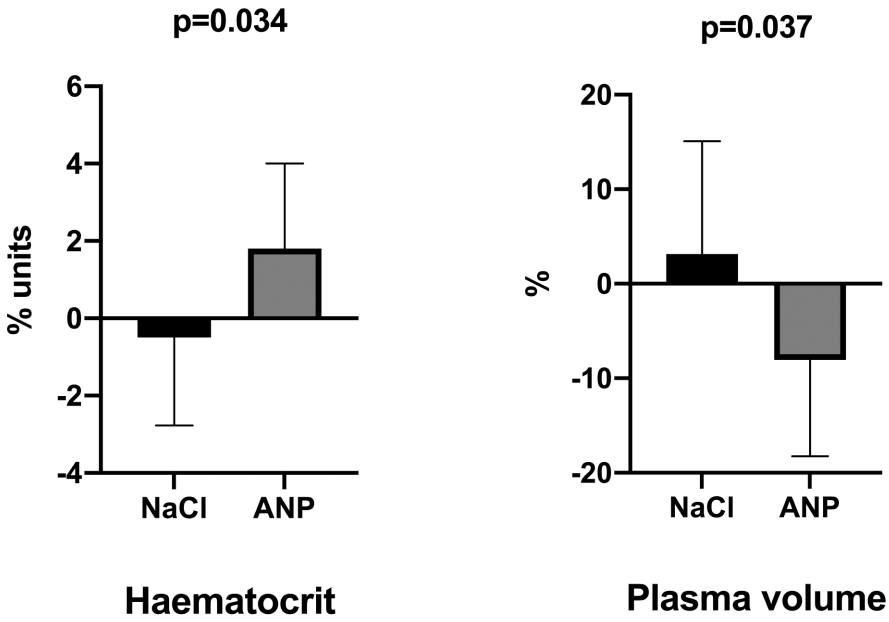

Figure 15 Change in arterial blood haematocrit and calculated plasma volume. Data are presented as mean ± SD

4.4 Effects of norepinephrine infusion on platelet function and clot formation in patients undergoing CABG (Paper IV)

4.4.1 Patients

The 24 patients included in Paper IV are the same as the 24 patients in Paper II. The demographics and baseline characteristics for the 24 patients allocated to the control and intervention groups in Paper IV are provided in Table 5.

	Control (n=12)	Intervention (n=12)
Age (years)	67 (64-69)	64 (55-69)
Body mass index (kg/m2)	28 (24.6-28.5)	25 (24.3-27.0)
MAP (mm Hg)	95 (91-103)	96 (85-102)
Haemoglobin (g/l)	139 (133-144)	134 (130-142)
Haematocrit (%)	41 (39-42)	39 (39-42)
Platelet count (x109/l)	204 (190-241)	256 (232-306)
Fibrinogen concentration (g/l)	2.9 (2.7-3.4)	3.4 (3.0-3.9)
Creatinine (µmol/l)	80 (74-89)	89 (75-97)
ASA treatment	12 (100%)	11 (92%)

Table 5. Patient demographics and baseline characteristics. ASA: acetylsalicylic acid. Values are median (25th-75th percentiles or number (proportion).

Comparing the control and intervention groups at baseline, there were no significant differences in platelet aggregation or clot formation.

4.4.2 Norepinephrine

In the intervention group, all patients but one received NE at a dose of median 0.09 (range 0-0.26) µg/kg/min at 50 minutes after anaesthesia induction. One patient received NE at a maximum dose of 0.2 µg/kg/min up to 40 minutes after anaesthesia induction. In the control group, seven patients needed NE at a maximal dose of 0.01-0.12 µg/kg/min at some time point to maintain MAP above 60 mm Hg. Four patients in the control group had a norepinephrine infusion at a dose of 0.03-0.12 µg/kg/min at 50 minutes after anaesthesia induction.

4.4.3 Effect of norepinephrine on platelet aggregation

In the intervention group, that received NE to maintain the preoperative MAP, the ADP-induced aggregation increased from 71 (53-94) U at baseline to 87 (70-103) U at 50 minutes after anaesthesia induction (p=0.023). In the control group, which received NE if MAP decreased below 60 mmHg, ADP-induced aggregation decreased from 85 (67-90) U at baseline to 72 (64-80) U at 50 minutes after anaesthesia induction (p=0.028). The change in ADP-induced aggregation from baseline to 50 minutes after anaesthesia induction was significantly different between the groups (p=0.002) (Figure 17).

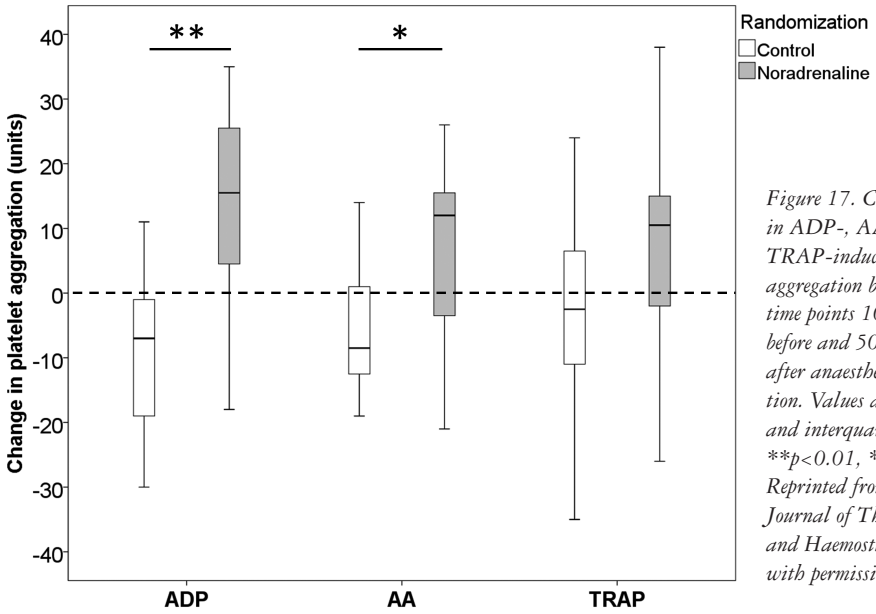

*Figure 17. Change in ADP-, AA-, and TRAP-induced platelet aggregation between the time points 10 minutes before and 50 minutes after anaesthesia induction. Values are median and interquartile range. **p<0.01, *p<0.05. Reprinted from Singh, Journal of Thrombosis and Haemosthasis 2019 with permission*

Between the two time points, neither the AA- nor the TRAP-induced aggregation changed in the intervention group (p=0.27 and p=0.12) or in the control group (p=0.12 and p=0.61). The change in AA-induced aggregation was however significant between the two groups (p=0.046) (Figure 17). No change in TRAP-induced aggregation was observed between the groups (p=0.12) (Figure 17).

Between the two time points, there was a significant effect of an increase of 0.01 µg/kg/min in the NE-infusion rate (up to 0.13 µg/kg/min) on the changes in ADP- and AA-induced aggregations; for ADP ΔLSM 2.68 (95% CI 1.06-4.30) (p=0.003) and for AA ΔLSM 1.79 (95% CI 0.80-2.78) (p=0.001) (Figure 18). Accordingly, the mean effect of a 0.01 µg/kg/min increase in NE-infusion rate on the change in ADP- and AA-induced aggregation was +2.68 U and +1.79 U, respectively. No significant effect was observed when the infusion rate was >0.13 µg/kg/min (Figure 18, Figure 19). No effect of NE-infusion rate was observed on TRAP-induced aggregation.

4.4.4 Effect of norepinephrine on clot formation

There was a significant increase in INTEM maximum clot firmness in the intervention group (p=0.009) while no significant change was noted in the control group (p=0.89). The change in INTEM maximum clot firmness from baseline to 50 minutes after anaesthesia induction was significantly different between the two groups (p=0.008).

Between the two time points there was no significant change in FIBTEM maximum clot firmness in the intervention group (p=0.12) while it significantly decreased in the control group (p=0.047).

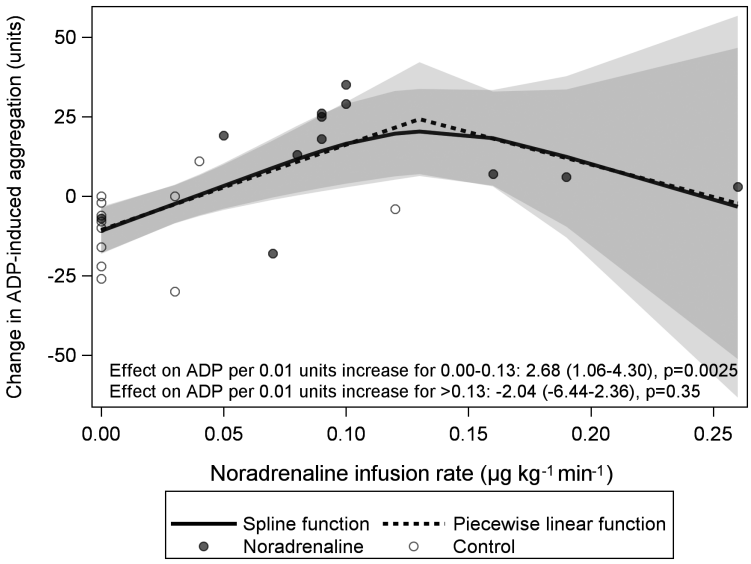

*Figure 18. Effect of NE-infusion rate on the change in ADP-induced platelet aggregation between the time points 10 minutes before and 50 minutes after anaesthesia induction. **

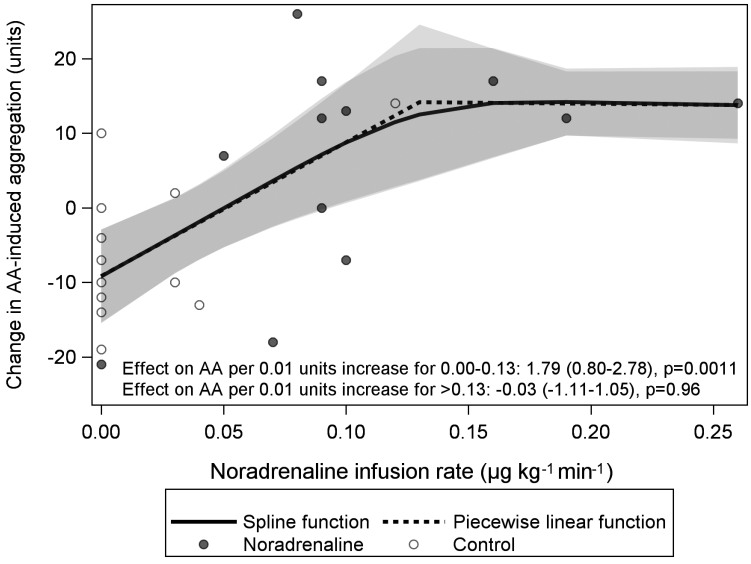

*Figure 19. Effect of NE-infusion rate on the change in AA-induced platelet aggregation between the time points 10 minutes before and 50 minutes after anaesthesia induction. **

** "Regression models with natural cubic splines (black lines) were used. The spline functions were simplified into piecewise linear functions (dotted lines) with a breaking point set at 0.13 µg/kg/min. Difference in Least Square Means (ΔLSM) with 95% Confidence Intervals (CI) are presented per 0.01 µg/kg/min increase in the NE-infusion rate".*[43]
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5. DISCUSSION

This thesis aimed to explore various aspects of anaesthesia induction-related hypotension and its prevention. We analysed the magnitude and dynamics of the hypotension-induced expansion of the plasma volume. For the first time, we looked at whether an increased PV secondary to perioperative hypotension leads to ANP release and EG degradation. Moreover, for the first time in a porcine model we investigated whether an ANP infusion caused degradation of the EG. In a sub-study, the effects of NE infusion on platelet function and clot formation were investigated on CABG patients treated with ASA.

5.1 Ethical issues

All studies were planned and predefined in advance. Ethical approval was obtained for studies I, II and IV from the Regional Ethical Review Board in Gothenburg. Studies II and IV were approved by the Sahlgrenska Radiation Safety Committee and by the Swedish Medical Products Agency. An appropriate notification was performed to Biobank Sweden and to the personal data law authority. The human studies were registered at www.clinicaltrials.com. All patients were given oral and written information before inclusion. All patients were cared for according to Good Clinical Practice and the Declaration of Helsinki.[38,39]

For study III, ethical approval was provided by the Ethics Committee on Animal Experiments at the University of Gothenburg. The animals received care in accordance with the Swedish Board of Agriculture regulations and common advice concerning research animals. The pigs lived in a peaceful environment where they were properly fed and taken care of by professional animal keepers. According to the regulations each pig had the company of at least one fellow pig during their remaining lifetime.

Patients in the intervention groups in studies I, II and IV were treated with NE infusion from the induction of anaesthesia in order to maintain MAP at the preoperative awake level. In study I, NE infusion was initially administered through a peripheral vein catheter until the central venous line was inserted. The peripheral line was checked for a proper backflow before induction in order to avoid subcutaneous NE infusion and the risk of tissue necrosis. The central line was checked for backflow and a central venous pressure curve before NE infusion was connected.

The patients in the intervention group received NE with no clinical need of NE. Reduced blood flow, secondary to NE-induced vasoconstriction, could be a potential threat. However, all patients had a normal heart function with an ejection fraction of at least 45%, and all, with a few exceptions, were operated in the morning and most probably normovolemic, factors that reduce NE-infusion hazards. Continuous ECG, oxygen saturation, cerebral oximetry as well as observation of lactate were monitored as safety indicators to reveal possible malperfusion due to NE-induced vasoconstriction. An increased left ventricular afterload, secondary to NE infusion could theoretically increase the cardiac work and therefore the myocardial oxygen consumption. On the other hand, NE infusion may increase the diastolic perfusion pressure improving the coronary perfusion and therefore supply the heart with more oxygen.

Patients in studies II and IV would normally have had their central venous line after anaesthesia induction. In studies II and IV both the central venous line and the pulmonary artery catheter were inserted in local anaesthesia before anaesthesia induction.

This is more inconvenient for the patients, but the procedural risks are the same. Ultrasound guidance was used in order to avoid accidental carotid artery puncture. Nevertheless, one patient had an uneventful accidental carotid artery puncture. Patients in studies II and IV would normally not have had a pulmonary artery catheter. Although complications secondary to pulmonary artery catheterisation are uncommon, any sort of a ventricular arrythmia could be dangerous for a patient suffering from coronary artery disease. No complications secondary to the right-heart catheterisation were noted. The pulmonary artery catheter was removed at the ICU before the patient was weaned from the respirator.

A total dose of 0.2 MBq [megabecquerel(s)] ^{125}I-albumin was given to each patient in study II. According to the guidelines of the World Health Organization (WHO), no iodine prophylaxis was needed for the patients. The radiation dose to the patient was very low, at the same level as obtained from a flight from Sweden to the USA. For safety reasons, the radiation level in the theatre was checked with a dosimeter for the first patients and was found to be negligible.

5.2 Haemodynamics

The gold standard methods, direct continuous intraarterial blood pressure measurement, continuous measurement of central venous pressure and intermittent measurements of cardiac index by the thermodilution technique via the pulmonary artery catheter were used.

The baseline, preinduction MAP for all 48 patients in studies I and II was 96 ± 9.0 mm Hg. According to the European Society of Cardiology/European Society of Hypertension, arterial hypertension guidelines, a blood pressure of 130–139/85–89 mm Hg is defined as higher normal.[44] The corresponding MAP would be 100-105 mmHg. Thus, although an immediate preinduction blood pressure usually poorly reflects an ambulatory blood pressure, the baseline MAP recorded in these studies can be considered reasonably normal.

In studies I and II, two distinct MAP strategies were compared (maintaining pre-operative MAP vs allowing MAP to decline to 60 mm Hg). Pressure levels were targeted with intravenous NE infusion.

Solely intraarterial and central vein pressure measurements cannot differentiate cardiac from vascular or combined causes of hypotension. Neither can mechanisms behind NE-maintained pre-operative MAP be interpreted without PAC measurements.

In study II, both the control group and the intervention group were equal considering baseline haemodynamic measurements including MAP, CVP, CI and SVR. As intended, the MAP during the trial was 62 ± 5 mm Hg in the control group and 93 ± 9 mm Hg in the intervention group.

In study II, the CVP increased in both groups. The increase in CVP could be explained by the increase in PV in the control group. In the intervention group, the α-adrenergic NE stimulation most probably caused a vasoconstriction of the venous capacitance vessels causing an increase in intrathoracic blood volume explaining the increased CVP.

In study II, both the CI and the SV decreased to the same extent with no difference between the groups. The reduced SV was most probably caused by anaesthesia-induced myocardial depression together with positive pressure ventilation. As no change was noted in heart rate, the reduced SV also explained the similar reduction in CI in both groups.

Data on the effects of NE on stroke volume and cardiac index are somewhat controversial. In a single-centre study on septic shock patients, dose titration of NE from MAP

65 to 85 mm Hg raised CI.[45] In vasoplegic cardiac surgery patients, an increase in MAP from 60 to 90 mm Hg by NE titration, increased the SV and the CI with no change in HR.[46] In a trial on uncomplicated postoperative cardiac surgery patients, NE titration increased MAP from 70 to 90 mmHg with no change in CI or HR.[47] In another trial on postoperative cardiac surgery patients, NE was found to have variable effects on cardiac output while increasing the MAP.[48] It was demonstrated that a SV-variation >8.7% reliably predicted an increased cardiac output in response to NE infusion.[48] In studies I and II, all patients were going through elective cardiac surgery and were most probably normovolemic at the time of anaesthesia induction. Thus, it is possible that their stroke volume variation was <8.7% explaining why their cardiac output did not increase by NE infusion.

In study II, 19 of 24 patients were preoperatively treated with β-blockers, the majority with metoprolol and a few with bisoprolol, which both are β_1-selective adrenoreceptor blockers. It is therefore possible that the NE-β_1 effect normally resulting in a slight positive inotropic effect of the myocardium was blunted by the β_1-selective adrenoreceptor blockers.

The fall in CI in the intervention group can also be explained by the negative impact of the high left ventricular afterload. This can, together with the anaesthesia-induced depression of myocardial performance and the β_1-selective adrenoreceptor blockers explain the fall in SV and CI, despite the inotropic effect of NE.

Taken together, reduced venous return (preload), reduced myocardial contractility and reduced systemic vascular resistance or different combinations of these can all induce post-induction hypotension. In the high-pressure intervention group, treated with NE infusion, the SVR increased significantly whereas it decreased significantly in the low-pressure control group. Thus, in studies I and II the higher MAP in the intervention group was primarily caused by a NE-induced increase in SVR.

5.3 Haematocrit and plasma volume

In all studies, haemoglobin was measured on a blood gas machine. Duplicates were used in the first study. The blood gas machine (RAPIDPoint 500) used in studies II-IV, at the cardiac surgery department in Gothenburg, has a measured coefficient of variation (CV) for estimation of tHb, between 0.4% and 0.7%. The corresponding measured CV for Hb at the routine haematology instruments at the clinical chemistry ward in Gothenburg is 2%. All blood gas syringes were accurately mixed when the blood gas was taken and just before the blood gas was analysed.

The (in study I and II) measured baseline Hct (%) 40 ± 4.1 and 41 ± 3.8 for women and men respectively, agrees with the normal values for women (35-46) and men (39-50).

In study II, baseline PV measurements were performed by, in both experimental and clinical settings, the well-established ^{125}I-albumin method.[5,49,50] The ^{125}I-albumin technique is widely used and often referred to as the gold standard method for measurements of PV. Based on a previous clinical study, suggesting that mixing of ^{125}I-albumin was 97% complete after 1 minute, a 5-minute period was chosen from injection to collection of blood sample.[50]

In study II, plasma volume was 2.5 ± 0.3 l and 3.4 ± 0.9 l for women and men respectively, measured by the ^{125}I-albumin technique. Volumes indexed for weight were 34 ± 4 ml/kg for women and 40 ± 10 ml/kg for men and agrees with the in literature normal values reported.

In studies I and II, the reduction in Hct at 10 minutes was mean -2.1 ± 0.8% units in the control groups and mean -0.3 ± 0.7% units in the intervention groups. The results are in line with previous studies showing a reduction in Hct of 2.7-2.8% units respectively 20 - 30 minutes after anaesthesia induction.[8,51]

The corresponding increase in calculated plasma volume was mean 12 ± 4.4% in the control groups and mean 2.6 ± 4.2% in the intervention groups. These studies show that the Hct decreased and the PV increased significantly more in the control groups compared to the intervention groups.

All patients had elective CABG surgery, normal heart function, no infection and all patients, expect a few had their surgery in the morning. Based on these assumptions we considered the patients normovolemic with a normal capillary permeability at the time of anaesthesia induction.

According to the 2-pore theory for transcapillary fluid exchange, fluid is transported across the capillary membrane by passive mechanisms through both the small and the large pores.[50] According to the classic Starling principle, transcapillary fluid exchange depends on the balance between hydrostatic and oncotic pressure gradients.[9] A sudden change in blood pressure, like the change from preoperative MAP of 96 ± 9.0 mm Hg to post anaesthesia-induction MAP of 62 ± 5 mm Hg in studies I and II, most probably decreased the capillary hydrostatic pressure causing an reabsorption from the interstitial to the intravascular space. The hydrostatic capillary pressure is determined by the MAP, by the post-/pre-capillary resistance ratio and by the CVP. A decreased hydrostatic capillary pressure can thus be the result of a decrease in MAP, in the post-/pre-capillary resistance ratio and in the CVP or a combination of these. Our assumption that anaesthesia induction-induced hydrostatic capillary pressure reduction causes a reabsorption of fluid from the tissues to the capillaries is supported by previous experimental and clinical studies showing that a sudden decrease in capillary hydrostatic pressure can transiently shift the steady state Starling equilibrium and cause a transcapillary fluid reabsorption.[52,53] According to the revised Starling principle interstitial fluid is returned to the blood stream by the lymphatic system and not by reabsorption.[54] It is, however, unlikely that the haemodilution with 400 ml within 10-30 minutes is explained by increased lymph return.

Changes in the disappearance rate of radioactive albumin after the induction is a function of both TER and PV expansion. In study II, we could not resolve changes in TER and changes in PV based on measurement of ^{125}I-albumin alone. Uncorrected TER was 22% per hour in the control group and 6.9% per hour in the intervention group. An adjustment of TER for dilution effects caused by increased PV was therefore performed. The corrected TER in study II, was 0% per hour in the control group and 6% per hour in the NE group. A TER of 5% per hour for albumin has previously been shown and is in line with our data in the group with maintained MAP.[55] The slightly higher TER in the intervention group in comparison to the control group is in compliance with a previous study showing significant positive correlation between the TER of albumin and blood pressure.[56]

The findings that serum albumin and the colloid osmotic pressure decreased to a greater extent in the control group compared to the intervention group in study II further support the observations of an increased PV in the control group. Whether the increased PV affected hormonal release of ANP and how this possibly affected the permeability of the EG has not previously been studied.

5.4 ANP and endothelial glycocalyx

In study II, we investigated whether a rapid increase of the PV due to anaesthesia-induced hypotension could release the cardiac hormone ANP and induce the shedding of EG components. In a porcine model, in Paper III, the effects of an ANP infusion on EG degradation were studied.

Shedding of the EG has been reported in patients undergoing cardiac surgery.[28,57] Patients undergoing on-pump CABG surgery have been reported to degrade the EG to a greater extent than patients undergoing off-pump CABG-surgery.[28] Both cardiac surgery and cardio pulmonary bypass (CPB) are independently suspected to activate a systemic inflammatory response leading to perioperative degradation of the EG.

Regarding volume loading, ANP release and EG degradation, there are conflicting results. Some studies reported that a volume loading stimulated ANP release, whereas others could not confirm the association. Some studies have reported an association between ANP release and EG degradation, whereas others have failed to confirm the association. For example, volume loading, as a blood-sparing procedure, has been shown to increase the release of ANP that has been suggested to cause EG degradation.[58] In another study, on post-operative patients with signs of hypoperfusion, a rapid infusion of 5% albumin caused an increase in ANP but no EG shedding.[59] In a third study, a 750 ml crystalloid fluid bolus degraded the EG without a significant increase in ANP.[60]

The important role of ANP in maintaining arterial blood pressure and intravascular volume homeostasis has been shown. Targeted deletion of the murine genes encoding for the ANP-peptide or its GC-A-receptor has been shown to lead to chronic arterial hypertension and hypervolemia.[61,62] Atrial wall stress is considered to be the major regulator of ANP secretion. The effects of ANP on endothelial and EG permeability regulating the intravascular volume homeostasis have still not been fully elucidated. Anaesthesia induction-related hypotension and PV changes could theoretically affect both the release of ANP and EG degradation components.

In study II, the PV increased significantly more in the control group (420 ml) compared to the intervention group (45 ml). MR-proANP increased significantly in the low-pressure group, but with no statistically significant difference between the groups. The CVP increased in both groups and was most probably a result of increased transcapillary reabsorption in the control group and due to NE-induced venoconstriction that caused an increased intrathoracic blood volume in the intervention group.

In addition to an increased preload causing a mechanical stretch of the atrium, an increased afterload has been reported to stimulate ANP secretion.[63] The SVR was significantly higher in the intervention group compared to the control group. The PAWP increased significantly in the intervention group, which was not seen in the control group. This was most likely an effect of the NE-induced increase in left-ventricular afterload. In spite of this increase in left-atrial pressure, only a modest and insignificant increase in MR-proANP was seen. Although there was a significant increase in MR-proANP in the control group, glycocalyx degradation, measured by the glycocalyx components hyaluronic acid and syndecan-1, was not found.

In study III, the ANP infusion did not degrade the EG measured by the glycocalyx components heparan sulphate proteoglycan and hyaluronic acid. This differs from previous ex vivo studies suggesting that ANP degrades the EG. A significant coronary venous washout of glycocalyx constituents and enhanced vascular permeability was observed

in isolated guinea pig hearts subjected to intracoronary perfusion with physiological concentrations of A-, B-, and C-type natriuretic peptide.[64,65] In study III, Hct increased, the calculated PV decreased and the COP increased in the ANP group and the changes were significantly different between the ANP group and the NaCl group. These results are in line with previous studies reporting an increased capillary permeability and reduced intravascular volume secondary to administration of recombinant ANP.[66]

Whether there was an ANP, via GC-A receptor, induced increase in microvascular endothelial permeability and thereby a decrease in PV cannot be clarified by our study setup. In study III, the pre-/post-capillary resistance ratio could have decreased due to an ANP-induced fall in precapillary resistance, thus explaining an increase in hydrostatic capillary pressure and a decrease in PV with or without a change in vascular permeability.[67]

5.5 Norepinephrine effects on platelet aggregation and clot formation

The effects of NE infusion on platelet aggregation (measured by impedance aggregometry) and clot formation (measured by rotational thromboelastometry) were investigated in study IV. NE infusion resulted in increased AA- and ADP- induced aggregation that was significantly different from the changes noticed in the control group. Furthermore, the INTEM maximum clot firmness increased significantly in the NE group. These findings suggest that NE infusion could clinically be used in order to improve platelet aggregation and clot firmness.

To the best of our knowledge, no previous studies have assessed NE-induced platelet aggregation and clot formation in ASA-treated patients undergoing CABG surgery. In a previous study, on healthy volunteers, platelet aggregation was improved by a NE infusion at a low and moderately high dose (0.03 and 0.14 μg/kg/min respectively).[68] This is in line with study IV, where a NE infusion rate up to 0.13 μg/kg/min improved AA- and ADP-induced platelet aggregation. A possible explanation for the improved platelet aggregation could be through NE-induced activation of α_{2A}- adreno receptor-mediated pathways.

In another study, on 24 healthy middle-aged men, studying NE infusion to mimic the effects of NE released during acute stress, a NE infusion of 0.07 μg/kg/min activated blood coagulation.[69] The improved INTEM maximum clot firmness, seen in study IV, that is physiologically correlated to fibrinogen and platelet contribution to the strength of the blood clot, could be explained by both the improved platelet aggregation but also by NE infusion-induced increase of coagulation factor VIII and fibrinogen.[69]

In a retrospective study on cardiac surgery patients on CPB, a low dose of NE infusion was associated with reduced haemodilution and intraoperative red cell transfusion.[70] In that study, fewer units of red cells were intraoperatively transfused in the NE group (0.2 ± 0.6 units/patient) compared with controls (0.53 ± 1.47).[70]

The observed NE infusion-related prothrombotic changes could be of clinical relevance, particularly with regard to patients treated with dual antiplatelet therapy and being in need of acute surgery.

5.6 Limitations and strengths

Limitations of the studies were the single-centre design and the lack of blinding of intervention in studies I, II and IV. Both studies III and IV were sub-studies of two randomised trials with other objectives. Hence neither studies III or IV were powered to

evaluate EG degradation in study III or haemostatic variables in study IV. The sample size is considered small in studies I, II and IV. Furthermore, the MAP-intervention protocol in studies I, II and IV referred to the time interval prior to CPB. Thus, changes in Hct, PV, ANP, EG and haemostasis during CPB and after CPB were not studied.

The design in studies I and II cannot distinguish between the effects of different blood pressure targets and the NE dose itself on the changes in PV. In study II, a repeated measures correlation showed, however, no correlation between NE and the change in PV, whereas the changes in MAP and PV were significantly correlated with each other. Furthermore, seven patients in the control group received NE at some timepoint during the experimental procedure and there was no difference in the PV change in the control group between patients receiving or not receiving NE.

In study II, a second dose of ^{125}I-albumin was given 45 minutes after anaesthesia induction in order to measure the actual PV at 50 minutes. However, this parameter could not be determined with high enough accuracy in the control group. The reason is that the PV increased after anaesthesia induction in an unknown manner in this group and the ^{125}I-activity remaining from the first administration could thus not be determined and subtracted from the total ^{125}I-activity in subsequent plasma samples. Furthermore, the assumption that steady state was obtained within 5 minutes after ^{125}I-albumin administration could not be ensured in both groups.

In study III, methylprednisolone was given as a pre-treatment before sternotomy in order to provide haemodynamic stability. Methylprednisolone may blunt the inflammatory cascade and thereby inhibit the potential EG degradation. Moreover, in study III, anaesthesia was maintained with isoflurane, that has immediate and delayed protective effects against cytokine-induced injury in endothelial cells.[71] Taken together, both methylprednisolone and isoflurane could have attenuated the degradation of the EG.

The strengths are that all studies were randomised, prospective studies, and study III was also blinded for the interventional team. The majority of outcome measurements were made blinded to treatment. Although studies I and II are separately considered as small studies, they are a result of the same basic experimental setup and regarding the results of Hct and PV, together interpreted as a medium-sized study. The external auditing support the reliability and scientific integrity of the data in study II. Study III, being a large animal study, included a relatively high number of pigs.

6. CONCLUSIONS

I. Anaesthesia induction-related hypotension caused a rapid increase in the plasma volume.

II. Maintaining blood pressure at pre-anaesthesia induction levels with norepinephrine infusion preserved the plasma volume.

III. Anaesthesia induction-related plasma volume expansion increased the release of atrial natriuretic peptide but had no effect on endothelial glycocalyx degradation.

IV. Exogenous administration of atrial natriuretic peptide did not cause endothelial glycocalyx degradation in an experimental porcine model.

V. Intraoperative infusion of clinically relevant norepinephrine doses in ASA-treated coronary artery bypass grafting patients improved platelet aggregation and clot formation.

7. FUTURE PERSPECTIVES

The goal of intraoperative blood pressure management research should be to define which blood pressure thresholds to target for and to develop preventive measures and treatment options for the common hypotensive condition. In order to accomplish this, the underlying mechanisms need to be understood and the possible causal relationship between perioperative hypotension and organ injury needs to be tested in randomised multi-centre trials.

In **Papers I** and **II** we found a strong association between a lower MAP and an increase in the plasma volume. Maintaining blood pressure at pre-anaesthesia induction levels with NE infusion preserved the plasma volume. The study period should be extended to the whole perioperative period. The outcome of systemic inflammatory response syndrome, organ dysfunction, infections and thromboembolic events, length of stay at the ICU and in the hospital as well as postoperative mortality should be addressed in the future along with safety outcomes of NE infusion.

In **Paper II** there was an association between increased PV and ANP release but with no effect on EG degradation. Although the change in ANP was significant in the control group, there was no difference between the groups. The negative result between the groups could still be due to an underpowered study and thus this should be addressed in a larger study.

In order to measure the actual PV at 50 minutes after anaesthesia induction a new baseline plasma sample should be collected before the second dose of ^{125}I-albumin, or another radiotracer, such as technetium-99m (^{99}mTc) labelled albumin should be administered as the second dose.

In **Paper III** the ANP infusion did not cause EG degradation, but decreased the PV. In this trial, methylprednisolone was used to provide stable cardiovascular conditions. Methylprednisolone may blunt the inflammatory cascade and thereby inhibit the potential degradation of glycocalyx induced by ANP. The study should therefore be repeated without methylprednisolone administration and ideally also as a pure clinical trial investigating the effects of ANP infusion only on the EG without any surgery.

In **Paper IV** norepinephrine infusion improved platelet aggregation and clot formation in ASA-treated CABG patients. The effect of norepinephrine or epinephrine infusion on platelet aggregation and clot formation should perioperatively be assessed on patients with ongoing DAPT.

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