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Evaluation of circulating exosomes in the course of surgical aortic valve replacement and coronary artery bypass grafting

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to my parents

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Abstract

In industrialized countries, calcific aortic valve disease is the most widespread valvular heart disease and is frequently associated with coronary artery disease. Over the last decade, research on extracellular vesicles (EVs) has developed enormously, focussing in particular on promising clinical applications, for example in cardiovascular diseases (CVDs). To date, literature has focused mainly on larger EVs, such as microvesicles, while the potential use of the smaller exosomes as biomarkers in CVDs remains largely unexplored.

The aim of this study is to investigate the development of circulating exosomes in the course of surgical aortic valve replacement (SAVR) and coronary artery bypass grafting (CABG) as well as to investigate the correlation between exosomes and established clinical parameters. Further, we examine whether exosome level variations may be associated with the aortic valve stenosis (AS) severity and potentially provide information about the postoperative clinical outcome.

Exosomes of patients undergoing SAVR and CABG were analysed by nanoparticle tracking analysis, characterized by CD63 Western blotting and correlation analyses with laboratory and echocardiographic parameters at two preoperative and three postoperative time points were performed.

Preoperative levels of circulating exosomes decreased significantly up to 24 hours postoperatively, followed by a significant recovery 7 days postoperatively. Also exosome-derived total protein content and CD63 revealed significant changes throughout the hospital stay for SAVR and CABG. Moreover, even though exosome levels did not seem to be influenced by AS severity, postoperative decreased exosome levels showed to be significantly associated with laboratory and echocardiographic parameters. In particular, our findings revealed that exosomes may be an indicator for postoperative outcome, *e.g.* for the development of a prosthesis-patient mismatch. Moreover, our findings led us to identify erythrocytes as a possible main source of circulating exosomes.

Collectively, circulating exosomes display a possible role as biomarkers to evaluate the postoperative outcome after SAVR and CABG.

Zusammenfassung

In Industrieländern ist die kalzifizierte Aortenklappenstenose (AS) die am weitesten verbreitete Herzklappenerkrankung und ist häufig mit der koronaren Herzerkrankung assoziiert. Die Forschung an extrazellulären Vesikeln (EVs) hat sich im letzten Jahrzehnt enorm entwickelt und konzentriert sich v.a. auf mögliche klinische Anwendungen, z.B. in kardiovaskulären Erkrankungen (engl.: cardiovascular diseases; CVDs). Bisher konzentrierte sich die Literatur hauptsächlich auf größere EVs, wie Mikrovesikel, während die potenzielle Verwendung der kleineren Exosomen als Biomarker in CVDs weitgehend unerforscht bleibt.

Ziel dieser Studie ist es, den Verlauf zirkulierender Exosomen im Laufe von chirurgischem Aortenklappenersatz (engl.: surgical aortic valve replacement; SAVR) und koronarer Bypassoperation (engl.: coronary artery bypass grafting; CABG) zu untersuchen, sowie eine mögliche Korrelation zwischen Exosomen und etablierten klinischen Parametern zu untersuchen. Darüber hinaus untersuchen wir, ob Variationen der Exosomenanzahl mit dem Schweregrad der AS assoziiert sein könnten und potenziell Informationen über das postoperative klinische Ergebnis liefern.

Exosomen von Patienten, die einen SAVR mit CABG erhalten haben, wurden zu zwei präoperativen und drei postoperativen Zeitpunkten mittels Nanopartikel-Tracking-Analyse analysiert, durch CD63 Western Blots charakterisiert und mit Labor- und echokardiographischen Parametern korreliert.

Die präoperative Anzahl zirkulierender Exosomen nahm signifikant bis 24 Stunden postoperativ ab, gefolgt von einer signifikanten Erholung 7 Tage postoperativ. Auch der gesamte exosomale Proteingehalt und CD63 zeigten signifikante Veränderungen im Laufe von SAVR und CABG. Obwohl die Exosomenanzahl nicht durch den AS-Schweregrad beeinflusst zu werden scheint, präsentierten postoperative verminderte Exosomenspiegel eine signifikante Assoziation mit Labor- und echokardiographischen Parametern. Unsere Ergebnisse zeigten insbesondere, dass Exosomen möglicherweise als Indikator für den postoperativen Verlauf dienen können, z.B. für die Entwicklung eines Patient-Prothesen Mismatches. Schließlich wurden Erythrozyten als mögliche Hauptquelle für zirkulierende Exosomen identifiziert.

Zirkulierende Exosomen stellen also potenzielle Biomarker für die Evaluation des postoperativen Ergebnisses nach SAVR und CABG dar.

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Abbreviations

If not otherwise specified, the International System of Units is used.

ACS	American Chemical Society
AI	aortic valve insufficiency
AMI	acute myocardial infarction
ANOVA	analysis of variance
APS	ammonium persulfate
AS	aortic valve stenosis
AST	aspartate transaminase / aminotransferase, also known as glutamic oxaloacetic transaminase
AV	aortic valve
AVR	aortic valve replacement
AVS	aortic valve sclerosis
AWd	anterior wall end-diastolic diameter
BD	Becton, Dickinson and Company
BMI	body mass index
BNP	Brain natriuretic peptide
BSA	body surface area
CABG	coronary artery bypass grafting
CAD	coronary artery disease, also known as coronary heart disease
CAVD	calcific aortic valve disease
CD	cluster of differentiation
CK	creatine kinase
cLVH	concentric left ventricular hypertrophy
CM	cOmplete^{TM} Mini solution
CPB	cardiopulmonary bypass

Cr	creatinine
CRF	case report form
CRP	C-reactive protein
cTn	cardiac troponin
CVD	cardiovascular disease
Da	Dalton
DC	detergent compatible
DTT	dithiothreitol
dP_m	(transvalvular) mean pressure gradient
dP_p	(transvalvular) peak pressure gradient
EACTS	European Association for Cardio-Thoracic Surgery
ECC	extracorporeal circulation
EDD	end-diastolic diameter
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
eLVH	eccentric left ventricular hypertrophy
euroSCORE	European System for Cardiac Operative Risk Evaluation
EOA	effective orifice area
EOA_i	effective orifice area indexed to the body surface area
ESC	European Society of Cardiology
EV	extracellular vesicle
Fig.	Figure
Fr	French scale or French gauge
g	gravitational force (when following a number without any spacing)
G	(Birmingham) Gauge

GOT	glutamic oxaloacetic transaminase, also known as aspartate transaminase / aminotransferase
GPT	glutamic pyruvic transaminase
Hb	haemoglobin
Hct	haematocrit
HRP	horseradish peroxidase
HSP	heat shock protein
ICU	intensive care unit
ILV	intraluminal vesicle
IMCU	intermediate care unit
INR	international normalized ratio
IVSd	intraventricular septal end-diastolic diameter
LDH	lactate dehydrogenase
LV	left ventricular
LVEDD	left ventricular end-diastolic diameter
LVEF	left ventricular ejection fraction
LVESD	left ventricular end-systolic diameter
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass indexed to the body surface area
M	molar
MHC	major histocompatibility complex
miRNA	micro ribonucleic acid
MP	microparticle
mRNA	messenger ribonucleic acid
MV	microvesicle

MVE	multivesicular endosome
MVB	multivesicular body
n.s.	not significant
NSTEMI	non-ST elevation myocardial infarction
NTA	nanoparticle tracking analysis
NYHA	New York heart association
p-value	probability value
PAGE	polyacrylamide gel electrophoresis
PCI	percutaneous coronary intervention, also known as percutaneous transluminal coronary angioplasty
PFO	patent foramen ovale
PLT	platelet, also known as thrombocyte
peri-op	perioperative / perioperatively / around the time of the surgery
post-op	postoperative / postoperatively / after surgery
pre-op	preoperative / preoperatively / before surgery
PPM	prosthesis-patient mismatch
PS	PhosSTOP^{TM} solution
PTCA	percutaneous transluminal coronary angioplasty, also known as percutaneous coronary intervention
PT	prothrombin time
PTT	partial thromboplastin time
PVDF	polyvinylidene fluoride
PWd	posterior wall end-diastolic diameter
RBC	red blood cell, also known as erythrocyte
RIPA	radioimmunoprecipitation assay
RM	repeated measures

RWT	relative wall thickness
SAVR	surgical aortic valve replacement
SD	standard deviation
SDS	sodium dodecyl sulfate
SI	*Système international d'unités* (International System of Units)
STEMI	ST elevation myocardial infarction
STS	Society of Thoracic Surgeons
t	time point
Tn	troponin
TTE	transthoracic echocardiography
TAVI	transcatheter aortic valve implantation, also known as percutaneous aortic valve implantation
TEM	tetraspanin-enriched microdomain
TEMED	tetramethylethylenediamine
TBS	tris-buffered saline
TBS-T	tris-buffered saline with Tween® 20
TSH	thyroid-stimulating hormone
TVR	tricuspid valve replacement
V_{max}	transvalvular peak velocity
WBC	white blood cell, also known as leucocyte

1 Introduction

1.1 Calcific aortic valve disease and coronary artery disease

In industrialized countries, calcific aortic valve disease (CAVD) is the most prevalent valvular heart disease amongst the elderly and is frequently associated with coronary artery disease (CAD) [1, 2]. The traditional open surgical treatments for these two heart diseases are surgical aortic valve replacement (SAVR) and coronary artery bypass grafting (CABG), respectively [1]. Alternative non-surgical interventions are, for CAVD, transcatheter aortic valve (AV) implantation (TAVI) and, for CAD, percutaneous coronary intervention (PCI), also termed percutaneous transluminal coronary angioplasty (PTCA) [3, 4].

1.1.1 CAVD and SAVR

Epidemiology and clinical manifestation. CAVD is the most common cause of AV stenosis (AS), with an incidence of 2 - 7% amongst the elderly (over 65) [5]. The prevalence of calcific AS approximates 2% in the population older than 65, it increases to about to 3% in patients over 75 and to around 4% in patients over 85 [5]. AV sclerosis (AVS), the preclinical manifestation of AS, has a prevalence of about 20 - 30% in the population older than 65, of about 35% in patients over 75 and of almost 50% in patients over 85 [5, 6].

CAVD is characterized by a slow progression over years (that can last up to 30 - 40 years) and an abrupt symptom development associated with high mortality [2, 7-9]. Classical symptoms due to a reduced pump function are: fatigue, palpitations, exertional dyspnoea, syncope, *angina pectoris* and congestive heart failure [10-14]. Further, sudden cardiac death risk is increased [10]. Various studies confirm that while untreated symptomatic AS displays a mortality up to 50% in 1 year, AVR can prevent this dramatic survival decline [8, 11, 15-17].

Remodelling. Increased pressure gradients due to lumen restriction at the valvular level is compensated through an initially beneficial LV hypertrophy (LVH), ("remodelling"), that can temporarily normalize wall stress and maintain systolic function [16]. Nevertheless, hypertrophy causes both an increase in the myocardial oxygen demand and a reduction of coronary blood flow [16]. These factors lead to myocardial ischemia and necrosis and, as a result, to interstitial fibrosis, so that a maladaptive, irreversible LV impairment is developed [12, 16]. Interestingly, several studies have revealed a so-called LV "reverse remodelling" (also referred to as LV mass - LVM - regression) after AVR, with a mass reduction of 30 - 40%

within 18 months postoperatively [16, 18, 19]. Noteworthily, this process apparently not only influences systolic and diastolic performance, but also long-term survival [16].

AS classification. Doppler echocardiography is considered as gold standard for AS staging [2]. On the basis of hemodynamic conditions, in particular of transvalvular peak velocity (V_{max}), mean pressure gradient (dP_m - the systolic pressure difference between left ventricle and aorta) and effective orifice area (EOA), it is possible to assess AS severity (see **Table 1**) [2, 20, 21]. The indication for AVR is then determined by evaluation of AS severity and LV systolic function, as well as by assessment of clinical symptoms [11].

Table 1: Schematic AS classification. Classification according to the 2017 Guidelines of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) [21].

AS severity	V_{max} (m/s)	EOA (cm^2)	dP_m (mmHg)
mild	< 4	> 1.5	< 25
moderate	< 4	1.0 - 1.5	25 - 39
severe	≥ 4	< 1	≥ 40

AS: aortic valve stenosis, V_{max}: transvalvular peak velocity, EOA: effective orifice area, dP_m: mean pressure gradient.

Prosthesis-patient mismatch. A known postoperative risk is the emergence of a prosthesis-patient mismatch (PPM) [19]. This occurs when EOA of the implanted valve is too small in relation to the body surface area (BSA) of the recipient patient, resulting in increased dP [19, 22]. The parameter used to define PPM is the EOA indexed to the BSA (EOA_i), defined by the following formula and expressed in cm^2/m^2 [22]:

$$EOA_i = \frac{EOA}{BSA} \qquad (1)\ EOA_i \text{ formula}$$

Since dP increases exponentially at EOA_i between 0.8 and 0.9 cm^2/m^2, the threshold for PPM has been established at 0.85 [22]. Specifically, values between 0.65 and 0.85 are considered as moderate PPM, while values < 0.65 are indicative for severe PPM [22]. PPM can result from a post-insertion tissue ingrowth and endothelialization, as well as from the anatomical conditions (in particular the annulus) sometimes disproportionately limiting the implantable prosthesis size [19].

PPM prevalence is relatively high (between 20 - 70% for moderate PPM and between 2 - 11% for severe PPM) and PPM has been shown to be associated with an adverse clinical outcome due to increased cardiac events, worse hemodynamic conditions and less LVM regression [22].

In particular, incomplete LVM regression after SAVR in patients with AS has been shown to be associated with impaired long-term clinical outcome [18]. Various risk factors for the development of a postoperative PPM - such as older age and greater BMI - have been described but strategies used until now to avoid its occurrence have resulted in conflicting results [18, 23]. Hence, some authors have advocated to calculate EOA_i during evaluation for SAVR and, if the development of PPM is likely, the patient's general condition should be evaluated to determine the overall risk to benefit ratio [22].

AVR: surgical procedure. Conventional AVR is an open-heart surgery in which the stenotic valve is removed and replaced with a new tissue or mechanical one [13]. It is traditionally performed via median sternotomy with aortic cross-clamping and cardioplegic cardiac arrest with the use of cardiopulmonary bypass (CPB), *i.e.* using a heart-lung machine [24].

Meanwhile, SAVR can also be performed as minimally invasive cardiac surgery (MIC-AVR) using several approaches [25, 26], including so-called "J" or "L" partial sternotomy and right anterior lateral mini-thoracotomy [25]. Even though MIC-AVR showed no difference in hard endpoints, *e.g.* hospital mortality, when compared to conventional SAVR, currently it is technically more challenging [25, 26].

An alternative to open-heart surgeries for patients at intermediate-high surgical risk is TAVI, that is increasingly being performed over the last decade [3, 8, 17, 27]. Nevertheless, a meta-analysis including more than 4,000 subjects revealed a TAVI overall survival ≥ 3 years worse than SAVR [28].

1.1.2 CAD and CABG

Epidemiology and clinical manifestation. CAD is the leading cause of death worldwide, with more than seven million deaths per year (about one third of all, not only of CVDs) [29]. Further, due to the increasing life length and the falling CAD mortality thanks to the constant treatment improvements, the prevalence of patients living with a CAD is continuously growing [29, 30]. Possible clinical manifestations of CAD are: silent ischemia, (non)-ST segment elevation myocardial infarction (NSTEMI / STEMI), unstable angina, arrhythmias, heart failure and sudden cardiac death [31].

CABG: surgical procedure. In CABG, one of the most frequent major surgeries, autologous small diameter blood vessels (*e.g.* internal thoracic artery, greater saphenous vein or radial artery) are used as grafts to bypass obstructed coronary arteries [4]. In a traditional on-pump

CABG, via median sternotomy the ascending aorta is occluded, the heart is arrested with a cardioplegic solution and CPB is used to guarantee perfusion and blood oxygenation [4]. The coronary artery is then incised distally to the atherosclerotic plaque and the bypass graft is sewn end-to-side to the incision [4]. When the left internal thoracic artery is used, the native inflow from the left subclavian artery can be maintained, otherwise the proximal end of the graft is anastomosed end-to-side to the ascending aorta or to another graft [4].

Meanwhile, CABG can also be performed off-pump, *i.e.* with the heart beating and without the use of a CPB and in this way manipulation of the ascending aorta can be minimized and thereby the risk of neurological complications reduced [32].

Different randomized trials have shown that especially patients with CAD of the left main coronary artery or with triple-vessel disease and reduced LV systolic function, including patients with diabetes, benefit from CABG as compared to competing transcatheter techniques [33]. Patients with a minor extent of CAD, a normal LV function and absence of major risk factors have been shown to experience no significant improvement in survival when receiving CABG and may therefore rather benefit from PCI, which can be directly performed during the catheter angiography, the diagnostic gold standard for CAD [4, 34, 35].

1.2 Exosomes

Over the last decade, research on extracellular vesicles (EVs) has become increasingly widespread [36]. Exosomes differ from other EV types, such as microvesicles (MVs) - also known as microparticles (MPs), ectosomes or shedding vesicles - and apoptotic bodies, because of their size, content, functions and formation process (**Fig. 1**) [37-39]. Their diameter has been described to be between 30 and 200 nm (and may depend on the origin cell type), rendering exosomes the smallest EV identified to date [40-43]. In fact, MVs are generally defined to be 0.1 to 1 μm and apoptotic bodies 1 to 4 μm [37, 44]. The present work is focused on exosomes, although it must be specified that an exact differentiation between exosomes and MVs is not always possible [45, 46].

Origin and content of exosomes. Exosomes were first described almost four decades ago as vesicles shed from neoplastic cells *in vitro* [47]. Already at that time it was hypothesized that these vesicles could have a physiological function *in vivo* and it was proposed to call them "exosomes" [47].

Exosomes have been identified in many different body fluids, *e.g.* blood, urine, saliva, pericardial fluid, ascites fluid, cerebrospinal fluid, amniotic fluid, breast milk, semen and bile [48, 49]. They transport both cytosolic components (like proteins, lipids and nucleic acids) and membrane proteins of the origin cell [48, 50, 51]. For example, they contain stress-associated heat shock proteins (HSPs), heteromeric G proteins, messenger ribonucleic acid (mRNA), microRNA (miRNA) and they exhibit membrane proteins such as major histocompatibility complex-II (MHC-II), integrins, cluster of differentiation 63 (CD63) and growth factors [50, 51]. The expressed surface receptors may also function for targeting specific effector cells [45].

Biogenesis of exosomes. The exosome release mechanism was first analysed with electron microscopy during maturation of sheep and rat reticulocytes to erythrocytes (also known as red blood cells - RBCs), a process that includes the recycling of reticulocyte transferrin receptors [52, 53]. It was observed how these vesicles form by budding inwards from the limiting membrane of early endosomes and multivesicular bodies / endosomes (MVBs / MVEs) and expose the endocytosed transferrin receptors on their membrane [48, 52, 54, 55]. The MVBs then fuse with the plasma membrane releasing the exosomes in the extracellular space [52, 53]. Once their destination is reached, exosomes deliver their cargo either by fusing directly with the target cell membrane, or by endocytosis and subsequent fusion with an endosome [45].

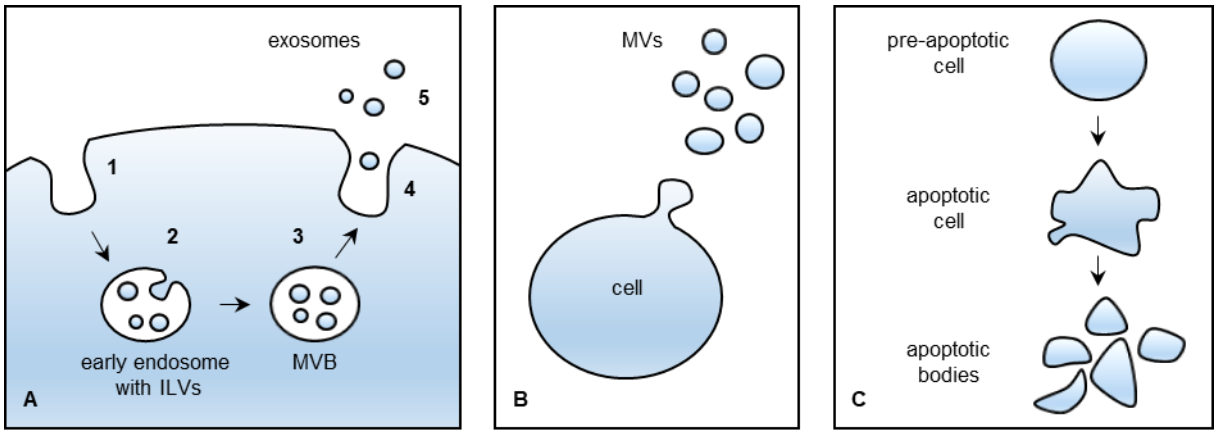

Figure 1: EV release. A: Exosomes are released following different steps: 1) early endosomes bud inwards from the plasma membrane; 2) so called intraluminal vesicles (ILVs) are formed by budding inwards from the endosome membrane; 3) the endosomes develop to multivesicular bodies (MVB); 4) the MVB fuse with the plasma membrane 5) releasing the ILVs, now called exosomes, in the extracellular space [48]. **B:** Microvesicles (MVs) are shed directly by budding outwards from the plasma membrane [48]. **C:** Apoptotic bodies form by cell fragmentation of the apoptotic origin cell [37].

Function of exosomes. The major exosome functions are listed in **Table 2**. The overall exosome role is intercellular communication: they transport origin cell components to target cell, thereby mediating paracrine and endocrine signalling [45, 51]. Potentially, exosomes can be secreted by every cell that contains MVBs [56].

A previous report has emphasized that dendritic cell-derived exosomes of 60 - 90 nm in diameter may suppress murine tumours by inducing a T-lymphocyte tumour specific immune response, suggesting a function as target cell suppressors [50, 57]. Further, exosomes are released by PLTs and seem to play a role in the PLT activation process, during which both exosomes and MVs are secreted [58]. Moreover, exosomes released from infected macrophages have been shown to trigger a pro-inflammatory response both *in vitro* and *in vivo*, while RBC-derived exosomes may boost an immune response only *in vitro* [59, 60].

Finally, it has been reported that exosomes released from (vascular) endothelial cells and cardiomyocytes play a role in the response to cellular stress (*e.g.* hypoxia, inflammation, hyperglycaemia and angiotensin-II release), mediating the transfer of stress signals to target cells by transporting a cargo that reflects the stress condition of their origin cell [51, 61]. In particular, exosomal endothelium-derived HSP70 seems to act as a pro-inflammatory cytokine and directly trigger the development of CVDs (in particular of atherosclerosis), suggesting a role in the regulation of vascular homeostasis and endothelial integrity [62, 63].

Table 2: Major exosome origin cells and functions.

Origin cell	Function	Reference
Origin cell (generally)	Intercellular communication	[51]
Reticulocyte	RBC maturation	[52, 53]
Dendritic cell	Target cell suppression	[50, 57]
PLT	PLT activation	[58]
Macrophage and RBC	Immune response	[59, 60]
(Vascular) endothelial cell and cardiomyocyte	Cellular stress response	[51, 61]

RBC: red blood cell, PLT: platelet.

1.2.1 Exosomes in CAVD, CAD and cardiac surgery

While MVs have already been intensively examined in respect to their role in atherogenesis and cardiovascular risk, exosomes have not yet been adequately investigated concerning their diagnostic and therapeutic use in CVDs [45, 63, 64]. Given the many similarities between MVs and exosomes, these two vesicle types may well share various biological functions [45]. Besides, whereas biomarkers in CAD have already been studied considerably, in CAVD they have hardly been explored yet [65, 66].

Clinical biomarkers of CVDs. In the last decade, brain natriuretic peptide (BNP) has increasingly been used both in risk stratification and in monitoring of valvular heart disease, since it correlates with myocardial wall stress and disease severity [67]. Further, MVs have meanwhile become known to be shed intensively under stress conditions and, in particular, in diseases involving increased shear stress, vascular injury, inflammation and thrombosis, serving both as biomarkers and as pathology mediators [37, 68, 69]. For example, it has been reported that MVs deriving from endothelium, leucocytes (white blood cells; WBCs) and PLTs are shed increasingly in patients with severe AS [68]. Nevertheless, other biomarkers enabling the identification of asymptomatic patients in initial disease stages may potentially add value to the current standard of diagnostic methods when screening for intervention [67].

Since exosomes transport the typical components of the origin cell with all embedded biological information and their concentration changes depending on the presence of a disease, exosomes may have a potential clinical value as biomarkers, for example in CAD [36, 39, 70]. Moreover, since exosomes may be considered as a “fingerprint” of the origin cell reflecting the cellular

pathological process of CVDs, they may represent a novel diagnostic tool, available simply by analysing body fluids such as blood, urine or saliva [63].

Interestingly, a study has shown that exosome concentration in blood was significantly increased immediately after CABG (before chest closure) for up to 48 hours, preceding the appearance of detectable high-sensitive cardiac troponin I (cTnI), the gold standard biomarker of myocardial damage [40]. Further, cTnI correlated positively with both the number of exosomes themselves and with the level of exosomal cardiac miRNAs, and the latter two also showed a positive association [40]. In fact, due to the use of CPB and of cardioplegic arrest, classical surgical procedures are inevitably associated with a variable myocardial damage [40, 71]. However, unlike current myocardial biomarkers, exosomes are not passive products of dying myocytes but are actively secreted and current knowledge suggests that exosomes may induce a response in target cells [40]. Therefore, exosomes are suggested to be directly involved in the regulation of physiological and pathological conditions [40]. Although exosomes are shed from many different types of cells, their increase after CABG greatly suggests myocardial cells as origin, since cardiac miRNAs were detected within them [40, 71, 72]. According to these studies, CABG seems to induce the trafficking of exosomes from the myocardium to the distinct organs [40]. Hence, exosomes could possibly be used as biomarkers to monitor response to cardiac surgery, maybe permitting a prediction of clinical outcome [40].

1.2.2 Exosome characterization

Exosomes have been reported to contain tetraspanins in highly enriched concentrations compared to their origin cells and CD63 in particular has often been used as an exosome marker [38, 42, 73]. Nevertheless, since tetraspanins are plasma membrane proteins, CD63 may be expressed also on other EVs [38]. CD63 was originally discovered as a membrane protein of activated PLTs that triggers PLT aggregation [74-76]. One emerging function of CD63 is transport regulation of its interaction partners, such as integrins, cell surface receptors and other tetraspanins [77].

The tetraspanin membrane protein family is characterized by four hydrophobic transmembrane domains linked by a small and a large extracellular loop with short cytoplasmic tails (N and C *terminus*), as well as by a small intracellular loop [77]. These proteins typically consist of 200 - 300 amino acids with three N-glycosylation sites and can interact with other membrane or cytosolic proteins to create multiprotein structures called tetraspanin-enriched microdomains

(TEMs) or “tetraspanin webs”, which are cell-type specific [77-80]. Interestingly, TEMs may play a role in the exosome formation, cargo selection and target cell binding [38]. Tetraspanins participate in different biological processes, such as cell activation, adhesion, differentiation, migration and fusion as well as signal transduction, coordinating multi-molecular signalling complexes [76-78]. Moreover, it has been reported that tetraspanins are involved in various processes such as vascular injury, angiogenesis, haemostasis and thrombosis [81].

1.3 Statement of purpose and hypothesis

The aim of this study is to investigate whether the perioperative course of established clinical parameters may correlate with the respective course of circulating exosomes in patients undergoing SAVR and CABG. Further, we aim at investigating whether exosome variation from preoperative to postoperative levels may provide information on the postoperative outcome and the risk of emerging PPM.

2 Materials and methods

2.1 Materials

2.1.1 Devices

The devices used for this work are listed in **Table 3**.

Table 3: Devices.

Device	Company
Centrifuge 5804 R	Eppendorf AG, Hamburg, Germany
Amersham™ Imager 600 chemiluminescence device	GE Healthcare Europe GmbH, Freiburg, Germany
Vivid S5 and S6 cardiovascular ultrasound machines	GE Healthcare Europe GmbH, Freiburg, Germany
Infinite M1000 PRO microplate reader	Tecan Group Ltd., Zürich, Switzerland
ZetaView® NTA instrument	Particle Metrix GmbH, Meerbusch, Germany

NTA: nanoparticle tracking analysis.

2.1.2 Materials, commercial solutions and consumables

Materials, commercial solutions and consumables used for this work are listed in **Table 4**.

Table 4: Materials, commercial solutions and consumables.

Material	Company
Acetone ACS reagent, ≥ 99.5%	Sigma Aldrich, Steinheim, Germany
40% acrylamide	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
APS	Sigma Aldrich, Steinheim, Germany
Ampuwa® water	Fresenius Kabi Deutschland GmbH, Bad Homburg vor der Höhe, Germany
Blotting paper Rotilabo 580 × 600 mm	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Bromophenol blue	Sigma Aldrich, Steinheim, Germany
CD63 antibody (H-193), sc-15362	Santa Cruz Biotechnology, Dallas, Texas, USA
cOmplete™ Mini Tablets	Roche, Basel, Switzerland

DC protein assay (reagent A + B)	Bio-Rad, Munich, Germany
DTT	Sigma Aldrich, Steinheim, Germany
ExoQuick® Exosome Precipitation Solution	System Biosciences SBI, Palo Alto, USA
Glycerol	Sigma Aldrich, Steinheim, Germany
Glycine	SERVA Electrophoresis GmbH, Heidelberg, Germany
Goat anti-rabbit IgG-HRP, sc-2004	Santa Cruz Biotechnology, Dallas, Texas, USA
Isopropanol	Millipore, Darmstadt, Germany
Methanol	VWR BDR Prolabo, Bruchsal, Germany
PageRuler Plus Prestained Protein Ladder	Thermo Fisher Scientific, MA, USA
PhosSTOP™ Tablets	Roche, Basel, Switzerland
Pierce Western Blot Transfer Buffer, 10×	Thermo Fisher Scientific, MA, USA
PVDF blotting membrane 0.45 µm	GE Healthcare Europe GmbH, Freiburg, Germany
Protein standard (bovine serum albumin solution 1.0 mg/mL)	Sigma Aldrich, Steinheim, Germany
RIPA buffer (cell lysis buffer)	Sigma Aldrich, Steinheim, Germany
Sodium chloride	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
SDS pellets	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Skim milk powder	Fluka, Buchs, Switzerland
SuperSignal West Femto Maximum Sensitivity Substrate	Thermo Fisher Scientific, MA, USA
TEMED	Bio-Rad, Munich, Germany
Tris base	Sigma Aldrich, Steinheim, Germany
Tris-Glycine SDS buffer 10× (running buffer)	Thermo Fisher Scientific, MA, USA
Tween® 20	Calbiochem, San Diego, USA

ACS: American Chemical Society, APS: ammonium persulfate, DC: detergent compatible, DTT: dithiothreitol, HRP: horseradish peroxidase, PVDF: polyvinylidene fluoride, RIPA: radioimmunoprecipitation assay, SDS: sodium dodecyl sulfate, TEMED: tetramethylethylenediamine.

2.1.3 Buffers and solutions

The buffers and solutions prepared for this work are listed in **Table 5**.

Table 5: Buffers and solutions.

Buffer / solution	Components	Quantity
0.5 M tris HCl pH 6.8	tris base	6.057 g
	distilled water	100 mL
1.5 M tris HCl pH 8.8	tris base	45.428 g
	distilled water	250 mL
	HCl / NaOH if necessary, until pH 8.8 reached	
Laemmli buffer 2× (loading buffer)	0.5 M tris HCl pH 6.8	2 mL
	10% SDS	4 mL
	glycerol	2 mL
	2 M DTT in distilled water	2 mL
	bromophenol blue	0.1 mg
Prepared RIPA buffer	RIPA buffer	2.15 mL
	PhosSTOP™ solution (1 tablet in 1 mL RIPA buffer)	250 µL
	cOmplete™ Mini solution (1 tablet in 2 mL RIPA buffer)	100 µL
10% SDS	SDS pellets	10 g
	distilled water	100 mL
TBS 10×	tris base	24 g
	sodium chloride	88 g
	distilled water	1 L
TBS-T 1×	TBS 10×	100 mL
	distilled water	900 mL
	Tween® 20	1 mL
Water-saturated isopropanol	isopropanol	80 mL
	distilled water	20 mL

SDS: sodium dodecyl sulfate, DTT: dithiothreitol, RIPA: radioimmunoprecipitation assay, TBS: tris-buffered saline, TBS-T: tris-buffered saline with Tween® 20.

2.2 Clinical study design

This work is based on a non-randomized, single-centre, descriptive, prospective, longitudinal study involving patients with a hemodynamically significant CAVD undergoing isolated SAVR or SAVR combined with CABG at the *Universitätsklinikum Düsseldorf* (University Hospital in Düsseldorf) between June 2015 and September 2016. The present work focused on the group of patients who received SAVR combined with CABG.

2.2.1 Clinical examination time points

Within the hospitalization period, study patients (see Paragraph 2.2.2 Patient screening and inclusion and exclusion criteria) were informed about the study at admission to the cardiosurgical ward. After a consulting interview, and both an oral and a written consent, patients received a copy of the patient information material that is attached in the appendix.

The standard course of a hospital stay is depicted in **Fig. 2**.

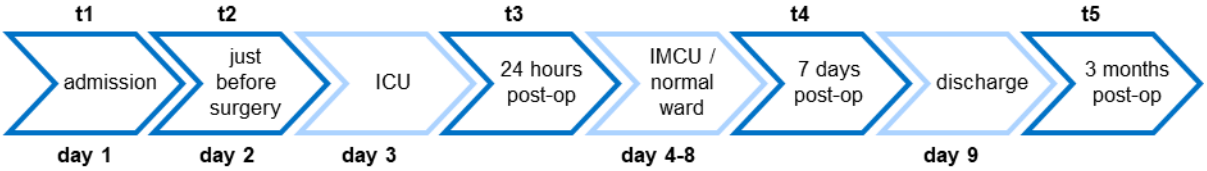

Figure 2: Standard hospital stay and clinical examination times points. Within the study framework, at fixed time points the patients underwent four examinations during their stay in hospital for elective surgical aortic valve replacement (SAVR) and coronary artery bypass grafting (CABG): t1 = admission day, t2 = just before surgery after induction of anaesthesia, t3 = 24 hours postoperatively, t4 = 7 days postoperatively and subsequently a follow-up examination 3 months after surgery (t5). t: time point, ICU: intensive care unit, post-op: postoperatively, IMCU: intermediate care unit.

Directly on the day of admission, or rather at the examination time point 1 (t1), a preoperative anamnesis of the patients was collected, study-specific blood samples (in addition to the standard blood samples of the ward) were taken and a preoperative transthoracic echocardiography (TTE) was performed. On the next day (t2), the operation took place and study-specific blood samples were collected directly after induction of anaesthesia. After the operation, patients were transferred to the intensive care unit (ICU). The third study-specific blood samples were taken on the first morning after the operation (t3). Depending on the postoperative progress, patients were then transferred to the intermediate care unit (IMCU) and further to the normal cardiosurgical ward. At the earliest on postoperative day 7 the patients were then discharged home or to a rehabilitation centre. Before discharge or more precisely on

postoperative day 7 (t4), for the fourth time study-specific blood samples (in addition to the standard blood samples) were taken and a second TTE was performed according to the standard of care protocols of the Department of Cardiac Surgery. Finally, at a follow-up examination 3 months postoperatively (t5), the last blood samples (in addition to the standard blood samples) were taken and a final TTE was performed.

2.2.2 Patient screening and inclusion and exclusion criteria

The eligibility criteria are listed in **Table 6**.

Table 6: Eligibility criteria.

Inclusion criteria	Exclusion criteria	
	primary	**secondary**
Hemodynamically significant AS requiring replacement	Severe valvular heart disease (> II) other than AS	Thrombotic embolism in the last 6 months
Informed written consent for participation in the study	Severe impairment of the LVEF (< 30%)	Autoimmune or inflammatory disease
Age ≥ 18	Peripheral artery occlusive disease > IIb (Fontaine stage)	Cancer
	AMI in the last 30 days	Dialysis

AS: aortic valve stenosis, LVEF: left ventricular ejection fraction, AMI: acute myocardial infarction.

2.2.3 Ethics committee approval

This study was approved by the Ethics Committee of the Medical Faculty of the Heinrich-Heine University in Düsseldorf under the number 3881 (04.09.2012 and 28.09.2017) and complies with the Declaration of Helsinki of the World Medical Association. Participation in this study had no effect on the patient's treatment nor on the choice of the valve prosthesis. The participation is voluntary and the consent may be withdrawn at any time, for any reason and without influence on the further medical care.

2.3 Data collection

All the clinical data were collected by means of standardized protocols and questionnaires, documented in patient-specific case report forms (CRFs, attached in the appendix) and then digitalized in a Microsoft Excel database. Protocol deviations were always recorded.

2.3.1 Patient history, demographic and perioperative parameters

Patient history and demographic data were collected on the admission day. European System for Cardiac Operative Risk Evaluation (euroSCORE) II and Society of Thoracic Surgeons (STS) score were calculated to estimate the individual operative risk.

Operative parameters were documented in the CRFs directly from the operative report and the perfusion report. All perioperative data, as well as the details concerning any possibly required blood transfusions, were documented using the patient's records, anaesthesia protocol, operative report as well as perfusion report. An example of the aforementioned is shown in the appendix.

2.3.2 Blood samples

Study-specific blood samples were taken at all five examination time points using BD (Becton, Dickinson and Company) Vacutainer® blood tubes always in the same order: first serum (5 mL), then citrate (2.7 mL), then heparin (3 mL) and lastly ethylenediaminetetraacetic acid (EDTA, 2 × 6 mL). The samples collected on the admission day, 7 days and 3 months postoperatively were taken using a 21G BD Vacutainer® butterfly-needle set in accordance with the study protocol (summarized in **Fig. 3**). Just before surgery after induction of anaesthesia and 24 hours postoperatively an 8.5 Fr jugular central venous catheter was used. Blood sampling was combined with therapeutically necessary blood sampling wherever possible.

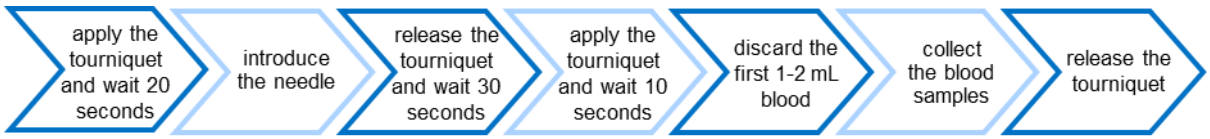

Figure 3: Blood sampling procedure.

The samples needed for the exosome quantification (the serum blood tubes) were processed directly after collection. The rest of the samples were stored at -80 °C until further use.

Calculation of the glomerular filtration rate. The estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft-Gault-formula and expressed in mL/min (2):

$$eGFR = \frac{\{[(140 - age) - weight]\}}{Cr * 72} * 0.85\ if\ female \quad \text{(2) Cockcroft-Gault-formula}$$

where Cr is creatinine [82].

2.3.3 Echocardiographic parameters

The echocardiographic data were acquired performing TTEs on the admission day, 7 days and 3 months postoperatively. A study-specific form (see **Table 7**) was used to determine study-specific parameters in addition to the routine ones.

Table 7: Echocardiographic parameters.

Parameter	Abbreviation	Unit
Left ventricular ejection fraction	EF	%
Left ventricular end-diastolic diameter	LVEDD	mm
Left ventricular end-systolic diameter	LVESD	mm
Intraventricular septal end-diastolic diameter	IVSd	mm
Anterior wall end-diastolic diameter	AWd	mm
Posterior wall end-diastolic diameter	PWd	mm
Transvalvular mean pressure gradient	dP_m	mmHg
Transvalvular peak pressure gradient	dP_p	mmHg
Transvalvular peak velocity	V_{max}	m/s
Effective orifice area	EOA	cm^2
Aortic valve insufficiency (I-IV)	AI	-

Calculation of V_{max}. V_{max} was calculated according to the recommendations of the European Association of Cardiovascular Imaging and the American Society of Echocardiography 2017 using the following formula [20]:

$$V_{max} = \sqrt{\frac{dP_p}{4}} \quad \text{(3) } V_{max} \text{ formula}$$

Calculation of shear stress. In accordance with existing literature [68] transvalvular shear stress was estimated according to the following formula:

$$transvalvular\ shear\ stress = \frac{V_{max}}{LVEF} \quad \text{(4) Transvalvular shear stress estimation}$$

where V_{max} is the transvalvular peak velocity and LVEF is the left ventricular ejection fraction.

Calculation of LVH parameters. LVM, LV mass indexed to the BSA (LVMI) and relative wall thickness (RWT) were calculated according to the recommendations of the European Association of Cardiovascular Imaging and the American Society of Echocardiography 2015 [83]. The LVM (g) was estimated using the end-diastolic LV cavity dimension and wall thickness with the following formula (5):

$$LVM = \frac{0.8\{1.04[(LVEDD + IVSd + PWd)^3 - LVEDD^3]\} + 0.6}{1000} \quad \text{(5) LVM formula}$$

where 1.04 is the specific gravity of the myocardium (g/cm^3), LVEDD the left ventricular end-diastolic diameter, IVSd the intraventricular septal end-diastolic diameter, PWd the posterior wall end-diastolic diameter.

The LVMI (g/m^2) allows to classify, according to sex, into mild (female: 96 - 108 g/m^2, male: 116 - 131 g/m^2), moderate (female: 109 - 121 g/m^2, male: 132 - 148 g/m^2) and severe (female: ≥ 122 g/m^2, male: ≥ 149 g/m^2) LVH and is defined by the following formula:

$$LVMI = \frac{LVM}{BSA} \quad \text{(6) LVMI formula}$$

The RWT allows to distinguish between eccentric (RWT ≤ 0.42) and concentric (RWT > 0.42) LV hypertrophy (eLVH and cLVH) and was calculated using the following formula (7):

$$RWT = \frac{2 * PWd}{LVEDD} \quad \text{(7) RWT formula}$$

where PWd is the posterior wall end-diastolic diameter and LVEDD the left ventricular end-diastolic diameter.

2.4 Exosome analysis

Directly after the blood sample collection, exosomes were isolated from the serum tube with ExoQuick® Exosome Precipitation Solution following the manufacturer's protocol and examined by nanoparticle tracking analysis (NTA) using a ZetaView® NTA device.

2.4.1 Isolation

The exosomes were isolated as described in **Fig. 4**. Briefly, the blood samples were centrifugated at 4 °C for 15 minutes at 1700g to obtain PLT-rich serum and then 300 µL of the latter were centrifugated in an Eppendorf safe-lock tube at 4 °C for 15 minutes at 3000g to achieve PLT-poor serum. Further, 63 µL of Exoquick® (a polymer that precipitates exosomes) were added to 250 µL of the serum supernatants, vortexed and incubated for 30 minutes on ice. The samples were then vortexed again and centrifugated for 30 minutes at 1500g. The supernatants were drawn up and discarded, while the tubes containing the pellets were centrifugated for 5 minutes at 1500g. Lastly, the remaining supernatants were drawn up, obtaining the final exosome pellets ready for resuspension.

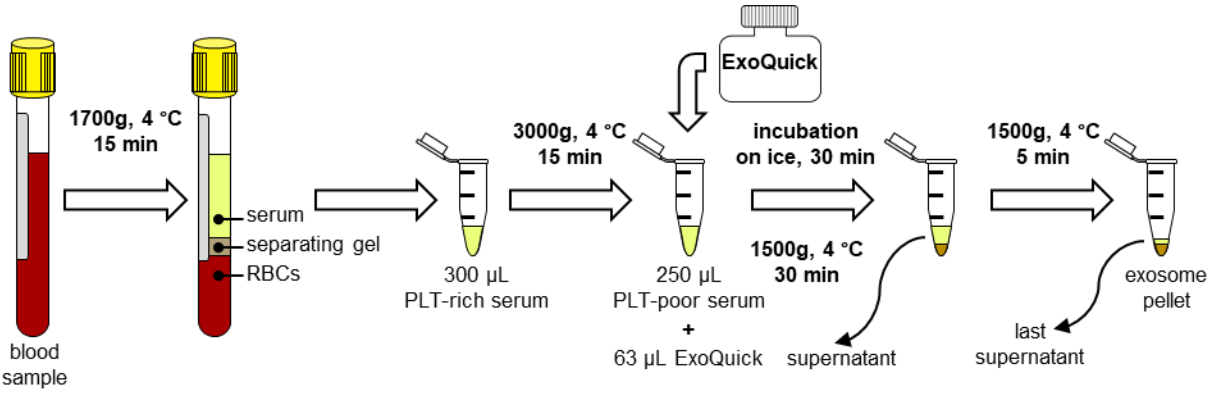

Figure 4: Exosome isolation. The exosomes were isolated using ExoQuick® Exosome Precipitation Solution following the illustrated centrifugation steps. After the last centrifugation, the remaining supernatant was drawn up, obtaining the final exosome pellet ready for resuspension. RBCs: red blood cells, PLT: platelet.

For the NTA, the exosome pellets were then resuspended in 30 µL of ultrapure water (Ampuwa®) in order to obtain the final exosome solution. To use an exosome concentration in millilitres, in further analyses the measured values (in 250 µL serum) were multiplied by 4 (250 µL × 4 = 1 mL), by 8.33 (250 µL serum / 30 µL ultrapure water) and by 250,000 (we used a 250,000 dilution - see Paragraph 2.4.2 NTA). For the Western blot analysis (see Section 2.5 Exosome characterization), the same exosome isolation protocol was applied, but using EDTA

blood tubes (or rather plasma instead of serum) with half of the above declared amounts. Exosome pellets were then resuspended following the respective protocol.

2.4.2 NTA

A study has shown that among different techniques to detect EVs - such as transmission electron microscopy, flow cytometry and resistive pulse sensing - NTA was able to detect the smallest size and could identify particles between 50 and 450 nm [43, 84].

ZetaView® NTA instrument. In this work, NTA was performed using a ZetaView® NTA device (**Fig. 5**). The microscope optical axis is focused on the cell channel, that is filled with the exosome solution, whilst being orthogonal to the laser beam [85]. When the particles are irradiated by the laser beam, the scattered light is orthogonally registered by the video camera, permitting the detection of the particles and the determination of their absolute number and size distribution [43, 85].

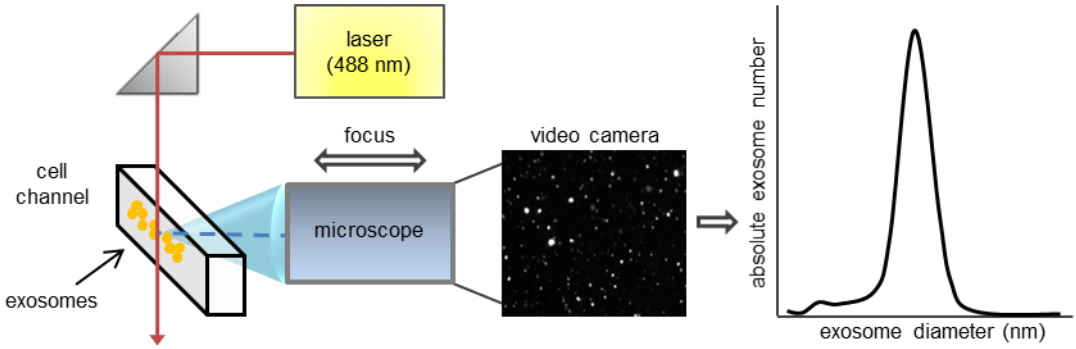

Figure 5: ZetaView® NTA instrument. When the exosomes are irradiated by the laser beam previously reflected at 90°, the scattered light is orthogonally registered by the video camera. In this way, the detected nanoparticles are directly visualized in a live-view screen and represented on a distribution curve [43, 85].

Settings. Before each measurement an automatic cell quality check was performed and technical parameters were set as illustrated in **Table 8**.

Cell channel was regularly cleaned thoroughly by flushing it with a 20% acetone solution in ultrapure water and the image was manually focused in order to always have a clear live-view screen with visible, unblurred particles. Further, after each measurement, the cell channel was thoroughly flushed with ultrapure water.

Table 8: ZetaView® NTA instrument technical parameters.

Parameter	Value / setting
Sensitivity	70
Shutter	70
Dilution	250,000
Number of experiments	3
Experiment type	size
Measurement positions	11
Cycles	10 per experiment

Analysis. To achieve a measurable concentration, a final dilution of about 1: 250,000 was attained in two steps. Firstly, 2.5 µL of the exosome solution isolated from the serum blood tube (see Paragraph 2.4.1 Isolation) were diluted in 247 µL of ultrapure water. Secondly, 10 µL of the obtained solution were further diluted with 25 mL of ultrapure water. At this point, 5 mL of the final solution were slowly injected through the inlet port by syringe in the NTA device, ensuring not to introduce air bubbles in the system that would alter the measurement. The particles could then be directly visualized and the measurement was started only when the particle drift reached a value < 30 µL/s, in order not to alter its validity.

Each measurement consisted of three experiments with ten cycles each. During each cycle, the particles were detected from eleven positions in the cell channel. Each experiment resulted in a distribution curve, that enabled the calculation of the mean and the standard deviation (SD) of the average number of particles, as well as of the particle size.

2.5 Exosome characterization

To characterize the circulating exosomes, Western blot analysis was performed targeting the exosome marker CD63 in samples isolated from the EDTA blood tubes (see Paragraph 2.4.1 Isolation). In order to obtain comparable measurements, the protein concentration of the exosome samples was determined after isolation and then the samples were diluted to achieve a standard protein content.

2.5.1 Modified Lowry protein assay

For the protein concentration measurement of the exosome samples, the Bio-Rad detergent compatible (DC) Protein Assay kit was used to perform a modified Lowry protein assay.

First of all, the exosome pellet isolated from the EDTA blood was resuspended in 100 µL of the previously prepared radioimmunoprecipitation assay (RIPA) buffer containing phosphatase- and protease-inhibitor cocktails (PhosSTOP™ and cOmplete™ Mini, see **Table 5**), in order to lyse the exosome membrane without denaturing the released proteins (**Fig. 6**).

For protein measurement, resuspended samples were diluted 1: 50 with distilled water and vortexed. Further, a serial dilution (0.0 - 1.0 mg/mL) was prepared using a bovine serum albumin solution (1.0 mg/mL) as protein standard and the readymade RIPA buffer and the amount of protein was determined at 750 nm using a microplate reader.

2.5.2 Protein sample preparation

The sample previously resuspended in the prepared RIPA buffer was diluted with distilled water to achieve a protein content of 2 µg/µL in a total volume of 100 µL (**Fig. 6**). Then 100 µL of Laemmli buffer 2× (see **Table 5**) were added to the sample resulting in a final concentration of 1 µg/µL protein content (and 200 mM DDT of the loading buffer). The tube was vortexed, denatured at 95 °C for 5 minutes and then placed directly on ice.

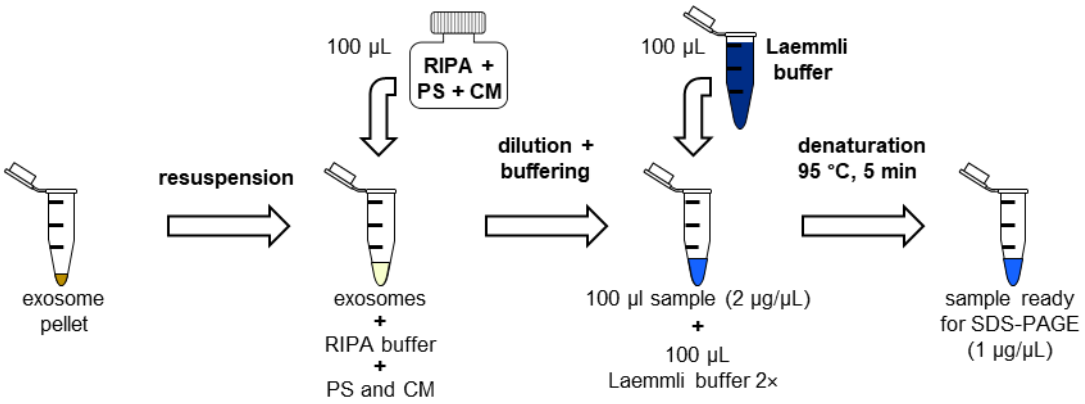

Figure 6: Sample preparation. The exosome pellet was resuspended in 100 µL of the previously prepared RIPA buffer containing phosphatase- and protease-inhibitor cocktails (PhosSTOP™ and cOmplete™ Mini solutions). In order to obtain comparable measurements, protein concentration was determined and samples were diluted to 2 µg/µL in a total volume of 100 µL. Then 100 µL of Laemmli buffer 2× were added to the sample, the tube was vortexed and denatured at 95 °C for 5 minutes, resulting in a final concentration of 1 µg/µL. RIPA: radioimmunoprecipitation assay, PS: PhosSTOP™ solution, CM: cOmplete™ Mini solution, SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

2.5.3 Gel casting and SDS-PAGE

Separating and stacking gels were prepared following the proportions illustrated in **Table 9** (quantity for 2 gels).

Table 9: Separating and stacking gels.

Components	Unit	Separating gel (12%)	Stacking gel (6%)
Double-distilled water	mL	8.6	5.8
1.5 M tris pH 8.8	mL	5	-
0.5 M tris pH 6.8	mL	-	2.5
40% acrylamide	mL	6	1.5
10% SDS	µL	200	100
TEMED	µL	40	20
10% APS	µL	200	100

SDS: sodium dodecyl sulfate, TEMED: tetramethylethylenediamine, APS: ammonium persulfate.

The protein ladder (5 µL per lane) and the exosome samples (10 µL per lane, *i.e.* a total amount of 10 µg protein content per lane) were loaded and gels were run for 15 minutes at 60 V and then for ~70 minutes at 150 V.

2.5.4 Western blot analysis

The blotting filter paper (8.5 × 6.5 cm) and the fiber pads were directly soaked in the 1× transfer buffer, while the membrane (polyvinylidene fluoride - PVDF, 0.45 µm) was first activated in methanol and then immersed in the buffer too. The gel and the membrane were sandwiched between the fiber pads and the paper, placed in the buffer tank, submerged in the buffer and an electrical field of 70 V was applied for 60 minutes, in order to transfer the proteins from the gel to the membrane.

Antibody staining. After the blotting, the membrane was incubated in a falcon tube on a tube roller with 10 mL of a 5% milk powder in tris-buffered saline with Tween® 20 (TBS-T) blocking solution (see **Table 5**) for 60 minutes. This way, the protein-free sites were blocked preventing nonspecific binding of the antibodies. The polyclonal primary antibody - CD63 (H-193), rabbit - was then diluted in the blocking solution 1: 500 and the membrane was incubated rotating overnight at 4 °C.

After incubation, the membrane was washed three times for five minutes with 10 mL TBS-T on a tube roller to eliminate the antibody excess. Subsequently, the incubation with the secondary antibody - goat anti-rabbit IgG-horseradish peroxidase (HRP) - directed against the primary one and diluted in TBS-T 1: 10,000 was carried out for 60 minutes in rotation at room temperature. Finally, the membrane was washed another three times.

Chemiluminescence detection. For signal detection, 1 mL of SuperSignal West Femto Maximum Sensitivity Substrate was pipetted on the membrane that was covered after a couple of minutes with a transparent film and detected in the chemiluminescence device. Protein band intensities were then quantified by densitometry analysis with the use of ImageJ (Version 1.50i).

2.6 Statistical analysis

Statistical analyses were performed with GraphPad Prism Software (GraphPad software, Inc., San Diego, USA) and Microsoft Excel (Version 16.0). The overall course of exosomes, protein content and CD63 was analysed with the use of repeated measures (RM) one-way analysis of variance (ANOVA). T test was used to compare data between patients receiving a biological and a mechanical valve, to compare data between two time points as well as to compare exosomes in female and male patients. Further, correlation between exosome levels and other variables was analysed by means of linear regression. P-values < 0.05 (*) were considered as statistically significant, values < 0.01 (**) as very significant, values < 0.001 (***) as highly significant, while values > 0.05 as not significant.

3 Results

3.1 Patient population

Of 250 patients undergoing isolated SAVR or SAVR combined with CABG, 204 were assessed for eligibility and 170 were finally enrolled in this study (**Fig. 7**). All patients gave written consent according to the study protocol and consent forms approved by the ethics committee. Of the 170 patients enrolled, 18 could only be followed up by phone or by contacting their general practitioner and 6 deceased before reaching the follow-up time point. Of the remaining 146 patients who completed study follow-up, 83 were treated with isolated SAVR, while 63 received a combined procedure with SAVR and CABG. Of these 63, further 4 patients had to be excluded for clinical reasons. This work focusses on the 59 patients undergoing SAVR combined with CABG who completed the study per protocol. Of these patients 53 received a biological valve, while 6 received a mechanical prosthesis.

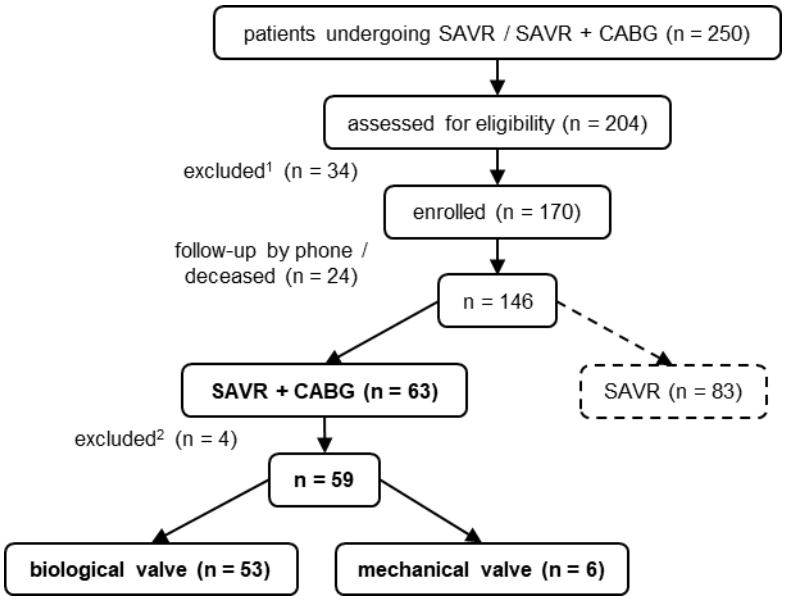

Figure 7: Patient population. The inclusion and exclusion criteria (see **Table 6**) were applied to the 250 patients undergoing SAVR or SAVR with CABG and accordingly 204 patients were assessed. Of these, 34 were excluded ([1]) for the following reasons: aortic valve insufficiency (AI) > grade 2 (n = 6), aortic root replacement (n = 11), no surgery (n = 2), TAVI (n = 8), others (n = 7). Additionally, 18 patients could only be followed up by phone or by contacting their general practitioner and were therefore ruled out from the study together with the 6 patients that deceased before the follow-up time point (n = 24). Of the 146 patients that completed the follow-up, 83 underwent an isolated SAVR, while 63 were treated with SAVR combined with CABG. Of the patients undergoing the combined surgery, 4 more had to be excluded ([2]) for clinical reasons (*i.e.* > 3 months spent on ICU or impossibility of taking a blood sample), so that 59 patients have completed the study per protocol: 53 received a bioprosthetic valve and 6 a mechanical one. SAVR: surgical aortic valve replacement, CABG: coronary artery bypass grafting.

3.1.1 Patient characteristics

Baseline characteristics and clinical history of the 59 patients treated with SAVR and CABG are listed in **Table 10.** As expected, there was a significant difference in age of patients receiving either prosthesis type, with patients receiving a mechanical valve being significantly younger. Of the 53 patients receiving biological valves 13 were female, while in the mechanical sub cohort only 1 female patient was present.

Patients in both subgroups were comparable in most of the analysed preoperative characteristics except for a significantly higher proportion of patients with hypercholesterinaemia in the group of biological valves. Further, half of the patients receiving a mechanical prosthesis were obese, as opposed to only a quarter of patients receiving biological prostheses.

Table 10: Baseline characteristics and clinical history of study patients.

Characteristic	**Biological valve (n = 53)**	**Mechanical valve (n = 6)**	**p-value**
Age (years)	74.1 ± 6.00	56.3 ± 7.56	**< 0.001**
Female sex	13 (24.5%)	1 (16.7%)	0.2272
Weight (kg)	82.7 ± 18.1	92.3 ± 24.7	0.2400
BMI class	-	-	0.0952
• Normal weight: 18.5 - 24.9	13 (24.5%)	1 (16.7%)	-
• Overweight: 25 - 29.9	27 (50.9%)	2 (33.3%)	-
• Obesity class I: 30 - 34.9	8 (15.1%)	2 (33.3%)	-
• Obesity class II: 35 - 39.9	3 (5.66%)	0 (0.00%)	-
• Obesity class III: ≥ 40	2 (3.77%)	1 (16.7%)	-
NYHA	-	-	0.1389
• Class I	2 (3.77%)	2 (33.3%)	-
• Class II	18 (34.0%)	0 (0.00%)	-
• Class III	29 (54.7%)	4 4 (66.7%)	-
• Class IV	4 (7.55%)	0 (0.00%)	-
Smoking status	-	-	0.1524
• Never	28 (52.8%)	0 (0.00%)	-
• Former	17 (32.1%)	2 (33.3%)	-
• Current	8 (15.1%)	4 (66.7%)	-

Diabetes mellitus	-	-	-
• Type I	1 (1.89%)	0 (0.00%)	0.4567
• Type II	14 (26.4%)	4 (66.7%)	0.2286
Cardiac decompensation	5 (9.43%)	0 (0.00%)	0.3922
Syncopation	2 (3.77%)	0 (0.00%)	0.4415
Pulmonary hypertension	11 (20.8%)	1 (16.7%)	0.2716
Cardiac arrhythmia	14 (26.4%)	2 (33.3%)	0.2018
Hypercholesterolemia	27 (50.9%)	2 (33.3%)	**0.0023**
Liver disease	2 (3.77%)	0 (0.00%)	0.4415
Arterial hypertension	45 (84.9%)	6 (100.0%)	0.3366
Lung disease	8 (15.1%)	1 (16.7%)	0.3339
Haemorrhage	0 (0.00%)	0 (0.00%)	-
Bicuspid AV	2 (3.77%)	2 (33.3%)	0.4390
euroSCORE II (%)	3.23 ± 1.98	2.17 ± 1.29	0.2073
STS score (%)	1.96 ± 0.71	2.01 ± 1.92	0.8961

BMI: body mass index, NYHA: New York heart association, AV: aortic valve, euroSCORE: European System for Cardiac Operative Risk Evaluation, STS: Society of Thoracic Surgeons. Data are presented as mean ± SD and the unit is given in brackets. When no unit is declared, the values refer to the absolute number of patients and in brackets the percentage of the total is given.

3.1.2 Blood count

Clinical laboratory parameters study patients are enumerated in **Table 11**. Predictably, there was a significant decrease of RBCs, Hb and Hct from before to after surgery as well as a significant increase from t4 to t5. Further, WBCs and CRP as well as Tnt und LDH increased significantly from t1 to t4 and then decreased from t4 to t5. On the contrary, CK decreased from before to after surgery. Moreover, PT, INR and PPT revealed a significant change from t1 to t5. The other parameters showed no or only slightly significant changes.

Table 11: Clinical laboratory parameters of study patients.

Parameter	t1	t4	t5	p-value (t1 - t4)	p-value (t1 - t5)	p-value (t4 - t5)
Hb (g/dL)	13.6 ± 1.89	10.6 ± 1.11	12.4 ± 1.57	**< 0.001**	**< 0.001**	**< 0.001**
Hct (%)	40.4 ± 4.80	32.2 ± 3.48	38.4 ± 4.28	**< 0.001**	**0.0193**	**< 0.001**
RBCs (10^6/µL)	4.49 ± 0.61	3.54 ± 0.37	4.47 ± 0.59	**< 0.001**	0.8796	**< 0.001**
WBCs (10^3/µL)	8.04 ± 2.58	10.6 ± 3.62	7.76 ± 2.46	**< 0.001**	0.5407	**< 0.001**
PLTs (10^3/µL)	257.7 ± 110.1	271.4 ± 110.4	253.1 ± 104.5	0.5005	0.8173	0.3574
TnT (ng/L)	26.8 ± 29.5	487.9 ± 860.8	25.4 ± 22.8	**< 0.001**	0.7663	**< 0.001**
CK (U/L)	131.3 ± 126.1	88.2 ± 64.8	95.1 ± 60.0	**0.0213**	**0.0489**	0.5439
LDH (U/L)	221.8 ± 72.6	344.0 ± 88.6	243.2 ± 48.5	**< 0.001**	0.0627	**< 0.001**
AST (U/L)	30.6 ± 27.4	47.8 ± 79.5	26.1 ± 8.71	0.1201	0.2307	**0.0398**
PT (%)	97.2 ± 18.4	90.4 ± 21.7	77.8 ± 30.6	0.0679	**< 0.001**	**0.0116**
INR	1.05 ± 0.27	1.19 ± 0.79	1.34 ± 0.58	0.2159	**< 0.001**	0.2456
PTT (s)	24.9 ± 3.89	29.3 ± 8.35	28.7 ± 6.15	**< 0.001**	**< 0.001**	0.6220
CRP (mg/dL)	0.86 ± 1.92	7.04 ± 5.10	0.84 ± 1.63	**< 0.001**	0.9465	**< 0.001**
Cr (mg/dL)	1.18 ± 0.63	1.09 ± 0.60	1.22 ± 0.60	0.4225	0.7302	0.2400
Urea (mg/dL)	39.8 ± 14.0	38.1 ± 21.5	47.3 ± 28.1	0.6102	0.0692	**0.0480**

Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, WBCs: white blood cells, PLTs: platelets, TnT: troponin T, CK: creatine kinase, LDH: lactate dehydrogenase, AST: aspartate transaminase / aminotransferase, PT: prothrombin time, INR: international normalized ratio, PTT: partial thromboplastin time, CRP: C-reactive protein, Cr: creatinine. Data are presented as mean ± SD and the unit is given in brackets.

The eGFR (calculated using the Cockcroft-Gault-formula, see Paragraph 2.3.2 Blood samples) of the study patients are enumerated in **Table 12**. In both groups, approximately 65% of the patients had a normal kidney function and only one single patient receiving a mechanical valve had a severe chronic kidney disease.

Table 12: eGFR of study patients.

Characteristic	**Biological valve (n = 53)**	**Mechanical valve (n = 6)**	**p-value**
eGFR	-	-	0.2247
• < 30 mL/min	0 (0.00%)	1 (16.7%)	-
• 30 - 60 mL/min	19 (35.8%)	1 (16.7%)	-
• > 60 mL/min	34 (64.2%)	4 (66.7%)	-

eGFR: estimated glomerular filtration rate. The values refer to the absolute number of patients and in brackets the percentage of the total is given.

3.1.3 Echocardiographic data

Patients in both subgroups were comparable in most of the analysed preoperative echocardiographic data except for LVH parameters (see **Table 13**). In fact, patients receiving a mechanical valve displayed a significantly greater LVM and LVMI. Further, patients receiving a biological valve tended to have a cLVH (RWT > 0.42), while patients receiving a mechanical valve an eLVH (RWT ≤ 0.42).

Table 13: Preoperative (t1) echocardiographic parameters of study patients.

Parameter	**Biological valve (n = 53)**	**Mechanical valve (n = 6)**	**p-value**
LVEF (%)	57.2 ± 8.28	55.0 ± 12.6	0.5615
LVEDD (mm)	48.7 ± 4.69	51.0 ± 5.80	0.2704
LVESD (mm)	34.1 ± 6.63	35.0 ± 4.82	0.7487
IVSd (mm)	13.5 ± 1.60	13.5 ± 1.00	> 0.999
AWd (mm)	11.5 ± 1.30	11.7 ± 0.57	0.7124
PWd (mm)	10.9 ± 1.05	11.3 ± 0.45	0.3625
dP_m (mmHg)	35.3 ± 10.8	36.0 ± 2.45	0.8757
dP_p (mmHg)	60.0 ± 17.1	60.8 ± 3.7	0.9101
V_{max} (m/s)	3.83 ± 0.59	3.90 ± 1.12	0.8046
EOA (cm^2)	0.79 ± 0.20	0.82 ± 0.12	0.7213
EOA_i (cm^2/m^2)	0.41 ± 0.10	0.42 ± 0.04	0.8103
shear stress[1]	0.07 ± 0.01	0.07 ± 0.01	> 0.999
LVM (g)	230.8 ± 49.7	327.0 ± 41.2	**< 0.001**
LVMI (g/m^2)	118.5 ± 20.5	164.5 ± 40.2	**< 0.001**
RWT	0.50 ± 0.06	0.40 ± 0.02	**< 0.001**
AI	-	-	0.2495
• Grade I	23 (43.4%)	3 (50.0%)	-
• Grade II	10 (18.7%)	2 (33.3%)	-
• Grade III / IV	0 (0.00%)	0 (0.00%)	-

LVEF: left ventricular ejection fraction, LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular end-systolic diameter, IVSd: intraventricular septal end-diastolic diameter, AWd: anterior wall end-diastolic

diameter, PWd: posterior wall end-diastolic diameter, dP_m: mean pressure gradient, dP_p: peak pressure gradient, V_{max}: transvalvular peak velocity, EOA: effective orifice area, EOA_i: effective orifice area indexed to the body surface area. [1]shear stress was estimated as V_{max} / LVEF (see Paragraph 2.3.3 Echocardiographic parameters), LVM: left ventricular mass, LVMI: left ventricular mass indexed to the body surface area, RWT: relative wall thickness, AI: aortic valve insufficiency. Data are presented as mean ± SD and the unit is given in brackets.

Similarly, both subgroups were comparable in most of the analysed postoperative echocardiographic data except for a significantly greater LVM and LVMI in the group of mechanical valves (see **Table 14**). However, postoperatively both subgroups tended to have an eLVH (RWT ≤ 0.42).

Table 14: Follow-up (t5) echocardiographic parameters of study patients.

Parameter	**Biological valve (n = 53)**	**Mechanical valve (n = 6)**	**p-value**
LVEF (%)	59.0 ± 7.51	56.7 ± 6.83	0.4766
LVEDD (mm)	49.0 ± 4.04	50.3 ± 7.69	0.5033
LVESD (mm)	32.4 ± 6.71	33.3 ± 7.26	0.7584
IVSd (mm)	12.8 ± 1.37	13.4 ± 1.56	0.3197
AWd (mm)	10.8 ± 1.13	11.6 ± 0.42	0.0928
PWd (mm)	10.4 ± 0.96	10.9 ± 0.89	0.2287
dP_m (mmHg)	9.40 ± 3.22	9.92 ± 3.58	0.7119
dP_p (mmHg)	17.8 ± 5.92	19.2 ± 8.33	0.6003
V_{max} (m/s)	2.09 ± 0.34	2.15 ± 0.45	0.6930
EOA (cm^2)	1.92 ± 0.18	1.93 ± 0.15	0.8964
EOA_i (cm^2/m^2)	1.01 ± 0.13	0.96 ± 0.17	0.3899
shear stress[1]	0.04 ± 0.01	0.04 ± 0.01	> 0.999
LVM (g)	217.0 ± 44.7	317.1 ± 59.4	**< 0.001**
LVMI (g/m^2)	112.3 ± 18.4	147.5 ± 32.6	**< 0.001**
RWT	0.40 ± 0.04	0.40 ± 0.03	> 0.999

LVEF: left ventricular ejection fraction, LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular end-systolic diameter, IVSd: intraventricular septal end-diastolic diameter, AWd: anterior wall end-diastolic diameter, PWd: posterior wall end-diastolic diameter, dP_m: mean pressure gradient, dP_p: peak pressure gradient, V_{max}: transvalvular peak velocity, EOA: effective orifice area, EOA_i: effective orifice area indexed to the body surface area. [1]shear stress was estimated as V_{max} / LVEF (see Paragraph 2.3.3 Echocardiographic parameters),

LVM: left ventricular mass, LVMI: left ventricular mass indexed to the body surface area, RWT: relative wall thickness. Data are presented as mean ± SD and the unit is given in brackets.

Echocardiographic parameters overall curse of the study patients is listed in **Table 15**. As expected, there was a significant decrease of dP_m, dP_p, V_{max}, and shear stress as well a significant increase of EOA, EOA_i from before to after surgery. Similarly, IVSd, AWd and PWd tended to decrease from before to after surgery. Further, LVM and LVMI decreased significantly from t1 to t4. The other parameters showed no significant changes.

Table 15: Echocardiographic parameters overall course.

Parameter	t1	t4	t5	p-value (t1 - t4)	p-value (t1 - t5)	p-value (t4 - t5)
LVEF (%)	57.0 ± 8.70	56.9 ± 7.13	58.8 ± 7.41	0.9625	0.2399	0.1751
LVEDD (mm)	48.9 ± 4.81	48.4 ± 4.81	49.2 ± 4.47	0.6121	0.7643	0.4096
LVESD (mm)	34.2 ± 6.44	34.2 ± 5.69	32.5 ± 6.71	0.9585	0.1707	0.1340
IVSd (mm)	13.5 ± 1.54	12.8 ± 2.26	12.8 ± 1.39	0.0586	**0.0149**	0.9682
AWd (mm)	11.6 ± 1.25	10.9 ± 1.68	10.9 ± 1.11	**0.0242**	**0.0035**	0.9200
PWd (mm)	10.9 ± 1.02	10.5 ± 1.47	10.4 ± 0.96	0.0983	**0.0053**	0.5754
dP_m (mmHg)	35.4 ± 10.4	10.6 ± 3.77	9.46 ± 3.27	**< 0.001**	**< 0.001**	0.0792
dP_p (mmHg)	60.1 ± 16.4	18.5 ± 6.81	18.0 ± 6.13	**< 0.001**	**< 0.001**	0.6498
V_{max} (m/s)	3.83 ± 0.57	2.12 ± 0.40	2.09 ± 0.35	**< 0.001**	**< 0.001**	0.7275
EOA (cm^2)	0.80 ± 0.20	1.98 ± 0.28	1.93 ± 0.18	**< 0.001**	**< 0.001**	0.2911
EOA_i (cm^2/m^2)	0.41 ± 0.10	1.01 ± 0.14	1.01 ± 0.13	**< 0.001**	**< 0.001**	> 0.999
shear stress[1]	0.07 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	**< 0.001**	**< 0.001**	0.3985
LVM (g)	240.6 ± 56.9	220.4 ± 54.4	227.3 ± 55.6	**0.0415**	0.2016	0.4771
LVMI (g/m^2)	123.1 ± 27.1	112.0 ± 26.7	116.0 ± 23.0	**0.0111**	0.1276	0.3041
RWT	0.40 ± 0.06	0.40 ± 0.08	0.40 ± 0.04	> 0.999	> 0.999	> 0.999

LVEF: left ventricular ejection fraction, LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular end-systolic diameter, IVSd: intraventricular septal end-diastolic diameter, AWd: anterior wall end-diastolic diameter, PWd: posterior wall end-diastolic diameter, dP_m: mean pressure gradient, dP_p: peak pressure gradient, V_{max}: transvalvular peak velocity, EOA: effective orifice area, EOA_i: effective orifice area indexed to the body surface area. [1]shear stress was estimated as V_{max} / LVEF (see Paragraph 2.3.3 Echocardiographic parameters), LVM: left ventricular mass, LVMI: left ventricular mass indexed to the body surface area, RWT: relative wall thickness. Data are presented as mean ± SD and the unit is given in brackets.

3.2 Exosome analysis and characterization

3.2.1 Exosome overall course

In patients receiving bioprosthetic valves, circulating exosomes decreased significantly from preoperative time points t1 and t2 to t3 (t1 - t3 $p < 0.001$, t2 - t3 $p < 0.01$) and increased from t3 to t4 ($p < 0.001$) and t5 ($p < 0.001$), returning approximately to the initial values (**Fig. 8A**). There was no significant correlation between t1 and t4 or t5, respectively. For patients with mechanical valve prostheses similar trends were observed, although statistical significance was not reached in this small cohort. Moreover, between the two valve sub cohorts no significant difference could be observed.

Further, we divided the patients in three AS severity subgroups according to the ESC and EACTS guidelines (see **Table 1**). Of the 53 patients receiving biological valves, 6 had a mild ($dP_m < 25$ mmHg), 30 a moderate (dP_m between 25 mmHg and 39 mmHg) and 17 a severe ($dP_m \geq 40$ mmHg) AS. All 6 patients receiving mechanical valves had a moderate AS. No significant difference could be detected between the three subgroups at any respective time point (**Fig. 8B**).

Noteworthily, all patients with $dP_m < 25$ mmHg (and therefore categorized the mild AS group), displayed an EOA < 1.5 cm^2, which actually corresponds to a moderate to severe AS (see **Table 1**). Further, it must be specified that patients with $dP_m < 25$ mmHg were all operated primarily due to severe CAD and the indication of SAVR was derived from morphological signs of valve degeneration in addition to the indicated hemodynamic parameters.

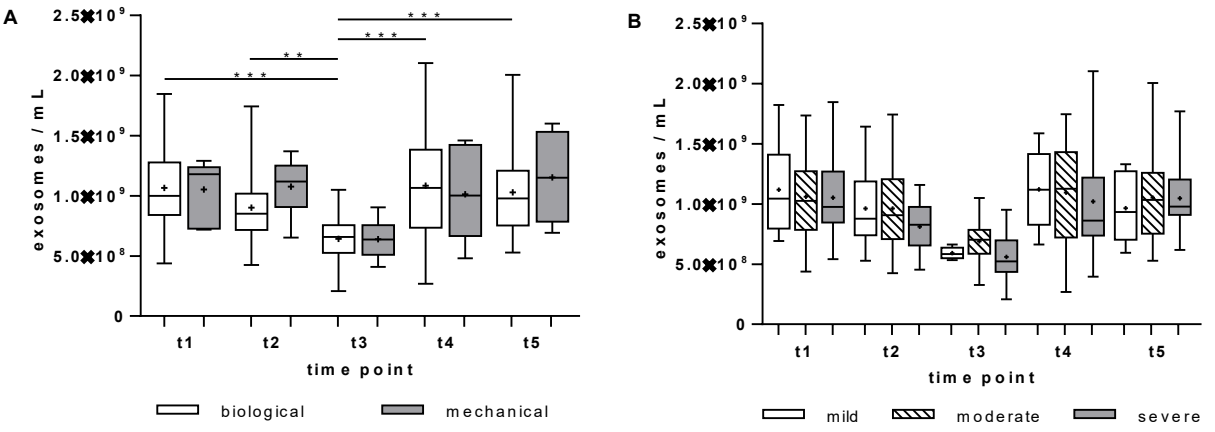

Figure 8: Exosome overall course. Blood samples were analysed at five time points: t1 = admission day, t2 = just before surgery, t3 = 24 hours postoperatively (post-op), t4 = 7 days post-op, t5 = 3 months post-op. Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). The exosome overall course is represented as box-and-whisker plots. Bottom and top of each box represent the first and third quartiles (*i.e.* 50%

of the values are included in the box), lines inside the boxes represent median values (= second quartile) and plus signs indicate respective mean values. Further, the ends of the whiskers represent the minimum and maximum of all data. P-values were calculated using ANOVA. * indicates $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Only significant correlations are marked. **A:** Exosome overall course in patients receiving biological (white boxes, n = 53) or mechanical (grey boxes, n = 6) aortic valve prosthesis. **B:** Course of circulating exosomes in patient sub cohorts stratified according to preoperative (pre-op) aortic valve stenosis (AS) severity: mild AS (white boxes, n = 6), moderate AS (striped boxes, n = 36) and severe AS (grey boxes n = 17).

3.2.2 Characterization of exosomal proteins

After exosome preparation, total protein content was measured using the DC Protein Assay. In patients receiving bioprosthetic valves, measured protein content decreased from preoperative values (t1 and t2) to first postoperative measurement t3 (t1 - t3 $p < 0.001$, t2 - t3 $p < 0.001$, **Fig. 9A**). However, in further course it increased from t3 to later time points ($p < 0.001$ for comparison to t4 or t5, respectively) and also showed a significant increase when t4 values were compared to t5 ($p < 0.05$). Finally, exosome protein content returned approximately to the initial values at t5. There was no significant correlation between t1 and t4 or t5, respectively. In patients receiving mechanical valves, similar trends were observed, although in this small cohort a statistical significance was only reached for comparison of t3 to t4 ($p < 0.05$). Between the two valve sub cohorts no significant difference could be observed. Further, no significant difference could be detected when data were stratified according to the preoperative AS severity (**Fig. 9B**).

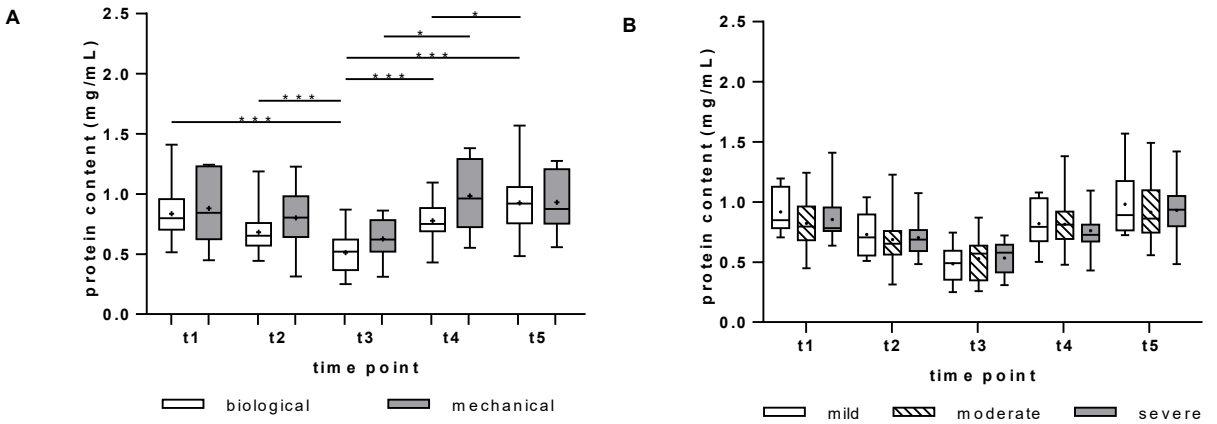

Figure 9: Course of exosome protein content. Blood samples were analysed at five time points: t1 = admission day, t2 = just before surgery, t3 = 24 hours postoperatively (post-op), t4 = 7 days post-op, t5 = 3 months post-op. Exosome protein content was measured using the DC Protein Assay. Values are represented as box-and-whisker plots with bottom and top of each box representing the first and third quartiles (*i.e.* 50% of the values are included in the box), lines inside the boxes representing median values (= second quartile) and plus signs indicating respective mean values.

Further, the ends of the whiskers represent the minimum and maximum of all data. P-values were calculated using ANOVA. * indicates $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Only significant correlations are marked. **A**: Exosome protein content in patients receiving biological (white boxes, n = 53) or mechanical (grey boxes, n = 6) aortic valve prosthesis. **B**: Course of exosome protein content in patient sub cohorts stratified according to preoperative (pre-op) aortic valve stenosis (AS): mild AS (white boxes, n = 6), moderate AS (striped boxes, n = 36) and severe AS (grey boxes, n = 17).

Nevertheless, considering all 59 patients at all time points together (t1 to t5), a significant positive correlation between circulating exosome concentrations and measured total protein content could be detected ($p < 0.001$, R^2: 0.04904, **Fig. 10**).

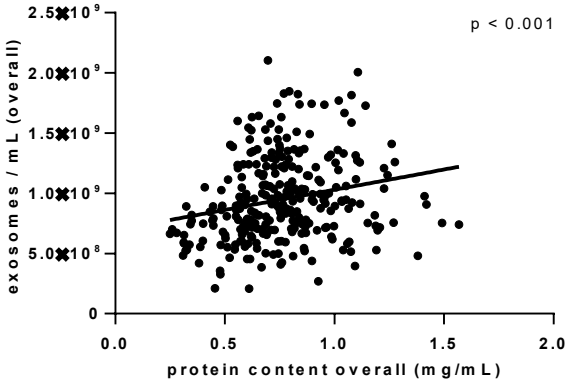

Figure 10: Overall correlation between circulating exosomes and protein content. Blood samples were analysed at five time points: t1 = admission day, t2 = just before surgery, t3 = 24 hours postoperatively (post-op), t4 = 7 days post-op, t5 = 3 months post-op. Exosome protein content was measured using the DC Protein Assay. P-values were calculated using linear regression.

Isolated exosomes were characterized by Western blot analysis to determine CD63 protein content. We identified two main CD63 bands, at 70 kDa and at 55 kDa, while other minor bands were detected at ca. 40 - 45 kDa and 20 kDa (**Fig. 11**).

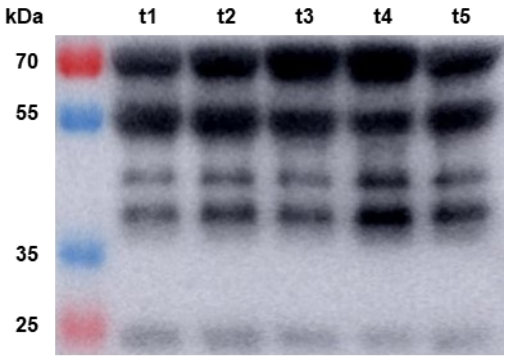

Figure 11: Representative CD63 Western blot bands. Blood samples were analysed at five time points: t1 = admission day, t2 = just before surgery, t3 = 24 hours postoperatively (post-op), t4 = 7 days post-op, t5 = 3 months post-op. CD63 protein content was evaluated by Western blot analysis. The numbers on the left indicate the size of the bands of the protein ladder in kDa (left lane).

When protein band intensities were quantified by densitometry analysis, we found changes in CD63 content along the course of the study. CD63 protein was identified with a strong signal for both major molecular sizes (70kD and 55kD) at all time points. Compared to t1 values, at t3 and at t4 CD63 expression of the 70 kDa band was significantly increased in patients receiving biological valves (**Fig. 12A**). On the other hand, at t5 CD63 expression was approximately equivalent to t1 and t2, suggesting a return of CD63 content to preoperative values 3 months postoperatively. In patients receiving a mechanical valve prosthesis, similar trends were observed, although a statistical significance was not achieved in this small cohort. In contrast, when the 55kDa band of CD63 was considered, there was no change at any time point for both valve type sub cohorts (**Fig. 12B**). Thereby, we found a different course for the individual CD63 bands in the perioperative period for patients undergoing a combined operation for SAVR and CABG. Further, there was no significant difference between the two valve type subgroups at any time point irrespective of the analysed individual protein band.

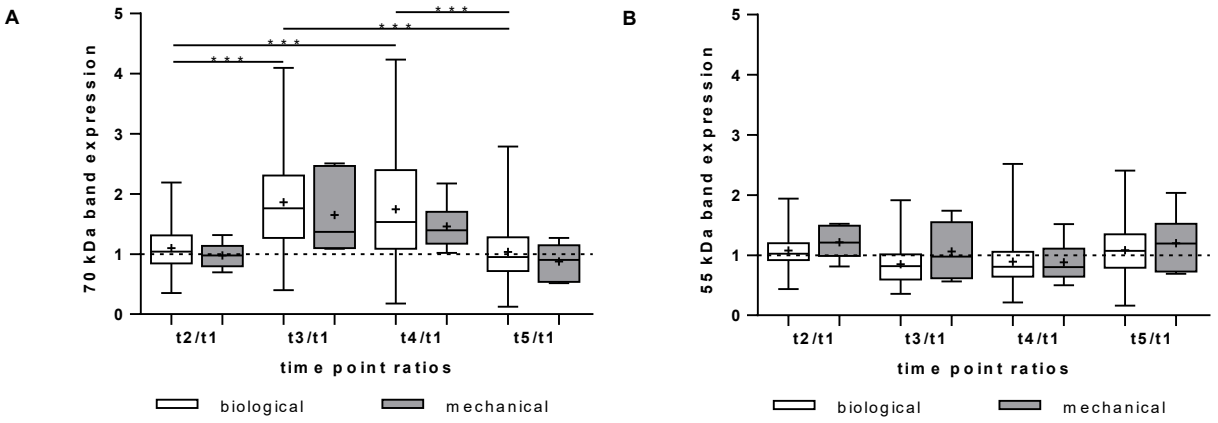

Figure 12: Analysis of CD63 expression depending on the molecular protein size and the implanted valve prosthesis type. Blood samples were analysed at five time points: t1 = admission day, t2 = just before surgery, t3 = 24 hours postoperatively (post-op), t4 = 7 days post-op, t5 = 3 months post-op. CD63 protein content was evaluated by Western blot analysis and individual protein bands were quantified by densitometric analysis. CD63 protein content at each time point is normalized to the respective value of t1. Values are represented as box-and-whisker plots. Bottom and top of each box represent the first and third quartiles (*i.e.* 50% of the values are included in the box), lines inside the boxes represent median values (= second quartile) and plus signs indicate respective mean values. Further, the ends of the whiskers represent the minimum and maximum of all data. When the ratio box includes the 1, the CD63 expression is approximately equivalent to t1. When the ratio box is above (or underneath) the 1, the CD63 expression is increased (or decreased) compared to t1. P-values were calculated using ANOVA. * indicates $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Only significant correlations are marked. **A:** CD63 expression of the 70 kDa CD63 band in patients receiving biological (white boxes, n = 53) or mechanical (grey boxes, n = 6) aortic valve prosthesis. **B:** CD63 expression of the 55 kDa CD63 band in patients receiving biological (white boxes, n = 53) or mechanical (grey boxes, n = 6) aortic valve prosthesis.

CD63 protein content was also analysed for all patients after stratification according to preoperative AS severity. Values measured at the respective study time points showed no significant difference in either band (**Fig. 13A** and **Fig. 13B**).

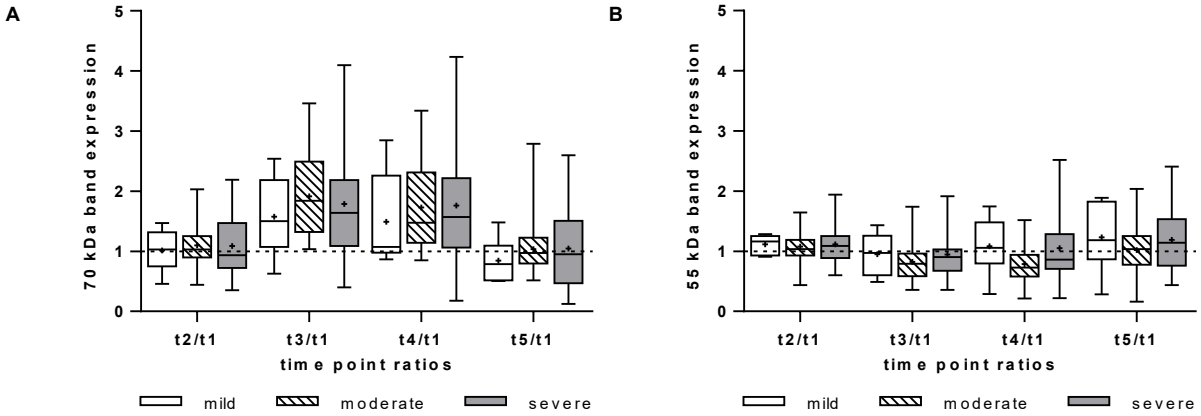

Figure 13: Overall CD63 expression of the 70 and the 55 kDa band in patients with mild, moderate or severe AS. Blood samples were analysed at five time points: t1 = admission day, t2 = just before surgery, t3 = 24 hours postoperatively (post-op), t4 = 7 days post-op, t5 = 3 months post-op. CD63 protein content was evaluated by Western blot analysis and individual protein bands were quantified by densitometric analysis. CD63 protein content at each time point is normalized to the respective value of t1. Values are represented as box-and-whisker plots. Bottom and top of each box represent the first and third quartiles (*i.e.* 50% of the values are included in the box), lines inside the boxes represent median values (= second quartile) and plus signs indicate respective mean values. Further, the ends of the whiskers represent the minimum and maximum of all data. When the ratio box includes the 1, the CD63 expression is approximately equivalent to t1. When the ratio box is above (or underneath) the 1, the CD63 expression is increased (or decreased) compared to t1. P-values were calculated using ANOVA. * indicates $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Only significant correlations are marked. **A:** CD63 expression of the 70 kDa CD63 band in patient sub cohorts stratified according to preoperative (pre-op) aortic valve stenosis (AS) severity: mild AS (white boxes, n = 6), moderate AS (striped boxes, n = 36) and severe AS (grey boxes n = 17). **B:** CD63 expression of the 55 kDa CD63 band in patient sub cohorts stratified according to pre-op AS severity: mild AS (white boxes, n = 6), moderate AS (striped boxes, n = 36) and severe AS (grey boxes n = 17).

3.3 Correlation between exosome levels and clinical parameters

3.3.1 Demographic parameters

In study patients, a gender-related significant difference of exosome levels could not be detected (**Fig. 14A**). Further, no significant correlation was observed between circulating exosomes and patient age or body mass index (BMI), respectively (**Fig. 14B** and **Fig. 14C**).

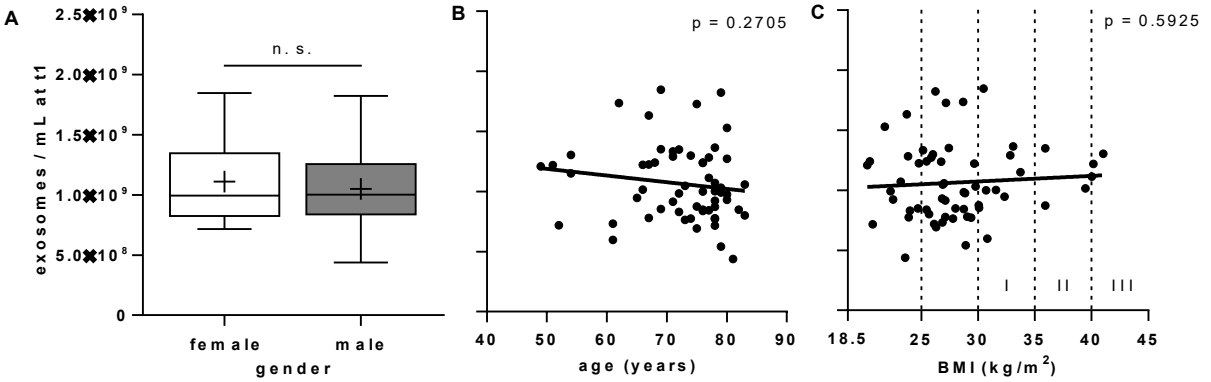

Figure 14: Circulating exosomes in female and male patients, correlated to patient age and to BMI. Blood samples were sampled on the admission day (t1) and exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). **A:** Circulating exosomes in female (white box, n = 13) and male (grey box, n = 40) patients at t1. The circulating exosomes are represented as box-and-whisker plots. Bottom and top of each box represent the first and third quartiles (*i.e.* 50% of the values are included in the box), lines inside the boxes represent median values (= second quartile) and plus signs indicate respective mean values. Further, the ends of the whiskers represent the minimum and maximum of all data. P-values were calculated using t test. n.s.: not significant. **B:** Correlation between circulating exosomes and patient age at t1. P-values were calculated using linear regression. **C:** Correlation between circulating exosomes and BMI at t1. P-values were calculated using linear regression. BMI: body mass index. BMI 18.5 - 24.9: normal weight; 25 - 29.9: overweight; 30 - 34.9: obesity class I; 35 - 39.9: obesity class II; ≥ 40: obesity class III.

3.3.2 Blood count

Of all determined clinical laboratory parameters, we analysed those values that are related either to exosomes themselves (such as RBCs, WBCs and PLTs, that are known to shed these nano-sized vesicles - see Section 1.2 Exosomes), or to myocardial / general tissue damage, *i.e.* troponin (Tn) T, creatine kinase (CK), aspartate transaminase / aminotransferase (AST), also known as glutamic oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH).

RBCs, Hb, Hct. Considering the time points altogether (t1, t4 and t5), a significant positive correlation between circulating exosomes and RBCs could be detected ($p < 0.05$, R^2: 0.03651, **Fig. 15A**). This correlation could also be observed analysing the single time points independently (t1: $p < 0.05$, R^2: 0.08306, **Fig. 15B**; t4: $p < 0.05$, R^2: 0.09146, **Fig. 15C**; t5: $p < 0.05$, R^2: 0.07981, **Fig. 15D**).

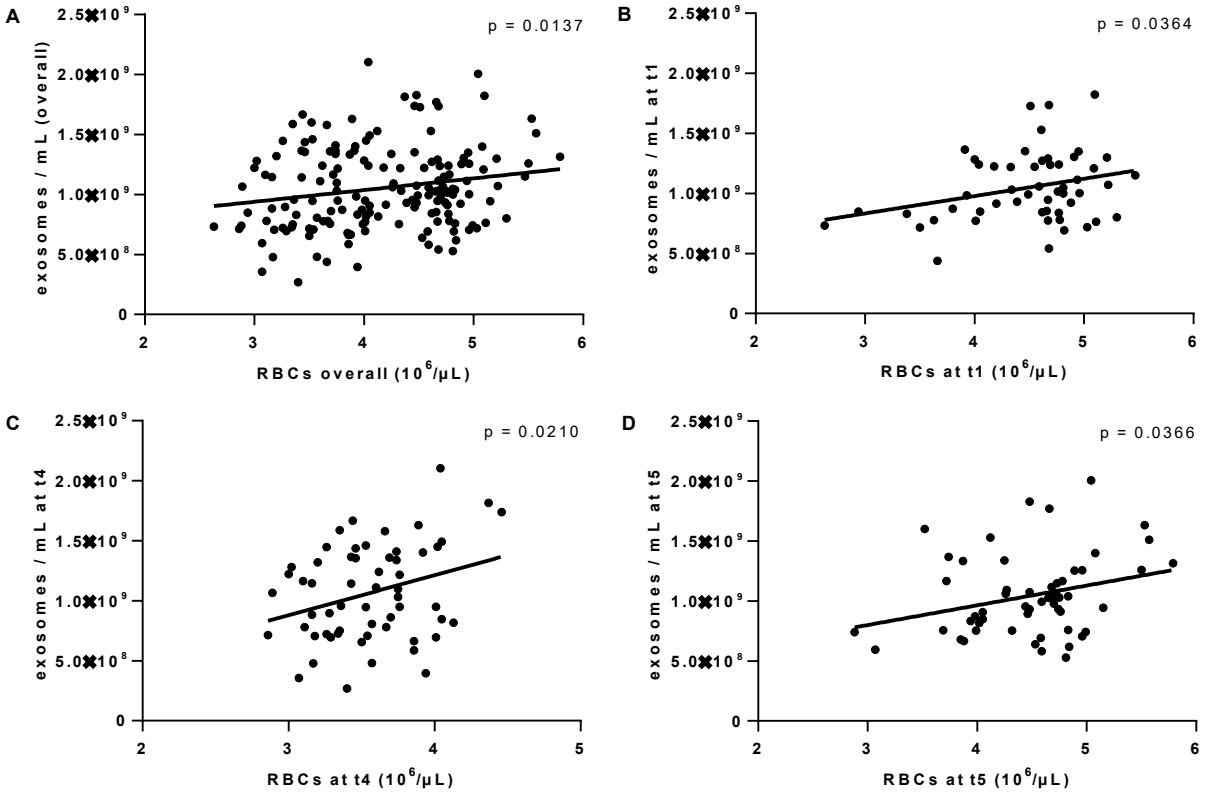

Figure 15: Correlation between circulating exosomes and RBCs. Blood samples were analysed at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. RBCs: red blood cells. **A:** Correlation between circulating exosomes and RBCs considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and RBCs at t1. **C:** Correlation between circulating exosomes and RBCs at t4. **D**: Correlation between circulating exosomes and RBCs at t5.

As expected, Hb and Hct revealed correlations similar to the pattern observed for RBCs. In fact, Hb displayed a significant positive correlation both considering the time points altogether ($p < 0.05$, R^2: 0.03274, **Fig. 16A**) and also when analysing single time points independently (t1: $p < 0.05$, R^2: 0.08518, **Fig. 16B**; t4: $p < 0.05$, R^2: 0.07779, **Fig. 16C**; t5: $p < 0.05$, R^2: 0.09220, **Fig. 16D**).

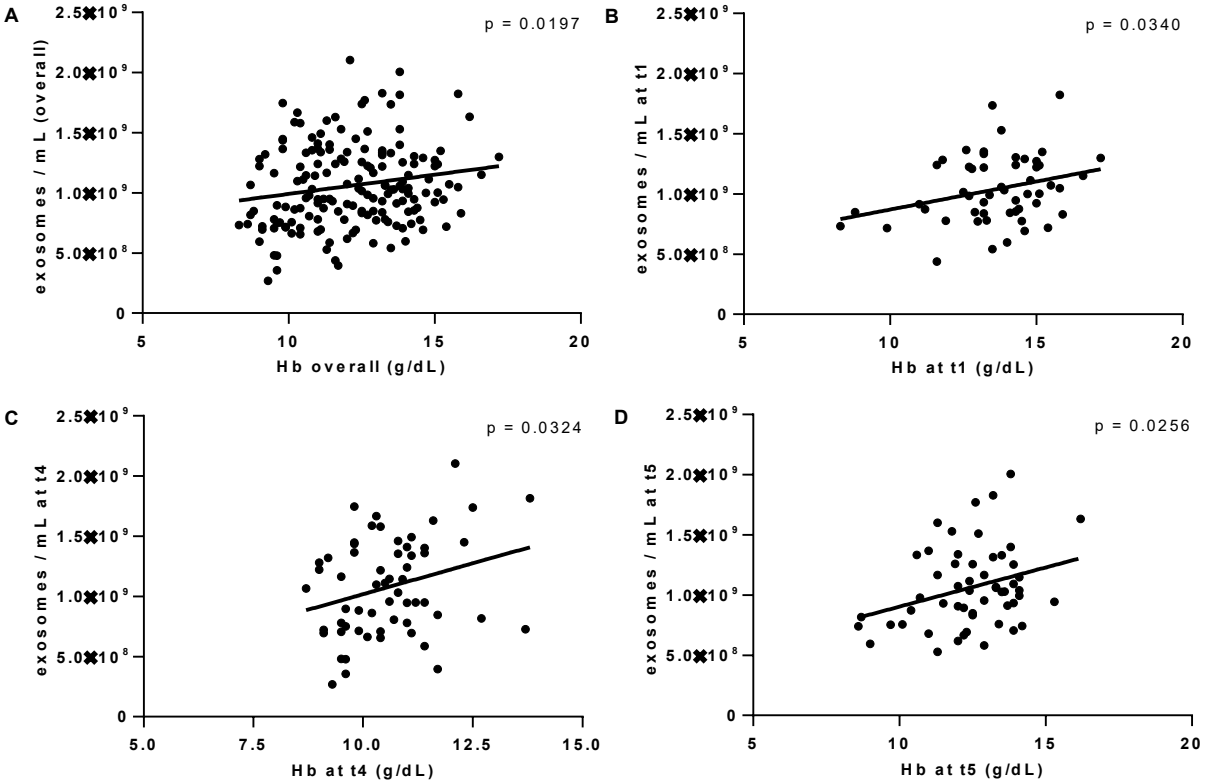

Figure 16: Correlation between circulating exosomes and Hb. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. Hb: haemoglobin. **A:** Correlation between circulating exosomes and Hb considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and Hb at t1. **C:** Correlation between circulating exosomes and Hb at t4. **D:** Correlation between circulating exosomes and Hb at t5.

Similarly, Hct displayed a significant positive correlation both considering the time points altogether ($p < 0.01$, R^2: 0.04003, **Fig. 17A**) and also when analysing the single time points independently (t1: $p < 0.05$, R^2: 0.07138, **Fig. 17B**; t4: $p < 0.05$, R^2: 0.1110, **Fig. 17C**; t5: $p < 0.01$, R^2: 0.1308, **Fig. 17D**).

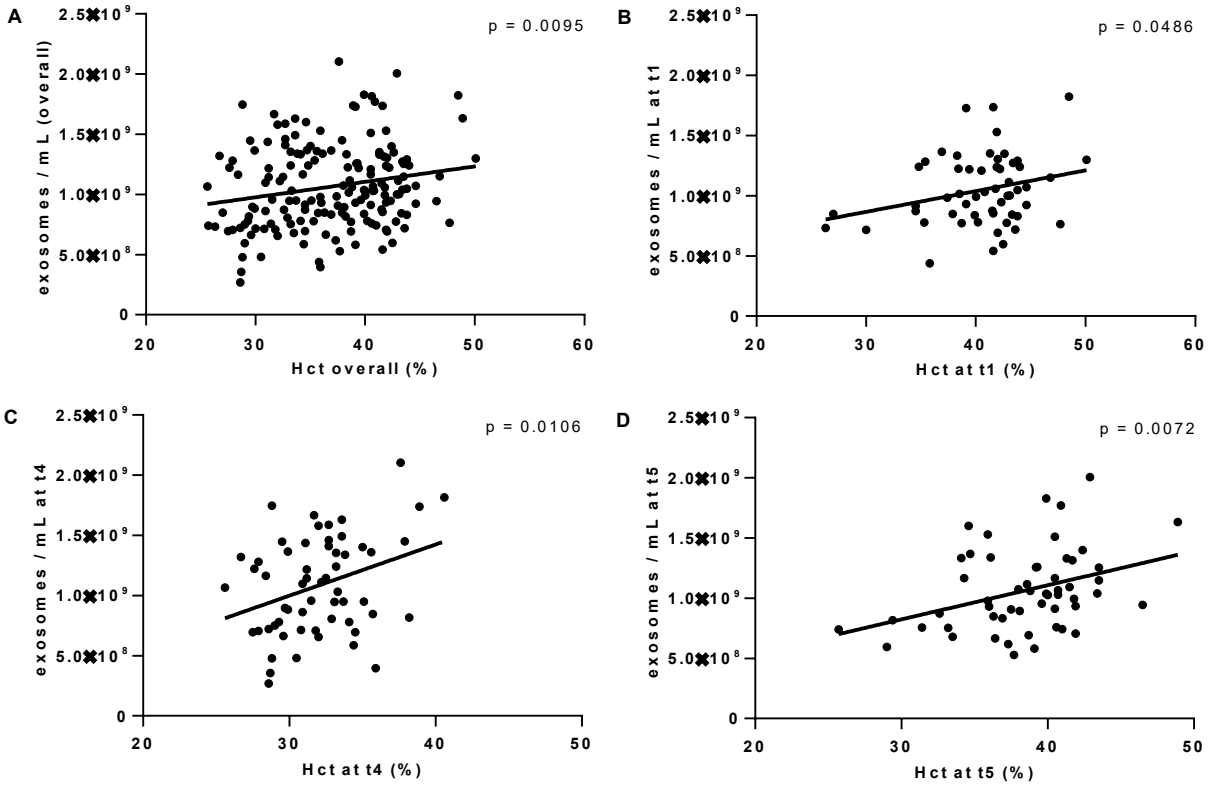

Figure 17: Correlation between circulating exosomes and Hct. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. Hct: haematocrit. **A:** Correlation between circulating exosomes and Hct considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and Hct at t1. **C:** Correlation between circulating exosomes and Hct at t4. **D:** Correlation between circulating exosomes and Hct at t5.

WBCs and PLTs. Between circulating exosomes and WBCs no significant correlation could be detected neither considering the time points altogether (**Fig. 18A**), nor analysing the single time points independently (**Fig. 18B-D**).

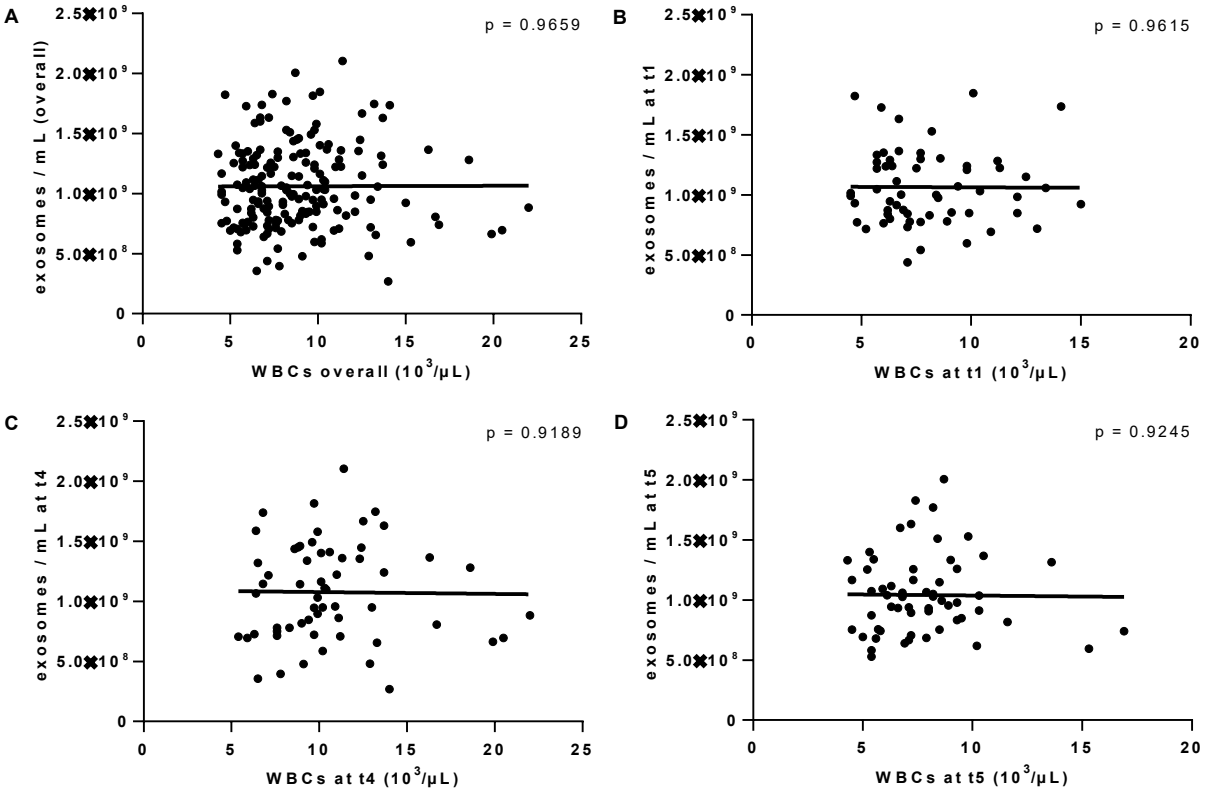

Figure 18: Correlation between circulating exosomes and WBCs. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. WBCs: white blood cells. **A:** Correlation between circulating exosomes and WBCs considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and WBCs at t1. **C:** Correlation between circulating exosomes and WBCs at t4. **D:** Correlation between circulating exosomes and WBCs at t5.

Like WBCs, also PLTs did not display a significant correlation neither considering the time points altogether (**Fig. 19A**), nor analysing the single time points independently (**Fig. 19B-D**).

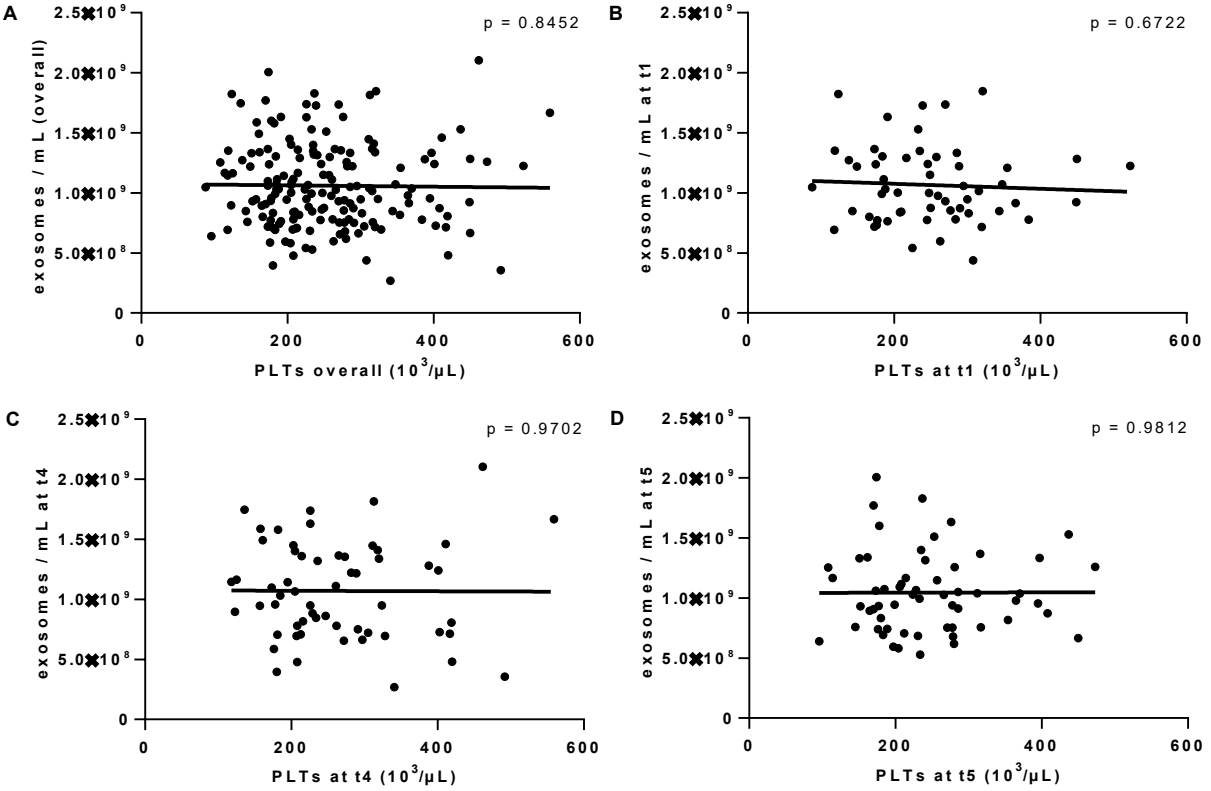

Figure 19: Correlation between circulating exosomes and PLTs. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. PLTs: platelets. **A:** Correlation between circulating exosomes and PLTs considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and PLTs at t1. **C:** Correlation between circulating exosomes and PLTs at t4. **D:** Correlation between circulating exosomes and PLTs at t5.

TnT, LDH, CK and AST. Between circulating exosomes and TnT no significant correlation could be detected neither considering the time points altogether (**Fig. 20A**), nor analysing the single time points independently (**Fig. 20B-D**).

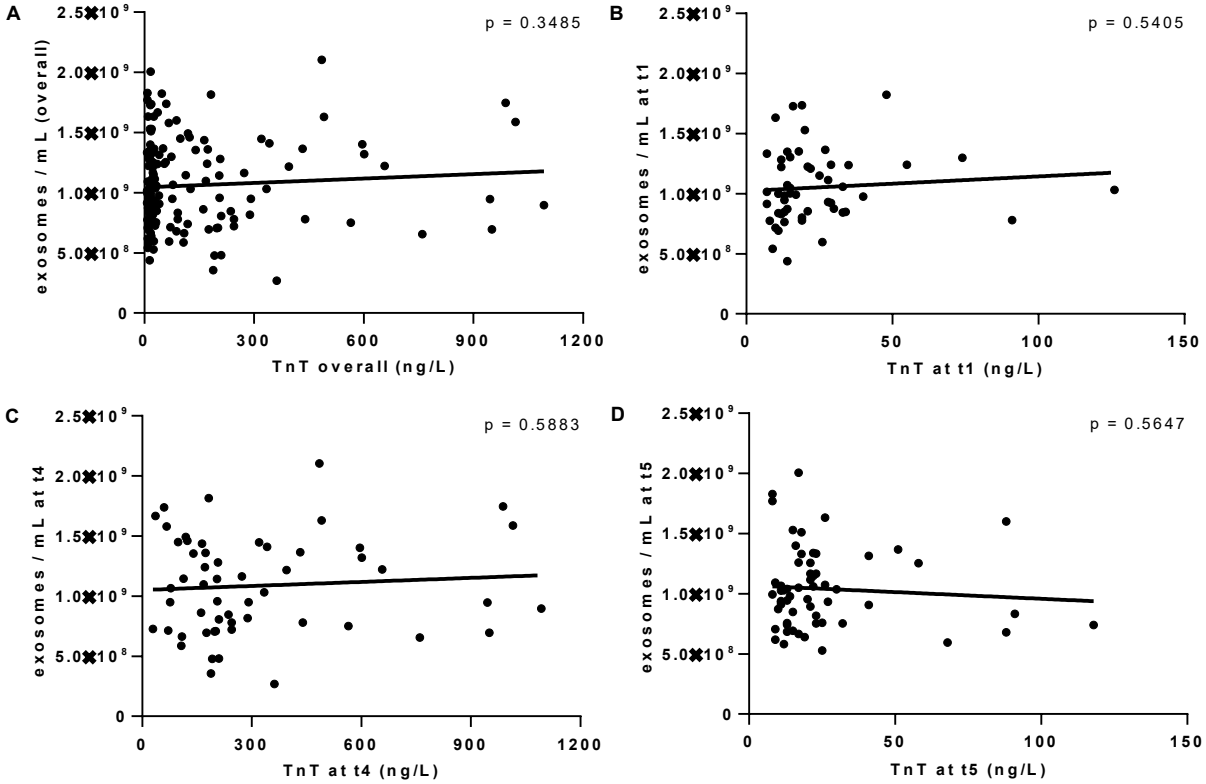

Figure 20: Correlation between circulating exosomes and TnT. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. TnT: troponin T. **A:** Correlation between circulating exosomes and TnT considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and TnT at t1. **C:** Correlation between circulating exosomes and TnT at t4. **D:** Correlation between circulating exosomes and TnT at t5.

Considering the time points altogether, no significant correlation between circulating exosomes and LDH could be detected (**Fig. 21A**). Nevertheless, even though analysing the single time points t1 and t4 no significant correlation was observed either (**Fig. 21B-C**), t5 did reveal a significant negative correlation ($p < 0.05$, R^2: 0.07365, **Fig. 21D**).

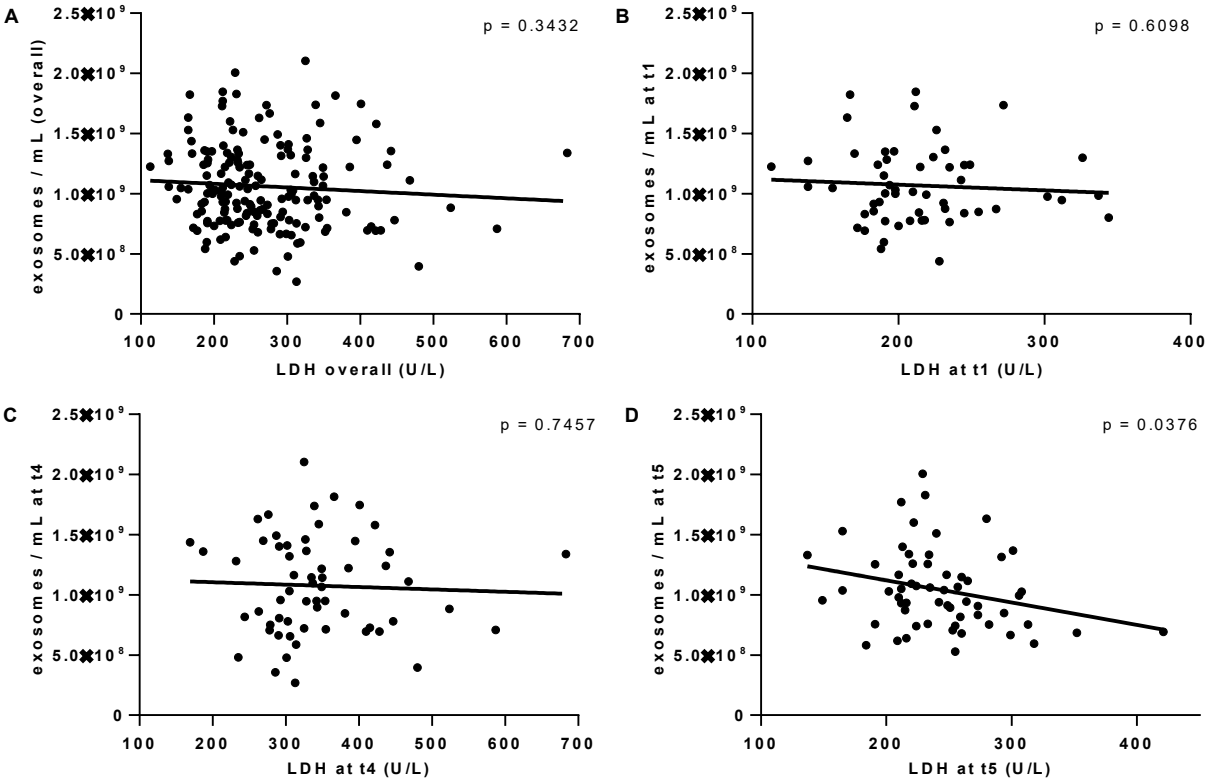

Figure 21: Correlation between circulating exosomes and LDH. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. LDH: lactate dehydrogenase. **A:** Correlation between circulating exosomes and LDH considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and LDH at t1. **C:** Correlation between circulating exosomes and LDH at t4. **D:** Correlation between circulating exosomes and LDH at t5.

CK displayed correlations similar to the pattern observed for LDH. In fact, considering the time points altogether, no significant correlation between circulating exosomes and CK could be detected (**Fig. 22A**). The analysis of the single time points t1 and t4 resulted in no significant correlation (**Fig. 22B-C**), but at t5 a significant negative correlation was observed ($p < 0.05$, R^2: 0.09217, **Fig. 22D**).

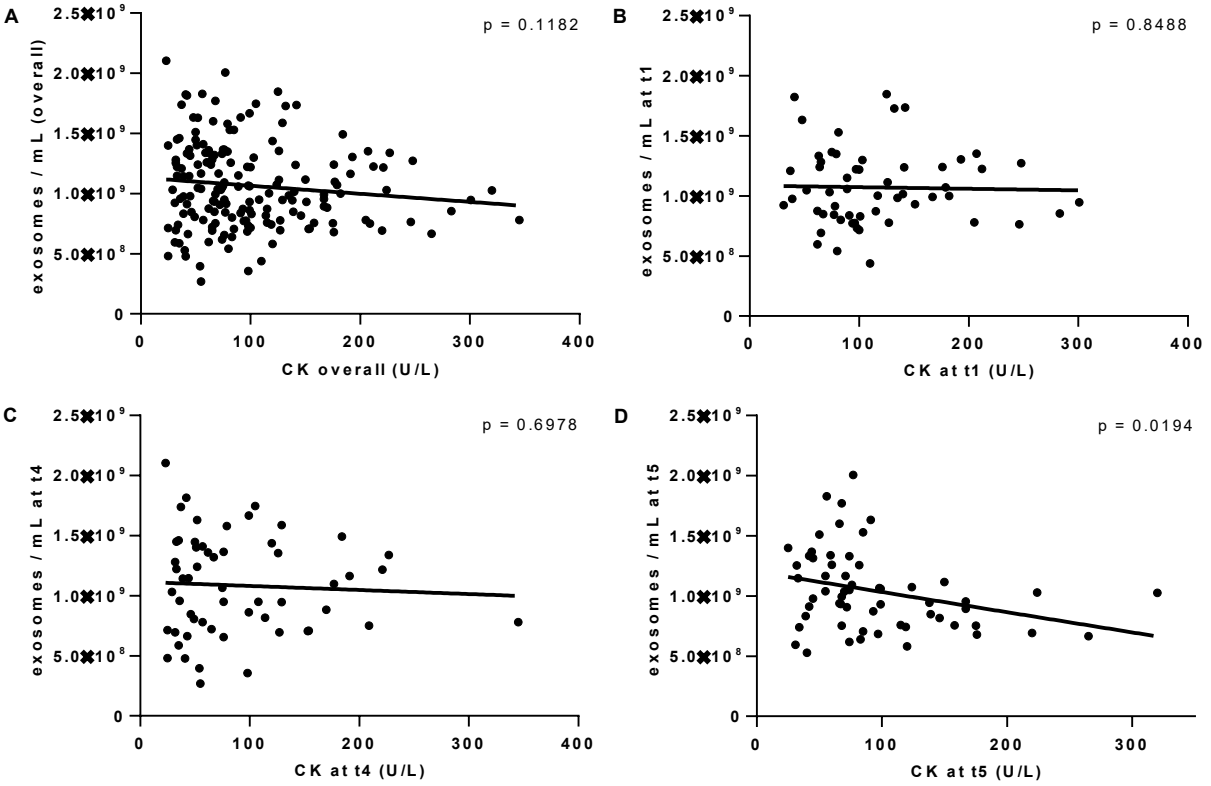

Figure 22: Correlation between circulating exosomes and CK. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. CK: creatine kinase. **A:** Correlation between circulating exosomes and CK considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and CK at t1. **C:** Correlation between circulating exosomes and CK at t4. **D:** Correlation between circulating exosomes and CK at t5.

Also AST displayed correlations similar to the pattern observed for LDH and CK. In fact, considering the time points altogether, no significant correlation between circulating exosomes and AST could be detected (**Fig. 23A**). The analysis of the single time points t1 and t4 resulted in no significant correlation (**Fig. 23B-C**), but at t5 a significant negative correlation was observed ($p < 0.05$, R^2: 0.07407, **Fig. 23D**).

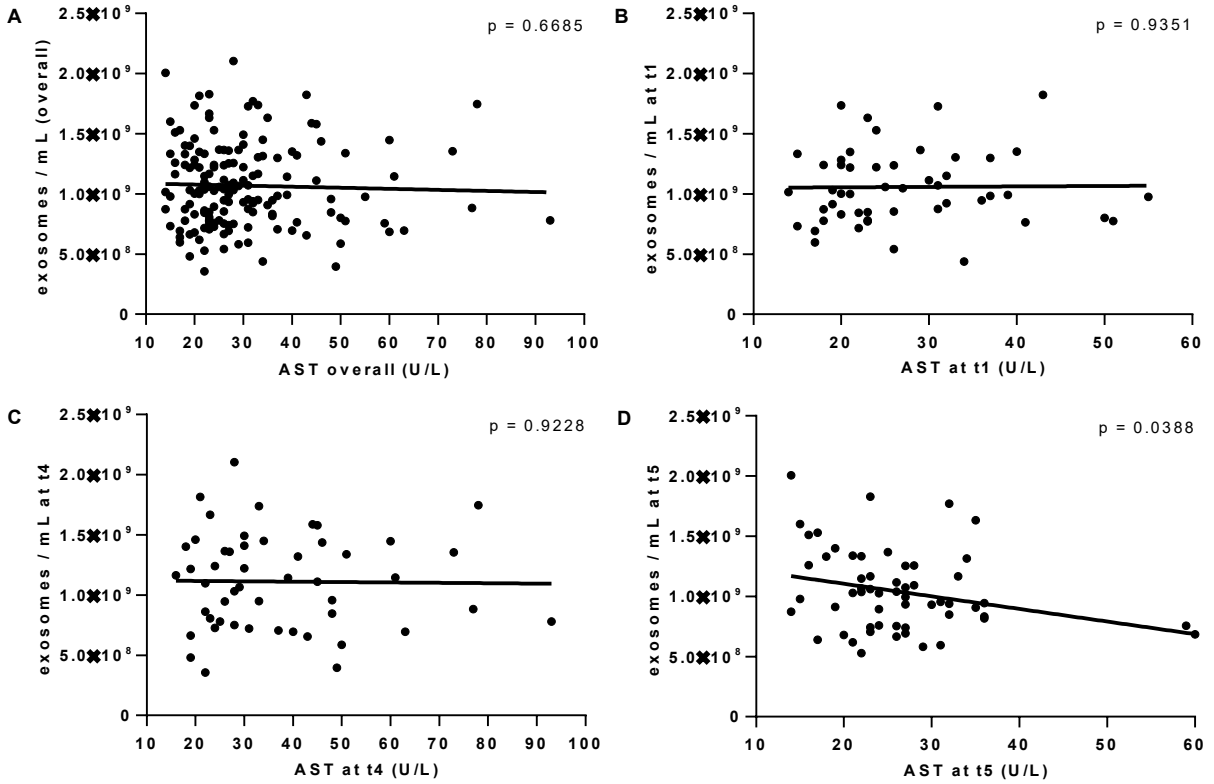

Figure 23: Correlation between circulating exosomes and AST. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. AST: aspartate transaminase / aminotransferase. **A:** Correlation between circulating exosomes and AST considering the time points altogether (t1, t4 and t5). **B:** Correlation between circulating exosomes and AST at t1. **C:** Correlation between circulating exosomes and AST at t4. **D:** Correlation between circulating exosomes and AST at t5.

3.3.3 Echocardiographic data

As previously stated, (see Section 1.3 Statement of purpose and hypothesis), in this work we aimed at examining whether variation of circulating exosomes levels in the perioperative course could possibly correlate with clinically relevant events. As such, we questioned whether exosome levels may provide information on hemodynamic changes after SAVR and CABG at the level of the aortic valve, *e.g.* normalization of pressure conditions or an emerging PPM. For this reason, we did not only analyse the correlation between circulating exosomes and echocardiographic parameters at the single time points. We also analysed the correlation considering the change in circulating exosomes from before the surgery to 3 months postoperatively (t5 / t1), rather than focussing only on absolute numbers of exosomes.

dPm, dPp, Vmax and EOA. Considering the single time points t1, t4 and t5, no significant correlation between circulating exosomes and dPm could be detected (**Fig. 24A-C**). Nevertheless, considering the exosome ratio (t5 / t1) and the dPm difference (t1 - t5), a significant positive correlation was observed ($p < 0.01$, R2: 0.1362, **Fig. 24D**). After surgery, the mean pressure gradient decreases and, the greater the decrease, the higher the exosome ratio (t5 / t1), *i.e.* the exosome number tends to increase from t1 to t5.

As previously specified, all patients with a $dP_m < 25$ mmHg (and therefore categorized the mild AS group), displayed an EOA < 1.5 cm^2, which actually corresponds to a moderate to severe AS (see **Table 1**). Further, patients with $dP_m < 25$ mmHg were all operated primarily due to severe CAD and the indication of SAVR was derived from morphological signs of valve degeneration in addition to the indicated hemodynamic parameters.

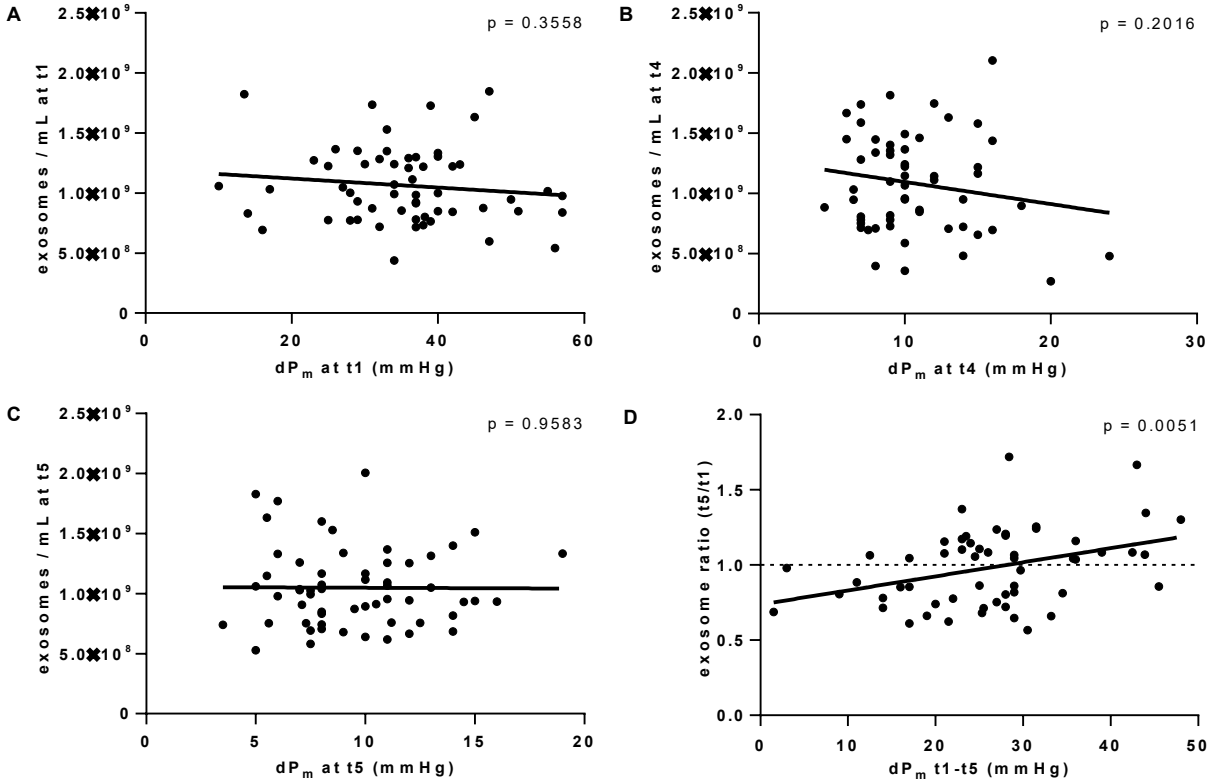

Figure 24: Correlation between circulating exosomes and dP_m. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. dP_m: mean pressure gradient. **A:** Correlation between circulating exosomes and dP_m at t1. **B:** Correlation between circulating exosomes and dP_m at t4. **C:** Correlation between circulating exosomes and dP_m at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the dP_m difference (t1 - t5).

As expected, dP_p and V_{max} revealed correlations similar to the pattern observed for dP_m. In fact, dP_p revealed no significant correlation considering the single time points t1, t4 and t5 **(Fig. 25A-C).** Further, considering the exosome ratio (t5 / t1) and the dP_p difference (t1 - t5), a significant positive correlation was observed ($p < 0.05$, R^2: 0.09545, **Fig. 25D**). After surgery, the peak pressure gradient decreases and, the greater the decrease, the higher the exosome ratio (t5 / t1), *i.e.* the exosome number tends to increase from t1 to t5.

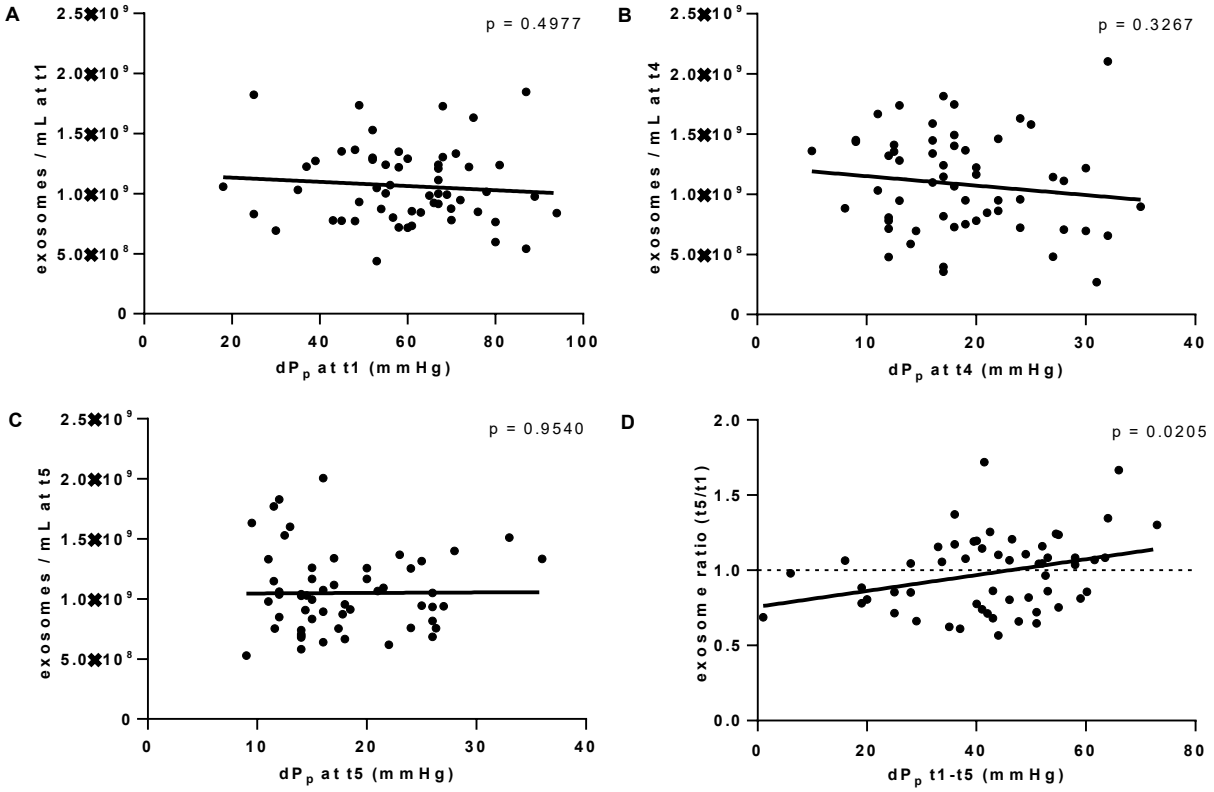

Figure 25: Correlation between circulating exosomes and dP_p. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. dP_p: peak pressure gradient. **A:** Correlation between circulating exosomes and dP_p at t1. **B:** Correlation between circulating exosomes and dP_p at t4. **C:** Correlation between circulating exosomes and dP_p at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the dP_p difference (t1 - t5).

Similarly, V_{max} revealed no significant correlation considering the single time points t1, t4 and t5 (**Fig. 26A-C**). Further, considering the exosome ratio (t5 / t1) and the V_{max} difference (t1 - t5), a significant positive correlation was observed ($p < 0.05$, R^2: 0.08291, **Fig. 26D**). After surgery, the transvalvular peak velocity decreases and, the greater the decrease, the higher the exosome ratio (t5 / t1), *i.e.* the exosome number tends to increase from t1 to t5.

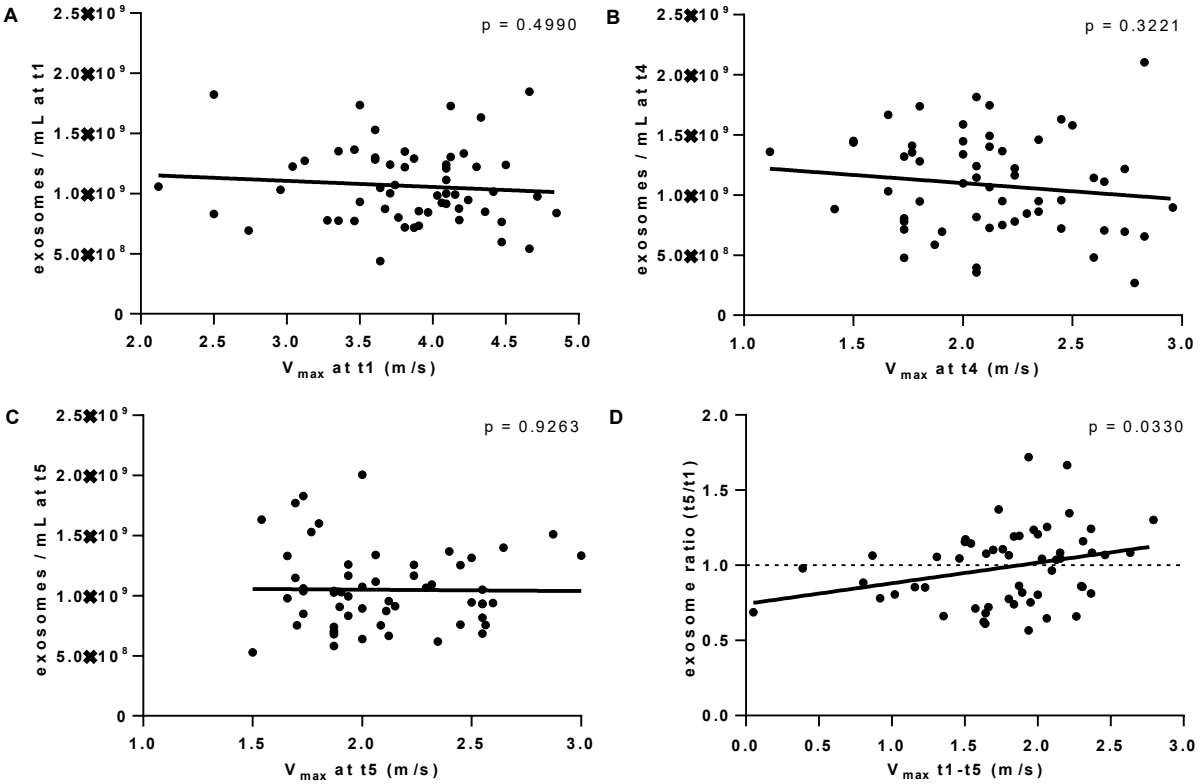

Figure 26: Correlation between circulating exosomes and V_{max}. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. V_{max}: transvalvular peak velocity. **A:** Correlation between circulating exosomes and V_{max} at t1. **B:** Correlation between circulating exosomes and V_{max} at t4. **C:** Correlation between circulating exosomes and V_{max} at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the V_{max} difference (t1 - t5).

EOA revealed the same correlations with circulating exosomes as previously observed for dP_m, dP_p and V_{max}. In fact, even though no significant correlation could be detected at each of the single time points t1, t4 and t5 (**Fig. 27A-C**), considering the exosome ratio (t5 / t1) and the EOA difference (t5 - t1), a significant positive correlation was observed ($p < 0.05$, R^2: 0.09314, **Fig. 27D**). After surgery, the EOA increases and, the greater the increase, the higher the exosome ratio (t5 / t1), *i.e.* the exosome number tends to increase from t1 to t5.

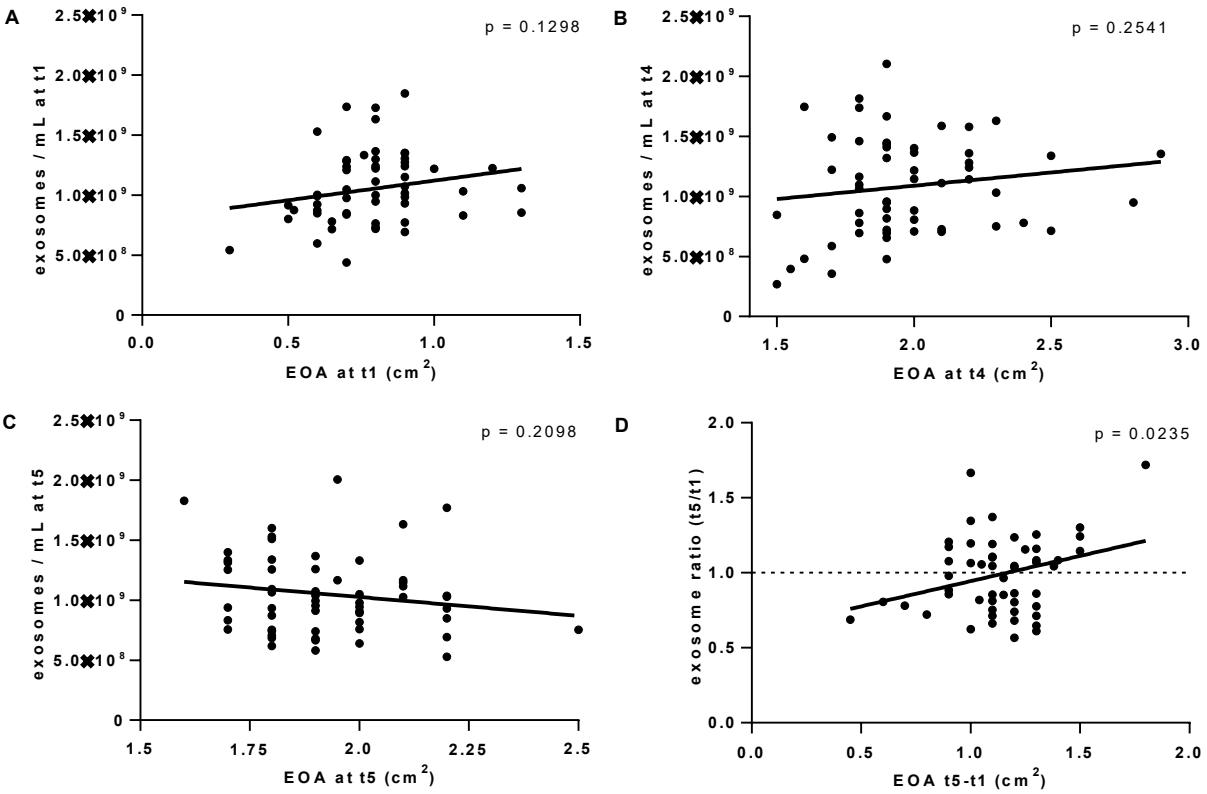

Figure 27: Correlation between circulating exosomes and EOA. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. EOA: effective orifice area. **A:** Correlation between circulating exosomes and EOA at t1. **B:** Correlation between circulating exosomes and EOA at t4. **C:** Correlation between circulating exosomes and EOA at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the EOA difference (t1 - t5).

Shear stress. Between circulating exosomes and shear stress no significant correlation could be detected neither at each of the single time points t1, t4 and t5 (**Fig. 28A-C**), nor considering the exosome ratio (t5 / t1) and the shear stress difference (t1 - t5, **Fig. 28D**). After surgery, shear stress decreases but the extent of decrease does not correlate with the exosome ratio (t5 / t1), *i.e.* with the change of the exosome number from t1 to t5.

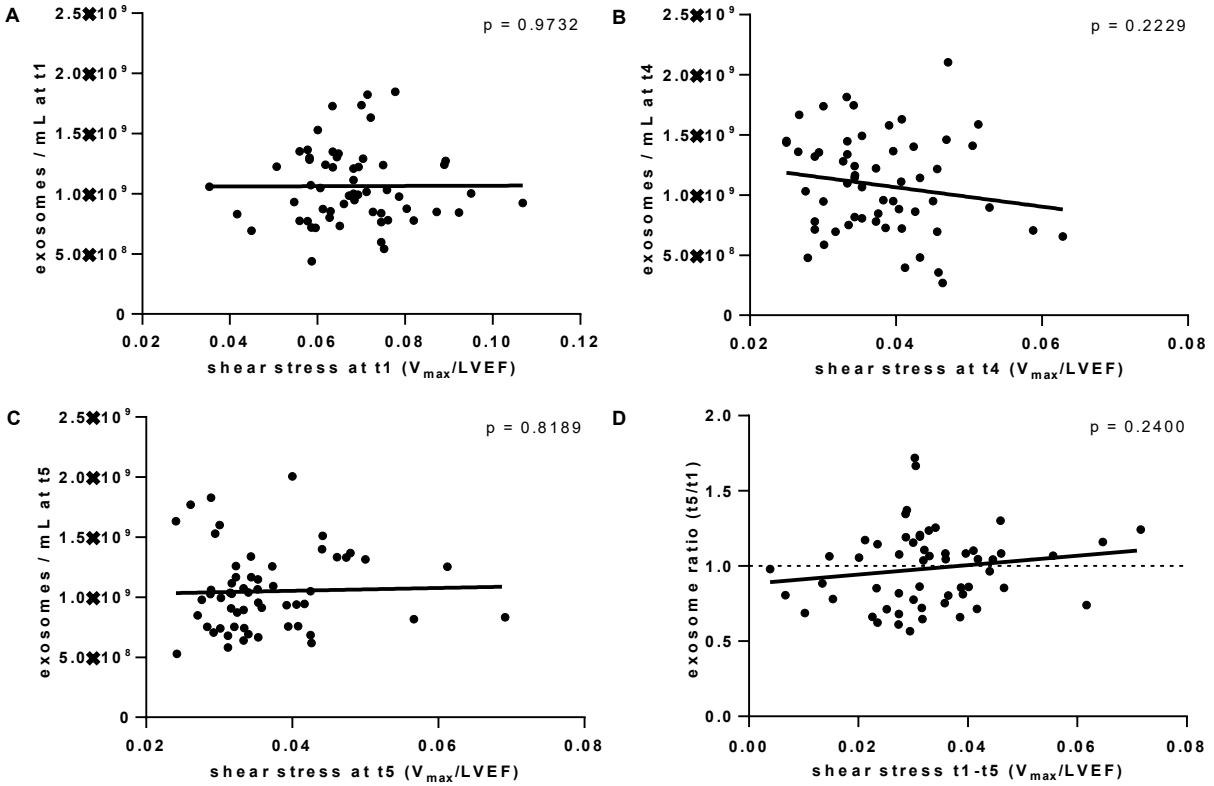

Figure 28: Correlation between circulating exosomes and shear stress (V_{max} / LVEF). Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. V_{max}: transvalvular peak velocity, LVEF: left ventricular ejection fraction. **A:** Correlation between circulating exosomes and shear stress at t1. **B:** Correlation between circulating exosomes and shear stress at t4. **C:** Correlation between circulating exosomes and shear stress at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the shear stress difference (t1 - t5).

LVH parameters. No significant correlation could be detected between circulating exosomes and LVM neither at each of the single time points t1, t4 and t5 (**Fig. 29A-C**), nor considering the exosome ratio (t5 / t1) and the LVM difference (t1 - t5), *i.e.* the LVM regression (**Fig. 29D**). In other words, the LVM change extent from before surgery to 3 months postoperatively did not correlate with the exosome ratio (t5 / t1), *i.e.* with the change of the exosome number from t1 to t5. Noteworthily, this graph shows that the LVM difference (t1 - t5) includes both positive and negative values, *i.e.* not all patients displayed a real LVM regression with (t1 - t5) > 0, but some had an impaired regression or even an increase of the LVM with (t1 - t5) < 0.

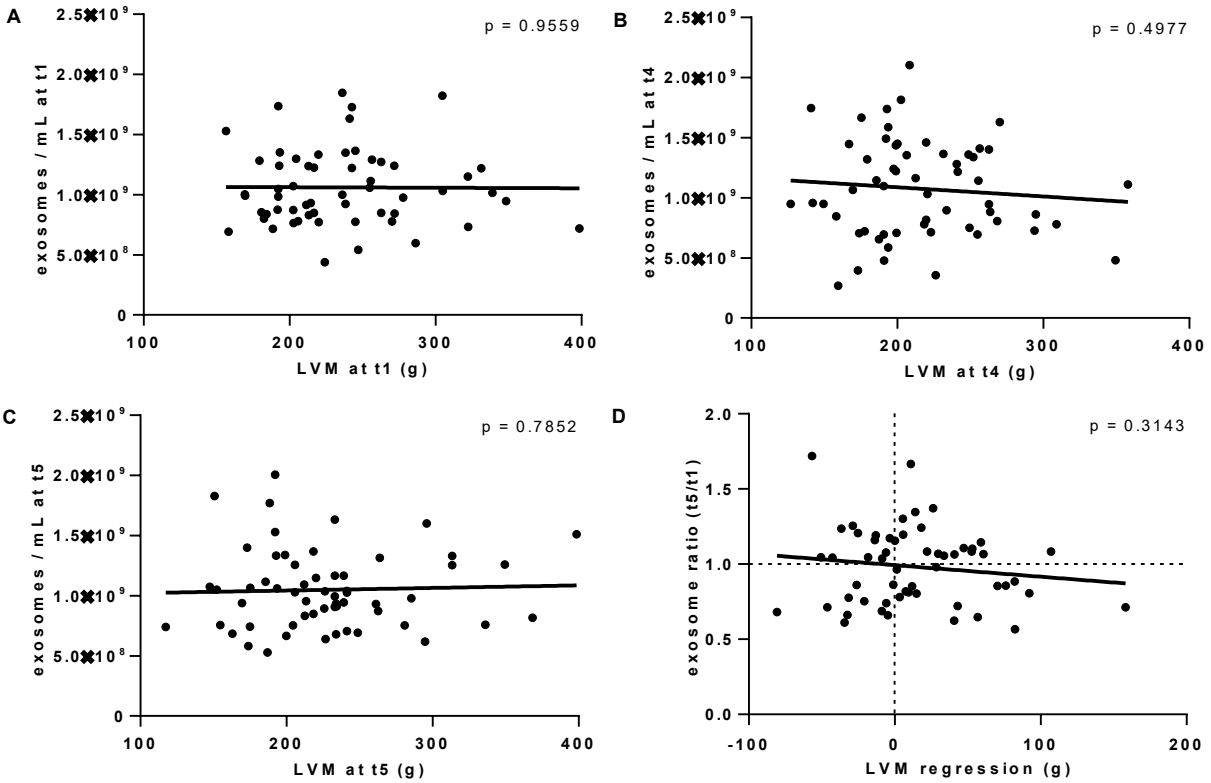

Figure 29: Correlation between circulating exosomes and LVM. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. LVM: left ventricular mass. **A:** Correlation between circulating exosomes and LVM at t1. **B:** Correlation between circulating exosomes and LVM at t4. **C:** Correlation between circulating exosomes and LVM at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the LVM difference (t1 - t5), *i.e.* the LVM regression.

Similarly to the findings regarding LVM, no significant correlation could be detected between circulating exosomes and LVMI neither at each of the single time points t1, t4 and t5 (**Fig. 30A-C**), nor considering the exosome ratio (t5 / t1) and the LVMI difference (t1 - t5), *i.e.* the LVMI regression (**Fig. 30D**). Further, similarly to what was observed for LVM, this graph shows that the LVMI difference (t1 - t5) includes both positive and negative values, *i.e.* not all patients displayed a real LVMI regression with (t1 - t5) > 0, but some had an impaired regression or even an increase of the LVMI with (t1 - t5) < 0.

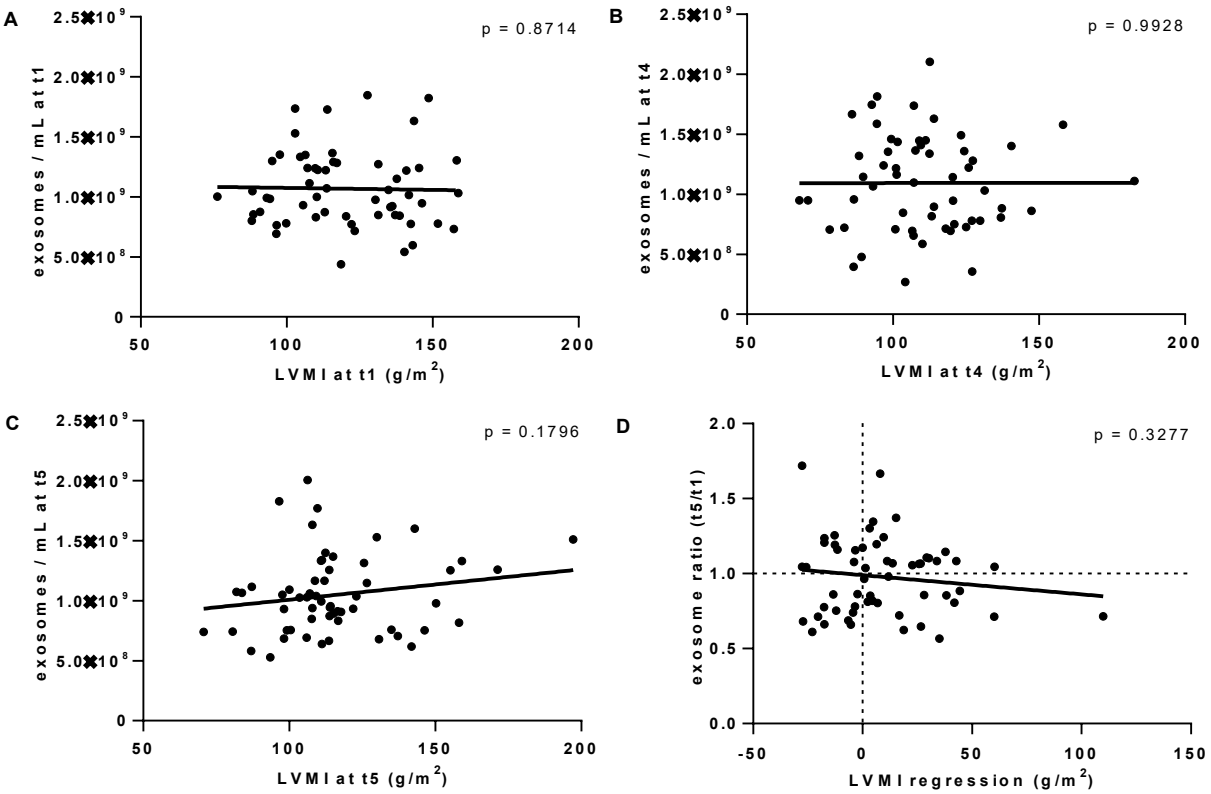

Figure 30: Correlation between circulating exosomes and LVMI. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. LVMI: left ventricular mass indexed to the body surface area. **A:** Correlation between circulating exosomes and LVMI at t1. **B:** Correlation between circulating exosomes and LVMI at t4. **C:** Correlation between circulating exosomes and LVMI at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the LVM difference (t1 - t5), *i.e.* the LVMI regression.

Similarly to the findings regarding LVM and the LVMI, no significant correlation could be detected between circulating exosomes and RWT neither at each of the single time points t1, t4 and t5 (**Fig. 31A-C**), nor considering the exosome ratio (t5 / t1) and the RWT difference (t1 - t5, **Fig. 31D**). Further, similarly to the LVM and LVMI, this graph shows that the RWT difference (t1 - t5) includes both positive and negative values.

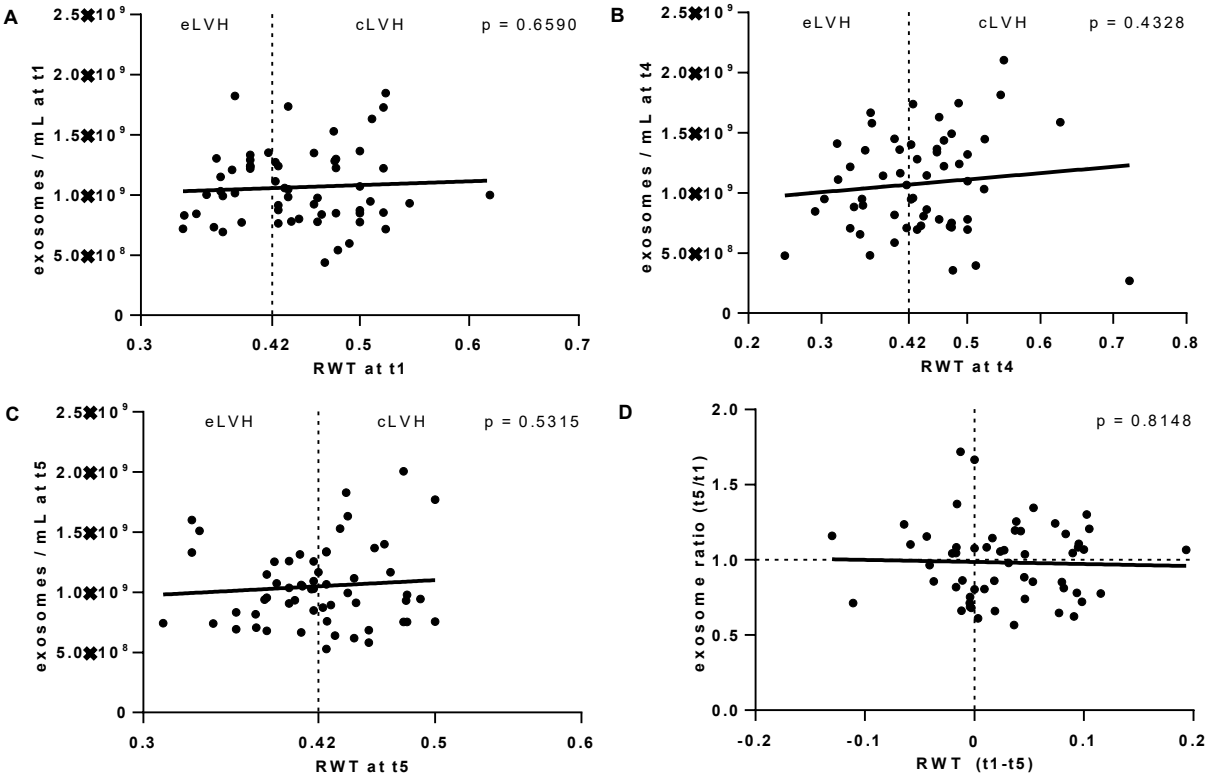

Figure 31: Correlation between circulating exosomes and RWT. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. RWT: relative wall thickness. RWT ≤ 0.42: eccentric left ventricular hypertrophy (eLVH); RWT > 0.42: concentric left ventricular hypertrophy (cLVH). **A:** Correlation between circulating exosomes and RWT at t1. **B:** Correlation between circulating exosomes and RWT at t4. **C:** Correlation between circulating exosomes and RWT at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the RWT difference (t1 - t5).

Age, BMI, EOA$_i$ and PPM. Both older age and greater BMI are risk factors for an emerging postoperative PPM [23] and, in this work, we wanted to investigate whether changes in circulating exosome numbers from preoperative to postoperative measurements could possibly provide information about an emerging PPM. For this reason, we did not only analyse the correlation between the absolute numbers of circulating exosomes and these two parameters (see Paragraph 3.3.1 Demographic parameters), but also the correlation considering changes in circulating exosomes from before surgery to 3 months postoperatively (t5 / t1).

While with patient age no significant correlation could be detected (**Fig. 32A**), BMI showed a significant negative correlation ($p < 0.01$, R^2: 0.1155) with the exosome ratio (t5 / t1, **Fig. 32B**). In patients with a lower BMI, the exosome ratio (t5 / t1) is by trend greater than 1, *i.e.* the exosome number tends to increase from t1 to t5. In overweight patients, on the other hand, the exosome ratio (t5 / t1) tends to be lower than 1, *i.e.* the postoperative circulating exosome levels tend to be lower than before surgery.

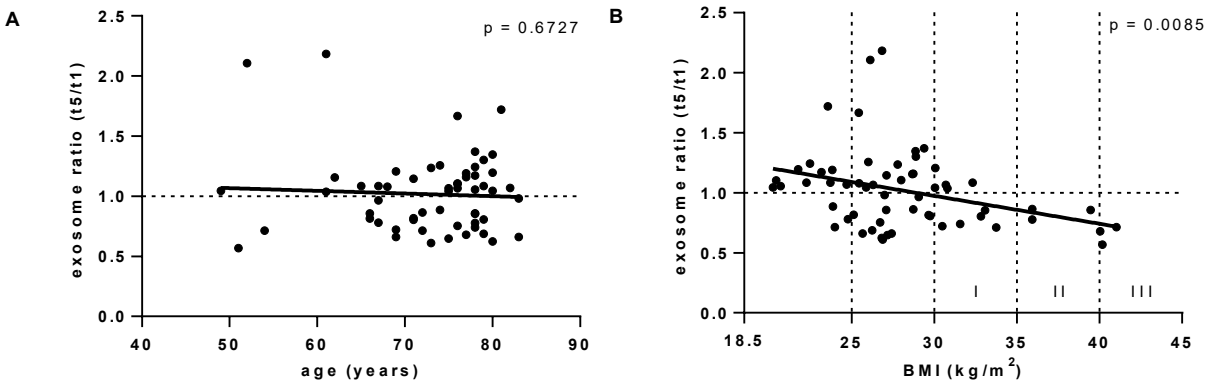

Figure 32: Correlation between the exosome ratio (t5 / t1) and patient age and BMI. Blood samples were sampled at t1 (admission day) and t5 (3 months postoperatively - post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). P-values were calculated using linear regression. **A:** Correlation between the exosome ratio (t5 / t1) and patient age. **B:** Correlation between the exosome ratio (t5 / t1) and BMI. BMI: body mass index. BMI 18.5 - 24.9: normal weight; 25 - 29.9: overweight; 30 - 34.9: obesity class I; 35 - 39.9: obesity class II; ≥ 40: obesity class III.

Considering the single time points t1, t4 and t5, no significant correlation between circulating exosomes and EOA_i could be detected (**Fig. 33A-C**). Nevertheless, considering the exosome ratio (t5 / t1) and the EOA_i difference (t5 - t1), a significant positive correlation was observed ($p < 0.001$, R^2: 0.1909, **Fig. 33D**). After surgery, EOA_i increases and, the greater the increase, the higher the exosome ratio (t5 / t1), *i.e.* the exosome number tends to increase from t1 to t5.

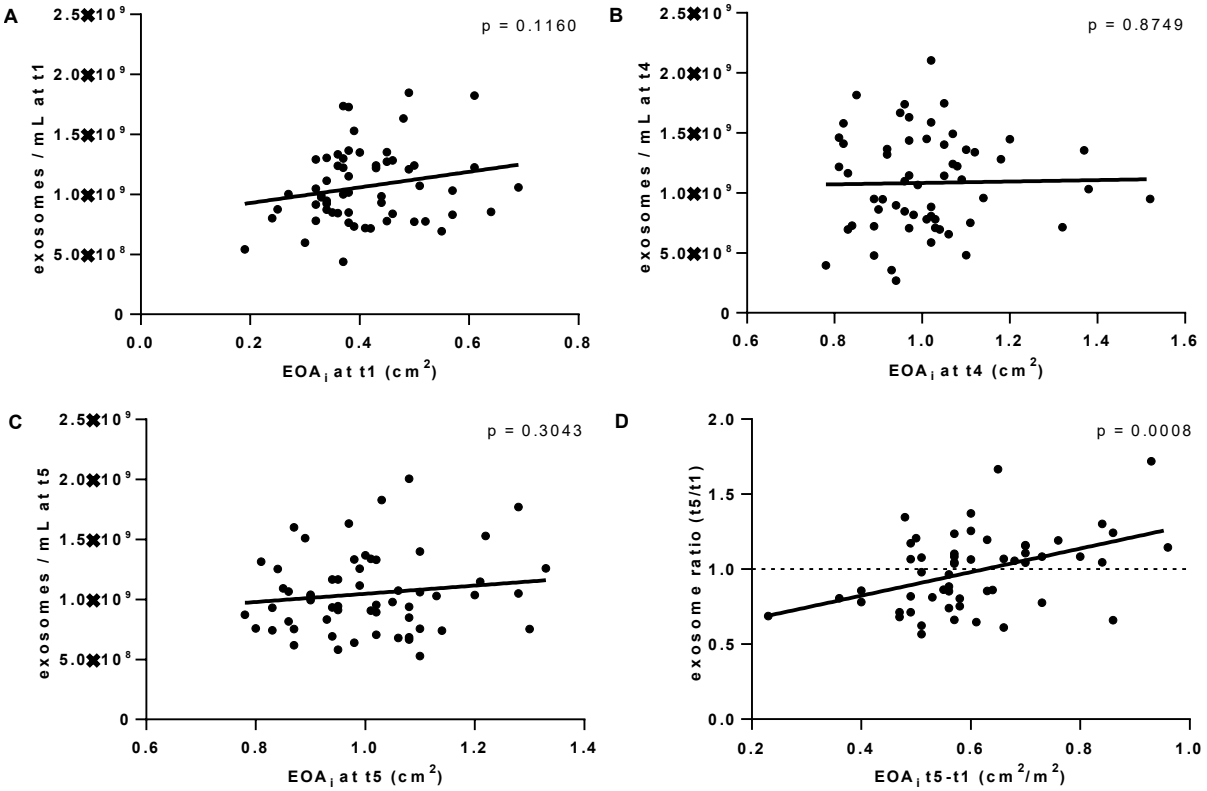

Figure 33: Correlation between circulating exosomes and EOA_i. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. EOA_i: effective orifice area indexed to the body surface area. **A:** Correlation between circulating exosomes and EOA_i at t1. **B:** Correlation between circulating exosomes and EOA_i at t4. **C:** Correlation between circulating exosomes and EOA_i at t5. **D:** Correlation between the exosome ratio (t5 / t1) and the EOA_i difference (t1 - t5).

In this study only very few patients had postoperative findings corresponding to PPM and all these patients had a just borderline moderate PPM with no patient experiencing a severe PPM after SAVR (**Fig. 34**). There was a significant positive correlation between the exosome ratio (t5 / t1) and the EOA_i at t5 ($p < 0.001$, R^2: 0.2701). In particular, this graph shows that all 7 patients with a postoperative PPM (by definition = $EOA_i < 0.85$ cm^2/m^2) present an exosome ratio (t5 / t1) < 1, *i.e.* PPM patients display fewer exosomes 3 months postoperatively than on the admission day.

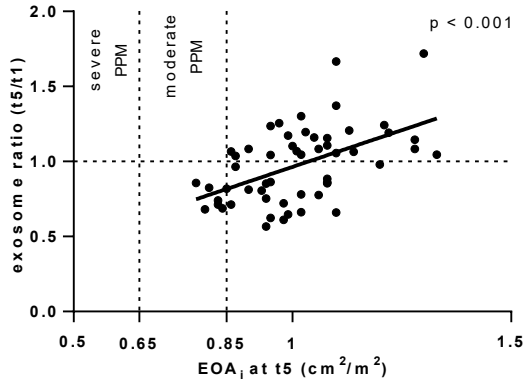

Figure 34: Correlation between the exosome ratio (t5 / t1) and EOA_i at t5. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. EOA_i: effective orifice area indexed to the body surface area. EOA_i 0.65 - 0.85 cm^2/m^2: moderate prosthesis-patient mismatch (PPM); $EOA_i < 0.65$ cm^2/m^2: severe PPM.

Considering the time points altogether (t1, t4 and t5), no significant correlation between circulating exosomes and echocardiographic data could be detected and the respective figures are attached as supplementary figures in the appendix.

4 Discussion

Over the last decade, research on the functions, the biology, the biochemistry and the promising clinical applications of EVs has developed enormously, focussing in particular on the potential of these EVs as biomarkers [36, 63, 86]. Nevertheless, presumably due to lack of a universal methodological approach and to the technical difficulties in analysing the small (nano-sized) exosomes, most studies concentrated on larger (micro-sized) MVs.

To the best of our knowledge, this is the first work providing an analysis of circulating exosomes in a prospective longitudinal study involving CAVD patients undergoing SAVR (combined with CABG). In particular, we investigated whether established clinical parameters correlate with circulating exosomes. Moreover, we aimed at examining whether exosome variation from preoperative to postoperative time points could possibly provide information about the clinical outcome.

Our findings suggest that circulating exosomes show a rapid turnover after major cell and tissue trauma, such as SAVR and CABG. Interestingly, our research revealed that neither AS severity, nor shear stress, nor the LV hypertrophic response are associated with exosome changes. On the other hand, our work reveals a potential role of exosomes as biomarkers to evaluate the postoperative outcome, in particular for the development of a PPM.

4.1 Course of exosome levels and exosomal proteins

Exosomes are known to be released both under physiological and pathological conditions [45]. In particular, exosomes are directly involved both in foam cell formation and in PLT activation in injured arteries and are therefore potential new biomarkers in CVDs [63].

In our study, patients receiving biological valves revealed a significant decrease of preoperative circulating exosomes up to 24 hours postoperatively and a following significant recovery from 24 hours postoperatively to 7 days and 3 months postoperatively, finally showing approximately the initial (preoperative) values. In patients receiving mechanical valves, similar trends could be detected. Statistically significant differences were absent, which may be due to the small sample size of this sub cohort. Remarkably, no significant difference could be observed between the two valve sub cohorts at any respective time point. Further, no significant difference could be detected between the three AS severity subgroups at any respective time point either. Therefore, exosomes are likely not generated as a response to the pathological progression of CAVD. Noteworthily, all 6 patients with a $dP_m < 25$ mmHg (and therefore

categorized the mild AS group), displayed an EOA < 1.5 cm^2, which actually corresponds to a moderate to severe AS (see **Table 1**). Further, it must be specified that the patients analysed in this work all underwent not only a SAVR but also a concomitant CABG and, therefore, the indication for surgery was given not only by CAVD but also by the concomitant CAD.

Exosomes are believed to enhance the healing process by delivering a specific cargo to the target cell [87]. More precisely, it has been reported that exosomes provide an organ protective effect and promote cell survival in pathological settings by monitoring inflammation, repairing injury, triggering angiogenesis, reducing fibrosis and remodelling [87, 88]. In particular, a previous work has emphasized that exosomes originated from hypoxic cardiac progenitor cells (endogenous stem-like heart cells) improved cardiac function and decreased pro-fibrotic gene expression, delineating exosomes as biological therapeutic tools triggering cardiac recovery [89]. Moreover, since the stem cell-derived exosome-mediated paracrine signalling is believed to be responsible for tissue regeneration, and because the general regenerative capacity of damaged cardiomyocytes is limited, exosomes are a promising approach in regenerative medicine for cardiac repair [87, 90].

Circulating exomes may therefore show a rapid turnover after major cell and tissue trauma, such as SAVR and CABG, to trigger cardiac recovery. Further, our findings suggest that, as opposed to MVs [68], exosome levels do not seem to be influenced by AS severity.

Total protein content. As expected, our findings revealed a significant correlation between circulating exosomes and their protein content. Moreover, the protein content of both valve type subgroups revealed an analogous course to the one shown by the exosomes themselves (a decrease from the preoperative values to 24 hours postoperatively was followed by a significant recovery, returning approximately to the initial levels). Further, like circulating exosomes themselves, no significant difference could be observed neither between the two valve sub cohorts nor between the three AS severity subgroups at any respective time point. To the best of our knowledge, no published study has analysed the course of the total protein content at different time points yet.

CD63. Western blot analysis showed that the expression pattern of CD63 changed significantly in the perioperative course. It is known that CD63 presents three N-glycosylation sites and, consequently, distinct glycosylation states [79, 91]. In fact, even though the ground state (the non-glycosylated form) has a molecular weight of 25 kDa, it has been reported that often higher-glycosylated forms are detected [92, 93]. We identified two main bands, at 70 kDa and at 55

kDa, presumably corresponding to two different glycosylation states. While the 55 kDa band remained more or less stable along the perioperative course, the 70 kDa band was tendentially increased in both valve sub cohorts 24 hours and 7 days postoperatively, while 3 months after surgery it was approximately equivalent to the levels before surgery. Further, like circulating exosomes themselves, no significant difference could be observed neither between the two valve sub cohorts nor between the three AS severity subgroups at any respective time point.

It has been reported that CD63 may promote myocardial fibrosis triggering collagen synthesis and that increased CD63 expression may be associated with symptomatic (carotid) stenosis [94, 95]. Moreover, factors such as PLT activation, alcohol or infection have been pointed out as elements that can influence the CD63 expression pattern [95-97]. Nevertheless, to date there is hardly any literature focused on the role of exosomal CD63 in CAVD and CAD or analysing the course of CD63 levels at different time points.

Our findings indicate that SAVR and CABG may somehow influence the CD63 expression pattern and lead to changes in the glycosylation states. Further, similarly to the exosome levels, the CD63 expression does not seem to be affected by the AS severity. Noteworthy, the detected postoperative variation in the CD63 expression pattern, possibly due to post-translational modifications followed by a normalization after three months, fits with the aforementioned significant perioperative change of circulating exosome levels and total protein content. In fact, CD63 expression normalizes 3 months after surgery while on the biochemical level the preoperative glycosylation state is re-established. Nevertheless, further research regarding the modulation of the CD63 expression is necessary to identify the biological explanation of our findings.

4.2 Correlation between exosomes and demographic parameters

Gender. Although gender-related differences in the number of circulating exosomes have hardly been investigated yet, it has been reported that exosomal cargo (in particular exosomal miRNA and specific proteins) presents variations depending on the sex [98, 99]. Our findings revealed no significant disparity. However, in our study more than 75% of all patients were men and, for this reason, a larger and more evenly balanced trial is needed to further investigate gender-related exosome differences.

Age and BMI. A previously published study has shown that not only the exosome concentration decreases with ageing, but also that this reduction could be due to increased internalization of the exosomes by WBCs [100]. In fact, exosome concentration is a consequence of the balance between particle release and particle internalization and the ageing process may affect this balance [100]. In any case, in current literature there is not yet a homogeneous conception about the role of exosomes in age or ageing. It has been reported that exosomes mediate both beneficial effects like tissue regeneration and deleterious effects like cellular senescence [101]. Even though our data showed no significant correlation between circulating exosomes and patient age, there was a weak trend towards lower exosome concentrations in older patients, in line with the here mentioned observations.

Further, a review has pointed out that exosomes are regularly produced by adipose tissue and that adipocyte-derived exosomes play a fundamental role in the development of obesity-related metabolic disorders such as diabetes and CVDs [41]. Nevertheless, our data showed no significant correlation between the absolute numbers of circulating exosomes and BMI.

4.3 Correlation between exosomes and blood count

Exosome-shedding blood cells. Blood cells such as RBCs, WBCs and PLTs are known to shed exosomes [48, 52, 58, 102]. Among the analysed blood count parameters, those with most significant correlations to measured exosome numbers turned out to be related to RBCs. We found a significant positive correlation between exosome levels and RBCs, Hb and Hct. Remarkably, these correlations were present preoperatively and persisted also 7 days and 3 months postoperatively. It has already been reported that, in circulating blood, RBCs are the major source of EV other than exosomes, *e.g.* MP [103], and we hypothesize that these blood cells may also be one of the main sources of circulating exosomes.

Further, exosomes play an important role in the immune response as well as in the PLT activation process [48, 58]. However, to the best of our knowledge, to date both WBC- and PLT-derived exosomes have been hardly investigated. In fact, even though different papers reported that PLT-derived EVs are the most abundant circulating particles, most sources refer specifically only to MVs and not to exosomes [104, 105]. In any case, our finding revealed that in contrast to RBCs, neither WBCs nor PLTs correlated significantly with circulating exosomes. This applies to analysis considering the time points altogether as well as the single time points.

Tissue damage parameters. TnT, CK, LDH and AST are established biomarkers for ischemia and tissue damage, which are regularly used in the clinical setting [106, 107].

Tn is a protein complex composed of three elements: TnC (calcium binding), TnI (ATPase inhibiting), and TnT (tropomyosin binding) [108]. While TnC exists only as skeletal muscle isoform, TnI and TnT have a specific cardiac isoform (cTnT and cTnI), which are therefore gold standard biomarkers of myocardial damage [40, 108]. In fact, cTn is superior to the other markers because of its cardiac specificity [106]. Further, cTn correlates with the clinical outcome: the greater the cTn level and the longer this marker is elevated, the more severe the myocardial damage and the poorer the clinical outcome [106]. Interestingly, a study revealed a significant positive correlation between cTn and both exosomes themselves and exosomal cardiac miRNAs after CABG [40].

CK is a biomarker for both myocardial and skeletal muscle injury and is used in the clinical setting for AMI diagnosis complementarily to other (more sensitive and specific) biomarkers such as cTn [106, 107].

LDH is an enzyme present in all human cells, including blood cells and cardiomyocytes, and it is released in the bloodstream during tissue damage [109]. In the clinical setting, LDH is regularly used in diagnostics and monitoring of various pathological conditions such as haemolysis, oncological diseases and AMI [110-113].

AST (also known as GOT) was the first diagnostic biomarker for AMI to be identified [108]. Nevertheless AST is not cardiac-specific, since it is present also in other cells such as skeletal myocytes and hepatocytes and is, in fact, also an established hepatic marker [106, 108, 114].

In this work we investigated whether the development of established clinical parameters (such as the above mentioned four tissue damage markers) may correlate with the course of circulating exosomes in the setting of SAVR and CABG. Considering the time points altogether, as well as at the single time points before surgery and 7 days postoperatively, none of the four parameters revealed a significant correlation. Nevertheless, LDH, CK and AST (but not TnT) increased significantly 3 months postoperatively with decreasing exosome levels.

These observations suggest that circulating exosomes may be involved in the development of the postoperative clinical outcome. Specifically, given the negative correlation between exosome levels and LDH, CK and AST, respectively, 3 months postoperatively, our findings may lead to the interpretation that higher circulating exosome levels could possibly be associated with a favourable postoperative outcome.

4.4 Correlation between exosomes and echocardiographic data

In this work we aimed at examining whether an exosome variation from before to after surgery could possibly provide information on emerging PPM. For this reason, we did not only analyse the correlation between circulating exosomes and echocardiographic parameters at the single time points, but also the correlation considering the perioperative change in circulating exosomes.

dP_m, dP_p, V_{max} and EOA. As mentioned above (see Section 4.1 Course of exosome levels and exosomal proteins), our findings revealed no significant difference within the three AS severity subgroups (determined according to the preoperative dP_m) neither regarding the exosome levels themselves nor regarding the exosome protein content. Accordingly, no significant correlation was detected between circulating exosomes and dP_m at any time point. Similarly, dP_p, V_{max} and EOA did not display a significant correlation with circulating exosomes. These findings are in accordance with the aforementioned considerations, *i.e.* exosome release is not triggered by severe AS or by the involved high pressure gradients or reduced EOA.

On the other hand, our data showed that after surgery the pressure gradients and the transvalvular peak velocity decreased and, the greater the decrease, the more the exosome number tended to increase from preoperative level to 3 months postoperatively. Further, our findings revealed that after surgery EOA increased and, the greater the increase, the more the exosome number tended to increase from preoperative level to 3 months postoperatively. Accordingly, increased postoperative exosome levels were associated with postoperative decreased pressure gradients and increased EOA, factors that seem to indicate a favourable postoperative clinical outcome. To the best of our knowledge this is the first work focussing on circulating exosomes to investigate the postoperative outcome.

However, even though our findings are in line with our aforementioned considerations, *i.e.* higher postoperative exosome levels seem to be associated with a favourable postoperative clinical outcome, current literature is not homogeneous regarding the association between clinical outcome and echocardiographic parameters such as dP and EOA. In fact, even though a recent study has reported that preoperative increased pressure gradients were associated with postoperative increased mortality, another study could not identify postoperative pressure gradients as a predictor for postoperative survival [115, 116]. Further research is required to investigate the association between the clinical outcome and the here mentioned postoperative

echocardiographic parameters as well as the correlation between the latter and circulating exosomes.

Shear stress. It has been reported that shear stress directly influences atherosclerosis [117, 118]. In particular, to date it is known that low (!) shear stress triggers plaque formation [119-121]. This can be partly explained using the Newtonian shear stress formula [119, 121]. In fact, shear stress is directly proportional to fluid velocity near the surface, *i.e.* with low shear stress the velocity is reduced and consequently the fluid residence time near the endothelium is increased [119]. Further, the fluid viscosity is inversely proportional to the shear rate, *i.e.* with low velocity gradient the viscosity is high [118, 121]. Besides, the most atherosclerotic-prone areas are vessel bifurcations, where the tangential frictional force acting on the endothelium, *i.e.* the shear stress, is weaker [120]. Moreover, it has been reported that high shear stress triggers the release of endothelial factors that inhibit the atherosclerotic process [121]. Shear stress is thus basically an athero-protective factor [117, 119-121].

Nevertheless, due to the reduced EOA, blood and blood cells passing through diseased AVs suffer higher shear stress compared to levels present in normal AVs [68]. It has been reported that in AS patients EVs - such as PLT-MVs - are increased and correlate with valvular shear stress [68]. Not only has it been shown that blood shear stress triggers MV formation, it has also been hypothesized that the released MV contribute to the progression of AS, creating a vicious circle [68]. Interestingly, in contrast to our expectations and as opposed to reported course and nature of larger MVs, our research findings showed that exosome secretion is not associated with altered shear stress in AS. In fact, on the one hand, no significant correlation could be detected between circulating exosomes and shear stress. On the other, while shear stress revealed a significant decrease from preoperatively to 7 days and 3 months postoperatively, exosomes displayed equivalent levels at the here mentioned time points. All these considerations indicate that exosome formation is not triggered by shear stress.

According to pertinent literature, exosome release seems rather to be induced by particular changes in cellular conditions, such as heightened intracellular calcium levels, potassium-induced cell depolarization, glutamatergic activity and receptor activation [48, 122-126], as well as by certain molecules like reactive oxygen species, p53 and Rab GTPases [102, 127-129]. Additionally, external stimuli like inflammation, hypoxia, heat stress and ethanol have shown to boost exosome shedding [51, 128, 130, 131].

LVH parameters. Generally, following SAVR LVH regresses. An incomplete LVM regression, as observed for patients with severe PPM, is associated with adverse clinical outcome [18, 23]. For this reason we investigated the correlation between circulating exosomes and LVH parameters, focussing in particular on patients with an impaired LVM regression at the latest follow-up time of this study, *i.e.* after three months. Nevertheless, in contrast to our expectations, our findings revealed no correlation between circulating exosomes and LVM, LVMI or RWT, respectively.

When exosomes and their interaction with myocytes are concerned, existing reports have focused on the role of exosomes in the development of myocyte hypertrophy [132]. However, a thorough research yielded no reports analysing the association between circulating exosome levels and LVH parameters. Our results suggest that exosome secretion is likely not associated with the LV hypertrophic response, but further investigation is needed to confirm this hypothesis.

Age, BMI, EOA_i and PPM. Both older age and greater BMI are known risk factors for postoperative PPM after SAVR [23]. In this work we aimed at investigating whether exosome variation from preoperative to postoperative status could possibly provide information about an emerging PPM. For this reason, we did not only analyse the correlation between circulating exosomes and the above mentioned two parameters at the single time points, but also the correlation considering the perioperative change in circulating exosomes.

While with age no significant correlation could be detected, the perioperative change in circulating exosomes significantly correlated with BMI. In particular, in patients with a lower BMI, exosome number tended to increase from preoperatively to 3 months postoperatively. In overweight patients, on the other hand, circulating exosome levels tended to be lower three months postoperatively than preoperatively. Even though further investigation is needed to identify the underlying biological functions, these findings appear to be in accordance with the general line of our observations, suggesting that postoperative decreased exosome levels may be related to a greater risk of adverse postoperative outcome.

Moreover, our data revealed that after surgery EOA_i increased and, the greater the increase, the more the exosome number tended to increase from preoperatively to 3 months postoperatively. Accordingly, high postoperative exosome levels seem to be associated with a favourable outcome after SAVR.

Further, our findings showed that 7 patients developed a moderate postoperative PPM. Interestingly, all of these patients had reduced circulating exosome levels three months postoperatively when compared to the respective preoperative levels. These findings reinforce our aforementioned considerations, *i.e.* reduced postoperative circulating exosome levels are associated with impaired clinical outcome three months after combined SAVR and CABG.

4.5 Limitations

The findings presented in this work should be considered in the light of a number of limitations.

First of all, sample size limitations present in this study impair the validity of adequate subgroup-analyses. The herein described findings related to PPM are limited by the fact that only very few patients in this study experienced postoperative PPM. Larger studies are needed to provide greater power for sub-cohort-analyses, *e.g.* on patients with PPM. Noteworthily, not all patients fitting the inclusion criteria of our study could be enrolled and some patients had to be excluded *ex post* for various reasons (see Section 3.1 Patient population).

Moreover, the results of this single-centre study warrant external validation. A multi-centre trial would be ideal to confirm the general validity of our statements.

Finally, our research is based on exosome isolation and analysis and, to date, there is no downstream universal methodological approach. In particular, conventional techniques either involve precipitation agents or ultracentrifugation steps and the actual major problem is that of isolating the exosomes selectively [133]. It has been reported that purified preparations likely contain both non-exosomal debris and also exosome aggregates. The latter particles may have contributed to the cluster of detected particles larger than 200 nm [133, 134]. However, we deliberately chose a method that uses a precipitation agent, since this approach seems to yield a higher degree of particles than the others [133].

4.6 Conclusions and perspectives

To the best of our knowledge, this is the first work providing an analysis of circulating exosomes in a prospective longitudinal study involving patients undergoing SAVR (in combination with CABG).

Our findings reveal that exosomes are permanently present in the circulation of the examined patients without any apparent influence of AS severity or LV hypertrophy on their perioperative course. In fact, our study reveals that AS is not a trigger for exosome secretion and that an impaired LVM regression does not correlate with circulating exosomes. Importantly, as opposed to larger MV, shear stress does not enhance exosome release.

Postoperative decreased exosome levels showed a significant association with echocardiographic parameters (such as the EOA_i) and certain clinical and laboratory parameters, *e.g.* BMI, CK, LDH and AST. In particular, our findings revealed that lower postoperative circulating exosome levels may be an indicator of adverse outcome after combined SAVR and CABG, *e.g.* development of PPM. Likewise, CD63 as a commonly used marker for exosomes showed a similar perioperative course. Further, our findings led us to identify RBCs as the possible main source of circulating exosomes.

To date, many pre-clinical studies have already highlighted the great potential of exosomes in diagnosis, therapy and assessment of prognosis of various diseases. However, currently we are still quite far from being able to use them in the clinical practice and draw information directly from their circulating levels.

Further investigation is necessary to explore possible mechanisms underlying our findings. Meanwhile, this work may contribute to a better understanding of the course of exosomes in the setting of surgical therapy for CAVD with concomitant CAD.

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Statement of authorship and disclosure

I hereby declare that this doctoral thesis is the result of my own work, unless otherwise acknowledged in the text. All references and verbatim extracts have been quoted, and all sources of information have been specifically acknowledged. This thesis has not been submitted for any other degree.

Moreover, I declare no competing interests of any kind.

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Appendix

Supplementary figures

Considering the time points altogether (t1, t4 and t5), no significant correlation was detected between circulating exosomes and dP_m, dP_p, V_{max}, or EOA (**Fig. 35A-D**).

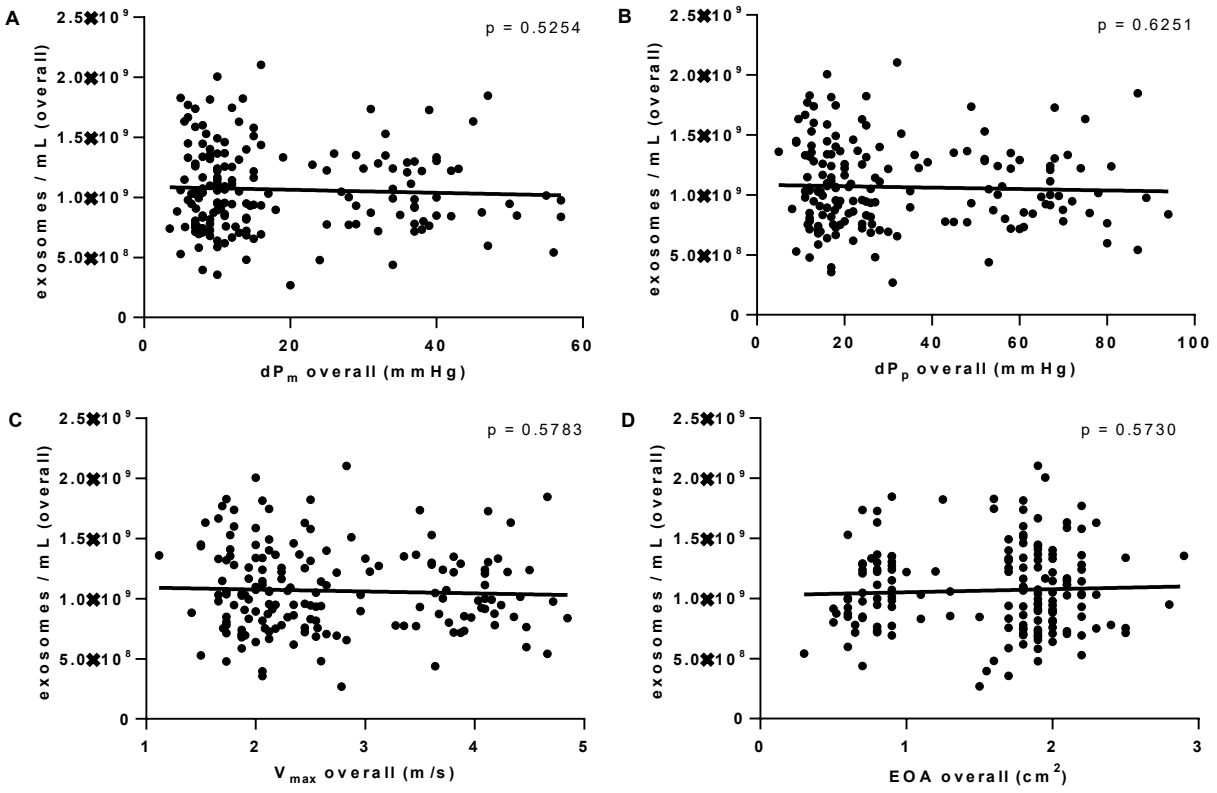

Figure 35: Overall correlation between circulating exosomes and dP_m, dP_p, V_{max}, and EOA. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. **A:** Overall correlation between circulating exosomes and dP_m (t1, t4 and t5). dP_m: mean pressure gradient. **B**: Overall correlation between circulating exosomes and dP_p (t1, t4 and t5). dP_p: peak pressure gradient. **C:** Overall correlation between circulating exosomes and V_{max} (t1, t4 and t5). V_{max}: transvalvular peak velocity. **D:** Overall correlation between circulating exosomes and EOA (t1, t4 and t5). EOA: effective orifice area.

Similarly, considering the time points altogether, no significant correlation was detected between circulating exosomes and shear stress, LVM, LVMI, RWT or EOA_i (**Fig. 36A-E**).

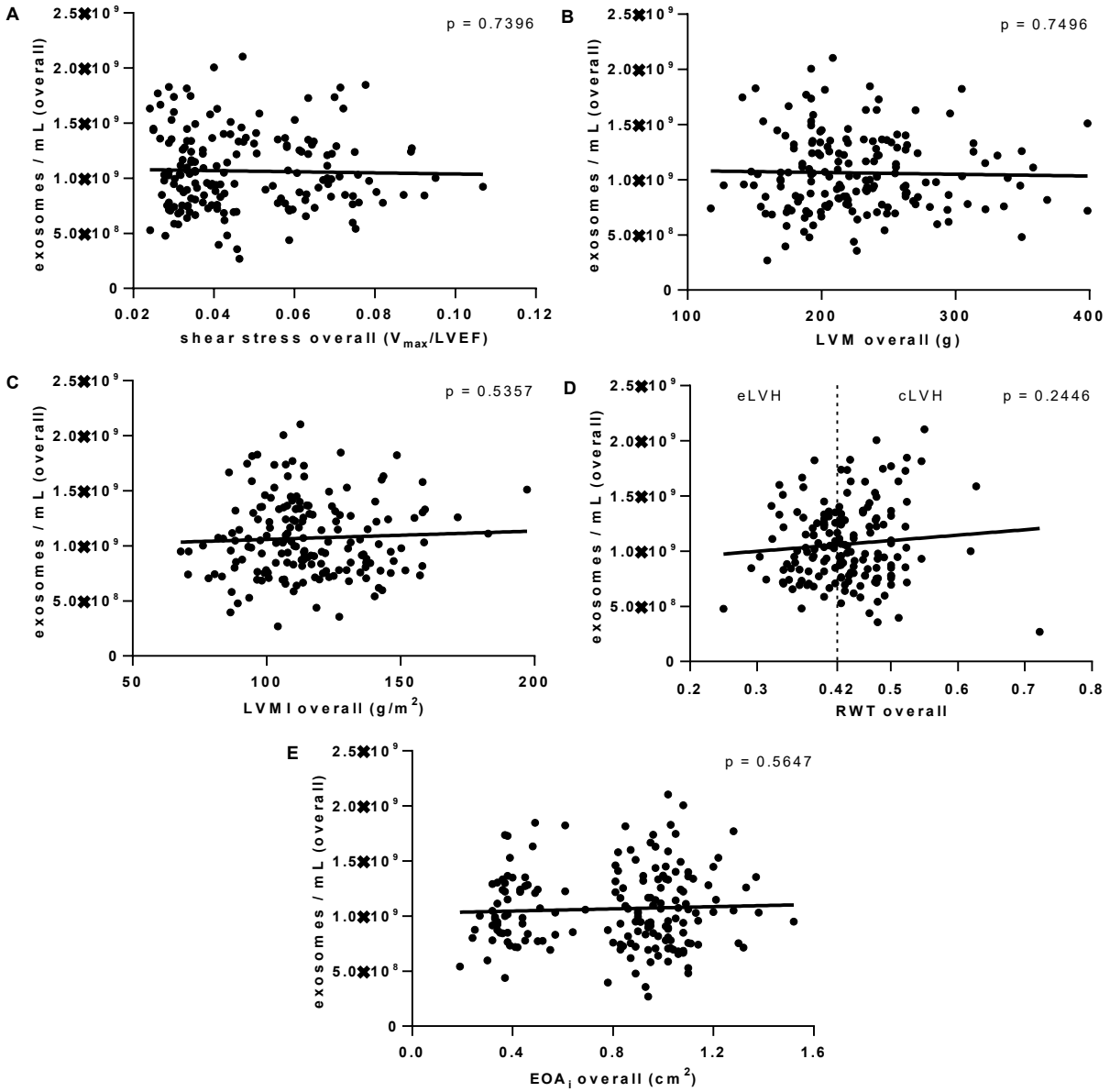

Figure 36: Overall correlation between circulating exosomes and shear stress, LVM, LVMI, RWT and EOA_i. Blood samples were sampled at t1 (admission day), t4 (7 days postoperatively - post-op) and t5 (3 months post-op). Exosomes were directly isolated and analysed by nanoparticle tracking analysis (NTA). Additionally, echocardiographic analysis was performed. P-values were calculated using linear regression. **A:** Overall correlation between circulating exosomes and shear stress (t1, t4 and t5). V_{max}: transvalvular peak velocity, LVEF: left ventricular ejection fraction. **B:** Overall correlation between circulating exosomes and LVM (t1, t4 and t5). LVM: left ventricular mass. **C:** Overall correlation between circulating exosomes and LVMI (t1, t4 and t5). LVMI: left ventricular mass indexed to the body surface area. **D:** Overall correlation between circulating exosomes and RWT (t1, t4 and t5). RWT: relative wall thickness. RWT ≤ 0.42: eccentric left ventricular hypertrophy (eLVH); RWT > 0.42: concentric left ventricular hypertrophy (cLVH). **E**: Overall correlation between circulating exosomes and EOA_i (t1, t4 and t5). EOA_i: effective orifice area indexed to the body surface area.

Perioperative data

Table 16: Perioperative parameters of study patients.

Parameter	Biological valve (n = 53)	Mechanical valve (n = 6)
Surgery duration (min)	247.1 ± 53.0	315.2 ± 19.7
ECC duration (min)	146.4 ± 33.2	183.0 ± 17.3
Aortic cross-clamping duration (min)	97.9 ± 26.0	125.8 ± 16.9
Ventilation duration (h)	11.0 ± 8.56	13.9 ± 14.1
Time spent at ICU (h)	42.7 ± 33.6	39.5 ± 28.8

ECC: extracorporeal circulation, ICU: intensive care unit. Data are presented as mean ± SD and the unit is given in brackets.

Table 17: Transfusion rates of study patients.

Transfusions	Biological valve (n = 53)		Mechanical valve (n = 6)	
	peri-op (t2 + t3)	post-op (t4 + t5)	peri-op (t2 + t3)	post-op (t4 + t5)
RBC unit	1.61 ± 1.70	0.11 ± 0.42	1.67 ± 2.32	0.17 ± 0.55
PLT unit	0.98 ± 1.10	0.00 ± 0.00	1.33 ± 1.84	0.00 ± 0.00
FFP unit	0.41 ± 1.01	0.00 ± 0.00	0.92 ± 1.89	0.00 ± 0.00

RBC: red blood cell, PLT: platelet, FFP: fresh frozen plasma, peri-op: perioperative time points, post-op: postoperative time points, t: time point. Data are presented as mean ± SD.

Table 18: Valve graft types of study patients.

Valve graft type	Biological valve (n = 53)	Mechanical valve (n = 6)
SJM Trifecta	13 (24.5%)	-
Edwards Magna Ease 3300 TFX	26 (49.1%)	-
Edwards Intuity Elite	9 (17.0%)	-
Edwards Perimount 2800	5 (9.43%)	-
SJM Regent AGN-751	-	5 (83.3%)
Medtronic Aortic AP 360	-	1 (16.7%)

The values refer to the absolute number of patients receiving the respective valve prosthesis type, the proportion relative to the entire sub-cohort is indicated in brackets.

Patient information material

Original German version:

Patienteninformation: Verlauf von zirkulierenden Mikropartikeln nach einem Aortenklappenersatz bei Patienten mit hochgradiger Aortenklappenstenose

Kurztitel: Mikropartikel bei AKE

Sehr geehrte Patientin, sehr geehrter Patient,

Ihr Arzt hat bei Ihnen eine hochgradige Aortenklappenstenose festgestellt, die eine operative Therapie durch die Implantation einer Herzklappenprothese erfordert. Die Wahl der Klappenprothese, die bei Ihnen implantiert werden soll, erfolgt nach rein medizinischer Indikation sowie nach Ihren Präferenzen und völlig unabhängig von der Teilnahme an dieser Studie.

Informationen zur klinischen Prüfung. *Sie werden gebeten, an einer klinischen Untersuchung teilzunehmen, in der von Ihnen entnommene Blutproben auf verschiedene laborchemische Werte hin untersucht werden sollen. Die Teilnahme an dieser Studie hat für Ihre medizinische Behandlung weder einen positiven noch einen negativen Effekt. Die Teilnahme an dieser Studie bedeutet für Sie lediglich, dass im Rahmen der therapeutisch routinemäßig indizierten Blutentnahmen an vier Zeitpunkten eine zusätzliche Abnahme von 20 mL erfolgt. Die Blutabnahme für diese Studie wird im zeitlichen Zusammenhang mit Blutabnahmen eingeplant, die im Rahmen Ihrer weiteren postoperativen Behandlung und Nachsorge notwendig sind, und denen Sie sich unabhängig von einer Teilnahme an dieser Studie unterziehen müssten. Darüber hinaus werden nur die üblichen Untersuchungen, denen Sie sich auch unterziehen müssten, wenn Sie nicht an der Untersuchung teilnehmen würden, vorgenommen. Drei Monate nach dem Eingriff werden Sie zu einem Nachsorgetermin gebeten, an dem die Funktion des Herzens im Verlauf untersucht werden soll. Ziel der Untersuchung ist es, einen detaillierten Einblick in den Verlauf von zell-sezernierten Mikropartikeln zu gewinnen. Diese Erkenntnisse könnten gegebenenfalls dazu beitragen, die heute angewandten Therapieverfahren zu optimieren und schonender zu gestalten.*

Risiken. *Durch Ihre Teilnahme an der klinischen Untersuchung sind Sie keinem erhöhten Operationsrisiko ausgesetzt. Die Blutentnahme erfolgt in der Regel im Rahmen einer Routine-Blutabnahme. Zu den Risiken der Blutabnahme gehört das Entstehen blauer Flecken im Bereich der Einstichstelle. Es besteht das sehr geringe Risiko einer lokalen oder allgemeinen Infektion.*

In extrem seltenen Fällen kann es zu einer Verletzung eines Hautnervs, evtl. sogar mit chronischem Verlauf, kommen.

Vertraulichkeit. *Damit Sie an der klinischen Prüfung teilnehmen können, muss Ihre Einwilligung vorliegen, nachdem Sie durch einen Arzt über Wesen, Bedeutung und Tragweite der klinischen Prüfung aufgeklärt worden sind und mit dieser Einwilligung zugleich erklären, dass Sie mit der im Rahmen der klinischen Prüfung erfolgenden Aufzeichnung von Krankheitsdaten durch die Klinik für Kardiovaskuläre Chirurgie und mit der Auswertung dieser Daten durch die Klinik für Kardiovaskuläre Chirurgie einverstanden sind. Sie selbst haben auch die Möglichkeit, Ihre für die Prüfung erhobenen Daten einzusehen. Die ärztliche Schweigepflicht und die Bestimmungen des Bundesdatenschutzgesetzes werden eingehalten. Es werden nur pseudonymisierte Daten* ausgewertet und ggf. auch nur in pseudonymisierter Form weitergegeben. Dritte erhalten keinen Einblick in Originalunterlagen.*

(Pseudonymisierung bedeutet Verschlüsselung von Daten / Proben ohne Namensnennung nur mit Nummern und ggf. mit dem Geburtsdatum codiert. Die Zuordnung der Daten oder Proben zu einer Person ist nur möglich, wenn hierfür der Schlüssel eingesetzt wird, mit dem die Daten pseudonymisiert wurden).*

Freiwillige Teilnahme. *Ihre Teilnahme an dieser klinischen Prüfung ist freiwillig. Sie können jederzeit Ihre Teilnahme verweigern, ohne dass das zu irgendeinem Zeitpunkt Nachteile oder den Verlust der ärztlichen Behandlung für Sie zur Folge hat. Auch kann Ihre Teilnahme an der klinischen Prüfung ohne Einwilligung abgebrochen werden, wenn eine Fortführung nach dem Ermessen Ihres Arztes oder der Ethik-Kommission nicht in Ihrem besten Sinne ist.*

Rücktrittsrecht. *Sie können Ihr Einverständnis jederzeit, ohne Angabe von Gründen und ohne Nachteile für Ihre weitere medizinische Versorgung, zurückziehen. Bei Rücktritt von der Studie kann auf Wunsch bereits gewonnenes Daten- / Probenmaterial vernichtet werden. Sie können sich beim Ausscheiden aus der Studie entscheiden, ob Sie mit der Auswertung des Materials bzw. Ihrer Studiendaten einverstanden sind oder nicht. Sollten Sie zu einem späteren Zeitpunkt Ihre Entscheidung ändern wollen, setzen Sie sich bitte mit dem Studienarzt in Verbindung.*

Vergütung für die Teilnahme. *Sie erhalten für die Teilnahme an der klinischen Prüfung keine finanzielle Entschädigung.*

Fragen zur klinischen Prüfung. *Wenn Sie der Meinung sind, dass nicht alle Fragen zur klinischen Prüfung zufriedenstellend beantwortet sind, wenden Sie sich bitte an den Prüfarzt*

Herrn PD Dr. med. Akhyari (Tel.: 0211-81-18331) oder an die entsprechende Abteilung des Krankenhauses. Wenn Sie Fragen zu Ihren Rechten als Teilnehmer an einer klinischen Prüfung haben, so können Sie sich jederzeit an uns wenden.

Einverständniserklärung. *Ich habe die schriftliche Patienteninformation erhalten, diese gelesen und voll inhaltlich verstanden, bzw. die Informationen zu meiner Teilnahme an der klinischen Studie Mikropartikel bei AKE sind mir vorgelesen worden, und ich erkläre mich freiwillig bereit zur Teilnahme an dieser Studie. Ich hatte die Gelegenheit, alle erdenklichen Fragen zu stellen und habe für mich zufriedenstellende Antworten erhalten. Ich weiß, dass meine Teilnahme an der klinischen Prüfung freiwillig ist und dass ich diese Einverständniserklärung jederzeit und ohne Angabe von Gründen widerrufen kann und dass eine Beendigung meiner Teilnahme an dieser klinischen Prüfung keinen Einfluss auf die weitere ärztliche Behandlung hat.*

Information und Einwilligungserklärung zum Datenschutz

1) Ich bin damit einverstanden, dass im Rahmen dieser Studie erhobene Daten, insbesondere Angaben über meine Gesundheit, erhoben, in Papierform oder auf elektronischen Datenträgern pseudonymisiert aufgezeichnet und gespeichert werden und dass die Studiendaten in anonymisierter Form für wissenschaftliche Darstellungen und Veröffentlichungen verwendet werden dürfen.

2) Ich bin darüber aufgeklärt worden, dass ich meine Einwilligung in die Aufzeichnung, Speicherung und Verwendung meiner Daten jederzeit widerrufen kann. Bei einem Widerruf werden meine Daten unverzüglich gelöscht.

3) Ich erkläre mich damit einverstanden, dass meine Daten nach Beendigung oder Abbruch der Studie 10 Jahre lang aufbewahrt werden. Danach werden meine personenbezogenen Daten gelöscht, soweit dem nicht gesetzliche, satzungsgemäße oder vertragliche Aufbewahrungs-fristen entgegenstehen.

Name des / der Patienten / -in (Druckbuchstaben oder Etikett); Unterschrift des / der Patienten / -in, Datum; Unterschrift des aufklärenden Arztes, Datum

Ich erkläre mich damit einverstanden, dass im Rahmen dieser Studie gewonnenes Material auch für spätere Untersuchungen zu kardialen Erkrankungen verwendet werden kann.

Düsseldorf, den (Datum); Name des Patienten, Unterschrift des Patienten

CRF

Original German version:

U1: Prä-OP-Daten

Laufende Nr. des Probanden, Patienten ID, Datum der Untersuchung (TTMMJJ)

A: Einschlusskriterien

Schriftliche Einwilligung des Patienten zur Teilnahme an dieser Studie, Alter ≥ 18 Jahre, hämodynamisch signifikante Aortenklappenstenose ad Ersatz

B: Primäre Ausschlusskriterien

Höhergradiges Klappenvitium anderer Klappen als der Aortenklappe (> II), Z.n. Myokardinfarkt innerhalb der letzten 30 Tage, peripher-arterielle Verschlusskrankheit (> Stadium IIb nach Fontain), Schwere Einschränkung der LV-Funktion (EF < 30%)

C: Sekundäre Ausschlusskriterien

Aktive maligne Erkrankung, Thromboembolische Ereignisse in den letzten 6 Monaten (unabhängig von Quelle), Autoimmunerkrankungen, Entzündungsprozesse, Dialyse

D: Patienteneinschluss / Einverständniserklärung

Patienteneinschluss (Ein- / Ausschlusskriterien zutreffend): Ja / Nein

Datum der Unterschrift (TTMMJJ)

E: Demografische Daten *(Daten vom Narkoseinleitungsprotokoll / Anästhesieprotokoll)*

Alter (Jahre), Größe (cm), Gewicht (kg), Oberfläche (m^2), Geschlecht: männlich / weiblich

F: Anamnese

Kardiale Dekompensation (Ja / Nein); KHK (Ja / Nein), bei Ja: Relevanz der KHK (nicht relevant / relevant -> Art der KHK); HTN (Ja / Nein); Lungenerkrankungen (Ja / Nein); Synkope (Ja / Nein); Nikotin (Ja / Nein / Ex-Nikotin); Hämorrhagie (Ja -> Hämorrhagie-Art: Magen Darm, Nasenblutung, bei nichtkardialen vor-OP / Nein); Diabetes mellitus (Typ I / Typ II / Nein); Pulmonale Hypertonie (Ja / Nein); Bekannte Herzrhythmusstörungen (Ja -> Herzrhythmusstörung-Art / Nein); Fettstoffwechselstörung (Ja / Nein); Lebererkrankungen (Ja / Nein); Sonstige Erkrankungen (Ja -> welche / Nein); Vorhandene Implantate mit Kontakt zum Blut (Herzschrittmacher / Aortenstent / Gefäß-prothesen / Sonstige -> welche); NYHA (I / II / III / IV / unbekannt); Herzkatheter (Ja -> wann / Nein)

G: Medikation *(Medikamente am Tag der Aufnahme)*

Katecholamin (Ja / Nein), Statine (Ja / Nein), ß-Blocker (Ja / Nein), ACE-Hemmer (Ja / Nein), AT1-Antagonisten (Ja / Nein), Ca-Antagonisten (Ja / Nein), Orale Antikoagulantien (Ja / Nein), Diuretika (Ja / Nein), Digitalis (Ja / Nein), Antiarrhythmika (Ja / Nein), Sonstige:

H: Untersuchung Blutdruck

RR Systolisch (mmHg), RR Diastolisch (mmHg)

I: EKG

Herzrhythmusstörung (Nein / atrial / AV-Block / ventrikulär / kombiniert), Herzfrequenz (bpm), Bemerkungen

J: Echokardiographie *(Erst-Echo, U1)*

EF (%), LVEDD (Enddiastolischer Diameter des linken Ventrikels, mm), LVESD (Endsystolischer Diameter des linken Ventrikels, mm), EDD Septum (Enddiastolische Wanddicke des Septums, mm), EDD Vorderwand (Enddiastolische Wanddicke der Vorderwand, mm), EDD Hinterwand (Enddiastolische Wanddicke der Hinterwand, mm), AK Gradient peak (Maximaler Druckgradient über der Aortenklappe, mmHg), AK Gradient mean (Mittlerer Druckgradient über der Aortenklappe, mmHg), EOA (Effektive Öffnungsfläche der Klappe, cm²), V_{max} (Max. Flussgeschwindigkeit über der Klappe, m/s), V_{max} (LVOT) (Max. Flussgeschwindigkeit im LVOT, m/s), Aortenklappeninsuffizienz (0 / I / II / III / IV / unbekannt), Aortenklappeninsuffizienz-Typ (Zentral / Paravalvulär / Kombiniert / Unbestimmt)

Datum der Untersuchung (TTMMJJ), Verantwortlicher Arzt

K: Labor

Hämoglobin (g/dL), Hämatokrit (%), Leukozytenzahl (1000/µL), Thrombozytenzahl (1000/µL), ACT - 1. Wert Anästhesiebogen (s), Quick (%), INR, PTT (s), CRP (mg/L), Kreatinin (mg/dL), Harnstoff (mg/dL), LDH (U/L), GOT (U/L), GPT (U/L), Lipase (U/L), Gesamtbilirubin (mg/dL), Kreatinkinase (U/L), Calcium (mmol/L), Natrium (mmol/L), Kalium (mmol/L), Troponin (ng/L), TSH (µL/U/L), Gesamteiweiß (g/dL)

L: Ereignisse / Protokollabweichungen

Sind unerwünschte Ereignisse aufgetreten? (Nein / Ja -> Formular "Unerwünschte Ereignisse"); sind Protokollabweichungen aufgetreten? (Nein / Ja -> Formular "Protokollabweichungen"); Datum (TTMMJJ), Verantwortlicher Arzt, Durchführende Person

U2: Intra-OP-Daten

Laufende Nr. des Probanden, Patienten ID, Datum der Untersuchung (TTMMJJ)

***A: Medikation** (Bei Ankunft auf Intensivstation)*

Katecholamin (Ja / Nein), Statine (Ja / Nein), ß-Blocker (Ja / Nein), ACE-Hemmer (Ja / Nein), AT1-Antagonisten (Ja / Nein), Ca-Antagonisten (Ja / Nein), Orale Antikoagulantien (Ja / Nein), Diuretika (Ja / Nein), Digitalis (Ja / Nein), Antiarrhythmika (Ja / Nein), Sonstige:

***B: Untersuchung Blutdruck** (OP- Ende; letzter Eintrag auf Anästhesiebogen)*

RR Systolisch (mmHg), RR Diastolisch (mmHg)

C: Transfusion (intraoperativ)

Transfusion erfolgt? (Ja / Nein); bei Ja: Details: Transfusion EK (Einheiten) / Transfusion TK (Einheiten), Transfusion FFP (Einheiten)

***D: EKG** (Bei Ankunft auf Intensivstation)*

EKG-Veränderungen (Ja -> welche? / Nein), Herzrhythmusstörung (Nein / atrial / AV-Block / ventrikulär / kombiniert), Herzfrequenz (OP- Ende; letzter Eintrag auf Anästhesiebogen, bpm), Bemerkungen

***E: Labor** (Bei Ankunft auf Intensivstation)*

Hämoglobin (g/dL), Hämatokrit (%), Leukozytenzahl (1000/µL), Thrombozytenzahl (1000/µL), ACT - letzter Wert Anästhesiebogen (s), Calcium (mmol/L), Albumin (g/dL), BZ - 1. Wert Anästhesiebogen (mmol/L), Kreatinkinase (U/L), LDH (U/L), Kreatinin (mg/dL), Quick (%), INR, PTT (s), Harnstoff (mg/dL), CRP (mg/L), HDL (mg/dL), LDL (mg/dL), Triglyceride (mg/dL), GOT (U/L), Troponin (ng/L), Phosphat (mmol/L)

F: OP-Daten

OP Datum (TTMMJJ), OP Dauer (Schnitt-Naht-Dauer, min), EKZ Dauer (Dauer der extrakorporalen Zirkulation, min), X-clamp Dauer (Dauer der Aortenklemmung, min)

G: OP-Daten

Plegieart (Brettschneider / Calafiore), Plegiemenge (mL), Implantationstechnik (intraanulär / supraanulär), Bikuspide Klappe (Ja, angeboren / Ja, sekundär / Nein), Kalzifizierung (Ja / Nein), Vegetation (Nein / alt / frisch), Prothesentyp (Trifecta / Mechanisch / Magna Ease /

Intuity / ...), Prothesengröße (mm), Erweiterungsplastik (Ja / Nein), Hämofiltration (mL), Kombinationseingriffe (Ja -> welche? / Nein), Operateur

H: Ereignisse / Protokollabweichungen

Sind unerwünschte Ereignisse aufgetreten? (Nein / Ja -> Formular "Unerwünschte Ereignisse"); sind Protokollabweichungen aufgetreten? (Nein / Ja -> Formular "Protokollabweichungen"), Datum (TTMMJJ), Verantwortlicher Arzt, Durchführende Person

U3: 1. Tag Post-OP

Laufende Nr. des Probanden, Patienten ID, Datum der Untersuchung (TTMMJJ)

A: Medikation

Katecholamin (Ja / Nein), Statine (Ja / Nein), ß-Blocker (Ja / Nein), ACE-Hemmer (Ja / Nein), AT1-Antagonisten (Ja / Nein), Ca-Antagonisten (Ja / Nein), Orale Antikoagulantien (Ja / Nein), Diuretika (Ja / Nein), Digitalis (Ja / Nein), Antiarrhythmika (Ja / Nein), Sonstige:

***B: Untersuchung Blutdruck** (24h pOP)*

RR Systolisch (mmHg), RR Diastolisch (mmHg)

C: Transfusion (seit Operation)

Transfusion erfolgt? (Ja / Nein); bei Ja: Details: Transfusion EK (Einheiten) / Transfusion ThK (Einheiten), Transfusion FFP (Einheiten)

D: EKG

EKG-Veränderungen (Ja -> welche? / Nein), Herzrhythmusstörung (Nein / atrial / AV-Block / ventrikulär / kombiniert), Herzfrequenz (bpm), Bemerkungen

E: Labor

Hämoglobin (g/dL), Hämatokrit (%), Leukozytenzahl (1000/µL), Thrombozytenzahl (1000/µL), Quick (%), INR, PTT (s), GOT (U/L), Kreatinkinase (U/L), Troponin (ng/L), LDH (U/L), Harnstoff (mg/dL), Kreatinin (mg/dL), CRP (mg/L)

F: Ereignisse / Protokollabweichungen

Sind unerwünschte Ereignisse aufgetreten? (Nein / Ja -> Formular "Unerwünschte Ereignisse"); sind Protokollabweichungen aufgetreten? (Nein / Ja -> Formular "Protokollabweichungen"), Datum (TTMMJJ), Verantwortlicher Arzt, Durchführende Person

U4: 7. Tag Post-OP

Laufende Nr. des Probanden, Patienten ID, Datum der Untersuchung (TTMMJJ)

A: Medikation

Katecholamin (Ja / Nein), Statine (Ja / Nein), β-Blocker (Ja / Nein), ACE-Hemmer (Ja / Nein), AT1-Antagonisten (Ja / Nein), Ca-Antagonisten (Ja / Nein), Orale Antikoagulantien (Ja / Nein), Diuretika (Ja / Nein), Digitalis (Ja / Nein), Antiarrhythmika (Ja / Nein), Sonstige:

B: Untersuchung Blutdruck

RR Systolisch (mmHg), RR Diastolisch (mmHg)

C: Transfusion *(seit letzter Kontrolle)*

Transfusion erfolgt? (Ja / Nein); bei Ja: Details: Transfusion EK (Einheiten) / Transfusion ThK (Einheiten), Transfusion FFP (Einheiten)

D: EKG

EKG-Veränderungen (Ja -> welche? / Nein), Herzrhythmusstörung (Nein / atrial / AV-Block / ventrikulär / kombiniert), Herzfrequenz (bpm), Bemerkungen

E: Intensiv-Daten

IABP-Dauer (h), Beatmung (h), Intensiv-Dauer (h), Gesamtdrainagevolumen (mL), Re-Intubation (Ja / Nein), Re-Thorakotomie (Ja / Nein)

F: Demografische Daten

Gewicht (kg), Oberfläche (m^2)

G: Echokardiographie *(Echo nach OP, U4)*

EF (%), LVEDD (Enddiastolischer Diameter des linken Ventrikels, mm), LVESD (Endsystolischer Diameter des linken Ventrikels, mm), EDD Septum (Enddiastolische Wanddicke des Septums, mm), EDD Vorderwand (Enddiastolische Wanddicke der Vorderwand, mm), EDD Hinterwand (Enddiastolische Wanddicke der Hinterwand, mm), AK Gradient peak (Maximaler Druckgradient über der Aortenklappe, mmHg), AK Gradient mean (Mittlerer Druckgradient über der Aortenklappe, mmHg), EOA (Effektive Öffnungsfläche der Klappe, cm^2), V_{max} (Max. Flussgeschwindigkeit über der Klappe, m/s), V_{max} (LVOT) (Max. Flussgeschwindigkeit im LVOT, m/s), Aortenklappeninsuffizienz (0 / I / II / III / IV / unbekannt), Aortenklappeninsuffizienz-Typ (Zentral / Paravalvulär / Kombiniert / Unbestimmt)

Datum der Untersuchung (TTMMJJ), Verantwortlicher Arzt

H: Labor

Hämoglobin (g/dL), Hämatokrit (%), Leukozytenzahl (1000/µL), Thrombozytenzahl (1000/µL), Quick (%), INR, PTT (s), CRP (mg/L), GOT (U/L), LDH (U/L), Natrium (mmol/L), Kalium (mmol/L), Troponin (ng/L), Harnstoff (mg/dL), Kreatinin (mg/dL), Kreatinkinase (U/L)

I: Ereignisse / Protokollabweichungen

Sind unerwünschte Ereignisse aufgetreten? (Nein / Ja -> Formular "Unerwünschte Ereignisse"); sind Protokollabweichungen aufgetreten? (Nein / Ja -> Formular "Protokollabweichungen"), Datum (TTMMJJ), Verantwortlicher Arzt, Durchführende Person

U5: 3 Monate Post-OP

Laufende Nr. des Probanden, Patienten ID, Datum der Untersuchung (TTMMJJ)

A: Demografische Daten

Gewicht (kg), Oberfläche (m^2)

B: Medikation

Statine (Ja / Nein), ß-Blocker (Ja / Nein), ACE-Hemmer (Ja / Nein), AT1-Antagonisten (Ja / Nein), Ca-Antagonisten (Ja / Nein), Orale Antikoagulantien (Ja / Nein), Diuretika (Ja / Nein), Digitalis (Ja / Nein), Antiarrhythmika (Ja / Nein), Sonstige:

C: Untersuchung Blutdruck

RR Systolisch (mmHg), RR Diastolisch (mmHg)

D: Transfusion *(seit letzter Kontrolle)*

Transfusion erfolgt? (Ja / Nein); bei Ja: Details: Transfusion EK (Einheiten) / Transfusion ThK (Einheiten), Transfusion FFP (Einheiten)

E: EKG

EKG-Veränderungen (Ja -> welche? / Nein), Herzrhythmusstörung (Nein / atrial / AV-Block / ventrikulär / kombiniert), Herzfrequenz (bpm), Bemerkungen

F: Anamnese

Kardiale Dekompensation (Ja / Nein); KHK (Ja / Nein), bei Ja: Relevanz der KHK (nicht relevant / relevant -> Art der KHK); HTN (Ja / Nein); Lungenerkrankungen (Ja / Nein);

Synkope (Ja / Nein); Nikotin (Ja / Nein / Ex-Nikotin); Hämorrhagie (Ja -> Hämorrhagie-Art: Magen Darm, Nasenblutung, bei nichtkardialen vor-OP / Nein); Diabetes mellitus (Typ I / Typ II / Nein); Pulmonale Hypertonie (Ja / Nein); Bekannte Herzrhythmusstörungen (Ja -> Herzrhythmusstörung-Art / Nein); Fettstoffwechselstörung (Ja / Nein); Lebererkrankungen (Ja / Nein); Sonstige Erkrankungen (Ja -> welche / Nein); Vorhandene Implantate mit Kontakt zum Blut (Herzschrittmacher / Aortenstent / Gefäß-prothesen / Sonstige -> welche); NYHA (I / II / III / IV / unbekannt); Herzkatheter (Ja -> wann / Nein)

***G: Echokardiographie** (Abschlussecho, U5)*

EF (%), LVEDD (Enddiastolischer Diameter des linken Ventrikels, mm), LVESD (Endsystolischer Diameter des linken Ventrikels, mm), EDD Septum (Enddiastolische Wanddicke des Septums, mm), EDD Vorderwand (Enddiastolische Wanddicke der Vorderwand, mm), EDD Hinterwand (Enddiastolische Wanddicke der Hinterwand, mm), AK Gradient peak (Maximaler Druckgradient über der Aortenklappe, mmHg), AK Gradient mean (Mittlerer Druckgradient über der Aortenklappe, mmHg), EOA (Effektive Öffnungsfläche der Klappe, cm²), V_{max} (Max. Flussgeschwindigkeit über der Klappe, m/s), V_{max} (LVOT) (Max. Flussgeschwindigkeit im LVOT, m/s), Aortenklappeninsuffizienz (0 / I / II / III / IV / unbekannt), Aortenklappeninsuffizienz-Typ (Zentral / Paravalvulär / Kombiniert / Unbestimmt)

Datum der Untersuchung (TTMMJJ), Verantwortlicher Arzt

H: Labor

Hämoglobin (g/dL), Hämatokrit (%), Leukozytenzahl (1000/µL), Thrombozytenzahl (1000/µL), Quick (%), INR, PTT (s), CRP (mg/L), Kreatinin (mg/dL), Harnstoff (mg/dL), GOT (U/L), Kreatinkinase (U/L), Troponin (ng/L), LDH (U/L), Gesamtbilirubin (mg/dL), Triglyceride (mg/dL), Calcium (mmol/L), Phosphat (mmol/L), HDL (mg/dL), LDL (mg/dL)

I: Ereignisse / Protokollabweichungen

Sind unerwünschte Ereignisse aufgetreten? (Nein / Ja -> Formular "Unerwünschte Ereignisse"); sind Protokollabweichungen aufgetreten? (Nein / Ja -> Formular "Protokollabweichungen"), Datum (TTMMJJ), Verantwortlicher Arzt, Durchführende Person

Ende der Nachbeobachtung

Laufende Nr. des Probanden, Patienten ID, Datum der letzten Studienuntersuchung (TTMMJJ)

A: Zeitpunkt

Prä-OP (U1), Intra-OP (U2), 1. Tag pOP (U3), 7. Tag pOP (U4), 3 Monate pOP (U5)

B: Details zum Ende der Nachbeobachtung

Hat der Patient die Studie planmäßig beendet? (Ja / Nein), bei Nein: Rücknahme der Patienteneinwilligung / Lost of Follow-Up / Abbruch der gesamten Studie / Explantation / Patient verstorben / Anderer Grund -> welcher?; bei Explantation: Explantationsdatum (TTMMJJ), Infektion -> Keim / nicht infektiös -> Grund); bei Patient verstorben: Todesdatum (TTMMJJ) -> Formular "Ereignisse" ausfüllen, Kardial arrhythmiebedingt / Kardial nicht arrhythmiebedingt / Nicht kardial / Unbekannt, Plötzlich / Nicht plötzlich / Unbekannt, Pre-operativ / Peri-operativ (≤ 30 Tage nach Impl.) / post-operativ (> 30 Tage nach Impl.)

Datum (TTMMJJ), Verantwortlicher Arzt, Durchführende Person(en)

Protokollabweichung

Laufende Nr. des Probanden, Patienten ID, Datum der Untersuchung (TTMMJJ)

A: Zeitpunkt

Prä-OP (U1), Intra-OP (U2), 1. Tag pOP (U3), 7. Tag pOP (U4), 3 Monate pOP (U5)

B: Klassifikation / Details

Die Protokollabweichung bezieht sich auf (bitte alle zutreffenden Felder ankreuzen - mindestens ein Feld muss angekreuzt sein): Einschluss (Ein- oder Ausschluss Kriterien / Patienteneinwilligung) -> Ethikkommission informieren; Vorgeschriebene Studientermine (Außerhalb des vorgeschriebenen Follow up Fensters / Nicht durchgeführt); Studien Prozeduren (Die Implantate entsprechen nicht den Protokollvorgaben / Andere Prozedur nicht Protokollkonform -> welche?)

Begründung für die Protokollabweichung (bitte genau ein Feld ankreuzen): Die Protokollabweichung folgte medizinischen Gründen bzw. Sicherheitserwägungen / Es gibt keinen medizinischen Grund für die Protokollabweichung.

Detaillierte Beschreibung der Protokollabweichung: (Freitext)

Datum (TTMMJJ), Leitender Arzt, Durchführende Person(en)

Unerwünschtes Ereignis

Laufende Nr. des Probanden, Patienten ID, Datum der Untersuchung (TTMMJJ)

A: Zeitpunkt

Prä-OP (U1), Intra-OP (U2), 1. Tag pOP (U3), 7. Tag pOP (U4), 3 Monate pOP (U5)

B: Art des Ereignisses *(kurze Beschreibung)*

(Bitte in Druckbuchstaben ausfüllen. Weitere Details bitte im Abschnitt G eintragen.)

C: Typ dieses Ereignis-Reports

Erstmeldung dieses Ereignisses / Ergänzende Informationen zu einem bereits gemeldeten Ereignis -> Datum des Ereignisses (TTMMJJ)

D: Schweregrad

Unerwünschtes Ereignis (jedes bei einer Versuchsperson auftretende ungünstige medizinische Ereignis) / Schweres unerwünschtes Ereignis / Anderes Ereignis bei dem ein Patient, Anwender oder Dritter hätte zu einem schwerwiegenden Schaden kommen können); bei Schweres unerwünschtes Ereignis: Tod -> Formular „Ende der Nachsorge" / Schwere Gesundheits-beeinträchtigung, die zu einer lebensbedrohenden Erkrankung oder Schädigung der Versuchsperson führte / zu einer dauernden Beeinträchtigung einer Körperstruktur oder -funktion führte / die Krankenhausaufnahme oder die Verlängerung eines bestehenden Krankenhausaufenthaltes erforderlich machte / zu einem medizinischen oder chirurgischen Eingriff führte, um eine dauernde Beeinträchtigung einer Körperstruktur oder -funktion zu verhindern / zur Schädigung eines Feten, zum Fetaltod, einer kongenitalen Fehlbildung oder einem Geburtsschaden führte

E: Ausgang des unerwünschten Ereignisses

Vollständig abgeklungen -> Datum (TTMMJJ) / Noch bestehend / Bleibende Schäden / Tod

F: Zusammenhänge

Besteht ein Zusammenhang mit ....? 1. ... dem Medizinprodukt -> Nein / Unwahrscheinlich / Möglich / Wahrscheinlich / Ja / Unbekannt; bei Möglich / Wahrscheinlich / Ja -> Angaben zum Medizinprodukt (Modellnummer, Seriennummer), Falls weitere Geräte betroffen sind, diese bitte auf der nächsten Seite als Freitext auflisten. 2. ... der Prüfung -> Nein / Unwahrscheinlich / Möglich / Wahrscheinlich / Ja / Unbekannt. 3. ...dem Verfahren -> Nein / Unwahrscheinlich / Möglich / Wahrscheinlich / Ja / Unbekannt. 4. Sonstigem (Zusammenhang angeben) -> Nein / Unwahrscheinlich / Möglich / Wahrscheinlich / Ja / Unbekannt)

G: Detaillierte Angaben zum unerwünschten Ereignis *(Freitext)*

Datum (TTMMJJ), Verantwortlicher Arzt, Durchführende Person(en)