Studying macro- and microvascular responses to major surgery to develop preventive strategies for perioperative complications
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1 Postoperative complications

Annually, almost 313 million surgical procedures are performed on patients worldwide (1). Five to 70% of patients experience postoperative complications, and within 30 days after surgery up to 4% of patients succumb to complications that are not immediately related to the surgical procedure or the underlying disease itself (2,3). Postoperative complications leading to morbidity and mortality are detrimental to the therapeutic benefit of surgeries and cause considerable suffering and a huge economic burden on health care systems worldwide (4). Postoperative complications can occur both related and unrelated to the surgical procedure or technique. To procure the benefit of surgery, postoperative complications have to be reduced as annual postoperative mortality numbers are predicted to rise up to twelve million patients in 2030 (5). This represents a global issue that weighs heavily on both individual patients and society as a whole and is destined to further escalate in an aging society. Molecular mechanisms underlying postoperative complications of the cardiovascular system that are unrelated to surgical procedure are studied in this thesis to foster development of preventive strategies.

1.1 The central role of the cardiovascular system

The cardiovascular system consists of the heart and blood vessels, and supplies all organs with blood to deliver oxygen and nutrients while removing waste products like CO₂ (6). Based on diameter and function, blood vessels are classified into five categories and either belong to the macrovasculature or microvasculature as illustrated in Figure 1. Arteries and veins are the largest vessels and belong to the macrovasculature, which connects organs to the heart. Arterioles, capillaries, and venules have a diameter of less than 100 µm and make up the microvasculature within organs and tissues (7). The microvasculature facilitates the exchange of oxygen, nutrients, and waste products within perfused tissues. For further reading, interested readers are referred to Chaudhry, Miao and Rehman (6).

An essential factor in physiological function of blood vessels is steady laminar shear stress, the force exerted by blood flow on vessel walls. By affecting the morphology of endothelial cells, shear stress directly influences vasoreactivity and can lead long-term to vascular morphogenesis and remodelling (11). Throughout the vascular tree, shear stress levels vary with highest levels measured in arteries (10 to 70 dynes/cm²) and lowest levels in veins (1 to 6 dynes/cm²) (12). Turbulent or oscillatory flow can
be a factor in pathogenesis of various diseases. Certain areas of the vasculature, i.e. inner curvatures and branch points, are prone to experience irregular shear stress, thus increasing the susceptibility of the vasculature to pathophysiological processes (13). Surgeries commonly affect haemodynamic factors like blood viscosity and erythrocyte percentage, and affect patterns and extent of shear stress pattern (14).

**Figure 1. Structures and composition of blood vessel types of the macro- and microvasculature.** Blood vessel types are grouped into macrovasculature, comprising arteries and veins, or microvasculature, including arterioles, capillaries, and venules. The wall of most blood vessels consists of three layers, namely tunica intima, tunica media and tunica adventitia (8). The most inner layer, the tunica intima, comprises a monocellular lining of endothelial cells, also known as the endothelium, a subendothelial layer of connective tissue and a fenestrated layer of elastic fibres, making up the internal elastic lamina. The middle layer, the tunica media, consists of vascular smooth muscle cells and extracellular matrix. The outer layer, the tunica adventitia, contains fibroblasts, progenitor cells, immune cells, microvessels and adrenergic nerves. The component portions vary for each tunica with vessel type. Capillaries, the smallest vessel type, represent an exception and consist solely of an endothelial cell layer, which is surrounded by pericytes and extracellular matrix (9). The endothelium of capillaries is continuous, fenestrated or sinusoid and determines which particle sizes traverse into the extravascular space. Fenestrated capillaries are predominant in organs like the small intestine and kidney to make them permeable to larger molecules. Adapted from Potente and Mäkinen (10) and reproduced with permission from Springer Nature. Created with BioRender.com. BM – basal membrane; EC – endothelial cell; SMCs – smooth muscle cells.
1.2 Vasculature in the postoperative setting

Surgical procedures induce a stress response that comprises a wide array of complex physiologic changes. Surgery-induced changes in blood flow can activate inflammatory response pathways. Alongside metabolic changes, systemic inflammatory response syndrome (SIRS) generally describes the response to infectious and non-infectious injuries. While SIRS can induce postoperative complications in the cardiovascular system, it can also affect other major organs such as the kidneys, liver and lungs (15). A better understanding of the role of the immune system involvement in and inflammatory responses to surgery may help to prevent or alleviate postoperative morbidity and mortality. The vasculature is a key component of the surgical stress response as, for instance, it facilitates the circulation of pro-inflammatory mediators that act on circulating immune cells as well as on the endothelium, which leads to prevalence of SIRS and eventually postoperative complications.

2 Part 1 – Predisposed macrovasculature in the postoperative context

The first part of this thesis focuses on arteries of the macrovasculature and how atherosclerotic burden can be lowered in the postoperative context. Atherosclerosis is the chronic inflammation of the arterial wall, which facilitates accumulation of lipids and macrophages resulting in plaque formation that leads to narrowing of the artery. Plaque formation is initiated upon activation of the endothelium by proinflammatory cytokines or cardiovascular risk factors, e.g. high plasma concentration of low-density lipoprotein (LDL) (16). Vessel regions that lack straight segments, especially bifurcations, curvatures, and valves, are more likely to induce disturbed flow, resulting in endothelial cells with predisposed susceptibility to pro-inflammatory stimuli. The activated endothelium expresses leukocyte adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), which allows interaction with leukocytes expressing very late antigen-4 (VLA4). Subsequently, leukocyte adherence to the endothelium is promoted to enable leukocytes transmigration into the tunica intima. There, monocytes mature into macrophages that exacerbate the pro-inflammatory environment by cytokine secretion, and form foam cells following excessive lipid uptake through LDL endocytosis. Foam cells can also originate from vascular smooth muscle cells (VSMCs), which migrated from the tunica media into the tunica intima. By producing extracellular matrix, VSMCs contribute to plaque stability by fibrous cap formation. Further hallmarks of atherosclerosis progression are – among others
– the proliferation of macrophages and VSMCs, pro- and anti-inflammatory cytokine production by various cell types and inefficient efferocytosis within the growing plaque (17). The latter results in debris accumulation from dead or dying cells and subsequently promotes the formation of a necrotic core of the atherosclerotic plaque. Atherosclerosis progression can be a matter of years and depends on the extent at which proliferation, cell death, clearance and inflammatory signalling occurs. Atherosclerosis in surgical populations is considered a “co-morbidity” that increases the susceptibility of macrovasculature to pathophysiological processes. Its mere presence increases the patient risk of having postoperative complications affecting various organs. Acute ischaemic events such as stroke or myocardial infarction present clinically following obstruction of blood flow due to atherosclerotic plaque rupture or erosion with thrombus apposition or critical stenosis of the conduit blood vessel (18). Surgical stress induces both pro- and anti-inflammatory responses targeted to remove destroyed tissue, which induces wound healing and ultimately leads to reconstitution of organ function (19–26). Pre-existing chronic inflammatory co-morbidities, such as atherosclerosis in large arteries, impair the body’s capacity to balance pro- and anti-inflammatory responses (27). In this setting, the pro-inflammatory response induced by surgical stress may promote atherosclerotic plaque rupture and thus, acute ischaemic events (28). The incidence of atherosclerosis is rising in an aging population, however, also affected numbers in the younger population are increasing (29). Therefore, therapeutic strategies are urgently required to lower the risk that the co-morbidity atherosclerosis may pose for patients scheduled for surgery. The importance of the immune system in cardiovascular disease (CVD) in general has been recognised in the last decades and novel therapies are emerging (30). Understanding the molecular mechanisms taking place in the macrovasculature will allow for personalised risk stratification and targeted treatments.

2.1 High-density lipoprotein in atherosclerosis predisposed vessels in the postoperative context

Patient-related risk for postoperative complications is influenced by the patient’s age, cardiovascular (CV) risk factors, established CVD, and comorbidities (31). Various pharmacological substances are available to lower the CVD risk in patients with and without established CVD. Their postoperative use is currently understudied due to patient population heterogeneity, cohorts of insufficient sizes, and confounding factors.
such as co-morbidities, ongoing medication regimens and factors related to the type of surgery (32).

Hyperlipidaemia, and particularly cholesterol accumulation, in blood is a CV risk factor for initiation of atherosclerosis and thus, CVD (33). Lipoproteins facilitate cholesterol transport through blood and are complex particles composed of apolipoproteins and lipids (34). Based on density, very low-density lipoprotein (VLDL), LDL, and high-density lipoprotein (HDL) are grouped, and their cholesterol content varies. LDL predominantly consists of apolipoprotein B, whereas apolipoprotein A-I are abundant in HDL. Pharmacological treatment with 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (statins) lowers the amount of circulating LDL cholesterol and effectively reduces patient risk of CVD (29). In patients with established CVD, statins are used as long-term treatment and continuation is recommended for these patients in the postoperative context (32). Current clinical data inadequately assess the benefit of initiating statins in the postoperative phase to reduce complications, as they are based on small patient cohorts and have ambiguous outcomes. Therefore, current ESC guidelines do not recommend statin initiation as routine therapy in the postoperative context of non-cardiac surgery (32).

HDL-binding cholesterol has been inversely correlated with the risk of atherosclerotic disease and thus represents a promising therapeutic target. Despite the fact that clinical trials aiming at elevating plasma HDL concentration have failed to benefit patients, the current understanding is that HDL function, and not quantity, is pivotal for lowering atherosclerotic burden (35). HDL plays a critical role in reverse cholesterol transport (revCT) (36). RevCT is initiated with the efflux of cholesterol from cholesterol-laden macrophages in the arterial wall and results in the transport of cholesterol to the liver for excretion. In the postoperative context, analysing the effect surgical stress has on lipoprotein levels and their function in a cohort of patients undergoing vascular surgery may allow for the development of therapeutic strategies to reduce postoperative complications.

2.2 Immunomodulatory approaches for treatment of atherosclerosis

Recent clinical trials have proven that inflammation is a key driver of atherosclerosis and thus, novel treatment options focusing on immunomodulation (i.e. Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) trial and colchicine trials) in addition to lipid modulation through statin therapy are emerging (37). The
cytokines interleukin-1β and interleukin-6 are at the centre of ongoing research and clinical trials that investigate immunotherapies targeting the innate immune system (33). These cytokines are released following pro-inflammatory stimulation, thereby eliciting cellular effects upon binding to cell-surface receptors of endothelial and immune cells. G-protein coupled receptors (GPCRs) are the largest family of cell surface receptors in the human body (38). G protein signalling pathways consist of four components, namely GPCRs, G proteins (with α, β and γ subunits), effectors and regulator of G protein signalling (RGS) proteins. Upon ligand binding, activation of GPCR-coupled signalling is facilitated by GDP to GTP exchange on the associated G protein, initiating dissociation of the Ga subunit from Gbg and the receptor. All G protein subunits can then interact with effectors, with subsequent activation of various intracellular processes. Additional to intrinsic GTPase activity, RGS proteins control the hydrolysis of GTP to GDP on Ga leading to reassociation of all G protein subunits and consequently, intracellular signalling is halted. GPCRs and all required signalling components have become valuable druggable targets. Up to 35% of marketed drugs target a member of this receptor class (38). The therapeutic potential of RGS proteins as regulators of GPCR signalling has already been recognised (39). At this moment, at least 20 RGS proteins have been identified and classified into seven different structural and functional families. Most RGS proteins interact with various proteins, except for members of the B/R4 family, which are solely acting on G-proteins. Thus, members of the B/R4 family are promising therapeutic targets for direct actions on GPCRs. One of its members, RGS5 may be implicated in atherosclerotic plaque development (40). However, the effect of RGS5 on atherogenesis and its therapeutic potential have not yet sufficiently been elucidated.

Part 2 – Microvasculature and the kidney in the postoperative context

The second part of the thesis focuses on the microvasculature and the role of the surgery-induced pro-inflammatory cytokine Tumour Necrosis Factor alpha (TNF-α) in acute kidney injury (AKI) as well as novel therapeutic options against TNF-α-induced endothelial pro-inflammatory activation. The microvasculature directly facilitates oxygen and nutrient transfer, waste product removal and blood flow regulation within all organ systems. Thus, reduced or dysfunctional microvascular perfusion is detrimental to an organ (7). Commonly, macro-haemodynamic targets are monitored during surgery without tracking the microcirculation. Tools to monitor microcirculation are a recent
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development and their use is not always feasible yet (41). To prevent postoperative complications, interventions are needed before, during and after surgery to maintain a healthy function of the microcirculation.

3.1 Surgical factors leading to endothelial dysfunction
As most inner layer of blood vessels, the endothelium encounters cellular and plasmatic components of circulating blood as well as alterations in shear stress, the tension blood flow exerts on the vessel wall. Steady laminar shear stress with longitudinal direction is required for the endothelium to maintain a quiescent, low proliferative and anti-inflammatory state. Surgery-induced alterations in shear stress and blood composition directly affect the endothelium by inducing endothelial inflammation and excessive permeability, ensuing a dysfunctional endothelium (42). Endothelial dysfunction underlies various cardiovascular and renovascular diseases. Particularly cardiac surgery that requires cardiopulmonary bypass (CPB) is prone to induce endothelial dysfunction and SIRS. This is commonly triggered by CPB-related factors, e.g. contact of blood with extracorporeal surfaces, and ischaemia/reperfusion injury in organs like the kidney (43). The kidney is a highly vascularised organ with an average blood flow of 4 mL/g tissue/min, resembling 25% of the cardiac output (44). The kidneys’ main functions are the filtration of blood to remove excess water, waste products, and toxins, and to balance electrolytes as well as acids and bases. Endothelial inflammatory activation and subsequent endothelial dysfunction may acutely impair kidney function, which eventually can lead to kidney failure and death. Postoperative AKI is a common complication that can lead to chronic kidney failure, dysrhythmias and myocardial damage, and subsequently to mortality (45,46).

3.2 Plasmatic components (pro- and anti-inflammatory cytokines) of circulating blood
Pro- and anti-inflammatory cytokines are plasmatic components of blood that are expressed and excreted by circulating immune cells and the activated endothelium. Cytokines are required in healthy organisms to develop the immune system and to maintain homeostasis. Sustained excessive pro-inflammatory cytokine presence is a trigger for endothelial inflammatory activation (47). This leads to expression of endothelial adhesion molecules, which facilitate leukocyte recruitment and tissue
infiltration. Postoperatively, the pro-inflammatory cytokine TNF-α is acutely elevated in plasma and has been linked to pathogenic systemic inflammation and vascular dysfunction (48). The extent to which plasmatic factors in combination with altered shear stress play a role in the induction of SIRS and subsequent kidney damage has not yet been sufficiently addressed, and adequate in vitro models are scarce.

3.3 TNF-α-induced intracellular signalling cascades as modulators and therapeutic targets

TNF-α is expressed by various cell types and exerts signals through interaction with TNF receptors (TNFR) 1 and 2. TNF-α can be membrane-bound or, following cleavage, in soluble form. Both forms interact with both receptors, although TNFR2 has a higher affinity for membrane-bound TNF-α. Intracellularly, key signal transcription pathways like NF-κB and MAPK are activated upon binding to both TNF receptors (49). TNFR1 contains a dead-domain, which enables initiation of apoptotic and necrotic cell death. Since TNF-α is centrally involved in virtually all aspects of immune homeostasis, direct interference with its signalling has delivered results hampered by adverse effects such as infections and malignancies. The presence of extensive studies and trials targeting TNF-α illustrate the difficulty of only targeting the adverse effect of excessive TNF-α-induced signalling (50). However, TNF-α inhibitors have yielded the desired effect of improving patient care as a treatment in several inflammatory pathologies including rheumatoid arthritis (51). Further research into intracellular pathway components is required to circumvent adverse effects of general TNF-α inhibitors.

Intracellularly, NF-κB and MAPK pathways are the known major signalling cascades induced by TNF-α. Crosstalk between these and other pathways is extensive and thus, selectively targeting intracellular signalling is a highly complex matter. The backbone of most signalling cascades are protein kinases, which reversibly phosphorylate substrates on serine, threonine, tyrosine, histidine, or aspartate residues (52). Thereby, signals are relayed downstream and protein activation or deactivation is achieved, commonly resulting in activation of transcription factors and subsequently, effects on cellular functions due to changes in gene expression patterns. At this moment, more than 500 protein kinases are known and it is estimated that more than two thirds of all encoded proteins undergo phosphorylation (53). Protein kinases downstream of TNF-α signalling in endothelial cells are promising therapeutic targets in order to modulate instead of completely blocking TNF-α-induced endothelial signalling (54).
4 Aim and outline of this thesis

As highlighted above, postoperative complications are affecting various organs and decrease the patient’s benefit of surgery. In healthy organisms, the vasculature facilitates organ blood flow for optimal supply-demand matching. Surgeries apply stress on the human organism and various factors affect how surgical stress is dealt with. Vessel- and organ-specific treatment options are required to minimise the incidence of postoperative complications. The general aim of this thesis is to advance the understanding of surgery-induced inflammatory processes involving the macro- and microvasculature in the context of postoperative complications with the overarching aim to improve the benefit of surgery for patients as illustrated in Figure 2.

![Figure 2. Macro- and microvascular responses underlying postoperative complications.](image)

The cardiovascular system is made up of the macro- and microvasculature, which facilitate exchange of nutrients and waste products. Surgical procedures are inducing a stress response, the extent of which depends on the state of the organ-specific vasculature. Macrovascular co-morbidities such as atherosclerosis predisposes the macrovasculature to postoperative complications. Atherosclerosis plaque rupture can precipitate thrombus formation, vessel occlusion and thereby eventually postoperative complications such as myocardial ischaemia. Surgery-induced systemic inflammation can lead to endothelial dysfunction and affect the microvasculature of the kidney. Subsequently, postoperative complications such as AKI can occur. Overall, postoperative complications lead to morbidity and can result in mortality. Created with BioRender.com.
In Part 1 of this thesis, research involving the macrovasculature during and after surgery will be addressed. In Chapter 2, the effect of surgical stress on lipoprotein levels, HDL function and the association with the occurrence of myocardial ischaemia are studied in a cohort of patients undergoing vascular surgery. Chapter 3 focuses on atherosclerosis, a co-morbidity commonly increasing patients’ risk for postoperative complications. GPCRs are the largest group of receptor types, and various GPCR-coupled signalling pathways have been associated with atherogenesis. RGS proteins are part of a negative feedback mechanism controlling GPCR signalling, and data suggest an involvement of RGS5 in cardiovascular pathophysiology. The role of RGS5 in atherosclerotic plaque composition and stability is studied in vivo. We hypothesise that RGS5 affects plaque composition and stability in apoE-deficient mice.

In Part 2 of this thesis, research involving the microvasculature during and after surgery will be addressed. This part is centred around microvascular endothelial inflammatory activation in the kidney and treatment approaches during and after surgery. In Chapter 4, the effect of shear stress and surgical patient plasma with elevated levels of pro-inflammatory cytokines on endothelial cells will be evaluated in vitro. We hypothesise that pro-inflammatory components of plasma, e.g. TNF-α, from cardiac surgery patients and altered shear stress induce endothelial inflammation in an endothelial in vitro model. Chapter 5 focuses on identification of therapeutic targets to attenuate TNF-α-induced endothelial inflammation in vitro. We hypothesise that pro-inflammatory components of plasma, e.g. TNF-α, from patients having cardiac surgery, and altered shear stress induce endothelial inflammation in an in vitro model. In Chapter 6, the main research findings are summarised, translation aspects discussed, future perspectives proposed and concluding remarks deduced.
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