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Cardiac microvascular dysfunction

Insights from COVID-19, myocardial infarction, and anthracycline-induced cardiotoxicity

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General Introduction

- Introduction of Microvascular Dysfunction
- COVID-19
- Myocardial Infarction
- Anthracycline-induced Cardiovascular Toxicity

Introduction

Microvascular structure and function

In the coronary circulation, the large epicardial coronary arteries, including the left and right coronary arteries and their major branches, run along the surface of the heart and function primarily as low-resistance conduits that provide adequate delivery of blood to the myocardium to ensure the sufficient perfusion. The intramyocardial blood vessels, that branch of the epicardial vessels into the myocardium, consist of prearterioles, arterioles, capillaries and venules. These vessels constitute the microcirculatory network, that plays a regulatory role in cardiac perfusion and, at the capillary level, enables oxygen and nutrient exchange with cardiac cells^[1,2]. Although stenosis or occlusion of the epicardial vessels, most often due to atherosclerosis or thrombus formation, can result in severe myocardial ischemia or infarction, they are not primarily involved in dynamic blood flow regulation. Instead, intramyocardial arterioles, which represent the principal site of coronary vascular resistance, are modulated by the cardiac cycle, with systolic compression and diastolic restoration of flow^[3]. Experimental studies have shown that retrograde systolic flow particularly reduces subendocardial perfusion and makes the deeper layers of myocardium particularly vulnerable to ischemia^[4].

In recent years, research has increasingly focused on the pathology of intramyocardial blood vessels, as coronary microvascular dysfunction has been recognized as an important contributor of myocardial ischemia and remodelling, independently of epicardial coronary artery disease. Clinically, primary microvascular angina is the main symptom in patients with coronary microvascular dysfunction^[5], and both structural and functional abnormalities of the microcirculation, such as endothelial dysfunction, impaired vasodilation, inflammation, remodelling, and rarefaction, have been documented across diverse conditions, including diabetes^[6], myocardial infarction^[7] and heart failure with preserved ejection fraction^[8,9]. These alterations may impair tissue perfusion, disrupt the local microenvironment, and promote adverse tissue remodelling, thereby contributing to disease progression and organ dysfunction, even in the absence of obstructive large-vessel disease. However, how these structural and functional alterations in the microvasculature evolve in relation to clinical outcomes remains incompletely understood.

Oxidative stress in endothelial dysfunction

The endothelium constitutes the innermost layer of the blood vessel walls and is composed of a single layer of endothelial cells. Together with the elastic lamina, it forms the tunica intima. Endothelial cells can secrete various vasoactive substances, such as nitric oxide (NO), endothelin-1 (ET-1), prostacyclin (PGI₂) etc., to regulate perfusion, vasodilation, vasoconstriction, platelet activity, coagulation, leukocyte adhesion, and angiogenesis. Consequently, the endothelium plays a vital role in the regulation of cardiovascular system function^[10]. The interruption of these vascular signal transduction and regulation processes usually leads to the occurrence of endothelial dysfunction^[11]. Oxidative stress had been proved a main mechanism of endothelial dysfunction, demonstrated by decreased NO bioavailability and eNOS activity, as well as increased reactive oxygen species (ROS) and reactive nitrogen species (RNS)^[12,13]. NADPH oxidase proteins (NOXes) are major sources of ROS in endothelial cells, where they function as signalling molecules that regulate vascular tone^[14]. Dysregulation of NOXes expression and activity under pathophysiological conditions are considered to be major contributors to oxidative stress^[15].

Endothelial inflammation and coagulation

Excessive accumulation of ROS induces endothelial activation, characterized by a phenotypic change in the affected cells from a quiescent, anti-inflammatory phenotype to an activated, pro-inflammatory state in response to stimuli^[16]. Activated endothelial cells show upregulation of adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin, to facilitate leukocyte recruitment. In parallel, secretion of cytokines and chemokines, such as IL-1 β , IL-8, TNF- α , MCP-1, is increased, leading to activation the NF- κ B signalling pathway^[17] and amplification of the inflammatory response. The upregulation of adhesion molecules and pro-inflammatory mediators increases vascular permeability and promotes the development of a pro-inflammatory- and pro-coagulant state. Endothelial inflammation is a recognized risk factor for the pathogenesis of cardiovascular disease^[18], and plays a critical role in the development of atherosclerosis^[19] and diabetes-associated vascular complications^[20]. Additionally, in certain multisystem inflammatory disorders^[21], oxidative stress-induced chronic inflammation promotes the activation of platelet and leukocytes through pro-inflammatory mediators. Increased platelet reactivity and lipid peroxidation heighten the risk of developing

a prothrombotic phenotype. In Behçet disease, coagulation driven by endothelial dysfunction has been implicated in the development of thromboembolism^[22].

AGE-RAGE pathway

The oxidative stress is intrinsically linked to advanced glycation end products (AGEs) formation. Elevated oxidative stress promotes AGE formation through both endogenous production and exogenous intake^[23]. Plasma AGE levels have been shown to be related to the severity and prognosis of chronic heart failure^[24,25], while AGE accumulation in the endothelium is known to induce senescence and barrier dysfunction^[26]. Moreover, AGEs can also form irreversible cross-links of extracellular matrix proteins such as collagens and elastin in the vascular basement membrane, resulting in increased vascular stiffness^[27]. AGE accumulation has been observed in microvasculature of patients with myocardial infarction^[28,29] and diabetes^[30], conditions that also show increased NOXes expression in the endothelium. This further highlights the mechanistic link between oxidative stress and AGEs pathology. In addition, AGEs-mediated cellular damage can activate the receptor of AGEs (RAGE), initiating the RAGE-AGE signalling cascade, which further amplifies oxidative stress and vascular injury^[31,32].

Vascular remodelling

Vascular physiological functions and structural remodelling depend on the coordinated communication between the various cell types, including endothelial cells, smooth muscle cells, as well as the fibroblasts surrounding the adventitia, which is the out layer of connective tissue that surround blood vessels. Disruption of this communication can influence the development of vascular diseases^[33,34]. Endothelial cells actively communicate with vascular cells through the release of a complex secretome of proteins and biomolecules, which is crucial for the regulation of neighbouring cells and shaping the tissue microenvironment. Under pathological conditions, this paracrine signalling is affected and can contribute to structural and functional remodelling of the vasculature^[35,36]. Additionally, cellular senescence plays a critical role in vascular dysfunction by inducing vascular structural and functional changes that contribute to development and progression of cardiovascular disease^[37,38]. Moreover, the senescence-associated secretory phenotype promotes inflammation, thrombosis, and vascular dysfunction through the release of cytokines and growth factors dysfunction^[39-41].

Emerging evidence indicates that vascular remodelling plays an important role in the development of vascular dysfunction. Notably, fibrotic remodelling of the microcirculation was shown to correlate with impaired coronary flow in patients with non-ischemic heart failure^[42]. Perivascular fibrosis, an important manifestation of vascular fibrotic remodelling, is characterized by the excessive accumulation of connective tissue surrounding blood vessels. This pathological change can impair microvascular dilatory function and subsequently lead to reduced tissue perfusion^[42] and has been recognized as an important predictor of cardiovascular morbidity and mortality^[43].

Amid the intricate mechanisms driving fibrosis, fibroblasts play a pivotal role. Upregulation of fibroblast activation protein (FAP) and α -smooth muscle actin (α -SMA) marks fibroblast activation and their transition to myofibroblasts, which are main processes in the pro-fibrotic activities. Emerging evidence also suggests that certain cardiac fibroblasts originate from vascular cells. For instance, endothelial cells undergoing endothelial-to-mesenchymal transition (EndMT) can acquire fibroblasts-like properties, contributing to the cardiac fibrosis^[43,44] as well as atherosclerosis progression^[45]. Similarly, vascular smooth muscle cells (vSMCs) can undergo phenotypic transitions, losing their contractile phenotype and adopting fibroblast-like features.

As the constituent cells of the medial layer of blood vessels, smooth muscle cells regulate vascular contraction and blood circulation. However, their phenotypic switching has been associated to chronic vascular diseases and fibrotic states, including atherosclerotic^[46,47] and aortic aneurysms^[48]. Through these processes, vascular cell plasticity contributes to vascular fibrosis and stiffness^[49,50].

This thesis investigates microvascular dysfunction across distinct pathological settings to elucidate the underlying mechanisms, as shown in **Figure 1**.

Part 1 describes the study of oxidative stress and inflammation contributing to cardiac microvascular endothelial injury in COVID-19 patients, focusing on NOXes (NOX2, NOX4 and NOX5) expression, and atrial inflammation with associated microvascular thrombogenicity.

Part 2 explores cardiac microvascular alterations in myocardial infarction (MI), focusing on structural remodelling processes, such as perivascular fibrosis,

fibroblast activation, vascular cells phenotypic shift, and the expression of FSTL3 under hypoxic and ischemic stress.

Part 3 examines doxorubicin (Dox)-induced cardiovascular injury, combining *in vitro* and *in vivo* models to investigate mechanisms of endothelial oxidative stress, endothelial-fibroblast crosstalk, and the resulting extracellular matrix remodelling and microvascular structural changes over both short- and long-term time frames.

Collectively, these studies aim to provide novel insights into the diverse manifestations of microvascular dysfunction in infectious, ischemic, and chemotherapy-induced cardiac injury.

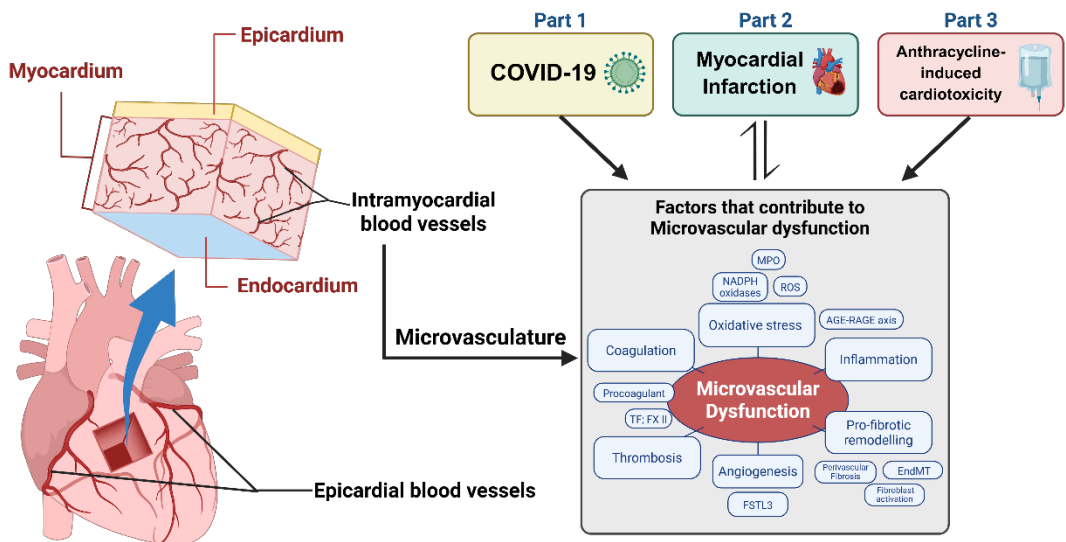


Figure 1. Overview of vascular structure and outline of this thesis.

Schematic representation of the vascular hierarchy from large arteries to the microvasculature. Microvascular dysfunction represents an pivotal pathogenic process influenced by multiple factors. This thesis investigates microvascular dysfunction in three contexts: Part 1: COVID-19, Part 2: Myocardial Infarction, and Part 3: Anthracycline-induced cardiotoxicity.

COVID-19

Coronavirus disease (COVID-19) is a highly contagious disease caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection. Its high replication rate and rapid transmission led to a global epidemic, resulting in nearly 7 million recorded deaths worldwide as of October 2023 ([COVID-19 Map - Johns](#)

[Hopkins Coronavirus Resource Center](#)). The coronavirus primarily attacks the respiratory system, causing symptoms that range from mild flu-like manifestations to severe pneumonia and acute respiratory distress syndrome (ARDS). However, COVID-19 was recognized a multisystem disease and early clinical evidence indicated that not only the lungs were effected, but also the cardiovascular, neurological and gastrointestinal systems, even triggering cytokine storms, systemic inflammation and multiple organ failure in critical and fatal cases.

The association between COVID-19 and cardiac complications is well established, with multiple studies documenting cardiac injury and stress in COVID-19 patients, as evidenced by elevated levels of key cardiac dysfunction biomarkers, such as troponin T concentrations (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP)^[51,52]. In addition to structural myocardial damage, dysfunction of cardiac microvasculature is increasingly recognized as a potential driver of COVID-19-induced cardiac injury. The vascular endothelium plays an important role in maintaining vascular homeostasis^[10]. A key mechanisms of coronavirus (SARS-CoV) infection is its interaction with the angiotensin-converting enzyme 2 (ACE2) receptor, which is abundantly expressed in vascular endothelial cells, particularly in cardiac blood vessels^[53]. This makes the cardiovascular system particularly susceptible to COVID-19-induced inflammatory responses, endothelial dysfunction, and vascular injury^[54], which may contribute to complications such as myocardial injury, stroke, and thrombosis. Indeed, significant cardiac microvascular involvement has been observed in COVID-19, with findings of microvascular thrombosis, endothelial cell damage and dysregulated pro-thrombotic protein expression in the intramyocardial vessels of COVID-19 patients, indicating the association of vascular dysfunction with cardiac damage and disease severity caused by COVID-19. NOXes-mediated oxidative stress is a key contributor to endothelial dysfunction under different pathophysiological conditions^{[15],[55,56]}. However, their involvement in COVID-19-induced cardiac microvascular dysfunction is not known. Therefore, in **Chapter 2**, we investigated the involvement of NOX2, NOX4 and NOX5 in COVID-19-induced cardiac microvascular dysfunction and oxidative stress in ventricles of deceased COVID-19 patients.

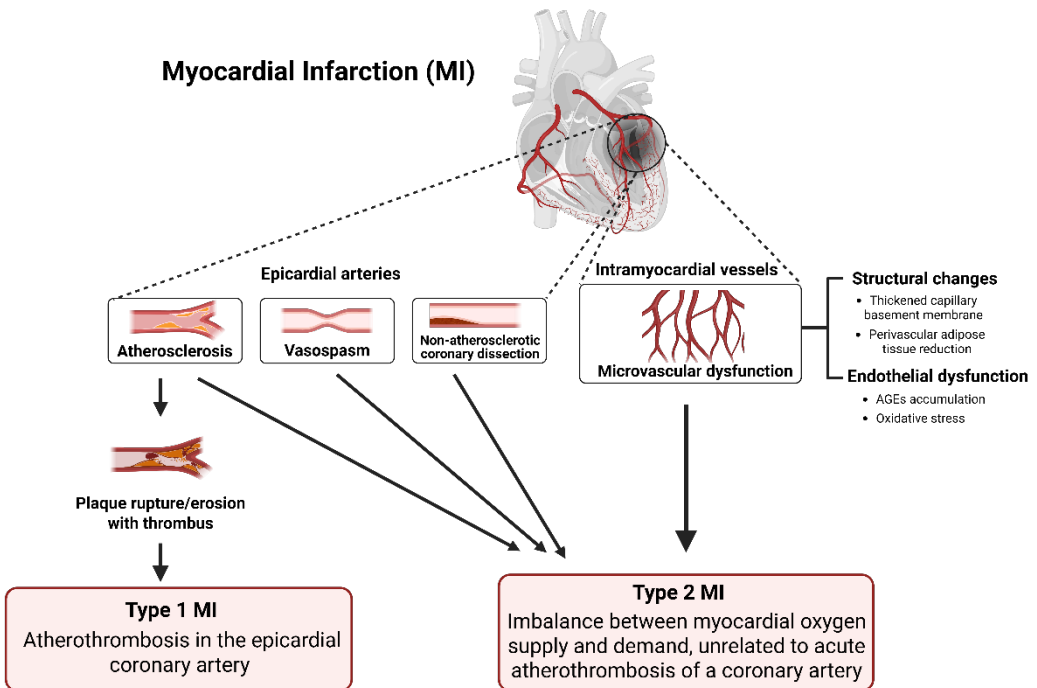
In addition to ventricular involvement, there is evidence that atrial pathology may also contribute to cardiac complications in COVID-19 patients. Atrial arrhythmias have been reported in hospitalized COVID-19 patients^[57], including cases of new onset atrial arrhythmias in individuals with no prior history of atrial arrhythmia,

which are generally associated with worse clinical outcomes^[58]. However, the pathological changes in atrial myocardium, adipose tissue and microvasculature remain largely unexplored. Therefore, in **Chapter 3**, we investigated atrial pathology in COVID-19 patients by analysing inflammation, cardiomyocyte injury, and microvascular lesions, providing insights into their potential role in COVID-19-induced atrial dysfunction.

Myocardial Infarction

Myocardial infarction (MI) is the most critical clinical outcome of coronary artery disease^[59], it is characterized by acute irreversible myocardial injury caused by reduced blood supply due to coronary artery obstruction. This pathological event is associated with poor clinical outcomes including arrhythmias, heart failure and death. It continues to be a major contributor to global morbidity and mortality^[60]. According to a meta-analysis, the mortality rate among individuals younger than 60 years old is approximately 3.9%, while it rises to 9.5% in those aged 60 years and older^[61]. MI is most commonly attributed to epicardial coronary artery disease, such as atherosclerosis, and is classified as type 1 MI (**Figure 2**). Traditional research has primarily focused on restoring the patency of epicardial coronary arteries to re-establish myocardial perfusion as the main therapeutic strategy. Studies have demonstrated a consistent decline in early mortality rate among MI patients, particularly within the first 30 days after onset, largely due to advances in acute management strategies, including coronary revascularization, antiplatelet therapy and lipid-lowering treatment, which are highly effective interventions in type 1 MI^[62,63]. However, the mortality rate beyond the initial 30-day period has shown only modest improvement over time. Currently, the chronic stage of MI, which refers to one year or more after MI onset, accounts for a larger proportion of overall MI-related mortality^[64]. Type 2 MI is caused by an imbalance in myocardial oxygen supply and demand^[65], without the presence of acute atherosclerotic thrombosis. Evidence indicates that the long-term mortality rate associated with type 2 MI is significantly higher than that of type 1 MI^[66]. This has led to growing research interest in factors beyond the epicardial coronary arteries in the pathophysiology of MI. Recently, the structural and functional alterations of intramyocardial microcirculation, namely the dysfunction of coronary microvasculature, have received increasing attention (**Figure 2**). A growing number of studies have shown that microvasculature dysfunction plays an important role in the onset and

progression of MI. Pathological studies in patients with MI have revealed various forms of microvascular dysfunction. These include endothelial dysfunction, characterized by excessive accumulation of advanced glycation end-products (AGEs) and oxidative stress in the endothelium of intramyocardial blood vessels^[28]; structural changes such as thickening of capillary basement membrane^[67] and a reduction in perivascular adipose tissue surrounding the intramyocardial branches of large coronary arteries^[68]. Notably, these alterations are independent of epicardial coronary artery stenosis and may precede the onset of infarction, suggesting that



microvascular dysfunction plays a significant contributory role in the risk of developing MI.

Figure 2. Pathophysiological mechanisms of type1 and type2 myocardial infarction (MI)

Type 1 MI is primarily caused by epicardial coronary artery disease, including atherosclerosis with plaque rupture/erosion, vasospasm, or non-atherosclerotic coronary dissection. Type 2 MI arises from an imbalance between myocardial oxygen supply and demand, which may result from reduced blood flow in large coronary vessels and is frequently associated with microvascular dysfunction. Structural alterations and endothelial dysfunction have been found to contribute to the microvascular dysfunction in MI patients.

Following MI, cardiac repair is typically divided into three phases. Within the first 3-6 hours after infarction, the affected ischemic region can be identified at autopsy

using nitro blue tetrazolium (NBT) staining, which visualizes the infarcted area. At this early stage, although the ischemic area is detectable by NBT staining, no visible histopathological changes are observed under light microscopy. Due to the irreversible necrosis of cardiomyocytes in the infarct core, multiple pro-inflammatory pathways are activated, leading to the sequential infiltration of various immune cells into the infarcted area to clear the necrotic tissue^[69]. This process occurs between 6 hours to 5 days after infarction and represents the second phase, the inflammatory phase. Herein, microscopically, extensive infiltration of inflammatory cells into the myocardium can be observed; Irreversible damage to cardiomyocytes at the centre of the ischemic region leads to necrosis, forming the infarct core. Surrounding the infarct core is a border zone of microscopically morphologically viable cardiomyocytes. Although apparently viable, due to a possibly less severe ischemia or timely reperfusion, the cells in these border zones are likely damaged as they have leaked cardiac enzymes. Subsequently, after clearance of the damaged myocardium, a third cardiac repair phase ensues wherein granulation tissue becomes visible within the infarct core under light microscopy. In this phase the infarct core undergoes increased angiogenesis and cardiac fibroblasts are activated, accompanied by upregulation of TGF- β , leading to the myofibroblast differentiation and the production of extracellular matrix proteins that promote scar formation, thereby facilitating structural repair of the infarcted myocardium^[64,70].

Vascular structural and functional changes, as well as vessel-associated pro-fibrotic activities, are critical to the process of cardiac repair following MI. However, their precise contributions to the development of MI and to the post-infarction repair process remain poorly understood. Therefore, **in Chapter 4**, we evaluated the perivascular fibrosis^[42] in cardiac tissue from patients at different phases of MI. In addition, we analysed the expression of fibroblast activation protein (FAP) in patients with myocardial infarction at various stages to investigate its potential roles throughout the course of post-infarction remodelling. Beyond structural alterations in the vasculature, phenotypic transitions of vascular cells, such as endothelial-to-mesenchymal transition^[43,71] and the conversion of smooth muscle cells into fibroblast-like cells^[47,72], are believed to contribute to vascular fibrosis. However, the extent to which these phenotypic changes occur in intramyocardial blood vessels of MI patients is poorly understood. Therefore, **in Chapter 4**, we also examined the

potential contributions of endothelial and smooth muscle cells to fibroblast-like phenotypic conversion at different stages post-MI onset.

In addition to vascular fibrotic remodelling, the role of endothelial cells in post-MI repair has attracted increasing attention. Follistatin-like 3 (FSTL3) is a glycoprotein that antagonizes members of the TGF- β superfamily^[73] and is known to be involved in apoptosis^[74], metabolic heart disease^[75], atherosclerosis^[76], angiogenesis^[77] and cardiac hypertrophy^[78]. Therefore, **in Chapter 5**, we investigated the dynamic transcriptional expression of FSTL3 at multiple time points (ranging from 0.5 hours to 7 days) following MI in a rat model, in order to explore its potential role in post-infarction cardiac remodelling. Furthermore, endothelial dysfunction and impaired angiogenesis are central contributors to adverse cardiac remodelling after myocardial infarction. Recent studies have suggested that FSTL3 may influence both endothelial function and angiogenic processes^[77]. To further examine this, we specifically analysed FSTL3 expression in intramyocardial endothelium of heart tissues from MI patients and investigated its secretion and expression under hypoxia stress in human umbilical vein endothelial cells (HUVECs) *in vitro*, with the aim of elucidating its potential role in vascular remodelling and endothelial repair post-infarction.

This part of the thesis investigates the dynamic changes in vascular fibrotic remodelling and the expression of related proteins in endothelial cells at different stages following MI, using cardiac tissue from MI patients. The aim is to enhance our understanding of how structural and functional alterations in the microvasculature, both before and after infarction, contribute to the onset of MI and influence the subsequent repair processes.

Anthracycline-induced Cardiovascular toxicity

Anthracyclines, a family of chemotherapeutic compounds^[79], are widely used for a variety of solid tumour and hematologic malignancies, including breast cancer, gastrointestinal, leukaemia, and lymphoma in pediatric and adult patients. The rapid advancement of anti-tumour therapy has greatly increased the survival time of cancer patients in recent years^[80]. However, their clinical utility is significantly limited by their cardiovascular toxicity^[81,82]. Anthracyclines are classified as type 1 agent cardiotoxic agents due to their capacity to cause irreversible cardiac damage, which can negatively affect long-term prognosis. The risk of toxicity is further

aggravated when combined with other anti-cancer therapies such as trastuzumab and radiotherapy. Among the most severe adverse effects is cardiotoxicity^[83]. The effect of anthracyclines on cardiomyocytes are well documented. Dose-related cardiomyocyte injury, apoptosis or even necrosis caused by anthracyclines are considered by many researchers to be the main cause of left ventricular dysfunction and even heart failure (**Figure 3**). However, increasing evidence suggests that anthracyclines also target the vascular system. Doxorubicin (Dox), the most widely used anthracycline, has been shown to impair vascular function and the microvasculature and circulatory system through direct toxicity to vascular cells.

An increased incidence of cardiovascular events in cancer survivors treated with anthracyclines has been reported in numerous clinical investigations, anthracycline chemotherapy has been shown to accelerate the original cardiovascular disease, especially in patients with high risk factors for cardiovascular disease^[84]. As a result, cancer survivors have a high probability of developing cardiovascular disease and at risk for premature cardiovascular events^[81]. Vascular damage and dysfunction induced by anthracyclines may therefore facilitate progressive cardiovascular deterioration in long-term cancer survivors. The side effects of anthracyclines are also critical on highly vascularized organs such as the skin. For instance Dox has been associated with impaired wound healing^[85], skin necrosis^[86], and reduced regenerative capacity^[87], further underscoring the broader systemic impact of anthracycline-induced vascular toxicity.

Dox has been shown to induce endothelial cell damage and alter the secretion of several endothelial factors that regulate microvascular tone and cardiac performance^[88,89]. In a previous study, we observed AGE N(epsilon)-(carboxymethyl)lysine (CML) accumulation in the cardiac microvasculature of mice with acute Dox-induced cardiotoxicity^[90]. Moriyama et al. further demonstrated that myocardial AGE accumulation correlated significantly with heart function and troponin-I levels and that treatment with the AGE inhibitor aminoguanidine counteracted Dox-induced cardiac AGE formation and improved heart function^[91]. Exogenous CML was also found to exacerbate the toxic effects of Dox in human cardiomyocytes in vitro^[92]. However, the impact of circulatory CML on Dox-induced vascular dysfunction, as well as the role of oxidative stress and the AGE-RAGE axis in these processes, remains unclear. Moreover, the absence of the NOX5 gene in rodent animal models limits the investigation of its function. Therefore in **chapter 6**, we investigated the impact of Dox and CML-bovine serum

albumin (CML-BSA) on endothelial oxidative stress and RAGE activation, specifically assessing NOX5 involvement in endothelial cells.

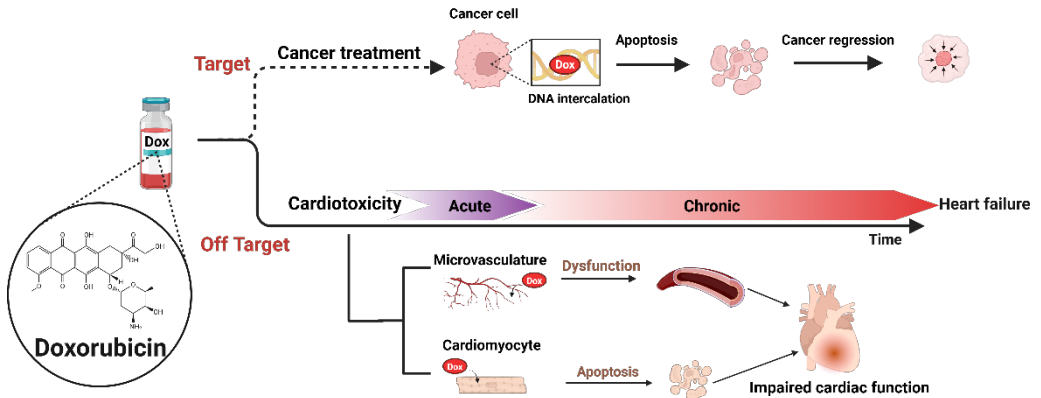


Figure 3. Introduction of Doxorubicin (Dox) and its cardiotoxicity

Doxorubicin (Dox), the most widely used anthracycline, is a cornerstone chemotherapeutic agent for both solid tumors and hematologic malignancies. Its anti-cancer efficacy is primarily mediated through DNA intercalation, topoisomerase II inhibition, and induction of apoptosis in cancer cells. However, off-target effects on cardiomyocytes and the microvasculature can cause irreversible cardiac damage, resulting in cardiotoxicity that manifests as both acute and chronic injury. Consequently, cancer survivors treated with Dox face an increased risk of cardiovascular disease and premature cardiovascular events, even in the long-term.

In addition to direct endothelial injury, recent studies have highlighted the significance of endothelial cells secretome, which consists of proteins and biomolecules released into extracellular space, that play dual roles in vascular homeostasis and disease^[35,93,94]. The endothelial secretome promotes tissue repair and endothelial function restoration under physiological conditions^[95,96], while it contributes to inflammation, thrombosis, and fibrosis under pathological stress^[39-41]. Dysregulated paracrine effects from endothelial cells may therefore contribute the adverse tissue remodelling observed after Dox exposure.

Fibroblasts, as key mediators of extracellular matrix (ECM) synthesis, angiogenesis, and tissue repair, are critical for maintaining vascular structure and homeostasis^[97]. Dysregulated fibroblast activity during chemotherapy can exacerbate fibrosis and disease progression. In Dox-induced cardiotoxicity models, activated fibroblasts are primary contributors to ECM accumulation and myocardial fibrosis^[98]. While in vitro studies showed that Dox induced pro-inflammatory responses and cellular senescence in fibroblasts^[99]. However, it remains unclear whether endothelial cells exhibit a fibrotic response under Dox treatment and how the altered endothelial

secretome influences fibroblast behaviour, and ECM remodelling. The impact of Dox on vascular-ECM crosstalk therefore warrants further investigation. In **chapter 7** we investigated the transition of endothelial cells toward a fibroblast-like phenotype following Dox treatment and examined the effects of the secretome from Dox-treated endothelial cells on fibroblast function over short-term (3 days) and long-term (21 days) periods.

While acute Dox-induced toxicity has been widely studied, anthracycline-induced cardiotoxicity has been recognized as a spectrum, including acute, early-onset chronic progressive, and late-onset chronic progressive. Notably, even low- or moderate cumulative doses of anthracyclines are associated with both subacute and chronic cardiovascular complications. Late-onset cardiotoxicity may not become clinically evident until 10–20 years after the first dose of cancer treatment^[100]. The Childhood Cancer Survivor Study revealed that long-term childhood cancer survivors are 15 times more likely to develop heart failure and 10 times more likely to develop coronary artery disease compared with matched sibling controls^[101]. Importantly, even decades after treatment childhood cancer survivors remain at increased risk (> three times more likely) to die of cardiac causes than the general population^[80,102]. Clinical studies have demonstrated that brachial artery endothelial function was found to be impaired for up to 20 years after therapy in anthracycline-treated cancer survivors^[103-105], suggesting long-term vascular dysfunction as a contributing factor.

In **chapter 8**, we investigated the short- and long-term effects of low-dose Dox treatment on microvascular dysfunction using a Wistar rat model, with a focus on endothelial dysfunction, inflammation, and cardiac remodelling in mediating progressive cardiac injury.

This part of the thesis aims to elucidate the vascular effects of anthracyclines and the underlying pathological mechanisms using complementary *in vitro* and *in vivo* models, thereby contributing to a more comprehensive understanding of anthracycline-induced cardiotoxicity.

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