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Platelet survival is associated with platelet surface expression of CXCR4 and CXCR7 and left ventricular functional recovery after ST-elevation myocardial infarction

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Meiner Familie

und

in Erinnerung an meinen Großvater

Klaus Ernst Hans Hugo Harffer

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Abbreviations

ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AP	Angina pectoris
Apaf-1	Apoptotic protease activation factor-1
ATP	Adenosine triphosphate
Bcl-2	B-cell lymphoma 2
BH	Bcl-2 homology domain
BMS	Bare-metal stent
CAD	Coronary artery disease
CCS	Canadian Cardiovascular Society
DD	Death domain
DES	Drug-eluting stent
DTS	Dense tubular system
ECG	Electrocardiography
FACS	Fluorescence-activated cell sorter
FADD	Fas-associated protein with death domain
FITC	Fluorescein isothiocyanate
FSC	Forward scatter
GMP	Guanosine monophosphate
GP	Glycoprotein
IP_3	Inositol triphosphate
LBBB	Left bundle branch block
LVEF	Left ventricular ejection fraction
MFI	Mean fluorescence intensity
MOMP	Mitochondrial outer membrane permeabilization
MRI	Magnetic resonance imaging
NO	Nitric oxide

NSTEMI	Non-ST-segment elevation myocardial infarction
OCS	Open canalicular system
PAR	Protease activation factor
PBS	Phosphate-buffered saline
PBS-Ca^{2+}	Phosphate-buffered saline with calcium
PCI	Percutaneous coronary intervention
PE	Phycoerythrin
PFA	Paraformaldehyde
PGI_2	Prostaglandin I_2 / Prostacyclin
PLCβ	Phospholipase C-β
PMT	Photomultiplier tube
PRP	Platelet-rich plasma
PS	Phosphatidylserine
PTCA	Percutaneous transluminal coronary angioplasty
SD	Standard deviation
SDF-1	Stromal-derived factor 1
SSC	Sideward scatter
STEMI	ST-segment elevation myocardial infarction
tBID	Truncated BID
TMRE	Tetramethylrhodamine ethyl ester
TNF	Tumor necrosis factor
Tpo	Thrombopoietin
TRAIL	TNF-related apoptosis-inducing ligand
vWF	Von Willebrand factor

1 Introduction

Responsible for 5.3% of all causes of death in Germany, acute myocardial infarction ranks in the second place of the ten most common causes of death, behind coronary heart disease [2]. Both of these diseases can be the result of coronary atherosclerosis and are only two different ways in which it manifests [3, 4]. Despite a long history of research and much effort to achieve knowledge, prevention, and therapy, further scientific efforts are still necessary. A closer look at the role of platelets in myocardial infarction has revealed that it can make a great contribution to cardiac repair mechanisms [5, 6].

1.1 Platelets

In recent years, knowledge about platelet function and fundamental mechanisms has increased rapidly [6]. Platelets are involved in many processes of tissue injury such as detection, inflammatory response, chemo attraction, angiogenesis, proliferation, remodelling, apoptosis and many more [6-9].

1.1.1 Morphology of platelets

Platelets play a crucial role in haemostasis [10]. They can be activated by various circumstances such as vessel injury, which leads to a change of their outer appearance and release of their content [10-12]. Having a diameter of 2.0 µm to 5.0 µm, the anuclear and discoid platelets are the smallest cellular content of circulating blood [13-15]. Under conditions of not being involved in endothelial lesion, thrombosis, haemostasis or another activating effect, their estimated lifetime is between 7 and 10 days [7, 15]. Current studies postulate that their lifespan might be mainly limited by the intrinsic pathway of apoptosis [7-9].

Platelets are fragments of their progenitors, the megakaryocytes, which primarily reside in the bone marrow [16-18]. They are subdivided into four zones: the peripheral zone, the sol-gel zone, the organelle zone, and the platelet membrane system [15, 19].

The peripheral zone consists of the plasma membrane with a lipid bilayer and a glycocalix with glycoprotein (GP) receptors, similar to other plasma membranes in the human body [15, 20]. However, unique for these blood cells is their rugose surface, similar to gyri and sulci in the brain, which is assumed to be an additional membrane, needed for platelet spreading after activation [15, 21]. The surface of these blood cells expresses a huge number of receptors, which are mostly involved in haemostasis and thrombosis, but also in other functions such as inflammation, tumor growth and angiogenesis [11, 22-27]. Important receptors for haemostasis are the GPiB-IX-V complex, the integrin $\alpha_{IIb}\beta_3$, the protease activation receptor (PAR) and the prostaglandin receptor [11, 15, 28-31]. One further receptor family of importance is the family of chemokine receptors, in particular its receptors CXCR4 and CXCR7 [11].

The sol-gel zone is responsible for platelets' shape, and contains the greatest amount of platelet volume, except for the organelles [15]. One component of the sol-gel zone is a circumferential band of microtubules [15, 32-37]. These microtubules stabilise the flat discoid shape of the platelets and act as a suspension for cell organelles [15, 32-37].

The organelle zone contains mitochondria and three types of secretory granules: α-granules, dense granules and lysosomes [12, 15]. The most numerous organelles in platelets are α-granules, which can release more than 300 types of proteins [12, 15, 38-41]. A selection of these proteins includes P-selectin, GPIba-IX-V, GPIIb/IIIa (also known as $\alpha_{IIb}\beta_3$) and GPVI [12, 40-44]. In addition, there are factors V, VII, IX, XIII, fibrinogen, von Willebrand factor (vWF), SDF-1, epidermal growth factor and many more [12, 40-44]. Dense bodies contain low molecular

weight ingredients such as calcium, adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, histamine and pyrophosphate [12, 15, 44].

The platelet membrane system consists of an open canalicular system (OCS) and a dense tubular system (DTS) [12, 15, 45-47]. The OCS is responsible for cell spreading upon platelet activation, and the DTS stores calcium, thus playing an important role in platelet activation [12, 15, 45-47].

1.1.2 Platelet activation

The physiological function of platelets is to detect a disrupted endothelium and exposed endothelial matrix after vessel injuries [48]. To prevent platelets that are not activated from adhesion to the vessel's endothelium, the endothelium releases nitric oxide (NO), prostacyclin (PGI_2) and endothelial ecto-ADPase [48, 49]. Nitric oxide is released upon shear stress and is well documented as an inhibitor of platelet aggregation [48, 50]. Prostacyclin is also released upon shear stress after cyclooxygenase-1-dependent production [48, 51]. Both of these increase levels of cyclic guanosine monophosphate (GMP) and cyclic adenosine monophosphate (AMP), respectively, which leads to the blockage of phospholipase C [52, 53]. Blocked phospholipase C decreases the intracellular level of Ca^{2+} and subsequently inhibits platelet activation [52, 53]. Endothelial ecto-ADPase mediates the metabolism of ADP, which is the most potent stimulator of platelet aggregation [48, 49, 54]. Since ADP is the most potent stimulator of platelet activation, decreased levels of ADP preserve platelets from being activated in healthy vessels [48, 49, 54].
Clot formation is a sequence of overlapping processes with no clearly defined stages [55]. Nevertheless, a three-stage model comprising initiation, extension and stabilization is described with the purpose to form a stable platelet clot after vessel injury [55].

The initiation step begins with a vessel injury. During this step, platelets form a plug to cover the vessel injury [55]. Detection of these injuries by circulating platelets leads to their adhesion. Participating agents are the GPIb-IX-V receptor complex and vWF, which can bind to collagen in the bare subendothelium even under high shear stress with a subsequent activation of GPVI and α2β1 [29, 48, 56]. With the activation of GPVI and α2β1, an intracellular cascade, including phospholipase C γ, IP_3 and Ca^{2+}, is initiated, leading to platelet activation [55, 57-60]. This cascade is depicted in **Figure 1**. Upon platelet activation, the platelet's

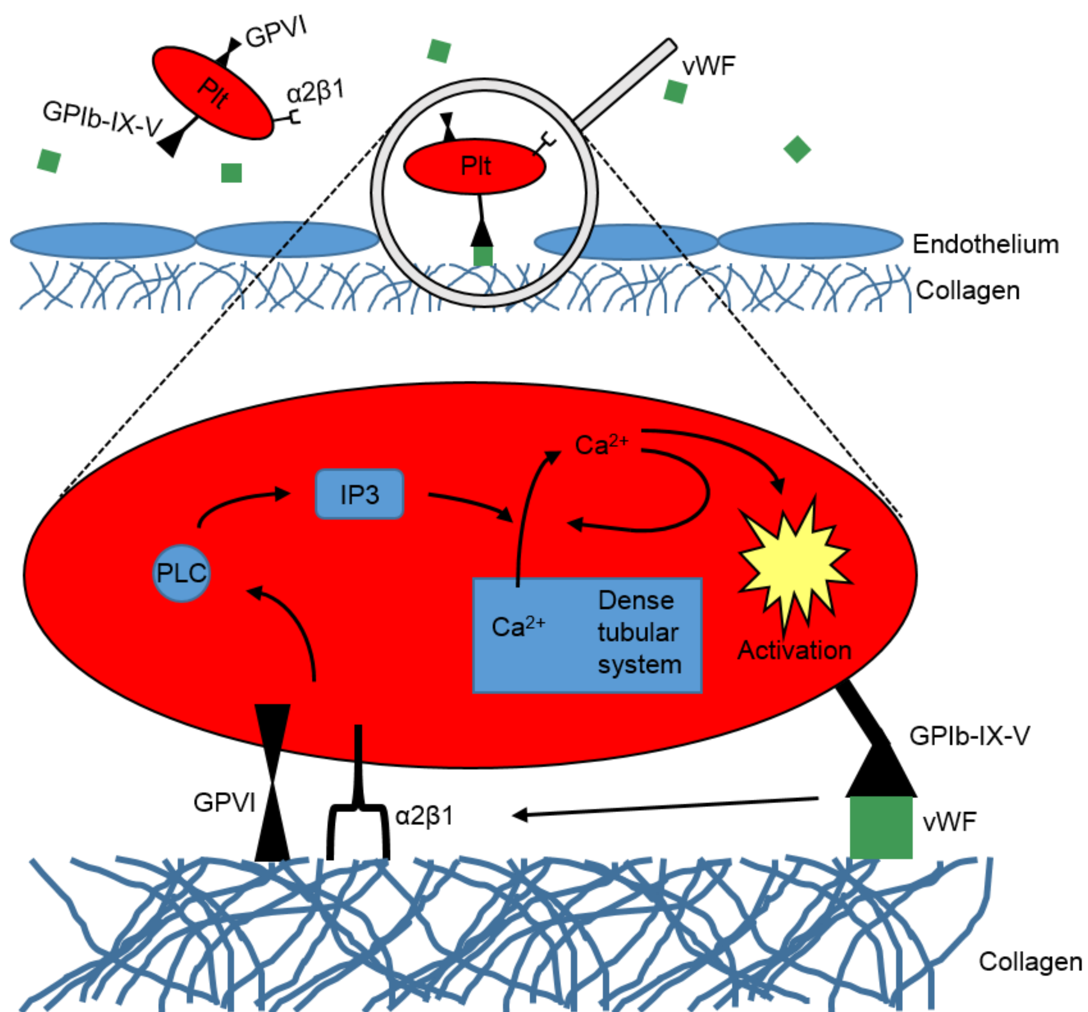

Figure 1: Initiation step after vessel injury in the endothelium. Plt: Platelet; vWF: von Willebrandt factor; PLC: Phospholipase C γ; IP3: Inositol triphosphate; GPIb-IX-V: Glycoprotein Ib-IX-V-platelet-surface receptor complex; GPVI: Glycoprotein VI-platelet surface receptor; α2β1: Platelet-surface receptor.
A: Depiction of platelet adhesion to collagen, mediated by GPIb-IX-V and vWF after endothelial injury. Figure modified after Chen J et al., 'Interactions of Platelets with Subendothelium and Endothelium', *Microcirculation*, 2005.
B: Platelet receptor cascade after GPIb-IX-V and vWF have tethered to collagen. Figure modified after Sangkuhl K et al., 'Platelet aggregation pathway', *Pharmacogenetics and Genomics*, 2011.

shape changes from a thin discoid cell into a rounded and vesicular cell with spines [57, 61]. Simultaneously, the granule's content is released into the extracellular space [59, 61]. These mechanisms are mediated by myosin phosphorylation, which leads to the reorganization of microtubules and actin filaments with a subsequent granule release [36, 45, 59, 61-63].

Release of the granule's content enables the second step of clot formation. The monolayer of platelets is enhanced by additional platelets and the procoagulant function of the platelets is elevated by auto-amplifying agents [55, 57]. These are phospholipase A_2, arachidonic acid, Cyclooxygenase-1, thromboxane A_2 and phospholipase C-β (PLCβ), all leading to a raise of intracellular Ca^{2+} [11, 55, 57, 64-66].

A further auto-amplifying agent is ADP, which is released from dense granules [12, 53, 55, 57]. Adenosine diphosphate auto-amplifies the activation signal by stimulating its G-protein-coupled receptors $P2Y_1$ and $P2Y_{12}$, thus activating PLCβ and increasing the plasma level of Ca^{2+} [11, 31, 55, 67].

The most potent activation amplifying agent is thrombin [55, 57]. As the inactive pre-stage prothrombin, it circulates in plasma [12, 68]. In a small amount, thrombin is already generated during the initiation step, due to prothrombin's exposure to the sub-endothelial expressed tissue factor [69, 70]. However, the vast majority of thrombin is produced during the step of clot formation as a second peak of thrombin generation [69, 71]: With the release of the granules' content, a large number of molecules is released, including factors V, VII, IX, and XIII [12, 41, 57]. In addition, after platelet activation phosphatidylserine (PS) is flipping from the inner leaflet of the platelet's membrane to the outer leaflet [8, 72, 73]. In the presence of PS and Ca^{2+}, coagulation factors V and X form the prothrombinase complex, which cleaves prothrombin to thrombin [57, 69, 70, 74]. Thrombin associates with the G-protein-coupled receptor PAR1, activating PLCβ and increasing intracellular Ca^{2+} [11, 55].

The third step of clot formation is called stabilization [55]. During this stage, a strong mesh of fibrin and vWF is formed within the platelets, which stabilizes the plug and forms a stable clot [55]. Besides fibrinogen and vWF, an essential role is played by the receptor $\alpha_{IIb}\beta_3$, which is released from the α-granules [12, 55, 75-77].

1.2 Coronary artery disease

1.2.1 Definition

There are a large number of known risk factors for developing coronary artery disease (CAD), including age, smoking, diabetes mellitus, hypertriglyceridemia, genetic predisposition and arterial hypertension [78, 79]. Atherosclerosis can affect the whole body, leading to strokes, peripheral artery disease or CAD [4, 80].

The main symptom of CAD is chest pain, called 'angina pectoris' (AP), which is caused by a mismatch of the myocardial supply and the demand for oxygen [78]. Coronary artery disease is categorized into stable AP and acute coronary syndrome (ACS). Stable AP appears in situations of physical or emotional stress and disappears at rest. The Canadian Cardiovascular Society (CCS) has published a classification to grade the severity of stable AP from grade 0 to grade IV [81]. If an AP appears *de novo*, has a CCS score of IV or increasing pain character, or does not disappear at rest, it is called an ACS.

Acute coronary syndrome describes a group of CAD manifestations which can be immediately life-threatening. These manifestations are not only distinguished by clinical signs but also by chemical parameters and the results of an electrocardiogram (ECG). Every ACS has to be treated as an emergency [82, 83].

The clinical manifestation ranges from an unstable angina pectoris as the simplest type of ACS to a non-ST-segment elevation myocardial infarction (NSTEMI) to a fulminant necrosis through all the layers of the myocardium, called an ST-segment elevation myocardial infarction (STEMI) [78, 84-89]. A brief overview to distinguish between these manifestations is depicted in **Table 1**. Even if not mandatory fatal, an unstable AP can be a precursor to a myocardial infarction and has to be treated quickly. Both a STEMI and NSTEMI can lead to sudden cardiac death and are commonly treated with interventional heart catheterization or a bypass operation [82, 89].

Table 1: Manifestations of CAD. Based on 'ESC guidelines on the management of stable coronary artery disease', 2013 [78, 84-89].

		CCS	Troponin	ECG
Stable AP		Unaltered I–III	Negative	Normal
ACS	Unstable AP	IV, raised or *de novo*	Negative	Normal, ST depression or T-inversion
	NSTEMI	IV, raised or *de novo*	Positive	Normal, ST depression or T-inversion
	STEMI	IV, raised or *de novo*	Positive	ST elevation or new LBBB > 20 min

1.2.2 History

Research into angina pectoris has a long history. The first clinical description was made by Heberden in 1772 [90, 91]. In 1812, Warren established the term 'angina pectoris', a combination of 'angina', meaning 'strangling', and 'pectus' meaning 'chest' [91, 92]. The first article reconciling the symptoms and pathology was published in 1879 by Hektoen, who concluded that the origin of angina pectoris is based on sclerotic changes in the coronary arteries, leading to a subsequent thrombosis [91, 93, 94]. The ECG was a further landmark in clinical cardiac research. The development of the ECG was based on findings of muscle electricity by Galvani in the 18th century, improved significantly by Einthoven, Wilson and Goldberger in the early 20th century [95-99]. Eventually, Pardee described the typical reactions recorded by an ECG during a myocardial infarction; in particular the elevation of the ST-segment, which became famous as 'Pardee's sign', and is known today as 'ST elevation' [100-102].

The first approach in therapy was made by Herrick in 1912 [91]. Considering stress as the main factor triggering ACS, he recommended absolute bed rest in calm rooms for several days to prevent patients experiencing physical and emotional stress [91, 103, 104]. Many ACS patients died from arrhythmias and sudden cardiac death, which led to establishment of coronary care units in 1961 [103]. These units enabled constant monitoring and thus instant cardiac massage in case of ventricular fibrillation, which considerably reduced the death rate after myocardial infarction [91, 103, 105]. The next progress in treating myocardial infarction was the development of the heart catheter.

Research into vessel catheterization had already been conducted as early as 1844, and advanced in 1929 with Forssmann's first catheterization of a healthy human heart – conducted on Forssmann himself [91, 106-109]. Thereafter, progress in new approaches to treating coronary stenosis developed rapidly. The first 'percutaneous transluminal coronary angioplasty' (PTCA) was performed by Grüntzig in 1977 [103, 107, 110-118]. Ten years later Dotter implanted the first coronary stent [103, 107, 110-112, 114, 118, 119].

Besides the treatment of ACS, research into risk factors gained greater consideration when the Framingham study started in 1948 [91, 120]. It was designed as a long-term epidemiological study about the development of cardiovascular diseases [120]. In this study, elevated blood pressure, elevated serum cholesterol, smoking and being male were indicated as risk factors and led to the development of primary and secondary prevention mechanisms [120-122].

1.2.3 Therapy

A STEMI is treated according to the 2014 guidelines on myocardial revascularisation of the European Society of Cardiology and the European Association for Cardio-Thoracic Surgery [123]. To minimize the myocardial ischemia duration, the revascularisation should be performed as fast as possible

and preferably within the first two to three hours after becoming symptomatic [123]. Hospital structures are commonly organized with the purpose to reduce the time between a patient's arrival and the primary percutaneous coronary intervention (PCI) to less than 60 minutes [123]. The preferred reperfusion strategy is a percutaneous catheter intervention in which the stenotic passage is dilated with a balloon catheter, followed by the implantation of an endoluminal stent [123, 124]. Primary stent implantation can be conducted with bare-metal stents (BMS) or drug-eluting stents (DES) [123]. Drug-eluting stents have indicated a reduced risk for restenosis, but an elevated risk for in-stent thrombosis [125]. The long-term outcome of the new generation of DES is slightly better than the BMS [126, 127]. For stent implantation via the arteries, the radial approach is preferred and associated with a lower risk of acute bleeding events [123, 128].

Today, revascularisation via fibrinolysis has nearly been replaced by primary PCI as the preferred approach to restore the myocardial oxygen supply [103, 123]. Fibrinolysis should only be considered as a pre-hospital therapy if the expected period of time between the first medical contact and catheter intervention is longer than 120 minutes, and on condition that the onset of symptoms was within the last 120 minutes [123]. After arrival in hospital, a subsequent PCI is still recommended [123].

Besides PCI, for patients with a STEMI, dual antiplatelet therapy with acetylsalicylic acid and a $P2Y_{12}$ receptor blocker is recommended, if possible before intervention [123].

1.2.4 Myocardial regeneration

1.2.4.1 Estimating recovery of ventricular function

The extent of myocardial damage after an infarction influences cardiac performance, which results in a reduced left ventricular ejection fraction (LVEF) [129]. After a STEMI, a LVEF of $<40\%$ is a reliable predictor to estimate short- and long-term mortality in terms of the development of adverse events [129, 130].

Due to heterogeneous healing and remodelling of the left ventricle, the early assessment of the LVEF has no reliable predictive value with regard to its long-term development [131, 132]. The best prognosis for long-term mortality can be assessed with the LVEF six months after a myocardial infarction, while studies are inconsistent concerning the benefit of early implantation of a cardioverter-defibrillator [133-136]. The current therapeutic regime to extend the long-term survival is the best medical treatment [137].

One approach to improving individual and early therapy is to search for early predictors of future LVEF outcomes [131]. Predictors of LVEF improvement are initial low levels of troponin and a low peak of creatine kinase [136, 138-141]. Another tool to estimate LVEF recovery is early evaluation of the acute infarct size via gadolinium-enhanced magnetic resonance imaging (MRI), which can detect a hibernating myocardium and is the best tool to predict long-term mortality, development of adverse events and recovery of LVEF [131, 142-150].

1.2.4.2 Platelet chemokines and the SDF-1-CXCR4/CXCR7 axis

In recent years, special attention has been given to chemokines and their influence on myocardial recovery. Chemokine proteins are a subgroup of cytokines, which can be secreted by many distinct cell types working as agents for cell communication [151]. They play a role as mediators in a wide range of processes such as inflammation, cardiovascular diseases, infections, survival, apoptosis, adhesion and many more [152].

Based on their molecular structure, chemokines can be divided into four groups: the CC, CXC, CX_3C and XC subfamilies [153]. Appending an ‘L’ for ‘ligand’ and a number, which represents the number of the corresponding chromosome nomenclature of its gene location, forms the official name of the chemokine [154]. The corresponding receptor is named with an ‘R’ as suffix [155]. Receptors are G-proteins coupled with seven transmembrane domains [155]. They have an affinity to more than one ligand and have another numeration system [154]. For example, suitable receptors for CXCL12 are CXCR4 and CXCR7 [154, 156]. Besides the official nomenclature, ligands also have commonly used names. For

example, CXCL12 is commonly called ‘SDF-1’, since it is a product of the chromosome ‘SCYB12’ with the gene ‘SDF-1’ [154].

The chemokine SDF-1 has a high affinity to CXCR4 and CXCR7, and is expressed by a number of different tissues [156-159]. It is involved in the development, homing, proliferation and survival of hematopoietic progenitor cells or in neuromodulation [156, 158, 159]. Its receptors are expressed in many cell types, including platelets [160]. In mesenchymal stem cells, expression of this type of receptor is elevated under hypoxic conditions [161]. Expression on the platelet’s surface remains on the same level without being affected by the platelet’s state of being activated or resting [162].

The chemokine SDF-1 is a key player in the recruitment of CXCR4-expressing cells from bone marrow to damaged tissue for the purpose of better regeneration [163]. This process has been demonstrated in myocardial infarction and strokes [156, 164, 165]. In patients with CAD, high platelet expression of SDF-1 is concomitant with high levels of CXCR4 and CXCR7 [162]. Patients with ACS also exhibit higher levels of CXCR7 expression compared to patients with stable CAD [162].

The SDF-1-CXCR4-CXCR7 axis has an influence on myocardial regeneration. In ACS patients with a high expression of the platelet SDF-1, an amelioration of the LVEF could be detected after three months [166]. In patients with symptomatic CAD, low levels of CXCR4 correspond to a low overall mortality compared to patients with high levels of CXCR4 [167]. High levels of the platelet CXCR7 in ACS patients indicate a correlation with an improvement of the LVEF after three months [162].

1.3 Apoptosis

‘Apoptosis’ comes from the Greek word ‘αποπτοσισ’, which can be translated as ‘pieces of leaves falling from the tree in autumn’ [168]. This translation is commonly regarded as a metaphor for the removal of unwanted cells [169]. It is a complex process of programmed cell death and plays a crucial role in multicellular organisms [169]. From the beginning of embryonic development until our death, apoptosis has an influence on our growth, our homeostasis, and the development of diseases [170-179].

1.3.1 Morphological changes in apoptotic cells

The morphological changes of apoptosis are well known. In 1974, Kerr noticed two discrete stages [180]. The first stage comprises the formation of apoptotic bodies and during the second stage, cell fragments undergo phagocytosis [180].

More specifically, the first stage begins with the condensation of the nuclei, DNA fragmentation and shrinkage of the cell [169, 172]. This is followed by nuclear fragmentation and cell fragmentation in small apoptotic bodies, keeping the cell organelles intact [169, 172, 180].

During the second stage, apoptotic bodies become phagocytosed by the surrounding cells [169, 172]. These cells are macrophages, but also intact cells of the surrounding tissue [180]. During this process, none of the intracellular components are released into the extracellular space, and all organelles remain intact [169, 181].

Another morphological feature of apoptosis is the exposure of phosphatidylserine (PS) at the outer cell membrane [7]. The negatively charged PS is an ingredient of the plasma membrane, and is localized on its inner leaflet [182-185]. Upon activation, it flips to the outer leaflet of the cell membrane. The degree of PS exposure is assumed to be cell-type-specific, but nevertheless is a common

feature of nearly all apoptotic cells [186, 187]. Phosphatidylserine serves as a decoy for macrophages, leading to the recognition and removal of apoptotic cells [183, 184].

1.3.2 A molecular view of apoptosis

The process of apoptosis involves many molecular processes and can be caused by a great number of different stimuli. These stimuli can originate from the extracellular space or mitochondrion, categorized as extrinsic and intrinsic pathways, respectively [169]. Stimuli using the extrinsic pathway are mediated by different ligands, activating death receptors such as TNF1, Fas, and DR4 [188]. The intrinsic pathway involves stress factors such as heat, radiation, hypoxia and anticancer drugs [189]. Both extrinsic and intrinsic pathways result in the induction of a caspase cascade [181]. Caspases are mediators of the proteolytic cleavage of the cell's components, leading to morphological changes during apoptosis [190]. At the same time, a subgroup of caspases mediates an inflammatory cell response [191, 192].

1.3.2.1 The extrinsic pathway

The extrinsic pathway is initiated by the activation of cell-surface death receptors [169, 193]. These receptors are transmembrane proteins from the tumor necrosis factor receptor (TNF) superfamily [169, 188]. The triggers are extracellular death ligands such as the Fas receptor, TNF-R1, DR4 (also called TRAILR1) or DR5 [169, 194]. There is a great number of other death ligands [169].

The activation of death receptors activates an intracellular signal cascade. The intracellular ligand associates with a protein with death domain (DD), which subsequently recruits the Fas-associated protein with death domain (FADD) [195-202]. This facilitates binding of the procaspase 8 to the FADD, which results in a self-activation of procaspase 8 to caspase 8, thus inducing the cascade of caspases [200, 203, 204]. **Figure 2** depicts a simplified extrinsic pathway, including its cross talk to the intrinsic pathway.

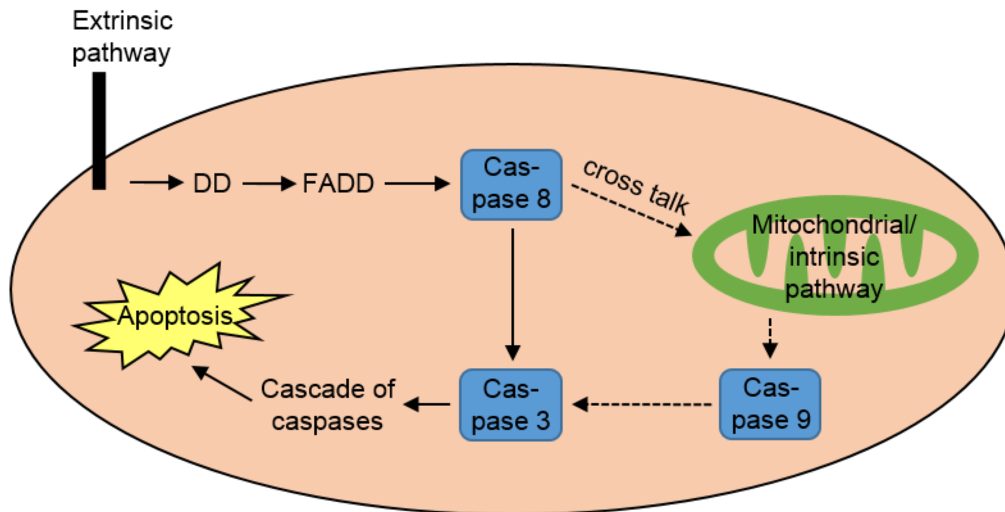

Figure 2: Extrinsic pathway of apoptosis. DD: Death domain; FADD: Fas-associated protein with death domain. The cross talk depicts the way from the extrinsic to the intrinsic pathway of apoptosis. Figure modified after Schultz D. R. et al., 'Apoptosis: Programmed Cell Death at a Molecular Level', Seminars in Arthritis and Rheumatism, 2003.

1.3.2.2 The intrinsic pathway

The intrinsic pathway is triggered by intracellular signals acting on intracellular targets [189]. Key players in this pathway are the mitochondria [189]. The mitochondria are organelles in eukaryotic cells, supplying them with chemical energy in the form of ATP [205, 206]. One crucial mitochondrial component of apoptosis is the protein cytochrome c [188]. Mitochondria consist of two membranes, an inner and an outer membrane [207]. The space between the outer and inner membranes is called the intermembrane space [188, 208]. In healthy cells, cytochrome c is stored in the intermembrane space [188, 208]. During apoptosis via the intrinsic pathway, cytochrome c is released into the cytosol [209].

Pro- and anti-apoptotic factors are located in the outer mitochondrial membrane, maintaining a state of equilibrium [169, 210]. With the ageing of the cell, anti-apoptotic factors withdraw, but the factors are also affected by stimuli such as toxins, radiation, free radicals, hypoxia, hyperthermia, or viral infections [189]. Disturbance of this state of equilibrium in favour of pro-apoptotic factors results in enhancement of mitochondrial outer membrane permeabilization (MOMP), which is attended by a reduction of the mitochondrial transmembrane potential

($\Delta\Psi_m$) [169, 211, 212]. Potential dropping is an early event of apoptosis and marks a point of no return, whereafter transduction of the apoptotic signal passes off within five minutes, regardless of its initiating signal strength [212-214]. The dropping membrane potential leads to an increase of membrane permeability and pro-apoptotic proteins such as cytochrome c are released into the cytosol [169, 215]. The main actors in the balance of pro- and anti-apoptotic factors are proteins from the B-cell lymphoma 2 (Bcl-2) family, so called due to its first description in research about follicular lymphomas [169, 216]. B-cell lymphoma 2 proteins are a large and heterogeneous family, which, on the one hand, keeps cells from apoptosis, and on the other hand, triggers apoptosis [217, 218]. They are commonly located in the outer mitochondrial membrane [169, 219]. Based on their molecular domain motifs and their functional role in apoptosis, they are divided into inhibitors of apoptosis, promotors of apoptosis and BH3-only proteins [7, 217, 218, 220-223]. The best known inhibitors are Bcl-2 and Bcl-x_L; the best known promotors are BAX and BAK [169, 209, 217, 218, 220, 224-228]. One BH3-only protein is BID, which is involved in extrinsic and intrinsic apoptosis [217].

With the initiation of apoptosis and the release of cytochrome c into the cytosol, the formation of the apoptosome is possible [169, 219, 229]. The apoptosome is a wheel-shaped enzyme complex, which serves as an activation tool for procaspase 9 [189, 230-233]. Upon release of cytochrome c, it binds to apoptotic protease activation factor 1 (Apaf-1), which allows an assembly of approximately seven Apaf-1 proteins to the apoptosome [189, 230-233]. The central structure of the apoptosome forms the procaspase 9 binding site, where initiator caspase 9 is activated, which initiates activation of downstream caspases [219, 231, 233-236]. **Figure 3** depicts an overview of intrinsic apoptosis.

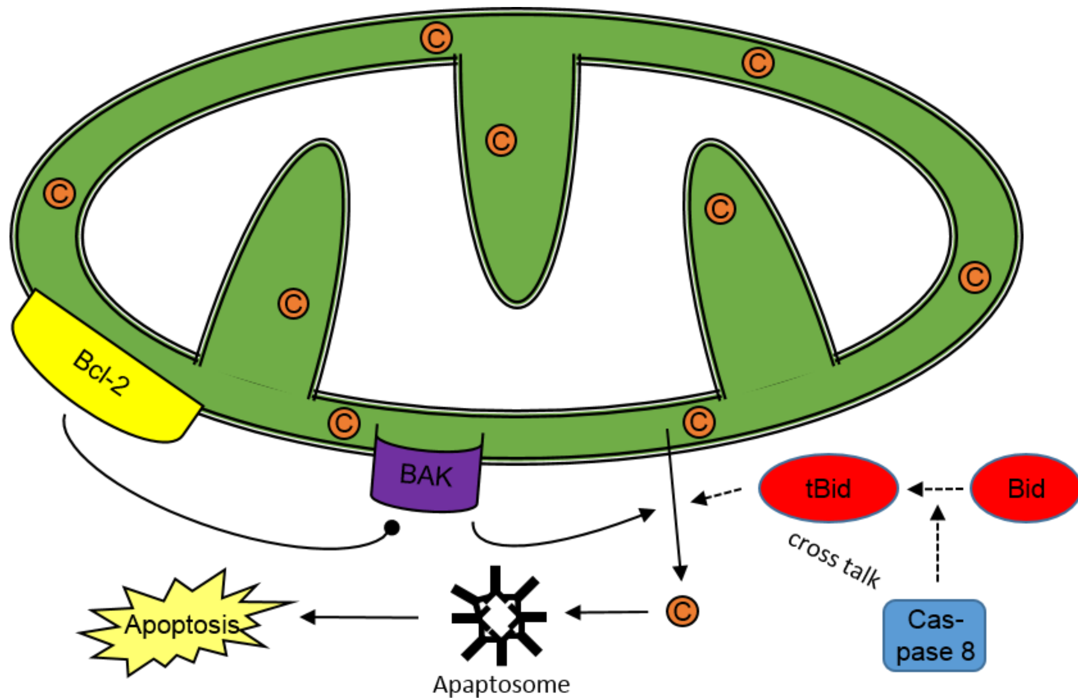

Figure 3: Intrinsic pathway of apoptosis. Bcl-2: B-cell lymphoma 2 are a group of anti-apoptotic proteins; BAK are a group of pro-apoptotic proteins; c: Cytochrome c release mediates formation of the apoptosome and thus apoptosis; The cross talk initiated by caspase 8 and by Bid and tBid links the extrinsic with the intrinsic pathway of apoptosis. Figure modified after Schultz D. R. et al., Apoptosis: 'Programmed Cell Death at a Molecular Level', Seminars in Arthritis and Rheumatism, 2003.

1.3.2.3 The caspases

Caspases are a group of proteolytic enzymes that play a key role in apoptosis [237]. The inactive pre-stage of the caspase is called the 'zymogen' or procaspase [192]. In nucleated cells they are expressed continuously, holding programmed cell death always ready to run [181, 238]. Activated caspases can cleave intracellular targets and help with the separation of the cell from its neighbours by cleaving the cell's skeleton [181, 239-244]. In addition, they activate other apoptotic enzymes with the purpose of supporting the caspase's depletion of the cell [243, 245-251]. All these caspase-triggered events are responsible for the morphological changes during apoptosis.

Depending on their role in the apoptotic cascade, caspases are divided into initiator and executioner (or downstream) caspases [252, 253]. Besides that, with caspases 1, 4, 5 and 11 a group of inflammation-regulating caspases is activated, leading to the release of inflammatory cytokines [191, 192]. The initiation of apoptosis is conducted by caspases 2, 8, 9 and 10 [192, 252, 254-256]. The

execution of apoptosis is conducted by caspases 3, 6 and 7 [192, 252, 254-256]. After activation, both the extrinsic and intrinsic pathways lead to activation of the caspase cascade by activating the executioner caspases, regardless of their trigger [169, 254]. Running the caspase cascade contains at least the activation of one initiator caspase, followed by the activation of one executioner caspase [255]. The change from procaspase to caspase is the result of the proximity-induced clustering to the dimeric active form [255].

Caspase 8 is capable of inducing a cross-talk between the intrinsic and extrinsic apoptotic pathways [189]. It cleaves the pro-apoptotic BH3-only protein BID, which is located in the cytosol [189, 257]. After cleavage, the henceforth called truncated BID (tBID) can translocate to the mitochondria, acting as a pro-apoptotic protein, thus releasing cytochrome c [254, 257]. With the release of cytochrome c, the mitochondrial pathway is activated, which amplifies the death receptor-induced signal [258].

1.3.3 Apoptosis in platelets

Apoptosis was formerly assumed to occur exclusively in nucleated cells, but platelets converting to apoptotic bodies was discovered as late as 1997, as a consequence of research into the short lifespan of platelets [180, 259-261]. Until the discovery that the intrinsic pathway plays an essential role in the platelet lifespan, several models about life restriction had been proposed and rejected [7-9, 262-269].

The first protein discovered to be involved in platelet lifespan was Bcl-x_L [7-9]. Further studies on platelets, taking a closer look at these particular apoptotic features, revealed that they contain caspase 3, caspase 9, Apaf-1 and cytochrome c [270]. Furthermore, morphological changes could be demonstrated in ageing platelets and upon activation of apoptosis as well [270-273]. This included an increasing exposure of PS and other phagocyte scavenger receptors

in ageing platelets with the simultaneous condensation of cytoplasm and the ability of mitochondrial membrane potential dropping [271-273].

The current model explaining the platelet lifespan is called the ‘molecular clock’ [7-9]. It assumes that degradation of Bcl-x_L occurs more quickly than degradation of BAK [7-9, 274, 275]. This model is substantially based on findings that depression of single pro- and anti-apoptotic proteins leads to an increase or reduction of platelet lifespan [7-9, 276, 277]. According to this model, platelet survival is mainly regulated by a balance of Bcl-x_L and BAK, leading to apoptosis after disturbance of this state of equilibrium in favour of BAK [7]. There is no evidence that platelets undergo an extrinsic pathway of apoptosis [7, 278]. Indeed, mRNA for the TRAIL receptors DR4 and DR5 has been found in platelets, but on the cell surface no expression of any corresponding protein like Fas has been detected [279].

2 Aims

Despite progress in the management and therapy of myocardial infarction, mortality is still high [2]. Unfortunately, there is little knowledge about prognostic markers indicating a prognosis of cardiac function [280, 281]. Indeed, studies have been conducted on the functional cardiac recovery in a population of ACS patients in general, but no special consideration has been given to STEMI patients in particular [166]. Recent studies on ACS patients have indicated a correlation between elevated levels of CXCR7 platelet surface expression and an improvement of the left ventricular ejection fraction (LVEF) within three months [160, 162]. In addition, there is evidence that CXCR4 and CXCR7 are closely associated with platelet survival. For this reason, a more precise view on the prognostic markers of this particular disease is necessary.
For this study, a population of patients with a diagnosis of acute STEMI was recruited. The aim was to investigate whether there is a relation between the six-month development of the LVEF on the one hand, and platelet survival on the other hand.

The study sought to achieve the following:

- To measure the course of the LVEF using thoracic MRI as a parameter for the cardiac function in STEMI patients;
- To assess apoptosis in platelets, including measuring their survival with tetramethylrhodamine ethyl ester (TMRE) and their apoptosis with annexin V;
- To investigate the association between markers of platelet apoptosis and the platelet surface expression of CXCR4 and CXCR7; and
- To investigate the association between platelet apoptosis and the course of the LVEF after a STEMI.

3 Materials and methods

3.1 Patient characteristics

This pilot study was conducted with patients who had experienced ST-segment elevation myocardial infarctions (STEMIs). The diagnosis and treatment were according to the guidelines of the Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology (ESC) [88]. All subjects were immediately treated with percutaneous transluminal coronary angioplasty (PTCA) in accordance with these guidelines [123]. This study was conducted in the Department of Cardiology of the University of Tübingen between August 2013 and June 2015 [1], and included 87 patients [1]. Blood was taken within 24 hours after intervention.

In each case 70 mL of blood was taken from a peripheral vein and immediately analysed for platelet survival and platelet apoptosis [1]. Contemporaneously, platelets were tested for the expression of the chemokine receptors CXCR4 and CXCR7 [1]. All samples were analysed with a fluorescence-activated cell sorter (FACS) [1]. Furthermore, the LVEF% was evaluated in each patient who had undergone a cardiac MRI during intrahospital stay, and six months later [1].

All subjects were informed in detail about the study and gave written informed consent. The study complies with the guidelines of the European Parliament and of the International Conference of Harmonization for good clinical practice, and with the Declaration of Helsinki [282-284]. The institutional ethics committee of the University of Tübingen gave its approval for this study (270/2011BO1).
The baseline characteristics are presented in **Table 2**. For 38 of the 87 subjects, no information about previous pharmacological therapy was available [1].

Table 2: Baseline characteristics. n: number of subjects; ±: standard deviation [1].

Basic data		
Number of subjects	87	
Number of males	63 (72.4 %)	
Age (mean ± SD)	60.8 ± 12.1	

Cardiovascular risk factors	**Yes**	**No**
Arterial hypertension	56 (64.4%)	31 (35.6%)
Hyperlipidaemia	23 (26.4%)	64 (73.6%)
Diabetes mellitus	20 (23.0%)	67 (77.0%)
Smoker	41 (47.1%)	39 (44.8%) ex: 7 (8.0%)
ACS in family	17 (19.5%)	70 (80.5%)
Prior CAD	18 (20.7%)	69 (79.3%)

Medication on admission	**Yes**	**No**
Acetylsalicylic acid	14 (16.1%)	73 (83.9%)
Clopidogrel	2 (2.3%)	85 (97.7%)
Prasugrel	1 (1.1%)	86 (98.9%)
Ticagrelor	3 (3.4%)	84 (96.6%)
Oral anticoagulant	2 (2.3%)	85 (97.7%)
ACE inhibitor	13 (14.9%)	74 (85.1%)
Angiotensin II receptor inhibitor	8 (9.2%)	79 (90.8%)
Calcium channel inhibitor	9 (10.3%)	78 (89.7%)
Beta blocker	20 (23.0%)	67 (77.0%)
Diuretic (thiazide, loop)	9 (10.3%)	78 (89.7%)
Potassium-sparing diuretic	4 (4.6%)	83 (95.4%)
Statin	12 (13.8%)	75 (86.2%)

3.2 Materials

Table 3 lists the used substances and agents. The consumable materials are listed in **Table 4**. The technical equipment is registered in **Table 5**.

Table 3: Used substances and agents.

Name	Description
PBS-Ca^{2+}	This study used Dulbecco's Phosphate-buffered Saline with $MgCl_2$ and $CaCl_2$, liquid, sterile-filtered, suitable for cell culture from Sigma-Aldrich, St. Louis, USA; Cat-No D8862.
PBS	This study used Dulbecco's Phosphate-buffered Saline, modifed, without calcium chloride and magnesium chloride, liquid, sterile-filtered, suitable for cell culture from Sigma-Aldrich, St. Louis, USA; Cat-No D8537.
PFA	This study used Paraformaldehyde (PFA) for synthesis, CAS-No 30525-89-4 from Merck-Schuchardt, 8011 Hohenbrunn, Germany; Cat-No 818715.
CXCR4	This study used anti-hCXCR4 PE conjugated from RD Systems Minneapolis, Minnesota 55413, USA; Cat-No FAB170P.
CXCR7	This study used anti-hCXCR7/RDC-1 PE conjugated from RD Systems, Minneapolis, Minnesota 55413, USA; Cat-No FAB42271P.
CD42b	This study used CD42b FITC conjugated from Beckman Coulter Company, 13276 Marseille, France; UNSPSC 41116015.
Thrombin	This study used Thrombin from human plasma, lyophilized from Roche, Basel, Switzerland; Cat-No 10602400001.
Annexin V	This study used Annexin V FITC conjugated from ImmunoTools, 26169 Friesoythe, Germany; Cat-No 314900013.
TMRE	In this study, TMRE was ordered from Invitrogen, Carlsbad, USA; Cat-No T669.

Table 4: Consumable materials.

Name	Producer	Material
S-Monovette 7.5 ml Z-Gel	Sarstedt	Polypropylene
S-Monovette 8.5 ml CPDA	Sarstedt	Polypropylene
Safe-lock microcentrifuge tubes 1.5ml	Eppendorf	Polypropylene
Cellstar tubes 15 ml	Greiner Bio-One	Polypropylene
Falcon tube 5 ml	Corning	Polystyrene
Pipette Research 100 – 1000 µl	Eppendorf	-
Pipette Research 10 – 100 µl	Eppendorf	-
Pipette Research 0.5 – 10 µl	Eppendorf	-
Pipette tips 1000 µl	Ratiolab	Polypropylene
Pipette tips 200 µl	Sarstedt	Polypropylene
Pipette tips 10 µl	Biozym Scientific	Polypropylene
Pasteur-plast pipets, 2,5 ml, 150 mm, graduated	Ratiolab	Polyethylene

Table 5: Technical equipment.

Equipment	Description
Centrifuge	This study used a Heraeus® Multifuge® 1S from Thermo Fisher Scientific, Waltham, MA, USA.
Automated Hematology Analyzer	This study used the Automated Hematology Analyzer KX-21N™ from Sysmex, Kobe, Japan.
Shaker	This study used a Vortexer IKA Vibrofix VF2 from IKA-Werke, Staufen im Breisgau, Germany.
FACS	This study used the FACSCalibur™ Flow Cytometer 342975 from Becton Dickinson Biosciences, Heidelberg, Germany.
FACS-Program	This study used a BD FACStation™ Mac Pro computer with a 2.8-GHz Quad-Core Intel® Xeon® processor and 2 GB RAM was used as a working station. As an analysis software, this computer ran with BD CellQuest™ Pro, Version 5.d.7f6b.
MRI	This study used a Magnetom Avanto from Siemens Healthcare, Erlangen, Germany.
MRI-Program	Thus study used cvi42 from Circle Cardiovascular Imaging Inc, Calgary, AB, Canada.

3.3 Methods

3.3.1 Study design

This study was designed as a prospective study. It was based on the parallel measurement of apoptosis and the expression of distinct chemokine receptors in platelets. Measurements were taken within the first 24 hours after PTCA stent implantation in patients with a diagnosis of STEMI. Analysis and course of the LVEF was conducted twice with MRI. Examination took place during initial hospitalization after the event, and six months later.

Blood samples were taken from the peripheral veins of the subjects. Measurements of platelet apoptosis in platelet-rich plasma (PRP) were taken for each subject. In a parallel measurement in whole blood, the platelets' expression of chemokine receptors was analysed. Experiments were conducted in the laboratories of the University of Tübingen, Department of Cardiology (Department of Internal Medicine III), where a constant temperature of 22°C could be ensured. All experiments were conducted at this temperature.

3.3.2 Preparation of chemokine dye

3.3.2.1 Preparation of a dilution with 1% paraformaldehyde

To prepare 500 ml of a 1% paraformaldehyde (PFA) dilution, 5 g of PFA was taken and dissolved in 400 ml of phosphate-buffered saline (PBS); 40 ml of sodium hydroxide (NaOH) (with 1 N equivalent concentration) was added. Afterwards, the liquid was stored in a dark environment. All these steps were conducted under a fume hood. In an environment of 65°C, this liquid was stirred continuously, until the liquid was clear. After being cooled down to room temperature, the pH of the liquid was reduced to 1.4 with hydrogen chloride (HCl) (with 1 M molar mass) and was filtered through a 0.2 µm filter.

3.3.2.2 Analysis of chemokines CXCR4 and CXCR7

For the analysis of the platelets' CXCR4 and CXCR7 expression, whole blood was taken. The blood sample was decanted into a CPDA-coated S-Monovette. Afterwards, 20 µl of the whole blood was put into a Cellstar tube and diluted with 980 µl of PBS.

Amounts of 35 µl of this dilution were transferred into each of two Falcon tubes. An amount of 5 µl of PBS was added to each Falcon tube to increase the level of dilution. Into each dilution, 5 µl of CD42b was added. An amount of 5 µl of CXCR4 was added to one of the Falcon tubes, and 5 µL of CXCR7 was added to the other Falcon tube. These two dilutions were mixed with the vortex mixer and incubated in a dark environment for 30 minutes.

The incubation of both samples was stopped with 300 µl of the prepared PFA dilution and was immediately analysed with FACS. **Figure 4** depicts a schematic illustration of the experimental setup for the expression of chemokine receptors.

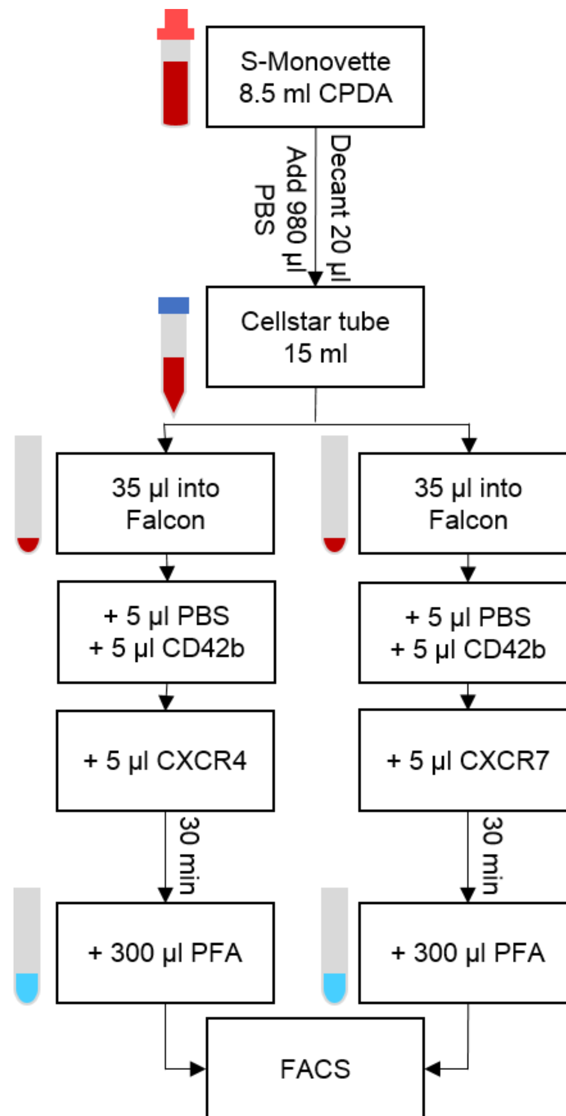

Figure 4: Preparation of chemokine dye.

3.3.3 Preparation of apoptosis dye

3.3.3.1 Preparation of platelet-rich plasma and of the dyes

The ordered thrombin was in a lyophilized state. In a preparatory step, it was dissolved in 2000 µL of PBS-Ca^{2+}. The ordered TMRE was delivered in a high concentration: 10 µL of the TMRE was diluted with 990 µL of PBS-Ca^{2+}.

After having taken a blood sample with a Z-Gel-coated S-Monovette, the sample was directly transferred into a Cellstar tube, which is appropriate for centrifugation. This sample was put into the centrifuge and was hydro-extracted with 1000 rounds per minute for 20 minutes. Subsequently, the supernatant PRP

was carefully transferred with a Pasteur pipette into another Cellstar tube. To ensure that only pure PRP would be transferred, 2 ml of PRP above the remaining effluent was not transferred.

In order to measure the platelet count in the PRP, 10 µL of the extracted PRP was transferred into a 1.5 ml Safe-lock microcentrifuge tube and diluted with 90 µL of PBS-Ca^{2+}. The platelet number in the resulting aliquot was measured with the Automated Hematology Analyzer. The platelet count was noted and the aliquot was rejected afterwards. With the measured platelet count, the final aliquot for the FACS analysis was formed. Via extrapolation, a PRP volume containing approximately 10^6 platelets was calculated. This volume was filled into each of four Falcon tubes. Afterwards, the prepared PRP was incubated with the other ingredients.

3.3.3.2 Induction of apoptosis and incubation with apoptosis-detecting agents

To approximate the whole potential of apoptosis in platelets, the samples were divided into two: One half to be incubated with thrombin and the other half to remain resting without thrombin.

Before the incubation of the platelets, an individually calculated volume of PBS-Ca^{2+} was added. The volume of PBS-Ca^{2+} depended on the added PRP volume in the step before, and also depended on the volume of the agents which had to be added afterwards. The purpose of this calculation was to configure a total volume of 50 µL for each tube. With this approach, it was guaranteed that the number and the concentration of platelets in each aliquot was the same. An amount of 5 µL of the thrombin dilution was added to two of the four Falcon tubes. To incubate this mixture, the samples were covered in a dark environment for 60 minutes. The two samples without added thrombin remained unaffected and were covered in a dark environment for 60 minutes as well. After incubation, 5 µL of TMRE was added t one of the thrombin-activated tubes and one of the resting tubes. Simultaneously, 4 µL of Annexin V was added to the remaining two tubes. These samples were incubated in a dark environment for 30 minutes. After

incubation, 400 μL of PBS-Ca^{2+} was added to each tube. Analysis was conducted immediately with FACS. **Figure 5** depicts a schematic illustration of the experimental setup for measuring apoptosis.

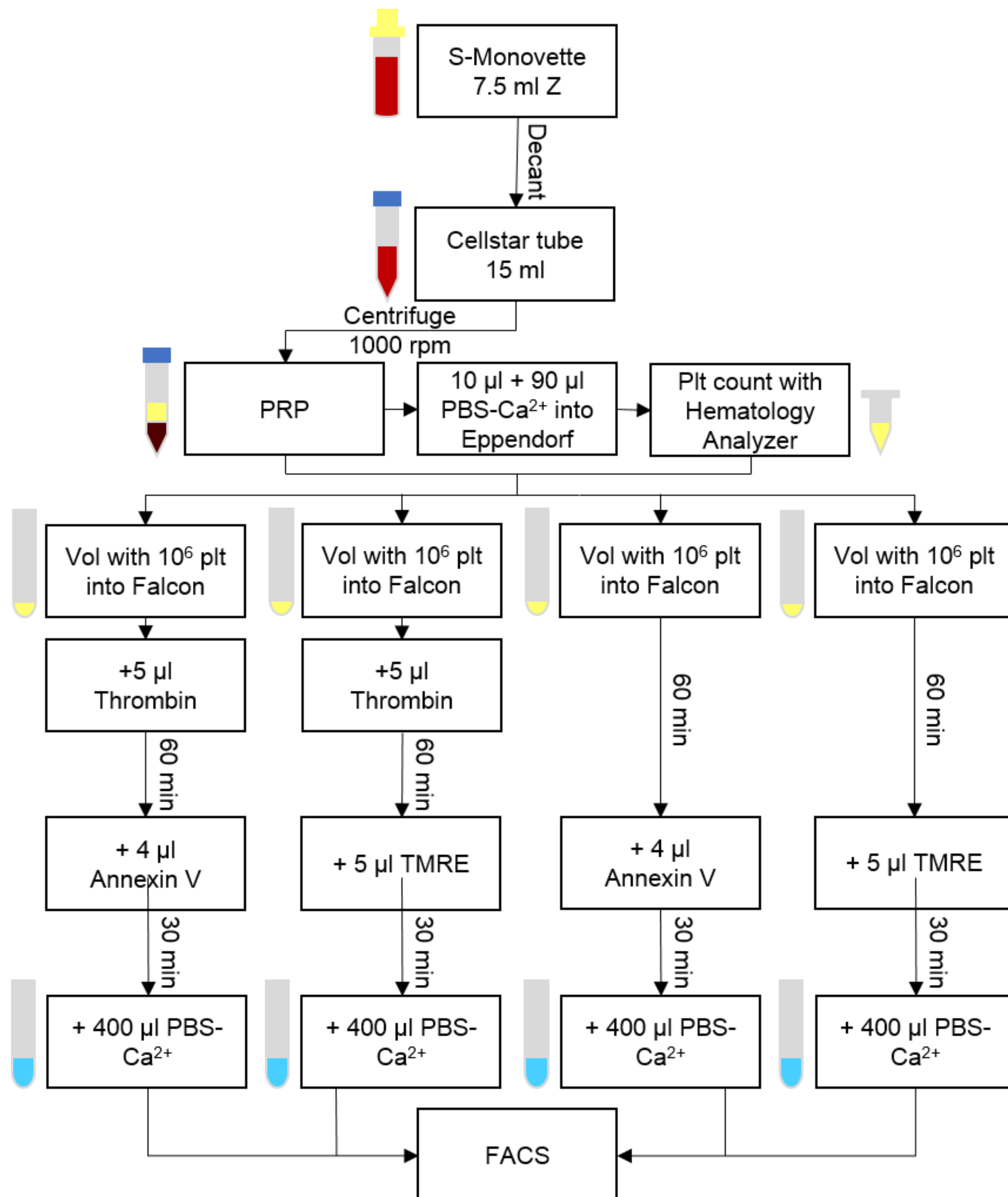

Figure 5: Preparation of apoptosis dye.

3.3.4 FACS analysis

The FACS that was used possesses two lasers with different wavelengths:
Blue laser: Air-cooled argon-ion laser, 15 mW with λ = 488 nm
Red laser: λ = 635 nm

This device has six detectors, which are calibrated for different wavelengths. The detectors are designed for forward scatter (FSC) and sideward scatter (SSC). Additional detectors for PE- and FITC-fluorescence, and for PerCP- and APC-fluorescence were installed. The fluorescence channels are called FL1 to FL4. Specifically, the used detectors and measured wavelengths are as follows:
FSC: Solid-state silicon detector
SSC: Photomultiplier tube (PMT)
FL1: Photomultiplier tube (PMT) for λ = 530 nm (FITC)
FL2: Photomultiplier tube (PMT) for λ = 585 nm (PE/PI)
FL3: Photomultiplier tube (PMT) for λ > 670 nm (PerCP)
FL4: Photomultiplier tube (PMT) for λ = 661 nm (APC)

3.3.5 MRI analysis and follow-up

To evaluate the recovery of myocardial function, subjects received two cardiac MRI examinations. The baseline evaluation was carried out during initial hospitalisation, at a median of three days after the acute event and under conditions of cardiorespiratory stability. The follow-up evaluation was carried out six months later. Both of these examinations were conducted using thoracic MRI.

For imaging, patients were placed in a supine position while breath was held in expiration. Imaging was ECG triggered. For acquiring images, the steady-state free precession technique was used. For evaluation of the LVEF, various axes were chosen. These axes were oblique images through the left ventricle, resulting in a four-chamber view, a two-chamber view, and a short axis scout. The LVEF was calculated with analysis software, in which the contours of the endocardium in end diastole and end systole, respectively, had to be drawn manually.

3.3.6 Statistical analysis

For the statistical analysis, SPSS statistics, version 24.0.0.1, from the IBM Corporation in Armonk, USA was used.

To determine the distribution of the samples, the data set was analysed with a graphical evaluation via histogram and box-plot.

The next step was to conduct a bivariate analysis: Since linear correlation requires linearity, a scatter plot was created for optical evaluation. Under conditions that the values were normally distributed, without any outliers, and that there was a linear correlation between the two samples, Pearson's correlation coefficient was calculated. Under conditions that the normal distribution had to be rejected or outliers were detected, Spearman's rank correlation coefficient was calculated.

The level of significance was determined as $p < 0.05$.

4 Results

A total of 87 patients were included in this study [1]. The mean fluorescence intensity (MFI) was measured in TMRE dyed platelets, Annexin V dyed platelets, CXCR4 dyed platelets, and CXCR7 dyed platelets. Platelets were sampled on the first day after the ST-segment elevation myocardial infarction (STEMI). In addition, the left ventricular ejection fraction (LVEF) was surveyed within a median of three days after the STEMI during hospital stay as a baseline, and six months later as a follow-up [1]. The LVEF was surveyed using thoracic MRI and under conditions of cardiorespiratory stability.

Since the values of the LVEF were evaluated twice and at two different dates in the same subjects, they were treated as related samples. The results were ratio scaled and thus different calculations, including division, were possible. The MFI results of the fluorescence-activated cell sorter (FACS) were ratio scaled as well.

The tetramethylrhodamine ethyl ester (TMRE) dyed platelets and Annexin V dyed platelets were both evaluated under resting conditions and under fully activated conditions simultaneously. **Table 6** presents a list and explanations for every calculation which was used for the final analysis. A similar approach was followed with the results of the LVEF evaluation. The precise approach of conducted calculations is also presented in **Table 6**.

Table 6: Values calculated. Explanations of the henceforth used terms of the results.

Value	Explanation/Calculation
TMRE MFI resting	MFI of TMRE dyed platelets under resting conditions
TMRE MFI thrombin	MFI of TMRE dyed platelets thrombin stimulated
Ratio platelet TMRE MFI resting/thrombin stimulated	$\frac{[\text{TMRE MFI resting}]}{[\text{TMRE MFI thrombin}]}$
Annexin V MFI resting	MFI of Annexin V dyed platelets under resting conditions
Annexin V MFI thrombin	MFI of Annexin V dyed platelets thrombin stimulated
Ratio platelet Annexin V MFI resting/thrombin stimulated	$\frac{[\text{Annexin V MFI resting}]}{[\text{Annexin V MFI thrombin}]}$
LVEF baseline	LVEF (%) evaluated immediately after event
LVEF follow-up	LVEF (%) evaluated six months after event
Ratio LVEF follow-up/baseline	$\frac{[\text{LVEF follow-up}]}{[\text{LVEF baseline}]}$
Platelet CXCR4 MFI	MFI of CXCR4 dyed platelets
Platelet CXCR7 MFI	MFI of CXCR7 dyed platelets

4.1 Descriptive statistics

4.1.1 Dropouts

There were manifold reasons for dropouts in this study. The most limiting factor was attendance at evaluation of the LVEF with thoracic MRI. Initially, 87 subjects had been recruited [1]. During the course of this study, 35.6% of the subjects rejected the first thoracic MRI evaluation and thus 56 subjects remained in the sample [1]. The second evaluation six months later was rejected by 37.5% of the remaining subjects [1]. Eventually, 35 subjects attended evaluation of the LVEF twice [1]. Comparing the baseline characteristics of these three groups, no structural difference was found [1]. In most cases, the reason for rejection of MRI evaluation was lacking motivation to participate in this study. Thus, the missing data were treated as missing at random.

Experimental failure occurred completely without regularity and was independent of the subject population. Eventually, all dropouts were regarded as part of the

overall collective and the reasons for dropout were coincidental [1]. Therefore, it did not harm the statistical analysis.

4.1.2 Evaluated results over time

4.1.2.1 Left ventricular ejection fraction

To quantify the alteration of cardiac function, MRI-based evaluation of the LVEF was conducted within a median of three days after the event as a baseline and six months later as a follow-up [1]. The images of one subject are depicted in **Figure 6**.

In 35 subjects, cardiac function could be evaluated twice [1]. The average LVEF at baseline was 53.09%, with a standard deviation of 9.12% and a maximum span between 38% and 71% [1]. The average LVEF at follow-up was 58.28%, with a standard deviation of 10.36% and a maximum span between 34% and 80% [1]. Both the baseline and follow-up LVEFs were assumed to be normally distributed.

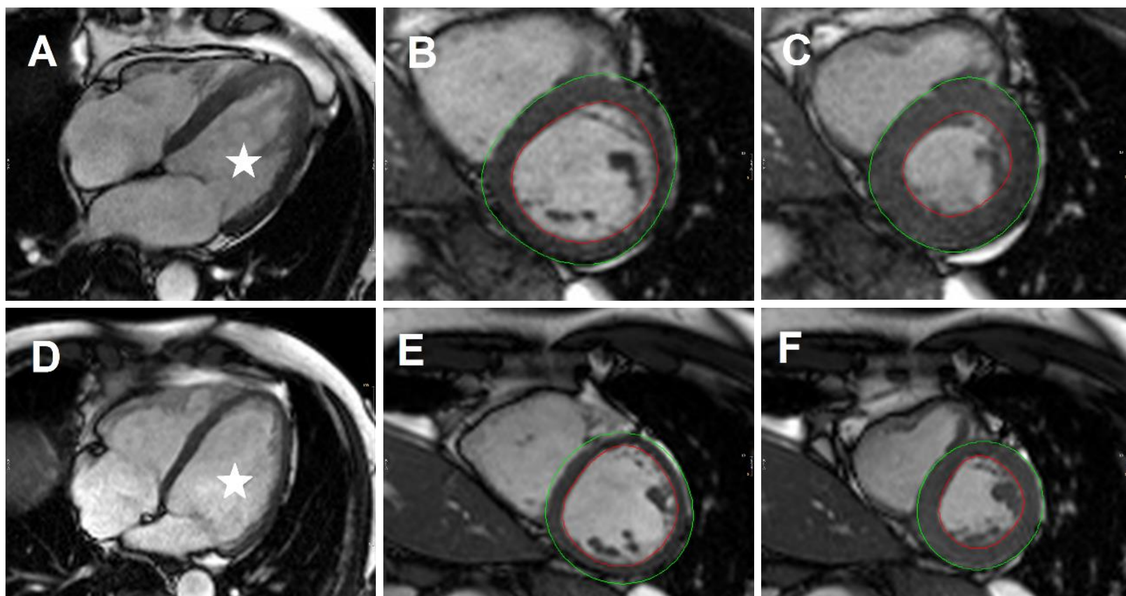

Figure 6: Measurement of LVEF baseline and follow-up. These images illustrate a typical result of the cardiac MRI. Here, imaging was conducted on a 58-year-old male patient with 52% LVEF at baseline and 58% LVEF at follow-up. **A – C:** Imaging at baseline; **D – F:** Imaging at follow-up; Star: left ventricular; green circle: pericardium of left ventricle; red circle: endocardium of left ventricle; **A & D:** four-chamber view; **B & E:** Short axis ventricle view, end diastolic in a middle layer of imaging; **C & F:** Short axis ventricle view, end systolic in a middle layer of imaging. These pictures were kindly provided by Patrick Krumm, M.D. from the Department of Radiology at the University Hospital Tübingen. White stars and letters were inserted for better explanation. For anonymization, the upper and lower edges of the picture were deleted [1].

4.1.2.2 Tetramethylrhodamine ethyl ester

To illustrate a typical result of the FACS analysis, the results of resting platelets incubated with TMRE are presented in **Figure 7**. The fluorescence intensity of each cell in a sample was measured with FACS. The output value for each sample was the mean fluorescence intensity (MFI). **Table 7** presents the average results of platelet TMRE MFI.

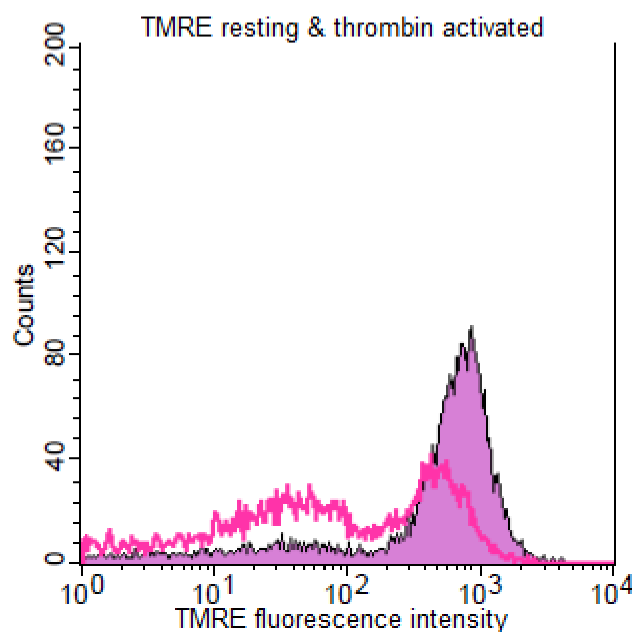

Figure 7: Fluorescence intensity of TMRE dyed platelets. The histogram indicates fluorescence intensity of TMRE dyed platelets. Graph with bright purple background illustrates fluorescence intensity of resting platelets and the dark purple line illustrates fluorescence intensity of thrombin-activated platelets. Both plots present the results sampled simultaneously in one patient.
In each plot, 10,000 events were counted. The intensity of fluorescence was detected with the second fluorescence channel of the photomultiplier tubes, calibrated for λ = 585 nm. The mean fluorescence intensity (MFI) in this exemplary measurement was, for **resting platelets:** 651.80 and for **thrombin activated platelets:** 218.78 [1].

Table 7: Results of TMRE dyed platelets. n: number of subjects; ±: standard deviation; IQR: interquartile range; MFI: mean fluorescence intensity; In TMRE dyed resting platelets normal distribution was assumed, but it had to be rejected both in TMRE dyed thrombin-activated platelets, and in the calculated ratio of resting and thrombin-activated platelets. The results of TMRE MFI resting are depicted with mean and standard deviations. Both the results of the thrombin dyed platelets and the ratio between the resting and the thrombin-activated platelets are depicted with the median and interquartile range [1].

	n	Results
TMRE MFI resting	47	652.26 ± 166.36
TMRE MFI thrombin stimulated	47	176.58 IQR 181.73
Ratio platelet TMRE MFI resting/thrombin stimulated	41	3.51 IQR 2.99

4.1.2.3 Annexin V

To illustrate a typical result of the FACS analysis, the results of the resting platelets incubated with Annexin V are presented in **Figure 8**. The fluorescence intensity of each cell in the sample was measured with FACS. The output values for each sample was the MFI. **Table 8** presents the average results of platelet Annexin V MFI.

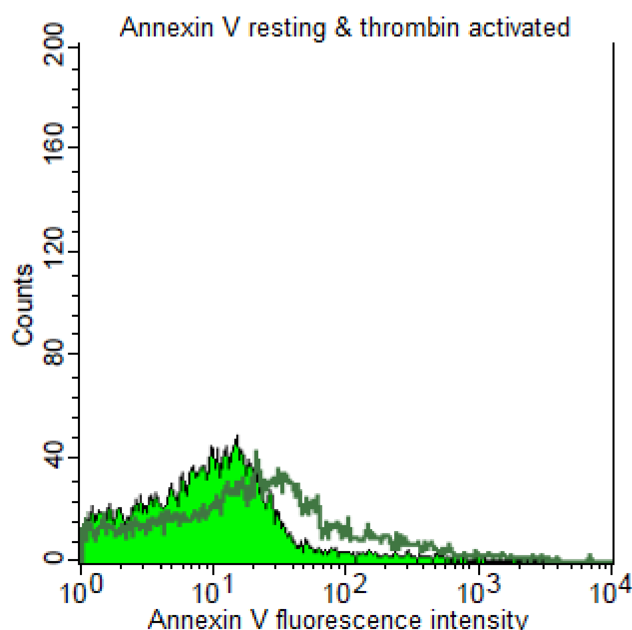

Figure 8: Fluorescence intensity of Annexin V dyed platelets. The histogram indicates the fluorescence intensity of Annexin V dyed platelets. The graph with bright green background indicates the fluorescence intensity of resting platelets and the dark green line indicates the fluorescence intensity of thrombin-activated platelets. Both plots illustrate the results sampled simultaneously in one patient.
In each plot, 10,000 events were counted. The intensity of fluorescence was detected with the first fluorescence channel of the photomultiplier tubes, calibrated for λ = 530 nm. The mean fluorescence intensity (MFI) in this exemplary measurement was, for **resting platelets:** 14.40 and for **thrombin-activated platelets:** 54.49 [1].

Table 8: Results of Annexin V dyed platelets. n: number of subjects; IQR: interquartile range; MFI: mean fluorescence intensity; none of the tested values of Annexin V dyed platelets was assumed as normally distributed. The results are depicted with the median and the interquartile range [1].

	n	Results
Annexin V resting (MFI)	59	12.69 IQR 7.09
Annexin V thrombin (MFI)	43	40.18 IQR 59.36
Ratio platelet Annexin V MFI resting/thrombin stimulated	43	0.35 IQR 0.45

4.1.2.4 Chemokine receptors

Parallel with TMRE and Annexin V, the expression of CXCR4 and CXCR7 receptors was measured. The platelets were dyed with fluorescent agents, specifically for these receptors. To illustrate a typical result of the FACS analysis, the results are illustrated in **Figure 9**. The average results of detected MFI are depicted in **Table 9**.

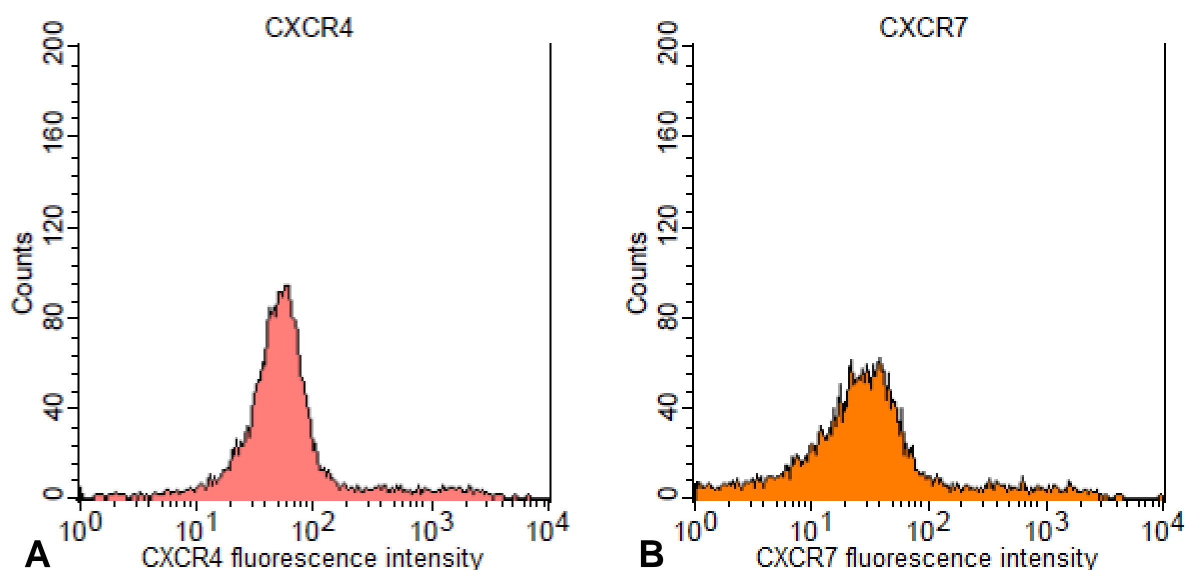

Figure 9: Fluorescence intensity of CXCR4 and CXCR7 dyed platelets. The histograms indicate the fluorescence intensity of CXCR4 and CXCR7 dyed platelets, respectively.
In each plot, 10,000 events were counted. The intensity of fluorescence was detected with the second fluorescence channel of the photomultiplier tubes, calibrated for λ = 585 nm. The mean fluorescence intensity (MFI) in this exemplary measurement was, for **A:** 97.22 and for **B:** 75.14 [1].

Table 9: Chemokine receptor results of the FACS analysis. n: number of subjects; ±: standard deviation; MFI: mean fluorescence intensity; all results were assumed to be normally distributed. The results are depicted with mean and standard deviations [1].

	n	Results
CXCR4 MFI	50	68.38 ± 21.56
CXCR7 MFI	53	56.08 ± 21.70

4.2 Exploratory data analysis

4.2.1 Association of chemokines and apoptosis

Bivariate analysis was conducted with the results of platelet chemokines with platelet apoptosis. The results are depicted in **Table 10**. The values of pure resting platelets dyed with apoptosis signalling agents revealed no significant correlation, but the ratio of resting and thrombin-stimulated platelets indicated a positive correlation for survival markers (TMRE), both with CXCR4 and CXCR7 [1]. For better presentation, the results are depicted in **Figure 10**. The ratio of resting and thrombin-stimulated platelets indicated a negative correlation for markers of apoptosis (Annexin V), both with CXCR4 and CXCR7 [1]. The results are depicted in **Figure 11**.

Table 10: Bivariate correlation of parameters of apoptosis with chemokines. MFI: mean fluorescence intensity; n: number of subjects; r: Pearson's correlation coefficient; ρ: Spearman's rank correlation coefficient; p: level of significance [1].

	Platelet CXCR4 MFI			**Platelet CXCR7 MFI**		
	Correlation	n	p	Correlation	n	p
Platelet TMRE MFI resting	r = -0.052	44	0.738	r = -0.216	45	0.154
Platelet Annexin V MFI resting	ρ = 0.121	60	0.121	ρ = 0.083	62	0.521
Ratio platelet TMRE MFI resting/thrombin stimulated	ρ = 0.365	41	0.019	ρ = 0.417	42	0.006
Ratio platelet Annexin V MFI resting/thrombin stimulated	ρ = -0.273	43	0.076	ρ = -0.401	45	0.006

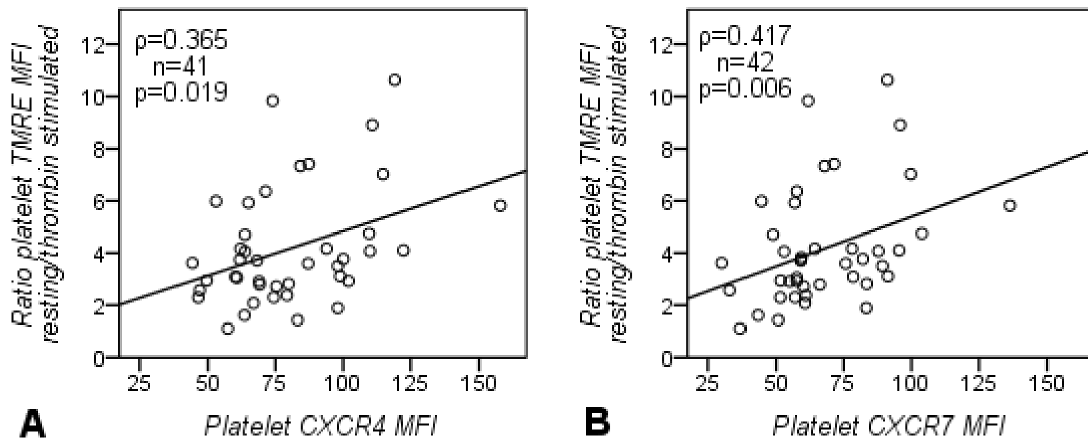

Figure 10: Association of the ratio of platelet TMRE MFI resting/thrombin stimulated with chemokine receptors. ρ: Spearman's rank correlation coefficient; n: number of subjects; p: level of significance; MFI: mean fluorescence intensity. **A:** with platelet CXCR4 MFI. **B:** with platelet CXCR7 MFI [1].

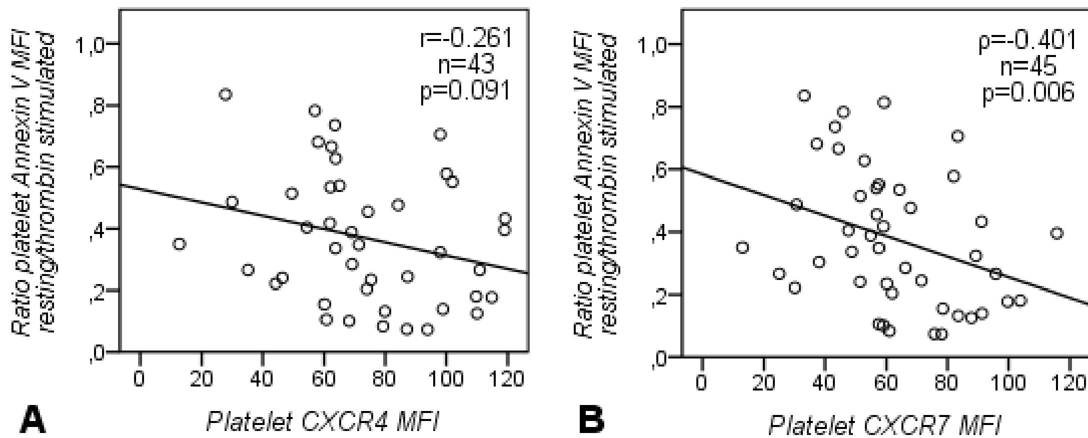

Figure 11: Association of the ratio of platelet Annexin V MFI resting/thrombin stimulated with chemokine receptors. r: Pearson's correlation coefficient; ρ: Spearman's rank correlation coefficient; n: number of subjects; p: level of significance; MFI: mean fluorescence intensity. **A:** with platelet CXCR4 MFI. **B:** with platelet CXCR7 MFI [1].

4.2.2 Association of LVEF and apoptosis

Bivariate analysis was conducted with the results of platelet apoptosis with the LVEF. It was tested whether there is an association between calculated apoptosis values on the one hand, and the LVEF at baseline or the ratio of the LVEF follow-up and the baseline on the other hand. The results are depicted in **Table 11**.

Table 11: Bivariate correlation of parameters of apoptosis with the LVEF. MFI: mean fluorescence intensity; n: number of subjects; r: Pearson's correlation coefficient; ρ: Spearman's rank correlation coefficient; p: level of significance [1].

	LVEF baseline			**Ratio LVEF follow-up/baseline**		
	Correlation	n	p	Correlation	n	p
Platelet TMRE MFI resting	r = -0.228	40	0.158	r = 0.006	28	0.976
Platelet Annexin V MFI resting	ρ = -0.293	52	0.030	ρ = -0.367	32	0.039
Ratio platelet TMRE MFI resting/thrombin stimulated	ρ = -0.188	36	0.273	ρ = 0.490	25	0.013
Ratio platelet Annexin V MFI resting/thrombin stimulated	ρ = -0.243	39	0.137	ρ = -0.028	25	0.895

The ratio of platelet TMRE MFI between resting and thrombin-stimulated platelets indicated a positive correlation with the ratio between follow-up and baseline of the LVEF. The results are depicted in **Figure 12**. There was no correlation between the ratio of the LVEF and the ratio of platelet Annexin V MFI resting/thrombin [1].

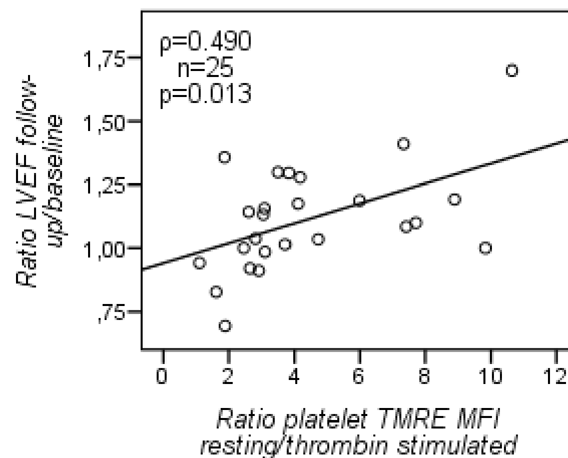

Figure 12: Association of the ratio of platelet TMRE MFI resting/thrombin stimulated with ratio of LVEF follow-up/baseline. ρ: Spearman's rank correlation coefficient; n: number of subjects; p: level of significance; MFI: mean fluorescence intensity [1].

There was significant correlation between the Annexin V MFI of resting platelets, both with the LVEF (%) at baseline and with the ratio between the follow-up and baseline LVEF as well [1]. The results are depicted in **Figure 13**.

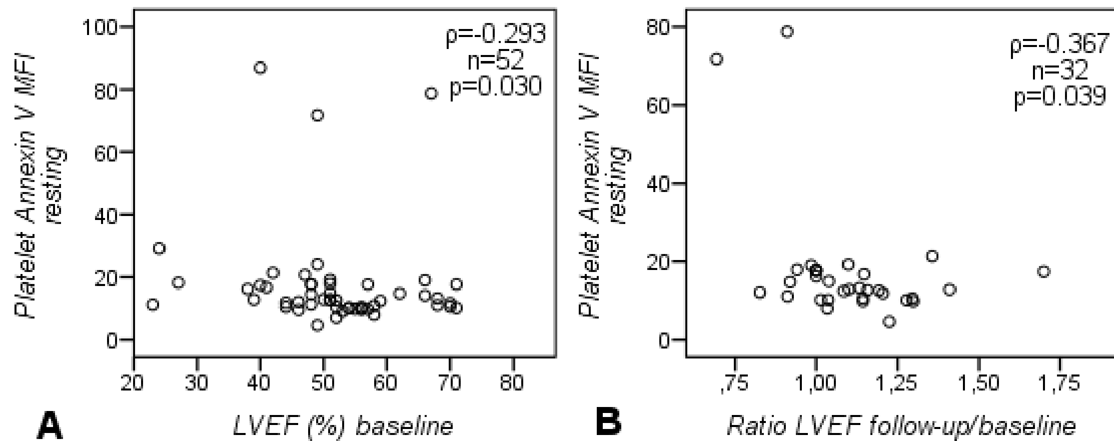

Figure 13: Association of platelet Annexin V MFI resting with LVEF. ρ: Spearman's rank correlation coefficient; n: number of subjects; p: level of significance; MFI: mean fluorescence intensity. **A:** Association with the LVEF at baseline. **B:** Association with the ratio between the follow-up and baseline of the LVEF [1].

5 Discussion

The major findings of this study are that platelets exhibit an expression of CXCR4 and CXCR7 after a STEMI. Chemokine receptors indicate no association with platelets, either dyed with TMRE or Annexin V [1]. However, the ratio between resting and thrombin-stimulated TMRE dyed platelets indicates a positive association with CXCR4 and CXCR7 [1]. Equally, the calculated ratio between resting and thrombin-stimulated Annexin V dyed platelets indicate a negative association with CXCR4 and CXCR7 [1].

Moreover, the ratio between resting and thrombin-stimulated TMRE dyed platelets exhibits a positive correlation with the six-month alteration of the LVEF, calculated as the ratio between the LVEF follow-up and baseline [1]. Pure resting platelets dyed with Annexin V or TMRE indicate no association to the LVEF or its recovery [1].

5.1 Methods

5.1.1 Patient collective

During the period of this study, nearly every patient with the diagnosis of a STEMI was interrogated to participate in this study and gave approval. Concerning baseline characteristics, we could not find any structural differences between dropouts and participants. Since dropouts only happened by coincidence, and abandonment or continuation revealed no regularity, this patient collective could be an approximate population of STEMI patients. Despite the small number of patients, the results of this study might have a high level of validity. A similar

approach concerning patient collective with a high quality was adopted elsewhere [285].

5.1.2 Evaluation of cardiac function using cardiac MRI

The LVEF is an established indicator of cardiac function after a STEMI and has a high predictive value concerning prognosis [129, 130]. Magnetic resonance imaging as a tool to evaluate cardiac function has one great disadvantage, which is poor approval by the subjects. Rejection during the course of this study caused a high percentage of dropouts. Conducting evaluation with transthoracic echocardiography is much easier, less expensive and has a higher rate of approval by the subjects. However, the quality of the results obtained in this manner is highly dependent on the expertise of the examiner. By comparison, imaging of the systolic function with cardiac MRI is of a very high quality and is accepted as the gold standard [286]. In the overall evaluation of the LVEF, cardiac MRI provides the best image quality and is the preferred technique [287]. In addition, it generates more supplementary information. For example, MRI gives exact information about the infarction size. As a consequence, a decision was made in favour of high-quality results instead of a larger sample size, and therefore cardiac MRI was chosen for the LVEF evaluation.

As an imaging technique, steady-state free precession imaging was used. This is commonly regarded as the superior technique for the generation of pictures with the highest quality [288, 289]. A similar approach has already been adopted successfully in other studies [166].

5.1.3 FACS analysis

With regard to flow cytometry, a well-established technique for the analysis of samples was used. In the laboratories of the University of Tübingen, Department of Cardiology (Department of Internal Medicine III), FACS is used frequently and there is a high level of expertise. For every single measurement, the same pattern

of settings was used. The FACS machine was cleaned properly after every use. In cases of contamination or failed dye, the measurement was repeated.

To summarize, the experimental setup concerning the FACS analysis guarantees a very high quality of data with high explanatory power and a low susceptibility to errors.

5.1.4 Agents

5.1.4.1 CXCR4, CXCR7 and CD42b

At the University of Tübingen, Department of Cardiology (Department of Internal Medicine III), usage of these agents to detect chemokine receptors on the platelet surface is well-established [167, 290]. CD42b, which is also called GPIb, is part of the GPIb/IX complex and thus part of the receptor for von Willlebrand factor [291]. Therefore, it might be an ideal target to detect platelets. The FACS calibration to obtain these results was similar to that of other studies [167].

5.1.4.2 Thrombin

The use of thrombin for the stimulation of platelets exhibits one disadvantage. Thrombin is commonly known as a potent platelet activator, called activation factor IIa [292, 293]. However, recent studies have revealed that thrombin is also capable of inducing platelet apoptosis [292, 294]. It can be detected both in the dropping of $\Delta\Psi_m$ and the exposure of PS [294]. Causing platelet activation or platelet apoptosis it is dependent on the concentration of thrombin. A low thrombin concentration leads to the activation of almost all platelets, whereas only a small percentage undergoes apoptosis [292, 293]. A high thrombin concentration leads to a high degree of apoptosis [292, 293]. To avoid this problem, in this study a high dose of thrombin was chosen. The purpose of the usage of thrombin was to calculate the ratio between unstimulated platelets and platelets under maximal stimulation.

5.1.4.3 TMRE

The lipophilic cation tetramethylrhodamine ethyl ester (TMRE) exhibits the maximum fluorescence as long as it can accumulate in mitochondria with intact membrane potential ($\Delta\Psi_m$) [295]. During apoptosis and the subsequent dropping of $\Delta\Psi_m$, TMRE externalizes and disperses in cytosol, thus reducing its fluorescence [295]. With the extent of fluorescence measured in the FACS analysis, the extent of the survival of apoptotic cells can be approximated, but this needs careful interpretation [296].

The TMRE MFI is not a direct value for the number of intact mitochondria. The more negative the mitochondrial $\Delta\Psi_m$ is, the more TMRE will accumulate and will be detected [296]. This value is dependent of the individual conditions of the subject's platelets and mitochondria. For this reason, it is recommended to conduct a complementary approach, which can be set in relation to the TMRE dye [296]. For this purpose, thrombin as a platelet-stimulating agent was chosen. According to former studies, it can be assumed that with the used dose of thrombin nearly all platelets undergo apoptosis [292].

The results were related to each other. The TMRE MFI of resting platelets represents the actual survival of the dye. This was divided by the MFI of TMRE dyed and thrombin-stimulated platelets, which represents the remaining fluorescence after maximum apoptosis and thus minimum possible survival. Consequently, a high ratio could represent an elevated relative survival of platelets, whereas a low ratio could represent a reduced relative survival of platelets.

5.1.4.4 Annexin V

Annexin V is well established in measuring apoptotic cells. In the presence of a physiological calcium concentration, it binds to expressed phosphatidyl serine (PS) [297]. Phosphatidyl serine is naturally located at the inner leaflet of the cell membrane [182, 276]. The process of flipping from the inner cell membrane to the outer membrane is called externalization, which is dependent on the release

of cytochrome c [182, 201, 276, 298, 299]. Cytochrome c release only occurs in apoptotic cells and is regarded as a general feature of this process [300]. After externalization, PS serves as a target for macrophages and the subsequent removal of apoptotic cells [183, 184, 298]. Consequently, a high MFI of Annexin V can be interpreted as a high level of platelets undergoing apoptosis.

This mechanism is similar in mammalian cell types [297]. Annexin V is a good and established tool to detect apoptotic cells in general. However, in the specific case of platelets, cytochrome c release is not the only mechanism leading to externalization of PS [8]. In platelets, PS is also exposed after activation, serving as a target for factors VIIIa, IXa and Ca^{2+} to form the tenase complex [8, 301]. The formation of the tenase complex leads to increased procoagulant activity of the platelets, which is an important step in platelet activation [298, 302]. This molecular overlap between activation and apoptosis is currently regarded as a process of activation-driven enhanced platelet senescence, with the purpose of the removal of thrombotic plaques after tissue healing [280].

One particular difference in the mechanisms of PS exposure is their dependence on Ca^{2+} [8, 276]. Apoptosis-driven PS exposure is dependent on the attendance of BAX or BAK and unaffected by the absence of Ca^{2+}, whereas activation-driven PS exposure needs extracellular Ca^{2+} and can be inhibited by Ca^{2+}-absorbing chelating agents [8, 259, 276]. In any case, with the used PBS-Ca^{2+} for dilution, the environment is already enriched with Ca^{2+}. Since Annexin V requires extracellular Ca^{2+} to detect PS, a calcium-rich environment is inevitable [297, 303]. In addition, Ca^{2+} is released by the platelets themselves [55]. For this reason, with PS exposure a definitive assertion whether the platelet is undergoing apoptosis or activation might be difficult [8]. Subsequently, a parallel detection of apoptosis and platelet activation is extremely likely, and an assessment of the measured MFI of Annexin V dyed platelets as apoptosis might not be possible. Rather, the results could represent a mixture of activation and apoptosis.
To adopt an approach comparable with TMRE evaluation, MFI of pure resting platelets dyed with Annexin V was set into relation with the Annexin V MFI of

thrombin-stimulated platelets. Following from the premise of an appropriate dose of thrombin, which leads to maximal apoptosis, the result of this ratio can roughly be interpreted as a percentage of the maximum apoptosis. Of course, it might be difficult to distinguish between Annexin V detected platelet activation and apoptosis, but at least in the thrombin-stimulated samples it can be expected that nearly all platelets are undergoing apoptosis. Thus, the ratio might represent the proportion of activated and apoptotic platelets to the maximum of the apoptotic platelets.

5.2 Results

5.2.1 Platelet apoptosis with chemokines

In this study, it has been demonstrated that TMRE dyed resting platelets indicate no correlation with CXCR4 or CXCR7 [1]. This is in accordance with the knowledge that the pure value of TMRE gives no hint of the actual survival of platelets, and that a reference value is needed [296]. Annexin V, which was chosen to measure platelet apoptosis, and thus to have an opposite approach to platelet survival, did not reveal any correlation with CXCR4 or CXCR7 [1]. Due to the presentation of PS in platelet activation and apoptosis as well, this result is not surprising [8, 183, 184, 298, 300, 301].

After having set the values into relation, significant results can be detected. It could be illustrated that the calculated TMRE ratio has a positive correlation with the expression of CXCR4 ($\rho = 0.365$, $n = 41$, $p = 0.019$) and CXCR7 ($\rho = 0.417$, $n = 42$, $p = 0.006$), respectively [1]. Inversely, the Annexin V ratio exhibits a negative association with these chemokine receptors ($\rho = -0.273$, $n = 43$, $p = 0.076$ for CXCR4 and $\rho = -0.401$, $n = 45$, $p = 0.006$ for CXCR7, respectively) [1]. The TMRE ratio, which represents platelet survival, has a positive correlation with chemokine receptors. The Annexin V ratio partially represents platelet apoptosis and has a negative correlation with chemokine receptors. These contrary results

are expected and can be interpreted as indicating the same result revealed with different tools [8, 292, 296, 297].

Here, platelet expression of CXCR4 and CXCR7 has a strong positive correlation with platelet survival (and, as a reverse argument, a strong negative correlation with platelet apoptosis) [1]. As a conclusion, platelet survival might be associated with platelet surface expression of CXCR4 and CXCR7 after a STEMI.

Both CXCR4 and CXCR7 are expressed permanently in every platelet, whilst the expression of CXCR4 is slightly higher [160]. After platelet activation and SDF-1 release, SDF-1-CXCR4 interaction leads to the homing of bone-marrow-derived cells to the place of the infarction, with the purpose of regeneration and further chemotactic recruitment [158, 163]. Upon SDF-1-CXCR4 interaction, expression of CXCR4 is decreased and expression of CXCR7 is increased [160]. The precise function of CXCR7 has not been revealed yet, but it could be demonstrated that the expression of CXCR7 is increased in mesenchymal stem cells under hypoxic conditions [160-162, 304]. It is also known that activated platelets have an influence on the regulation of apoptosis and the survival in surrounding cells via paracrine mechanisms [6, 305]. For example, SDF-1-driven CXCR4 suppresses apoptosis in bone marrow stromal cells [305, 306]. Subsequently, SDF-1 has been detected as a promoter of platelet survival [281, 290]. It could be demonstrated that the interaction of CXCR7 with the macrophage migration inhibitory factor, which is a chemokine-like ligand and released after platelet activation, increases the survival of platelets [280, 307, 308]. To sum up, with these results, it might be assumed that the influence on apoptotic processes is not only limited to surrounding cells, but acts on platelets too. At least the measured correlation provides evidence to suggest that there might be a crosstalk between chemokine expression and platelet survival. This assumption could indicate an approach for further studies.

The TMRE ratio has a slightly higher correlation coefficient with CXCR7 than with CXCR4 [1]. Similarly, the correlation coefficient of the Annexin V ratio with

CXCR7 is considerable higher than with CXCR4 [1]. The difference in the correlation coefficients between CXCR4 and CXCR7 could be in accordance with a result from a former study conducted on renal progenitor cells [304]. This study revealed that not the expression of CXCR4, but rather the expression of CXCR7 is responsible for the survival of cells after SDF-1 induction [304]. In addition, it could be demonstrated that patients with ACS, compared with healthy subjects, exhibit increased platelet levels of CXCR7, but not of CXCR4 [160, 162]. These studies provide no evidence as to whether patients with ACS do exhibit higher levels of platelet survival as well [160, 162]. In this particular study, no assertion regarding this effect is possible, but it might be an interesting avenue for further studies.

It is also probable that the difference in correlation coefficients could have no meaning. The difference between the correlation coefficients of CXCR4 and CXCR7 each with the TMRE ratio is only slight [1]. Since both of these exhibit a strong positive correlation with either chemokine receptors, there is strong evidence that there is an association. To determine a possible difference, the sample size is too small. In further studies, a greater patient collective should be evaluated concerning this feature.

A considerable difference only occurs in the Annexin V ratio. This could be caused by the overlap of Annexin V as an indicator both for apoptosis and platelet activation. Perhaps in future studies, using a more appropriate test construction, this problem could be solved. In the meantime, the conclusion is that Annexin V is not an ideal marker to detect apoptosis in platelets.

5.2.2 Association of LVEF and apoptosis

The TMRE dyed platelets under resting conditions do not indicate any correlation either with the baseline LVEF or with the ratio between the follow-up and baseline LVEF [1]. These results are conclusive, according to the depicted limitations of TMRE under resting conditions [296]. Conversely, the Annexin V dyed platelets

under resting conditions indicated a significant correlation coefficient, each with the baseline LVEF ($\rho = -0.293$, $n = 52$, $p = 0.030$), and the LVEF ratio between the follow-up and the baseline ($\rho = -0.367$, $n = 32$, $p = 0.039$) [1]. However, the graphic examination of these results in **Figure 13** reveals that there is actually only a very weak correlation [1]. Thus, there might not be any association between the Annexin V dyed platelets under resting conditions, neither with the baseline LVEF nor with the ratio of the LVEF. These results are in accordance with the limitations of Annexin V under resting conditions as well [8, 183, 184, 298, 300, 301]. With $\rho = 0.490$, $n = 25$, $p = 0.013$, the TMRE ratio indicates a strong and significant correlation with the LVEF ratio between the follow-up and the baseline, whereas the ratio of Annexin V exhibits no correlation [1]. Annexin V has the same limitations as depicted above [8, 183, 184, 298, 300, 301]. The findings on TMRE suggest that platelet survival might be associated with left ventricular functional recovery after a STEMI [1].

It has previously been demonstrated that interaction with CXCR4, driven by SDF-1, leads to the chemotactic recruitment of bone-marrow-derived cells and other cells with the purpose of regeneration [6, 158, 163]. A study in ACS subjects has revealed that elevated levels of platelet CXCR7 are associated with an amelioration of the LVEF [162]. A study in patients with CAD has revealed that patients with low platelet CXCR4 at baseline demonstrate poor overall mortality [167]. In addition, it has been demonstrated that the interaction of SDF-1 with CXCR4 and CXCR7 promotes platelet survival [281, 290]. Since the results from this current study suggest a crosstalk between platelet survival and the expression either of CXCR4 or CXCR7, the recognition that elevated survival levels are associated with a good prognosis might be a conclusive deduction.

6 Zusammenfassung

Hintergrund: Eine hohe Expression der thrombozytärer Chemokinrezeptoren CXCR4 und CXCR7 ist bei Patienten mit Koronarer Herzkrankheit mit gesteigerter myokardialer Reparatur und reduzierter Langzeitmortalität verbunden. Gleichzeitig verlängert sich das Überleben der normalerweise nach Aktivierung in Apoptose übergehenden Thrombozyten nach Stimulierung thrombozytärer CXCR7-Rezeptoren. Gegenstand dieser Studie ist die Frage, ob in einem Kollektiv aus STEMI-Patienten das thrombozytäre Überleben auch mit der Expression der thrombozytären Chemokin-Rezeptoren CXCR4 und CXCR7 assoziiert ist und ob eine Beziehung zwischen thrombozytärem Überleben mit der funktionalen Erholung der LV-Funktion besteht.

Methoden: Die Studie wurde an einem Kollektiv von 87 Patienten mit der akuten Diagnose eines ST-Hebungsinfarktes (STEMI) direkt nach erfolgter perkutaner Koronarintervention durchgeführt. Die Messung der linksventrikulären Ejektionsfraktion (LVEF) wurde mittels MRT während des Krankenhausaufenthalts und 6 Monate später durchgeführt. Mittels Durchflusszytometrie wurde die mittlere Fluoreszenz-Intensität (MFI) der thrombozytären Oberflächenrezeptoren CXCR4 und CXCR7 bestimmt. Außerdem erfolgte mit dieser Technik die Messung der thrombozytären Apoptose mittels Annexin V, sowie des thrombozytären Überlebens mittels TMRE zum einen nach erfolgter Stimulation mittels Thrombin zum anderen ohne Thrombin.

Ergebnisse: Ohne Stimulierung mittels Thrombin zeigt sich kein Zusammenhang zwischen der thrombozytären Apoptose mit der Expression der Chemokinrezeptoren oder der funktionalen Erholung der LV-Funktion.

Das Verhältnis der MFI von Thrombin-stimulierten zu unstimulierten Annexin V gefärbten Thrombozyten korreliert negativ mit der Expression von CXCR4 (ρ = -0.273, n = 43, p = 0.076) und CXCR7 (ρ = -0.401, n = 45, p =0.006). Analog reagiert das entsprechende Verhältnis TMRE gefärbter Thrombozyten positiv mit CXCR4 (ρ = 0.365, n = 41, p = 0.019) und CXCR7 (ρ = 0.417, n = 42, p = 0.006). Außerdem besteht eine positive Korrelation zwischen dem Verhältnis aus stimulierten und unstimulierten TMRE gefärbten Thrombozyten mit der Verbesserung der LVEF von der initialen Messung zur LVEF nach 6 Monaten (ρ = 0.490, n = 25, p = 0.013).

Schlussfolgerung: Mit dieser Studie konnte gezeigt werden, dass eine hohe thrombozytäre Expression sowohl von CXCR4 als auch CXCR7 mit einem gesteigerten Überleben der Thrombozyten nach STEMI einhergehen. Außerdem konnte gezeigt werden, dass ein gesteigertes thrombozytäres Überleben unmittelbar nach STEMI mit einer gesteigerten Erholung der linksventrikulären Funktion nach 6 Monaten einhergeht. Der Zusammenhang mit der Expression von CXCR4 und CXCR7 kann dabei einen Einfluss haben, eine andere Ursache ist mit dieser Studie jedoch nicht auszuschließen.

7 References

1. Rath D, Chatterjee M, Meyer CL, Tekath N, Krumm P, Adams C, et al. Relative survival potential of platelets is associated with platelet CXCR4/CXCR7 surface expression and modulates clinical outcome following STEMI. Thrombosis and haemostasis. 2018.
2. Sterbefälle insgesamt 2013 nach den 10 häufigsten Todesursachen der ICD-10: Statistisches Bundesamt Deutschland; [30.03.2014]. Available from: https://www.destatis.de/DE/ZahlenFakten/GesellschaftStaat/Gesundheit/Todesursachen/Tabellen/SterbefaelleInsgesamt.html.
3. Olin JW, Sealove BA. Peripheral Artery Disease: Current Insight Into the Disease and Its Diagnosis and Management. Mayo Clinic proceedings. 2010;85(7):678-92.
4. Klingenberg R, Hasun M, Corti R, Lüscher TF. Clinical Manifestations of Atherosclerosis. In: Wick G, Grundtman C, editors. Inflammation and Atherosclerosis. Vienna: Springer Vienna; 2012. p. 39-58.
5. Vogel S, Chatterjee M, Metzger K, Borst O, Geisler T, Seizer P, et al. Activated platelets interfere with recruitment of mesenchymal stem cells to apoptotic cardiac cells via high mobility group box 1/Toll-like receptor 4-mediated down-regulation of hepatocyte growth factor receptor MET. The Journal of biological chemistry. 2014;289(16):11068-82.
6. Gawaz M, Vogel S. Platelets in tissue repair: control of apoptosis and interactions with regenerative cells. Blood. 2013;122(15):2550-4.
7. Josefsson EC, Dowling MR, Lebois M, Kile BT. Chapter 3 - The Regulation of Platelet Life Span A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 51-65.
8. Kile BT. The role of apoptosis in megakaryocytes and platelets. British Journal of Haematology. 2014;165(2):217-26.
9. Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, et al. Programmed Anuclear Cell Death Delimits Platelet Life Span. Cell. 2007;128(6):1173-86.
10. Coller BS. Foreword - A Brief History of Ideas About Platelets in Health and Disease A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. xix-xliv.
11. Clemetson KJ, Clemetson JM. Chapter 9 - Platelet Receptors A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 169-94.
12. Flaumenhaft R. Chapter 18 - Platelet Secretion A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 343-66.
13. Latger-Cannard V, Fenneteau O, Salignac S, Lecompte TP, Schlegel N. Platelet morphology analysis. Methods in molecular biology (Clifton, NJ). 2013;992:207-25.

14. Bizzozero G. Über einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. Archiv path Anat Physiol klin Med. 1882;90:261–332.
15. White JG. Chapter 7 - Platelet Structure A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 117-44.
16. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood. 1993;81(11):2844-53.
17. Patel SR, Hartwig JH, Italiano JE. The biogenesis of platelets from megakaryocyte proplatelets. Journal of Clinical Investigation. 2005;115(12):3348-54.
18. Andrews RK, Berndt MC. Chapter 10 - The GPIb-IX-V Complex A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 195-213.
19. Italiano Jr JE, Hartwig JH. Chapter 2 - Megakaryocyte Development and Platelet Formation A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 27-49.
20. White JG. Platelet Morphology. The Circulating Platelet: Academic Press; 1971. p. 45-121.
21. White JG, Escolar G. Current concepts of platelet membrane response to surface activation. Platelets. 1993;4(4):175-89.
22. Vieira de Abreu A, Rondina MT, Weyrich AS, Zimmerman GA. Chapter 36 - Inflammation A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 733-66.
23. Weyrich AS, Zimmerman GA. Platelets: signaling cells in the immune continuum. Trends in Immunology. 2004;25(9):489-95.
24. Ware J, Jain S. Chapter 38 - Tumor Growth and Metastasis A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 803-10.
25. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. Nat Rev Cancer. 2011;11(2):123-34.
26. Lakka Klement G, Shai E, Varon D. Chapter 24 - The Role of Platelets in Angiogenesis A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 487-502.
27. Gimbrone MA, Aster RH, Cotran RS, Corkery J, Jandl JH, Folkman J. Preservation of Vascular Integrity in Organs perfused in vitro with a Platelet-rich Medium. Nature. 1969;222(5188):33-6.
28. Bledzka K, Pesho MM, Ma Y-Q, Plow EF. Chapter 12 - Integrin αIIbβ3 A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 233-48.
29. Pollitt AY, Hughes CE, Watson SP. Chapter 11 - GPVI and CLEC-2 A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 215-31.
30. Zhang P, Covic L, Kuliopulos A. Chapter 13 - Protease-Activated Receptors A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 249-59.
31. Cattaneo M. Chapter 14 - The Platelet P2 Receptors A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 261-81.

32. Kenney DM, Linck RW. The cystoskeleton of unstimulated blood platelets: structure and composition of the isolated marginal microtubular band. Journal of cell science. 1985;78:1-22.
33. Hartwig JH. Chapter 8 - The Platelet Cytoskeleton A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 145-68.
34. White JG, Krivit W. An Ultrastructural Basis for the Shape Changes Induced in Platelets by Chilling. Blood. 1967;30(5):625-35.
35. White JG. Effects of colchicine and Vinca alkaloids on human platelets. I. Influence on platelet microtubules and contractile function. Am J Pathol. 1968;53(2):281-91.
36. Escolar G, Krumwiede M, White JG. Organization of the actin cytoskeleton of resting and activated platelets in suspension. Am J Pathol. 1986;123(1):86-94.
37. White JG, Krumwiede M. Further studies of the secretory pathway in thrombin-stimulated human platelets. Blood. 1987;69(4):1196-203.
38. Sixma JJ, Slot JW, Geuze HJ. Immunocytochemical localization of platelet granule proteins. Methods in enzymology. 1989;169:301-11.
39. King SM, Reed GL. Development of platelet secretory granules. Seminars in cell & developmental biology. 2002;13(4):293-302.
40. Berger G, Masse JM, Cramer EM. Alpha-granule membrane mirrors the platelet plasma membrane and contains the glycoproteins Ib, IX, and V. Blood. 1996;87(4):1385-95.
41. Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, McRedmond JP, et al. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. Blood. 2004;103(6):2096-104.
42. Suzuki H, Murasaki K, Kodama K, Takayama H. Intracellular localization of glycoprotein VI in human platelets and its surface expression upon activation. British Journal of Haematology. 2003;121(6):904-12.
43. Cramer E, Savidge G, Vainchenker W, Berndt M, Pidard D, Caen J, et al. Alpha-granule pool of glycoprotein IIb-IIIa in normal and pathologic platelets and megakaryocytes. Blood. 1990;75(6):1220-7.
44. Reed GL, Fitzgerald ML, Polgar J. Molecular mechanisms of platelet exocytosis: insights into the "secrete" life of thrombocytes. Blood. 2000;96(10):3334-42.
45. White JG. Interaction of Membrane Systems in Blood Platelets. The American Journal of Pathology. 1972;66(2):295-312.
46. Escolar G, Leistikow E, White J. The fate of the open canalicular system in surface and suspension- activated platelets. Blood. 1989;74(6):1983-8.
47. Gerrard JM, White JG, Peterson DA. The platelet dense tubular system: its relationship to prostaglandin synthesis and calcium flux. Thrombosis and haemostasis. 1978;40(2):224-31.
48. Rumbaut RE, Thiagarajan P. Platelet Adhesion to Vascular Walls. Platelet-Vessel Wall Interactions in Hemostasis and Thrombosis. San Rafael (CA): Morgan & Claypool Life Sciences; 2010.
49. Marcus AJ, Safier LB, Broekman MJ, Islam N, Fliessbach JH, Hajjar KA, et al. Thrombosis and inflammation as multicellular processes:

significance of cell-cell interactions. Thrombosis and haemostasis. 1995;74(1):213-7.
50. Radomski MW, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet (London, England). 1987;2(8567):1057-8.
51. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature. 1976;263(5579):663-5.
52. Schwarz UR, Walter U, Eigenthaler M. Taming platelets with cyclic nucleotides. Biochem Pharmacol. 2001;62(9):1153-61.
53. Jurk K, Kehrel BE. Platelets: Physiology and Biochemistry. Seminars in thrombosis and hemostasis. 2005;31(04):381-92.
54. Marcus AJ, Broekman MJ, Drosopoulos JH, Islam N, Alyonycheva TN, Safier LB, et al. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. The Journal of clinical investigation. 1997;99(6):1351-60.
55. Brass LF, Newman DK, Wannermacher KM, Zhu L, Stalker TJ. Chapter 19 - Signal Transduction During Platelet Plug Formation A2 - Michelson, Alan D. Platelets (Third Edition): Academic Press; 2013. p. 367-98.
56. Chen J, López JA. Interactions of Platelets with Subendothelium and Endothelium. Microcirculation. 2005;12(3):235-46.
57. Sangkuhl K, Shuldiner AR, Klein TE, Altman RB. Platelet aggregation pathway. Pharmacogenetics and Genomics. 2011;21(8):516-21.
58. Mangin P, Yuan Y, Goncalves I, Eckly A, Freund M, Cazenave JP, et al. Signaling role for phospholipase C gamma 2 in platelet glycoprotein Ib alpha calcium flux and cytoskeletal reorganization. Involvement of a pathway distinct from FcR gamma chain and Fc gamma RIIA. The Journal of biological chemistry. 2003;278(35):32880-91.
59. Rumbaut RE, Thiagarajan P. Platelet Aggregation. Platelet-Vessel Wall Interactions in Hemostasis and Thrombosis. San Rafael (CA): Morgan & Claypool Life Sciences; 2010.
60. Varga-Szabo D, Braun A, Nieswandt B. Calcium signaling in platelets. Journal of Thrombosis and Haemostasis. 2009;7(7):1057-66.
61. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and functions. Platelets. 2001;12(5):261-73.
62. Fox JE. Cytoskeletal proteins and platelet signaling. Thrombosis and haemostasis. 2001;86(1):198-213.
63. Daniel JL, Molish IR, Rigmaiden M, Stewart G. Evidence for a role of myosin phosphorylation in the initiation of the platelet shape change response. The Journal of biological chemistry. 1984;259(15):9826-31.
64. Gijon MA, Leslie CC. Regulation of arachidonic acid release and cytosolic phospholipase A2 activation. Journal of leukocyte biology. 1999;65(3):330-6.
65. Wong DA, Kita Y, Uozumi N, Shimizu T. Discrete role for cytosolic phospholipase A(2)alpha in platelets: studies using single and double mutant mice of cytosolic and group IIA secretory phospholipase A(2). J Exp Med. 2002;196(3):349-57.

66. FitzGerald GA. Mechanisms of platelet activation: thromboxane A2 as an amplifying signal for other agonists. The American journal of cardiology. 1991;68(7):11b-5b.
67. Cattaneo M. Platelet P2 receptors: old and new targets for antithrombotic drugs. Expert Review of Cardiovascular Therapy. 2007;5(1):45-55.
68. Cohen L, McKinnell J, Puglisi V, Greco A, Nardi M, Lee M, et al. Prothrombin synthesis in the adult and fetal liver. Thrombosis and haemostasis. 1997;78(6):1468-72.
69. Mann KG, Brummel K, Butenas S. What is all that thrombin for? Journal of Thrombosis and Haemostasis. 2003;1(7):1504-14.
70. Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. Journal of Thrombosis and Haemostasis. 2005;3(8):1800-14.
71. Brummel-Ziedins KE, Pouliot RL, Mann KG. Thrombin generation: phenotypic quantitation. Journal of Thrombosis and Haemostasis. 2004;2(2):281-8.
72. Bevers EM, Comfurius P, van Rijn JL, Hemker HC, Zwaal RF. Generation of prothrombin-converting activity and the exposure of phosphatidylserine at the outer surface of platelets. Eur J Biochem. 1982;122(2):429-36.
73. Bevers EM, Comfurius P, Zwaal RFA. Platelet procoagulant activity: physiological significance and mechanisms of exposure. Blood Reviews. 1991;5(3):146-54.
74. Sims PJ, Wiedmer T, Esmon CT, Weiss HJ, Shattil SJ. Assembly of the platelet prothrombinase complex is linked to vesiculation of the platelet plasma membrane. Studies in Scott syndrome: an isolated defect in platelet procoagulant activity. The Journal of biological chemistry. 1989;264(29):17049-57.
75. Ma YQ, Qin J, Plow EF. Platelet integrin αIIbβ3: activation mechanisms. Journal of Thrombosis and Haemostasis. 2007;5(7):1345-52.
76. Wang J-h. Pull and push: Talin activation for integrin signaling. Cell Res. 2012;22(11):1512-4.
77. Niiya K, Hodson E, Bader R, Byers-Ward V, Koziol J, Plow E, et al. Increased surface expression of the membrane glycoprotein IIb/IIIa complex induced by platelet activation. Relationship to the binding of fibrinogen and platelet aggregation. Blood. 1987;70(2):475-83.
78. Task Force M, Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. European heart journal. 2013;34(38):2949-3003.
79. Meyer J, Breithardt G, Erbel R, Erdmann E, Gohlke H, Hanrath P, et al. Leitlinie: Koronare Herzkrankheit / Angina pectoris. Zeitschrift fur Kardiologie. 1998;87:907-11.
80. Vinereanu D. Risk factors for atherosclerotic disease: present and future. Herz. 2006;31 Suppl 3:5-24.
81. Campeau L. Letter: Grading of angina pectoris. Circulation. 1976;54(3):522-3.

82. Hamm CW, Deutsche Gesellschaft fur Kardiologie- Herz- und K. Guidelines: acute coronary syndrome (ACS). 1: ACS without persistent ST segment elevations. Zeitschrift fur Kardiologie. 2004;93(1):72-90.
83. Braunwald E. Unstable angina. A classification. Circulation. 1989;80(2):410-4.
84. Gurm HS, Topol EJ. The ECG in acute coronary syndromes: new tricks from an old dog. Heart (British Cardiac Society). 2005;91(7):851-3.
85. Inbar R, Shoenfeld Y. Elevated cardiac troponins: the ultimate marker for myocardial necrosis, but not without a differential diagnosis. The Israel Medical Association journal : IMAJ. 2009;11(1):50-3.
86. Daubert MA, Jeremias A. The utility of troponin measurement to detect myocardial infarction: review of the current findings. Vascular Health and Risk Management. 2010;6:691-9.
87. Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, et al. ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction—Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 1999 Guidelines for the Management of Patients With Acute Myocardial Infarction). Journal of the American College of Cardiology. 2004;44(3):671-719.
88. Steg PG, James SK, Atar D, Badano LP, Lundqvist CB, Borger MA, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevationThe Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology (ESC). European heart journal. 2012;33(20):2569-619.
89. Hamm CW. Guidelines: Acute coronary syndrome (ACS). II: Acute coronary syndrome with ST-elevation. Zeitschrift fur Kardiologie. 2004;93(4):324-41.
90. Heberden W. Some account of a disorder of the breast. Medical Transactions. 1772;2:59-67.
91. Nabel EG, Braunwald E. A Tale of Coronary Artery Disease and Myocardial Infarction. New England Journal of Medicine. 2012;366(1):54-63.
92. Warren J. Remarks on Angina Pectoris. The New England Journal of Medicine, Surgery and Collateral Branches of Science. 1812;1(1):1-11.
93. Hajar R. Coronary Heart Disease: From Mummies to 21(st) Century. Heart Views : The Official Journal of the Gulf Heart Association. 2017;18(2):68-74.
94. Hektoen L. Embolism of the left coronary artery; sudden death. Medical Newsletter. 1892:61:210.
95. Kirby RS, Withington S, Darling AB, Kilgour FG. Engineering in History. New York, Toronto, London: McGraw-Hill Book Company Inc.; 1956. 530 p.
96. AlGhatrif M, Lindsay J. A brief review: history to understand fundamentals of electrocardiography. Journal of community hospital internal medicine perspectives. 2012;2(1).

97. Einthoven W. Ueber die Form des menschlichen electrocardiogramms. Pflügers Arch Eur J Physiol. 1895;60(3):101-23.
98. Burch GE. History of precordial leads in electrocardiography. European journal of cardiology. 1978;8(2):207-36.
99. Goldberger E. A simple, indifferent, electrocardiographic electrode of zero potential and a technique of obtaining augmented, unipolar, extremity leads. American Heart Journal. 1942;23(4):483-92.
100. Klingfield P. Harold Ensign Bennet Pardee. Clin Cardiol. 2005;28:396–8.
101. Pardee HB. An electrocardiographic sign of coronary artery obstruction. Archives of Internal Medicine. 1920;26(2):244-57.
102. Fye WB. A history of the origin, evolution, and impact of electrocardiography. The American journal of cardiology. 1994;73(13):937-49.
103. Braunwald E. The treatment of acute myocardial infarction: the Past, the Present, and the Future. European heart journal Acute cardiovascular care. 2012;1(1):9-12.
104. Herrick JB. Landmark article (JAMA 1912). Clinical features of sudden obstruction of the coronary arteries. By James B. Herrick. Jama. 1983;250(13):1757-65.
105. Julian DG. Treatment of cardiac arrest in acute myocardial ischaemia and infarction. The Lancet. 1961;278(7207):840-4.
106. Espinosa RE, Vlietstra RE, Mann RJ. J. B. A. Chauveau, E. J. Marey, and their resolution of the apex beat controversy through intracardiac pressure recordings. Mayo Clinic proceedings. 1983;58(3):197-202.
107. Mueller RL, Sanborn TA. The history of interventional cardiology: cardiac catheterization, angioplasty, and related interventions. Am Heart J. 1995;129(1):146-72.
108. Meyer JA. Werner Forssmann and catheterization of the heart, 1929. The Annals of thoracic surgery. 1990;49(3):497-9.
109. Forssmann W. Die Sondierung des Rechten Herzens. Klin Wochenschr. 1929;8(45):2085-7.
110. Payne MM. Charles Theodore Dotter: The Father of Intervention. Tex Heart Inst J. 2001;28(1):28-38.
111. Dotter CT, Judkins MP. Transluminal Treatment of Arteriosclerotic Obstruction. Description of a new Technic and a Preliminary Report of Its Application. Circulation. 1964;30:654-70.
112. Knoebel SB, Sherry S. The origin of thrombolytic therapy. Journal of the American College of Cardiology. 1989;14(4):1085-92.
113. Chazov EI, Matveeva LS, Mazaev AV, Sargin KE, Sadovskaia GV, Ruda MI. Intracoronary administration of fibrinolysin in acute myocardial infarct. Terapevticheskii arkhiv. 1976;48(4):8-19.
114. Dotter CT, Frische LH, Judkins MP, Mueller R. The “Nonsurgical” Treatment of Iliofemoral Arteriosclerotic Obstruction. Radiology. 1966;86(5):871-5.
115. Grüntzig A, Hopff H. Perkutane Rekanalisation chronischer arterieller Verschlüsse mit einem neuen Dilatationskatheter. Dtsch med Wochenschr. 1974;99(49):2502-5.

116. Grüntzig A. Die perkutane Rekanalisation chronischer arterieller Verschlüsse (Dotter-Prinzip) mit einem neuen doppellumigen Dilatationskatheter. Fortschr Röntgenstr. 1976;124(01):80-6.
117. Grüntzig A. Transluminal Dilatation of Coronary-Artery Stenosis. The Lancet. 1978;311(8058):263.
118. Schatz RA. A view of vascular stents. Circulation. 1989;79(2):445-57.
119. Sigwart U, Puel J, Mirkovitch V, Joffre F, Kappenberger L. Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. The New England journal of medicine. 1987;316(12):701-6.
120. Mahmood SS, Levy D, Vasan RS, Wang TJ. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. The Lancet.383(9921):999-1008.
121. Dawber TR, Moore FE, Mann GV. II. Coronary Heart Disease in the Framingham Study. International journal of epidemiology. 2015;44(6):1767-80.
122. Dawber TR, Kannel WB, Revotskie N, Stokes J, 3rd, Kagan A, Gordon T. Some factors associated with the development of coronary heart disease: six years' follow-up experience in the Framingham study. American journal of public health and the nation's health. 1959;49:1349-56.
123. Task Force m, Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, et al. 2014 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS)Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). European heart journal. 2014;35(37):2541-619.
124. Nordmann AJ, Hengstler P, Harr T, Young J, Bucher HC. Clinical outcomes of primary stenting versus balloon angioplasty in patients with myocardial infarction: a meta-analysis of randomized controlled trials. The American journal of medicine. 2004;116(4):253-62.
125. Finn AV, Nakazawa G, Joner M, Kolodgie FD, Mont EK, Gold HK, et al. Vascular Responses to Drug Eluting Stents. Importance of Delayed Healing. 2007;27(7):1500-10.
126. James SK, Stenestrand U, Lindback J, Carlsson J, Schersten F, Nilsson T, et al. Long-term safety and efficacy of drug-eluting versus bare-metal stents in Sweden. The New England journal of medicine. 2009;360(19):1933-45.
127. Kassimis G, Banning AP. Is it time to take bare metal stents off the catheter laboratory shelf? European heart journal. 2016;37(45):3372-5.
128. Romagnoli E, Biondi-Zoccai G, Sciahbasi A, Politi L, Rigattieri S, Pendenza G, et al. Radial versus femoral randomized investigation in ST-segment elevation acute coronary syndrome: the RIFLE-STEACS (Radial Versus Femoral Randomized Investigation in ST-Elevation Acute Coronary Syndrome) study. Journal of the American College of Cardiology. 2012;60(24):2481-9.
129. Kelly MJ, Thompson PL, Quinlan MF. Prognostic significance of left ventricular ejection fraction after acute myocardial infarction. A bedside radionuclide study. British Heart Journal. 1985;53(1):16-24.

130. Ng VG, Lansky AJ, Meller S, Witzenbichler B, Guagliumi G, Peruga JZ, et al. The prognostic importance of left ventricular function in patients with ST-segment elevation myocardial infarction: the HORIZONS-AMI trial. European heart journal Acute cardiovascular care. 2014;3(1):67-77.
131. Larose E, Rodés-Cabau J, Pibarot P, Rinfret S, Proulx G, Nguyen CM, et al. Predicting Late Myocardial Recovery and Outcomes in the Early Hours of ST-Segment Elevation Myocardial Infarction. Traditional Measures Compared With Microvascular Obstruction, Salvaged Myocardium, and Necrosis Characteristics by Cardiovascular Magnetic Resonance. 2010;55(22):2459-69.
132. Christian TF, Behrenbeck T, Gersh BJ, Gibbons RJ. Relation of left ventricular volume and function over one year after acute myocardial infarction to infarct size determined by technetium-99m sestamibi. The American journal of cardiology. 1991;68(1):21-6.
133. Nijland F, Kamp O, Verhorst PMJ, de Voogt WG, Visser CA. Early prediction of improvement in ejection fraction after acute myocardial infarction using low dose dobutamine echocardiography. Heart (British Cardiac Society). 2002;88(6):592-6.
134. Moss AJ, Zareba W, Hall WJ, Klein H, Wilber DJ, Cannom DS, et al. Prophylactic Implantation of a Defibrillator in Patients with Myocardial Infarction and Reduced Ejection Fraction. New England Journal of Medicine. 2002;346(12):877-83.
135. Hohnloser SH, Kuck KH, Dorian P, Roberts RS, Hampton JR, Hatala R, et al. Prophylactic Use of an Implantable Cardioverter–Defibrillator after Acute Myocardial Infarction. New England Journal of Medicine. 2004;351(24):2481-8.
136. Dagres N, Hindricks G. Risk stratification after myocardial infarction: is left ventricular ejection fraction enough to prevent sudden cardiac death? European heart journal. 2013;34(26):1964-71.
137. Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJJ, Cuddy TE, et al. Effect of Captopril on Mortality and Morbidity in Patients with Left Ventricular Dysfunction after Myocardial Infarction. New England Journal of Medicine. 1992;327(10):669-77.
138. Codolosa JN. Novel Predictors of Left Ventricular Systolic Function Recovery in Patients with ST Elevation Myocardial Infarction who Underwent Reperfusion Therapy. Journal of Clinical & Experimental Cardiology. 2013;04(02).
139. Solomon SD, Glynn RJ, Greaves S, Ajani U, Rouleau JL, Menapace F, et al. Recovery of ventricular function after myocardial infarction in the reperfusion era: the healing and early afterload reducing therapy study. Annals of internal medicine. 2001;134(6):451-8.
140. Guo RW, Yang LX, Liu B, Qi F, Wang XM, Guo CM, et al. Effect of sex on recovery of ejection fraction in patients with anterior ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. Coronary artery disease. 2014;25(2):133-7.
141. Pain TE, Jones DA, Rathod KS, Gallagher SM, Knight CJ, Mathur A, et al. Influence of female sex on long-term mortality after acute coronary

syndromes treated by percutaneous coronary intervention: a cohort study of 7304 patients. Coronary artery disease. 2013;24(3):183-90.
142. Wu E, Ortiz JT, Tejedor P, Lee DC, Bucciarelli-Ducci C, Kansal P, et al. Infarct size by contrast enhanced cardiac magnetic resonance is a stronger predictor of outcomes than left ventricular ejection fraction or end-systolic volume index: prospective cohort study. Heart (British Cardiac Society). 2008;94(6):730-6.
143. Klug G, Metzler B. Assessing myocardial recovery following ST-segment elevation myocardial infarction: short- and long-term perspectives using cardiovascular magnetic resonance. Expert Review of Cardiovascular Therapy. 2013;11(2):203-19.
144. Merlos P, López-Lereu MP, Monmeneu JV, Sanchis J, Núñez J, Bonanad C, et al. Long-term Prognostic Value of a Comprehensive Assessment of Cardiac Magnetic Resonance Indexes After an ST-segment Elevation Myocardial Infarction. Revista Española de Cardiología (English Edition). 2013;66(8):613-22.
145. Eitel I, Desch S, Fuernau G, Hildebrand L, Gutberlet M, Schuler G, et al. Prognostic Significance and Determinants of Myocardial Salvage Assessed by Cardiovascular Magnetic Resonance in Acute Reperfused Myocardial Infarction. Journal of the American College of Cardiology. 2010;55(22):2470-9.
146. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation. 1990;81(4):1161-72.
147. Rahimtoola SH. The hibernating myocardium. American Heart Journal. 1989;117(1):211-21.
148. Braunwald E, Kloner RA. The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation. 1982;66(6):1146-9.
149. Kim RJ, Wu E, Rafael A, Chen E-L, Parker MA, Simonetti O, et al. The Use of Contrast-Enhanced Magnetic Resonance Imaging to Identify Reversible Myocardial Dysfunction. New England Journal of Medicine. 2000;343(20):1445-53.
150. Wu E, Judd RM, Vargas JD, Klocke FJ, Bonow RO, Kim RJ. Visualisation of presence, location, and transmural extent of healed Q-wave and non-Q-wave myocardial infarction. The Lancet. 2001;357(9249):21-8.
151. Zhang J-M, An J. Cytokines, Inflammation and Pain. International anesthesiology clinics. 2007;45(2):27-37.
152. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annual review of immunology. 2004;22:891-928.
153. Levine SJ. Cytokines A2 - Lennarz, William J. In: Lane MD, editor. Encyclopedia of Biological Chemistry. Waltham: Academic Press; 2013. p. 613-21.
154. Murphy PM, Baggiolini M, Charo IF, Hébert CA, Horuk R, Matsushima K, et al. International Union of Pharmacology. XXII. Nomenclature for Chemokine Receptors. Pharmacological Reviews. 2000;52(1):145-76.
155. Nomenclature IWSoC. Chemokine/chemokine receptor nomenclature. Cytokine. 2003;21(1):48-9.

156. Janowski M. Functional diversity of SDF-1 splicing variants. Cell Adhesion & Migration. 2009;3(3):243-9.
157. Secchiero P, Celeghini C, Cutroneo G, Di Baldassarre A, Rana R, Zauli G. Differential effects of stromal derived factor-1 alpha (SDF-1 alpha) on early and late stages of human megakaryocytic development. The Anatomical record. 2000;260(2):141-7.
158. Wright LM, Maloney W, Yu X, Kindle L, Collin-Osdoby P, Osdoby P. Stromal cell-derived factor-1 binding to its chemokine receptor CXCR4 on precursor cells promotes the chemotactic recruitment, development and survival of human osteoclasts. Bone. 2005;36(5):840-53.
159. Gillette JM, Larochelle A, Dunbar CE, Lippincott-Schwartz J. Intercellular transfer to signalling endosomes regulates an ex vivo bone marrow niche. Nature cell biology. 2009;11(3):303-11.
160. Chatterjee M, Rath D, Gawaz M. Role of chemokine receptors CXCR4 and CXCR7 for platelet function. Biochemical Society transactions. 2015;43(4):720-6.
161. Liu H, Xue W, Ge G, Luo X, Li Y, Xiang H, et al. Hypoxic preconditioning advances CXCR4 and CXCR7 expression by activating HIF-1alpha in MSCs. Biochem Biophys Res Commun. 2010;401(4):509-15.
162. Rath D, Chatterjee M, Borst O, Muller K, Stellos K, Mack AF, et al. Expression of stromal cell-derived factor-1 receptors CXCR4 and CXCR7 on circulating platelets of patients with acute coronary syndrome and association with left ventricular functional recovery. European heart journal. 2014;35(6):386-94.
163. Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal cell-derived factor-1alpha plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. Circulation. 2004;110(21):3300-5.
164. Wang Y, Deng Y, Zhou GQ. SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. Brain Res. 2008;1195:104-12.
165. Ma J, Ge J, Zhang S, Sun A, Shen J, Chen L, et al. Time course of myocardial stromal cell-derived factor 1 expression and beneficial effects of intravenously administered bone marrow stem cells in rats with experimental myocardial infarction. Basic research in cardiology. 2005;100(3):217-23.
166. Geisler T, Fekecs L, Wurster T, Chiribiri A, Schuster A, Nagel E, et al. Association of platelet-SDF-1 with hemodynamic function and infarct size using cardiac MR in patients with AMI. European journal of radiology. 2012;81(4):e486-90.
167. Rath D, Chatterjee M, Borst O, Müller K, Langer H, Mack AF, et al. Platelet surface expression of stromal cell–derived factor-1 receptors CXCR4 and CXCR7 is associated with clinical outcomes in patients with coronary artery disease. Journal of Thrombosis and Haemostasis. 2015;13(5):719-28.
168. Duque-Parra JE. Note on the origin and history of the term “apoptosis”. The Anatomical Record Part B: The New Anatomist. 2005;283B(1):2-4.

169. Singh N, Bose K. Apoptosis: Pathways, Molecules and Beyond. In: Bose K, editor. Proteases in Apoptosis: Pathways, Protocols and Translational Advances. Cham: Springer International Publishing; 2015. p. 1-30.
170. Saunders JW, Jr. Death in embryonic systems. Science. 1966;154(3749):604-12.
171. Meier P, Finch A, Evan G. Apoptosis in development. Nature. 2000;407(6805):796-801.
172. Lodish H, Berk A, Kaiser CA, Krieger M, Bretscher A, Ploegh H, et al. Molecular Cell Biology. 7 ed. Byrd ML, editor. New York: Parker, K.A.; 2012.
173. Levy R, Seifer-Aknin I. [Apoptosis during spermatogenesis and in ejaculated spermatozoa: importance for fertilization]. Annales de biologie clinique. 2001;59(5):531-45.
174. Goodnow CC. Balancing immunity and tolerance: deleting and tuning lymphocyte repertoires. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(6):2264-71.
175. Minamide S, Naora H, Adachi M, Okano A, Naora H. Apoptosis as a mechanism of skin renewal: Le(y)-antigen expression is involved in an early event of a cell's commitment to apoptosis. Histochemistry and cell biology. 1995;103(5):339-43.
176. Lippens S, Hoste E, Vandenabeele P, Agostinis P, Declercq W. Cell death in the skin. Apoptosis : an international journal on programmed cell death. 2009;14(4):549-69.
177. Greenberg PL. Apoptosis and its role in the myelodysplastic syndromes: implications for disease natural history and treatment. Leukemia research. 1998;22(12):1123-36.
178. Mullauer L, Gruber P, Sebinger D, Buch J, Wohlfart S, Chott A. Mutations in apoptosis genes: a pathogenetic factor for human disease. Mutation research. 2001;488(3):211-31.
179. Eguchi K. Apoptosis in autoimmune diseases. Internal medicine (Tokyo, Japan). 2001;40(4):275-84.
180. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. British journal of cancer. 1972;26(4):239-57.
181. Raff M. Cell suicide for beginners. Nature. 1998;396(6707):119-22.
182. Cooper GM. Structure of the Plasma Membrane. The Cell: A Molecular Approach. 2nd ed. Sunderland (MA): Sinauer Associates Inc.; 2000. p. 689.
183. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. Journal of Immunology. 1992;148(7):2207-16.
184. Verhoven B, Schlegel RA, Williamson P. Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. The Journal of Experimental Medicine. 1995;182(5):1597-601.
185. Castegna A, Lauderback CM, Mohmmad-Abdul H, Butterfield DA. Modulation of phospholipid asymmetry in synaptosomal membranes by

the lipid peroxidation products, 4-hydroxynonenal and acrolein: implications for Alzheimer's disease. Brain Research. 2004;1004(1–2):193-7.
186. Lee SH, Meng XW, Flatten KS, Loegering DA, Kaufmann SH. Phosphatidylserine exposure during apoptosis reflects bidirectional trafficking between plasma membrane and cytoplasm. Cell Death Differ. 2013;20(1):64-76.
187. Fadeel B, Gleiss B, Hogstrand K, Chandra J, Wiedmer T, Sims PJ, et al. Phosphatidylserine exposure during apoptosis is a cell-type-specific event and does not correlate with plasma membrane phospholipid scramblase expression. Biochem Biophys Res Commun. 1999;266(2):504-11.
188. Schultz DR, Harrington WJ, Jr. Apoptosis: programmed cell death at a molecular level. Seminars in arthritis and rheumatism. 2003;32(6):345-69.
189. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicologic pathology. 2007;35(4):495-516.
190. Nicholson DW, Thornberry NA. Caspases: killer proteases. Trends in biochemical sciences. 1997;22(8):299-306.
191. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Micro. 2009;7(2):99-109.
192. Cade CE, Clark AC. Caspases – Key Players in Apoptosis. In: Bose K, editor. Proteases in Apoptosis: Pathways, Protocols and Translational Advances. Cham: Springer International Publishing; 2015. p. 31-51.
193. Jin Z, El-Deiry WS. Overview of cell death signaling pathways. Cancer Biol Ther. 2005;4(2):139-63.
194. Ashkenazi A, Dixit VM. Apoptosis control by death and decoy receptors. Current opinion in cell biology. 1999;11(2):255-60.
195. Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. The Journal of biological chemistry. 1995;270(14):7795-8.
196. Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of fas and initiates apoptosis. Cell. 1995;81(4):505-12.
197. Jiang Y, Woronicz JD, Liu W, Goeddel DV. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science. 1999;283(5401):543-6.
198. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-κB activation. Cell. 1995;81(4):495-504.
199. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell. 1996;84(2):299-308.
200. Wang S, El-Deiry WS. TRAIL and apoptosis induction by TNF-family death receptors. Oncogene. 2003;22(53):8628-33.
201. Ashkenazi A. Death Receptors: Signaling and Modulation. Science. 1998;281(5381):1305-8.
202. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, et al. The receptor for the cytotoxic ligand TRAIL. Science. 1997;276(5309):111-3.

203. Boldin MP, Goncharov TM, Goltsev YV, Wallach D. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell. 1996;85(6):803-15.
204. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell. 2003;114(2):181-90.
205. van der Giezen M. Mitochondria and the Rise of Eukaryotes. BioScience. 2011;61(8):594-601.
206. Siekevitz P. Powerhouse of the Cell. Scientific American. 1957;197(1):131 - 44.
207. Frey TG, Mannella CA. The internal structure of mitochondria. Trends in biochemical sciences. 2000;25(7):319-24.
208. Frey TG, Renken CW, Perkins GA. Insight into mitochondrial structure and function from electron tomography. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2002;1555(1–3):196-203.
209. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science. 1997;275(5303):1132-6.
210. Shoemaker AR, Mitten MJ, Adickes J, Ackler S, Refici M, Ferguson D, et al. Activity of the Bcl-2 Family Inhibitor ABT-263 in a Panel of Small Cell Lung Cancer Xenograft Models. Clinical Cancer Research. 2008;14(11):3268-77.
211. Kroemer G. Mitochondrial control of apoptosis: an introduction. Biochemical and Biophysical Research Communications. 2003;304(3):433-5.
212. Zamzami N, Marchetti P, Castedo M, Zanin C, Vayssière JL, Petit PX, et al. Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. The Journal of Experimental Medicine. 1995;181(5):1661-72.
213. Chipuk JE, Bouchier-Hayes L, Green DR. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. Cell Death Differ. 2006;13(8):1396-402.
214. Goldstein JC, Waterhouse NJ, Juin P, Evan GI, Green DR. The coordinate release of cytochrome c during apoptosis is rapid, complete and kinetically invariant. Nature cell biology. 2000;2(3):156-62.
215. Heiskanen KM, Bhat MB, Wang H-W, Ma J, Nieminen A-L. Mitochondrial Depolarization Accompanies Cytochrome cRelease During Apoptosis in PC6 Cells. Journal of Biological Chemistry. 1999;274(9):5654-8.
216. Bosga-Bouwer AG, van Imhoff GW, Boonstra R, van der Veen A, Haralambieva E, van den Berg A, et al. Follicular lymphoma grade 3B includes 3 cytogenetically defined subgroups with primary t(14;18), 3q27, or other translocations: t(14;18) and 3q27 are mutually exclusive. Blood. 2003;101(3):1149-54.
217. Aouacheria A, Brunet F, Gouy M. Phylogenomics of Life-Or-Death Switches in Multicellular Animals: Bcl-2, BH3-Only, and BNip Families of Apoptotic Regulators. Molecular Biology and Evolution. 2005;22(12):2395-416.
218. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol. 2008;9(1):47-59.

219. Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR. The BCL-2 family reunion. Mol Cell. 2010;37(3):299-310.
220. Tait SWG, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol. 2010;11(9):621-32.
221. Borner C. The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. Molecular Immunology. 2003;39(11):615-47.
222. Villunger A, Labi V, Bouillet P, Adams J, Strasser A. Can the analysis of BH3-only protein knockout mice clarify the issue of /`direct versus indirect/' activation of Bax and Bak[quest]. Cell Death Differ. 2011;18(10):1545-6.
223. Willis S, I Fletcher J, Kaufmann T, van Delft M, Chen L, E Czabotar P, et al. Apoptosis Initiated When BH3 Ligands Engage Multiple Bcl-2 Homologs, Not Bax or Bak2007. 856-9 p.
224. Susin SA, Zamzami N, Castedo M, Hirsch T, Marchetti P, Macho A, et al. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. J Exp Med. 1996;184(4):1331-41.
225. Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, et al. Prevention of Apoptosis by Bcl-2: Release of Cytochrome c from Mitochondria Blocked. Science. 1997;275(5303):1129-32.
226. Sedlak TW, Oltvai ZN, Yang E, Wang K, Boise LH, Thompson CB, et al. Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. Proc Natl Acad Sci U S A. 1995;92(17):7834-8.
227. Yin XM, Oltvai ZN, Korsmeyer SJ. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature. 1994;369(6478):321-3.
228. Wei MC, Zong W-X, Cheng EH-Y, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: A Requisite Gateway to Mitochondrial Dysfunction and Death. Science. 2001;292(5517):727-30.
229. Ferraro E, Fuoco C, Strappazzon F, Cecconi F. Apoptosome Structure and Regulation. In: Cecconi F, D'Amelio M, editors. Apoptosome: An up-and-coming therapeutical tool. Dordrecht: Springer Netherlands; 2010. p. 27-39.
230. Hill MM, Adrain C, Duriez PJ, Creagh EM, Martin SJ. Analysis of the composition, assembly kinetics and activity of native Apaf-1 apoptosomes. Embo j. 2004;23(10):2134-45.
231. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, et al. Cytochrome c and dATP-Dependent Formation of Apaf-1/Caspase-9 Complex Initiates an Apoptotic Protease Cascade. Cell. 1997;91(4):479-89.
232. Reubold TF, Eschenburg S. A molecular view on signal transduction by the apoptosome. Cellular signalling. 2012;24(7):1420-5.
233. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. Mol Cell. 2002;9(2):423-32.
234. Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. Autoactivation of Procaspase-9 by Apaf-1-Mediated Oligomerization. Molecular Cell. 1998;1(7):949-57.
235. Yuan S, Yu X, Topf M, Ludtke SJ, Wang X, Akey CW. Structure of an Apoptosome-Procaspase-9 CARD Complex. Structure.18(5):571-83.

236. Green DR. Apoptotic Pathways: The Roads to Ruin. Cell. 1998;94(6):695-8.
237. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, et al. Human ICE/CED-3 Protease Nomenclature. Cell. 1996;87(2):171.
238. Weil M, Jacobson MD, Coles HS, Davies TJ, Gardner RL, Raff KD, et al. Constitutive expression of the machinery for programmed cell death. The Journal of Cell Biology. 1996;133(5):1053-9.
239. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol. 2004;5(11):897-907.
240. Göhring F, Schwab BL, Nicotera P, Leist M, Fackelmayer FO. The novel SAR-binding domain of scaffold attachment factor A (SAF-A) is a target in apoptotic nuclear breakdown. The EMBO Journal. 1997;16(24):7361-71.
241. Lazebnik YA, Takahashi A, Moir RD, Goldman RD, Poirier GG, Kaufmann SH, et al. Studies of the lamin proteinase reveal multiple parallel biochemical pathways during apoptotic execution. Proceedings of the National Academy of Sciences. 1995;92(20):9042-6.
242. Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, et al. Caspase-3-Generated Fragment of Gelsolin: Effector of Morphological Change in Apoptosis. Science. 1997;278(5336):294-8.
243. Wolf BB, Green DR. Suicidal Tendencies: Apoptotic Cell Death by Caspase Family Proteinases. Journal of Biological Chemistry. 1999;274(29):20049-52.
244. Cohen GM. Caspases: the executioners of apoptosis. Biochemical Journal. 1997;326(1):1-16.
245. Cardone MH, Salvesen GS, Widmann C, Johnson G, Frisch SM. The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. Cell. 1997;90(2):315-23.
246. Rudel T, Bokoch GM. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. Science. 1997;276(5318):1571-4.
247. Lee N, MacDonald H, Reinhard C, Halenbeck R, Roulston A, Shi T, et al. Activation of hPAK65 by caspase cleavage induces some of the morphological and biochemical changes of apoptosis. Proc Natl Acad Sci U S A. 1997;94(25):13642-7.
248. Emoto Y, Manome Y, Meinhardt G, Kisaki H, Kharbanda S, Robertson M, et al. Proteolytic activation of protein kinase C delta by an ICE-like protease in apoptotic cells. The EMBO Journal. 1995;14(24):6148-56.
249. Datta R, Kojima H, Yoshida K, Kufe D. Caspase-3-mediated cleavage of protein kinase C theta in induction of apoptosis. The Journal of biological chemistry. 1997;272(33):20317-20.
250. Cryns V, Yuan J. Proteases to die for. Genes & Development. 1998;12(11):1551-70.
251. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature. 1998;391(6662):43-50.

252. Galluzzi L, López-Soto A, Kumar S, Kroemer G. Caspases Connect Cell-Death Signaling to Organismal Homeostasis. Immunity. 2016;44(2):221-31.
253. Wang ZB, Liu YQ, Cui YF. Pathways to caspase activation. Cell biology international. 2005;29(7):489-96.
254. Ghavami S, Hashemi M, Ande SR, Yeganeh B, Xiao W, Eshraghi M, et al. Apoptosis and cancer: mutations within caspase genes. Journal of Medical Genetics. 2009;46(8):497-510.
255. Salvesen GS, Riedl SJ. Caspase Mechanisms. Programmed Cell Death in Cancer Progression and Therapy. Dordrecht: Springer Netherlands; 2008. p. 13-23.
256. Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene. 2008;27(48):6194-206.
257. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998;94(4):491-501.
258. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer. 2002;2(4):277-88.
259. Leytin V. Apoptosis in the anucleate platelet. Blood Reviews. 2012;26(2):51-63.
260. Jacobson MD, Burne JF, Raff MC. Programmed cell death and Bcl-2 protection in the absence of a nucleus. The EMBO Journal. 1994;13(8):1899-910.
261. Vanags DM, Orrenius S, Aguilar-Santelises M. Alterations in Bcl-2/Bax protein levels in platelets form part of an ionomycin-induced process that resembles apoptosis. Br J Haematol. 1997;99(4):824-31.
262. Duke WW. The Rate of Regeneration of Blood Platelets. The Journal of Experimental Medicine. 1911;14(3):265-73.
263. Coller BS. Historical perspective and future directions in platelet research. Journal of thrombosis and haemostasis : JTH. 2011;9 Suppl 1:374-95.
264. Stefanini M, Dameshek W. Collection, Preservation and Transfusion of Platelets. New England Journal of Medicine. 1953;248(19):797-802.
265. Mustard JF, Rowsell HC, Murphy EA. Platelet Economy (Platelet Survival and Turnover)*. British Journal of Haematology. 1966;12(1):1-24.
266. Murphy EA, Robinson GA, Rowsell HC, Mustard JF. The pattern of platelet disappearance. Blood. 1967;30(1):26-38.
267. Murphy EA, Francis ME. The estimation of blood platelet survival. I. General principles of the study of cell survival. Thrombosis et diathesis haemorrhagica. 1969;22(2):281-95.
268. Murphy EA, Francis ME. The estimation of blood platelet survival. II. The multiple hit model. Thrombosis et diathesis haemorrhagica. 1971;25(1):53-80.
269. Murphy EA. The estimation of blood platelet survival. III. The robustness of the basic models. Thrombosis et diathesis haemorrhagica. 1971;26(3):431-48.
270. Wolf BB, Goldstein JC, Stennicke HR, Beere H, Amarante-Mendes GP, Salvesen GS, et al. Calpain functions in a caspase-independent manner

to promote apoptosis-like events during platelet activation. Blood. 1999;94(5):1683-92.

271. Brown SB, Clarke MC, Magowan L, Sanderson H, Savill J. Constitutive death of platelets leading to scavenger receptor-mediated phagocytosis. A caspase-independent cell clearance program. The Journal of biological chemistry. 2000;275(8):5987-96.

272. Pereira J, Soto M, Palomo I, Ocqueteau M, Coetzee LM, Astudillo S, et al. Platelet aging in vivo is associated with activation of apoptotic pathways: studies in a model of suppressed thrombopoiesis in dogs. Thrombosis and haemostasis. 2002;87(5):905-9.

273. Rand ML, Wang H, Bang KWA, Poon KSV, Packham MA, Freedman J. Procoagulant surface exposure and apoptosis in rabbit platelets: association with shortened survival and steady-state senescence. Journal of Thrombosis and Haemostasis. 2004;2(4):651-9.

274. Bertino AM, Qi XQ, Li J, Xia Y, Kuter DJ. Apoptotic markers are increased in platelets stored at 37°C. Transfusion. 2003;43(7):857-66.

275. Kodama T, Takehara T, Hikita H, Shimizu S, Shigekawa M, Li W, et al. BH3-only Activator Proteins Bid and Bim Are Dispensable for Bak/Bax-dependent Thrombocyte Apoptosis Induced by Bcl-xL Deficiency: MOLECULAR REQUISITES FOR THE MITOCHONDRIAL PATHWAY TO APOPTOSIS IN PLATELETS. Journal of Biological Chemistry. 2011;286(16):13905-13.

276. Schoenwaelder SM, Yuan Y, Josefsson EC, White MJ, Yao Y, Mason KD, et al. Two distinct pathways regulate platelet phosphatidylserine exposure and procoagulant function. Blood. 2009;114(3):663-6.

277. Josefsson EC, James C, Henley KJ, Debrincat MA, Rogers KL, Dowling MR, et al. Megakaryocytes possess a functional intrinsic apoptosis pathway that must be restrained to survive and produce platelets. The Journal of Experimental Medicine. 2011;208(10):2017-31.

278. Li J, Xia Y, Bertino AM, Coburn JP, Kuter DJ. The mechanism of apoptosis in human platelets during storage. Transfusion. 2000;40(11):1320-9.

279. Plenchette S, Moutet M, Benguella M, N'Gondara JP, Guigner F, Coffe C, et al. Early increase in DcR2 expression and late activation of caspases in the platelet storage lesion. Leukemia. 2001;15(10):1572-81.

280. Chatterjee M, Borst O, Walker B, Fotinos A, Vogel S, Seizer P, et al. Macrophage migration inhibitory factor limits activation-induced apoptosis of platelets via CXCR7-dependent Akt signaling. Circulation research. 2014;115(11):939-49.

281. Chatterjee M, Seizer P, Borst O, Schonberger T, Mack A, Geisler T, et al. SDF-1alpha induces differential trafficking of CXCR4-CXCR7 involving cyclophilin A, CXCR7 ubiquitination and promotes platelet survival. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2014;28(7):2864-78.

282. European Parliament and of the Council of 4th April 2001 on the approximation of the laws, regulations and administrative provisions of the member states relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. Med Etika Bioet. 2002(9):12-9.

283. ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice. Journal of postgraduate medicine. 2001;47(3):199-203.
284. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. Cardiovascular research. 1997;35(1):2-3.
285. Nilsson L, Szymanowski A, Swahn E, Jonasson L. Soluble TNF receptors are associated with infarct size and ventricular dysfunction in ST-elevation myocardial infarction. PloS one. 2013;8(2):e55477.
286. Leong DP, De Pasquale CG, Selvanayagam JB. Heart failure with normal ejection fraction: the complementary roles of echocardiography and CMR imaging. JACC Cardiovascular imaging. 2010;3(4):409-20.
287. Bellenger NG, Burgess MI, Ray SG, Lahiri A, Coats AJS, Cleland JGF, et al. Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance. Are they interchangeable? European heart journal. 2000;21(16):1387-96.
288. Carr JC, Simonetti O, Bundy J, Li D, Pereles S, Finn JP. Cine MR angiography of the heart with segmented true fast imaging with steady-state precession. Radiology. 2001;219(3):828-34.
289. Bogaert J, Eitel I. Role of cardiovascular magnetic resonance in acute coronary syndrome. Global Cardiology Science & Practice. 2015;2015(2):24.
290. Chatterjee M, von Ungern-Sternberg SNI, Seizer P, Schlegel F, Büttcher M, Sindhu NA, et al. Platelet-derived CXCL12 regulates monocyte function, survival, differentiation into macrophages and foam cells through differential involvement of CXCR4–CXCR7. Cell Death & Disease. 2015;6(11):e1989.
291. Slupsky JR, Kamigutt AS, Rhodes NP, Cawley JC, Shaw ARE, Zuzel M. The Platelet Antigens CD9, CD42 and Integrin αIIbβIIIa Can be Topographically Associated and Transduce Functionally Similar Signals. European Journal of Biochemistry. 1997;244(1):168-75.
292. Leytin V, Allen DJ, Lyubimov E, Freedman J. Higher thrombin concentrations are required to induce platelet apoptosis than to induce platelet activation. British Journal of Haematology. 2007;136(5):762-4.
293. Van Rijn J, Rosing J, Van Dieijen G. Activity of Human Blood Platelets in Prothrombin and in Factor X Activation Induced by Ionophore A23187. European Journal of Biochemistry. 1983;133(1):1-10.
294. Leytin V, Allen DJ, Mykhaylov S, Lyubimov E, Freedman J. Thrombin-triggered platelet apoptosis. Journal of thrombosis and haemostasis : JTH. 2006;4(12):2656-63.
295. Scaduto RC, Grotyohann LW. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. Biophysical Journal. 1999;76(1 Pt 1):469-77.
296. Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA. Mitochondrial membrane potential probes and the proton gradient: a practical usage guide. BioTechniques. 2011;50(2):98-115.
297. Dachary-Prigent J, Freyssinet JM, Pasquet JM, Carron JC, Nurden AT. Annexin V as a probe of aminophospholipid exposure and platelet

membrane vesiculation: a flow cytometry study showing a role for free sulfhydryl groups. Blood. 1993;81(10):2554-65.
298. Dasgupta SK, Argaiz ER, Mercado JEC, Maul HOE, Garza J, Enriquez AB, et al. Platelet senescence and phosphatidylserine exposure. Transfusion. 2010;50(10):2167-75.
299. Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, LaFace DM, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. The Journal of Experimental Medicine. 1995;182(5):1545-56.
300. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. Cell Death Differ. 2006;13(9):1423-33.
301. Zwaal RFA, Comfurius P, Van Deenen LLM. Membrane asymmetry and blood coagulation. Nature. 1977;268(5618):358-60.
302. Solum NO. Procoagulant expression in platelets and defects leading to clinical disorders. Arteriosclerosis, thrombosis, and vascular biology. 1999;19(12):2841-6.
303. Meers P, Mealy T. Calcium-dependent annexin V binding to phospholipids: stoichiometry, specificity, and the role of negative charge. Biochemistry. 1993;32(43):11711-21.
304. Mazzinghi B, Ronconi E, Lazzeri E, Sagrinati C, Ballerini L, Angelotti ML, et al. Essential but differential role for CXCR4 and CXCR7 in the therapeutic homing of human renal progenitor cells. J Exp Med. 2008;205(2):479-90.
305. Stellos K, Gawaz M. Platelets and stromal cell-derived factor-1 in progenitor cell recruitment. Seminars in thrombosis and hemostasis. 2007;33(2):159-64.
306. Lataillade JJ, Clay D, Bourin P, Herodin F, Dupuy C, Jasmin C, et al. Stromal cell-derived factor 1 regulates primitive hematopoiesis by suppressing apoptosis and by promoting G(0)/G(1) transition in CD34(+) cells: evidence for an autocrine/paracrine mechanism. Blood. 2002;99(4):1117-29.
307. Bernhagen J, Krohn R, Lue H, Gregory JL, Zernecke A, Koenen RR, et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med. 2007;13(5):587-96.
308. Strussmann T, Tillmann S, Wirtz T, Bucala R, von Hundelshausen P, Bernhagen J. Platelets are a previously unrecognised source of MIF. Thrombosis and haemostasis. 2013;110(5):1004-13.

8 Erklärung zum Eigenanteil

Diese Dissertationsschrift wurde in der Medizinischen Universitätsklinik und Poliklinik Abteilung Innere Medizin III unter der Betreuung von Herrn Prof. Dr. med. Tobias Geisler verfasst. Die Konzeption der Studie erfolgte in Zusammenarbeit mit Herrn Prof. Dr. med. Tobias Geisler (als Leiter der Forschungsgruppe), Herrn Dr. med. Dominik Rath, Frau Dr. rer. nat. Madhumita Chatterjee und Herrn Dr. med. Patrick Krumm.

Die Erhebung der Labordaten (exemplarisch dargestellt in **Figure 7** – **Figure 9**) wurde nach Einarbeitung durch die Labormitarbeiterinnen Frau Dr. rer. nat. Madhumita Chatterjee und Frau Lydia Laptev in gemeinsamer Arbeit von Frau Nina Tekath und mir eigenständig durchgeführt.
Die Auswertung der MRT-Befunde (dargestellt in **Figure 6**) wurde in Kooperation und nach Einarbeitung durch Herrn Dr. med. Patrick Krumm aus der Abteilung für Radiologie am Universitätsklinikum Tübingen von mir eigenständig durchgeführt.
Die statistische Auswertung (inklusive der Darstellungen in **Figure 10** – **Figure 13** und **Table 7** – **Table 11**) wurde nach Einarbeitung von Herrn Dr. med. Dominik Rath und nach Beratung durch Frau Aline Naumann vom Institut für Biometrie durch mich eigenständig durchgeführt.

Die Ergebnisse dieser Dissertationsschrift waren Teil einer Publikation unter Leitung von Herrn Dr. med. Dominik Rath, welche in Zusammenarbeit mit Frau Dr. rer. nat. Madhumita Chatterjee, Frau Nina Tekath, Herrn Dr. med. Patrick Krumm, Herrn Dr. med. Constatnin Adams, Herrn Dr. med. Oliver Borst, Frau Dr. med. Karin Müller, Herrn Dr. med. Michael Droppa, Herrn Dr. med. Konstantin Nikolaou, Herrn Dr. med. Joachim Riethmüller, Herrn Prof. Dr. med. Meinrad Gawaz, Herrn Prof. Dr. med. Tobias Geisler und mir verfasst wurde. Die

Publikation wurde unter dem Titel *Relative survival potential of platelets is associated with platelet CXCR4/CXCR7 surface exposure and functional recovery following STEMI* im Oktober 2018 im *Atherosclerosis* veröffentlicht. Die Grafiken **Figure 6** – **Figure 13** wurden von mir für diese Publikation angefertigt, wobei das Bildmaterial für **Figure 6** freundlicherweise von Herrn Dr. med. Patrick Krumm aus der Abteilung für Radiologie des Universitätsklinikums Tübingen bereitgestellt und von mir graphisch angepasst wurde.
Die in dieser Publikation angefertigten Grafiken und Statistiken wurden von mir erstellt und mit dem Einverständnis des Erstautors und des korrespondierenden Autors für diese Dissertation verwendet.

Ich versichere hiermit, die Dissertationsschrift selbständig verfasst und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Tübingen, den 25. März 2018

Christian Lennart Meyer

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Attempto! – Ich wage es!

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