Expression of SDF-1 Receptors CXCR4 and CXCR7 on Circulating Platelets of Patients with Acute Coronary Syndrome and Association with Left Ventricular Functional Recovery

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Abbreviations

ACD: Acid-citrate-dextrose

ACS: Acute coronary syndrome

ADP: Adenosine diphosphate

AMP: Adenosine monophosphate

BMS: Bare metal stent

BSA: Bovine serum albumin

CAD: Coronary artery disease

CD: Cluster of differentiation

CPDA: Citrate-phosphate-dextrose-adenine

CYP: Cytochrome

DAG: Diacylglycerol

DES: Drug eluting stent

EDTA: Ethylenediaminetetraacetic acid

ELISA: Enzyme-linked immunosorbent assay

FACS: Fluorescence activated cell sorting

Fc: Fragment crystallisable

FITC: Fluorescein isothiocyanate

GLUT: Glucose transporter

GP: Glycoprotein

HEPES: Hydroxyethyl-piperazineethanesulfonic acid

HIF-1α: Hypoxia-inducible factor 1-alpha

IP: Immunoprecipitation

IP_3: Inositol triphosphate

JAM: Junctional adhesion molecules

LVEF: Left ventricular ejection fraction

MFI: Mean fluorescence intensity

mRNA: Messenger ribonucleic acid

NO: Nitric oxide

NSTEMI: Non-ST-segment elevation myocardial infarction

PBS: Phosphate buffered saline

PBSF: Pre-B-cell growth stimulating factor

PCI: Percutaneous coronary intervention

PCR: Polymerase chain reaction

PE: Phycoerythrin

PECAM: Platelet endothelial cell adhesion molecule

PGI: Prostaglandin I

PRP: Platelet rich plasma

RMP: Renal multipotent progenitor cell

RT: Room temperature

SDF-1: Stromal cell-derived factor 1

SDS-PAGE: Sodium-dodecyl sulfate polyacrylamide gel electrophoresis

SEM: Standard error of mean

SLAM: Signalling lymphocyte adhesion molecule

STEMI: ST-segment elevation myocardial infarction

TPO: Thrombopoietin

TRAP: Thrombin receptor agonist peptide

vWf: Von Willebrand factor

Introduction

Coronary artery disease

Coronary artery disease: From the past to the present

“Remarks on Angina Pectoris” by John Warren, M.D., appeared in 1812 as the first article in the first issue of *The New England Journal of Medicine and Surgery*. Warren’s description of angina pectoris derived from the Latin term angina, “infection of the throat”; from the Greek ἀγχο′νη, “strangling”; and from the Latin pectus, “chest”.[1] At this time pathogenesis of angina pectoris was not known. Heberden was the first to clinically describe “Angina” in 1772.[2] However, it took almost a century until pathologists considered coronary arteries and their thrombotic occlusion as cause of angina pectoris.[3] In 1879, the pathologist Hektoen concluded, that myocardial ischemia was caused by coronary artery thrombosis.[4] In the early 20th century, Herrick suggested absolute bed rest as the treatment of choice for patients suffering from angina pectoris. Furthermore, clinicians began to use the electrocardiogram for diagnosis of myocardial infarction.[5,6] The management of people suffering an acute myocardial infarction stayed pretty much the same until the middle of the 20th century.[3] However, during the economic rise of the United States of America it was noticed, that obviously healthy men were affected by consequences of an acute myocardial infarction (AMI), meaning death or disability. Thus, in 1948 the National Heart, Lung and Blood Institute (NHLBI) started the Framingham Heart Study. The purpose of this study was to investigate the development of myocardial disease by studying the lifestyles of inhabitants in the town Framingham in Massachusetts. “Factors of risk in development of coronary heart disease”; this was the title of the institutes’ first findings dealing with cardiovascular risk factors. The study demonstrated that elevated blood

pressure and serum cholesterol levels were risk factors for developing coronary artery disease (CAD) and in consequence myocardial infarction.[7]

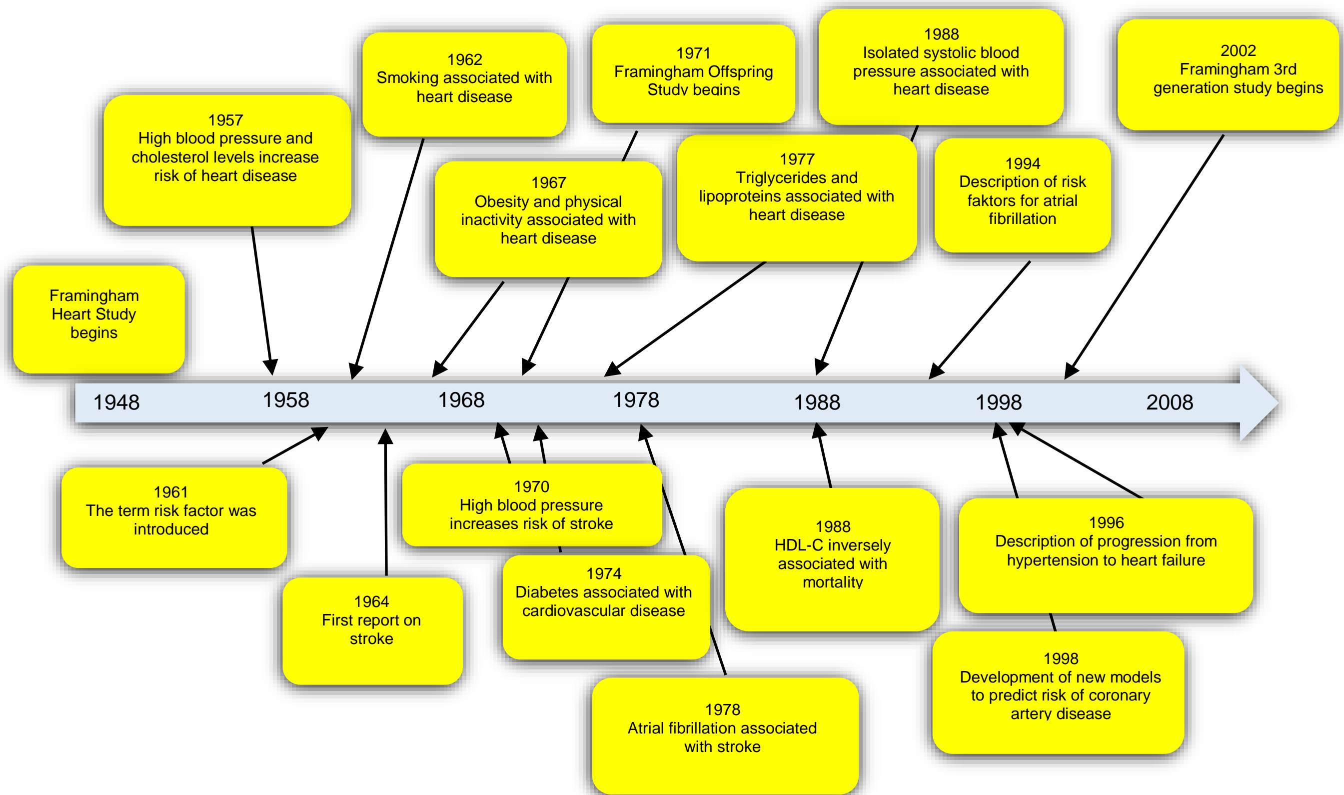
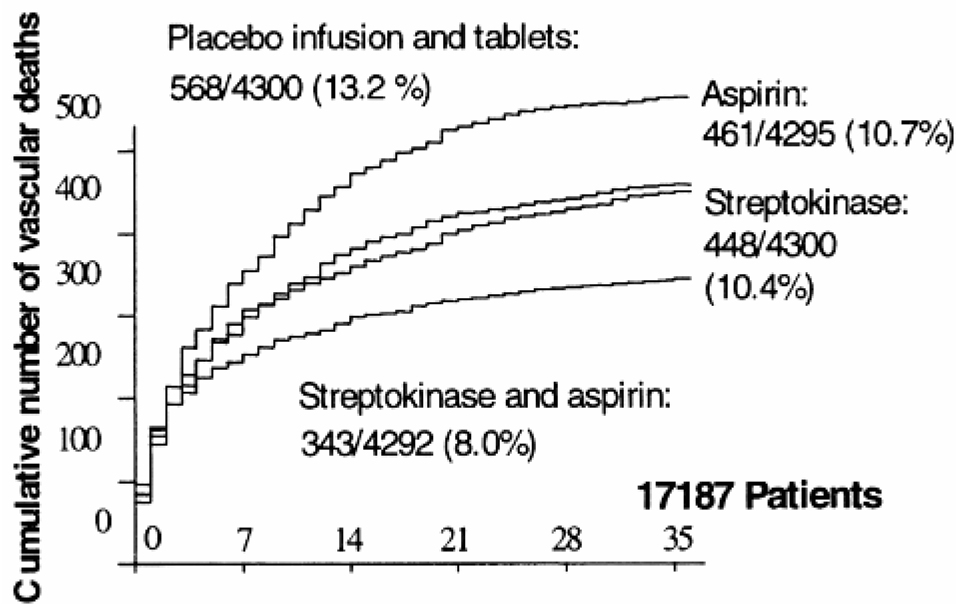


Figure modified after O'Donnell CJ et al. Cardiovascular risk factors. Insights form Framingham Heart Study. 2008.

With the identification of coronary risk factors, the concept of prevention was introduced. Until the year 1961, patients with AMI were prescribed with bed rest. However, many of these patients died within their stay in hospital probably due to fatal arrhythmias. Approximately 30% of patients after AMI died in hospital. This risk was reduced by half by introduction of early chest pain units, back then known as coronary care units. Those units featured monitoring by electrocardiogram and external defibrillation.[3] Early in the 20th century, the French physiologist Bernard started to do cardiac catheterization on animals.[8] These experiments led to the first cardiac

catherization procedure in humans, performed by Forssman in 1929.[9] He actually did the procedure on himself. This self-experiment in turn led to the exploration of cardiac hemodynamics by Cournand and Richards. These three men won the Nobel Prize in Physiology and Medicine in 1956. Cardiac catheterization was the initial step for the development of coronary arteriography in 1958.[10] Those two procedures became gold standard for diagnosis of myocardial function (MI) and vessel anatomy and provided the fundament for coronary artery bypass surgery.[3] The field of invasive cardiology soon emerged, built on the pioneering work of Dotter and Judkins, although Grüntzig is considered the father of percutaneous interventional cardiology.[3,11] Initially, the technique of choice was balloon angioplasty, which was soon improved by implantation of bare metal- and drug eluting stents (BMS, DES). In the 1970s, “the” strategy for reopening a thrombotic occlusion of a coronary artery was the application of a fibrinolytic agent, such as Streptokinase. Fibrinolysis reduced mortality.[12,13] The addition of acetyl salicylic acid (ASA) led to a further reduction in mortality as shown in the Second International Study of Infarct Survival (ISIS-2).[14]



Results from the ISIS-2 trial showing that administration of ASA and streptokinase led to a decreased incidence of vascular death in patients after AMI. Figure taken from the ISIS-2 Collaborative Group. 1988.

Coronary angioplasty and stenting, together with newer, more potent platelet inhibitors (e.g. $P2Y_{12}$- and glycoprotein IIb-IIIa platelet–receptor blockers), further reduced intra-hospital mortality.[3] In patients suffering from severe impairment of left ventricular ejection fraction (LVEF%) after MI, implantation of pace makers (especially with biventricular stimulation), defibrillators (to prevent fatal arrhythmia) and assist devices (as the ultimate ratio and bridging therapy to transplantation) have further improved prognosis.[15,16,17]

Stable CAD

Definition and pathophysiology

Stable CAD is a clinical syndrome most commonly caused by myocardial ischemia and characterized by discomfort in the chest, jaw, shoulder, back, or arms. These

symptoms are typically provoked by exercise or emotional stress and are relieved by rest or nitroglycerin. Less typically, discomfort may occur in the epigastric area.[18] Although the most common causes of myocardial ischemia are atherosclerotic lesions in the coronary arteries, it also occurs in dilated- and hypertrophic cardiomyopathy, aortic stenosis etc.[18] The cause of myocardial ischemia is an imbalance between myocardial oxygen supply and consumption. Oxygen saturation in peripheral blood and myocardial oxygen extraction are stable factors in this mechanism under most circumstances. However, the amount of blood and therefore oxygen delivered to the myocardium depends to a great extent on the diameter of the lumen of the coronary arteries. The diameter decreases with progression of atherosclerotic lesions in the coronary vessel and in consequence may be completely occluded. These alterations decrease- or in the worst case cut off the oxygen supply to certain areas of the myocardium, leading to symptoms of angina pectoris and a minor- to severe impairment of myocardial function. Exercise or emotional stress cause the heart to consume more oxygen. This results in increased heart rate, myocardial contractility- and wall stress. Ischemia-induced sympathetic activation can further increase the severity of ischemia through a variety of mechanisms including a further increase of myocardial oxygen consumption and coronary vasoconstriction, a vicious circle.[18] Angina pectoris is probably caused by adenosine, which is released by the ischemic myocardial tissue and stimulates A1 receptors on cardiac nerve endings.[19] Ischemia is followed by reversible contractile dysfunction known as “stunning”. Intermittent condition of ischemia may lead to chronic- but still reversible dysfunction of the myocardium called “hibernation”. A brief episode of ischemia may also lead to “preconditioning” of the myocardium. This causes an enhanced resistance to further ischemic conditions.[18] In physiological conditions, coronary arteries may lower

vascular resistance to a great amount, leading up to a 6-fold increased perfusion under maximum exercise. Reduction in the luminal diameter by atherosclerotic plaques reduces the normal ability of the coronary vascular bed to reduce its resistance during maximal exercise with the consequence of ischemia dependent on the degree of obstruction and myocardial oxygen demands. When luminal obstruction is 40%, the maximal flow during exercise can usually be maintained. But luminal diameter reduction of 50% may be associated with ischemia when coronary blood flow becomes inadequate to meet cardiac metabolic demand during exercise or stress.[20,21]

Acute coronary syndrome (ACS)

Definition and pathophysiology

ACS represents a life-threatening manifestation of atherosclerosis. It is usually caused by acute thrombosis induced by a ruptured or eroded atherosclerotic coronary plaque, with or without concomitant vasoconstriction, causing a sudden and critical reduction in blood flow.[22] Arteritis, trauma, dissection, thromboembolism, congenital anomalies, cocaine abuse, or complications of cardiac catheterization are rare causes of ACS. The cardinal clinical symptom leading to diagnosis is angina pectoris. Electrocardiography is used to classify patients in two different subgroups.

> Non-ST-Elevation-ACS:
>
> Patients with acute angina pectoris but without persistent ST-segment elevation in the electrocardiogram; These patients have persistent- or transient ST-segment depression or T-wave inversion, flat T waves, pseudo-normalization of T waves, or no ECG changes at presentation. Depending on cardiac-troponin (cTn) (a marker for cardiac necrosis) the diagnosis is further specified in Non-

ST-Elevation-Myocardial-Infarction (NSTEMI), if troponin values are elevated, or unstable angina, if troponin values present in normal range.[22] An increased cTn concentration is defined as a value exceeding the 99th percentile of a normal reference population.[23,24]

ST-Elevation-Myocardial-Infarction (STEMI):

Patients with acute angina pectoris and persistent (>20 min) ST-segment elevation; This condition usually reflects a total occlusion of a coronary artery. The therapeutic objective is to achieve rapid, complete, and sustained reperfusion by primary angioplasty or fibrinolytic therapy.[22]

Platelets

Discovery of platelets, their properties and therapies

The first to report about platelets was Bizzozero, a pioneer in platelet research. He discovered platelets using intravascular microscopy and in vitro flow chamber experiments in 1881-1882.[25,26] Bizzozero was the first one to correctly identify the role of platelets in hemostasis and thrombosis and to describe bone marrow megakaryocytes.[27] Wright identified the megakaryocyte as the precursor cell of the platelet.[28,29,30] In 1886, Osler established that platelets contribute to human thrombotic disorders by discovering them in atherosclerotic aortic lesions and on diseased heart valves.[31] Many important clinical disorders were described in the late 19th and the early 20th century, including immune thrombocytopenia, May–Hegglin anomaly, thrombocytopenic haemorrhage, Glanzmann thrombasthenia, thrombotic thrombocytopenic purpura, von Willebrand disease and Bernard–Soulier syndrome.[25] Histologic examination of sites of vascular injury in animals by light microscopy and

later electron microscopy led to discovery of platelet adhesion and aggregation, followed by degranulation, the loss of distinct borders between platelets, and platelet thrombus contraction.[32,33] By investigation of serum it was found that thrombin is a strong platelet activator and that platelets are secretory cells that store and release the vasoactive serotonin.[25,34] Observation of platelet adhesion to connective tissue further led to the finding that collagen is a potent platelet activator.[31,35] Extensive studies of clot retraction established that platelets contain actin and myosin. Platelets were the first non-muscle cells discovered to possess these functions.[36,37] Several therapies were invented in the early 20th century like platelet transfusion in cases of severe thrombocytopenia in 1910.[38] It took nearly 35 years before platelet transfusion therapies were improved significantly, encouraged by increased mortality due to bleeding caused by radiation of nuclear weapons.[39,40] Bleeding death caused by radiation therapy and the implementation of new chemotherapeutics led to further improvement of transfusion therapy. Therefore, platelet transfusion became crucial for success of chemotherapy.[25,41] Splenectomy was introduced in 1916 based on the recommendation of Kaznelson, who was a medical student in Prague.[42,43] Furthermore, corticosteroid therapy for immune thrombocytopenia was invented in the middle of the 20th century.[44] In 1994, purification and cloning of thrombopoietin (TPO) was established by several groups using the murine myeloproliferative leukaemia virus.[45] TPO increases platelet count in animals and humans by enhancing both megakaryocyte proliferation and survival.[25] The endothelial lining of blood vessels prevents platelet interactions with the vessel wall by both synthesizing platelet inhibitors and masking subendothelial platelet-adhesive proteins. In 1976 and 1977 Moncader and Weksler demonstrated that endothelial cells secrete prostaglandin I_2 (prostacyclin).[46,47] Subsequently endothelial cells were found to release the platelet

inhibitor nitric oxide (NO), which acts synergistically with prostacyclin.[48,49,50] Finally, an endothelial ADPase was discovered by Marcus et al. which is capable of rapidly metabolising the platelet activator ADP to its inactive form AMP.[51,52] Platelet receptors were initially characterized on biochemical responses and later on their ability to release intracellular Ca^{++}.[53,54] Understanding of platelet receptors was significantly improved by invention of the sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which paved the way for detailed biochemical assessment and therefore was a great help in receptor characterization- and purification.[55,56] The development of fluorescence-activated cell sorting (FACS) allowed analysis of the binding of antibodies to individual platelets and even to small platelet microparticles, which are biologically active and support blood coagulation.[57,58,59,60,61] In the late 20th century diagnosis of disorders due to platelet receptors was revolutionized by introduction of the polymerase chain reaction (PCR) which allowed studying of small mRNA.[62] In arterial thrombosis, the role of platelets for a long time was subject of speculation. This changed in 1967 by pioneering work of Mustard et al. who demonstrated ischemia and arrhythmias in pig hearts after injection of ADP.[63,64] The antithrombotic effects of ASA in many animal- and experimental models led to a systematic assessment of antithrombotic effects of ASA in humans.[62,65] In 1974, it was reported that thienopyridine and furopyridine had anti-inflammatory effects and inhibited platelet aggregation after administration of ADP in rats.[66] The 2-chloro thienopyridine derivative, later named ticlopidine, was found to be a potent prodrug inhibitor of platelet aggregation. It selectively inhibits ADP-induced platelet aggregation and prevents fibrinogen binding to $\alpha IIb\beta 3$.[25] Ticlopidine was found to be superior to aspirin especially in secondary prevention of vascular events.[25] Most importantly however, it was shown that the combination of ASA and ticlopidine

prevented from instent thrombosis after coronary stenting.[67] On the other hand, ticlopidine was demonstrated to have severe side effects like significant neutropenia in about 1% of patients.[68] In 1998, clopidogrel, another thienopyridine was approved for clinical use. The efficacy in secondary prevention and prevention of instent thrombosis in combination with ASA was similar to ticlopidine while clopidogrel having a significantly better toxicity profile.[69,70,71] Prasugrel was the third thienopyridine approved for clinical use. It achieves greater platelet inhibition than clopidogrel and it is unaffected by the CYP2C19 variants that seriously impair the effect of clopidogrel. In clinical studies prasugrel demonstrated both increased antithrombotic efficacy and increased risk of bleeding compared to clopidogrel, with the elderly and those with low body weight or a history of previous stroke or transient ischaemic attack at greatest risk of bleeding. Therefore, prasugrel is nowadays reserved for patients with acute coronary syndromes and tolerable bleeding risk.[72] Ticagrelor, a non-thienopyridine $P2Y_{12}$ inhibitor, is in clinical use in the European Union since 2010 and in the United States of America since 2011, respectively. Ticagrelor is an oral-acting, direct inhibitor that has rapid onset- and offset of action.[25] It demonstrated antiplatelet effects in patients who did not respond to clopidogrel and greater overall antithrombotic efficacy than clopidogrel in a pivotal study.[73,74] αIIbβIII (GP IIb/IIIa) antagonists like abciximab, eptifibatide and tirofiban are antithrombotic drugs that exert their platelet inhibitory effects by ligand binding to GP IIb/IIIa.[25] These drugs, that have to be administered intravenously, show potent antithrombotic effects but on the other hand may cause severe bleeding complications.[25] They demonstrated efficacy in reducing ischaemic complications of PCI and the treatment of ACS.[75,76,77] However, due to the high risk of haemorrhage GP IIb/IIIa antagonists are reserved for patients with the highest risk of thrombosis.[25]

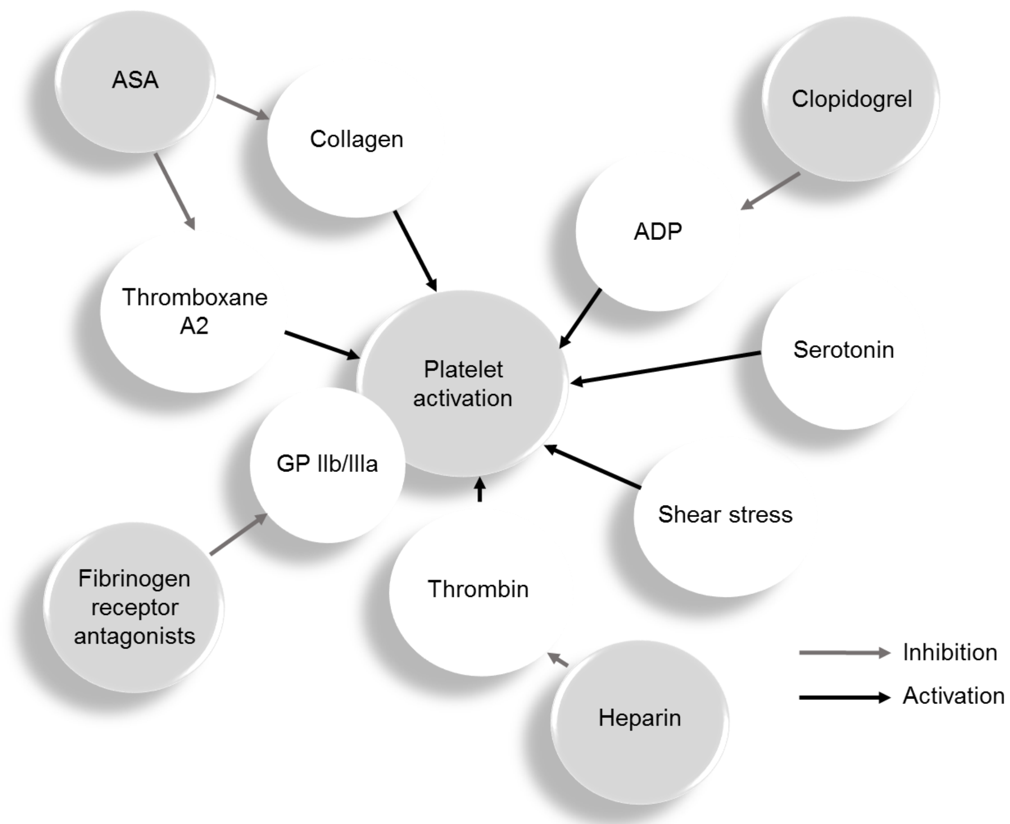


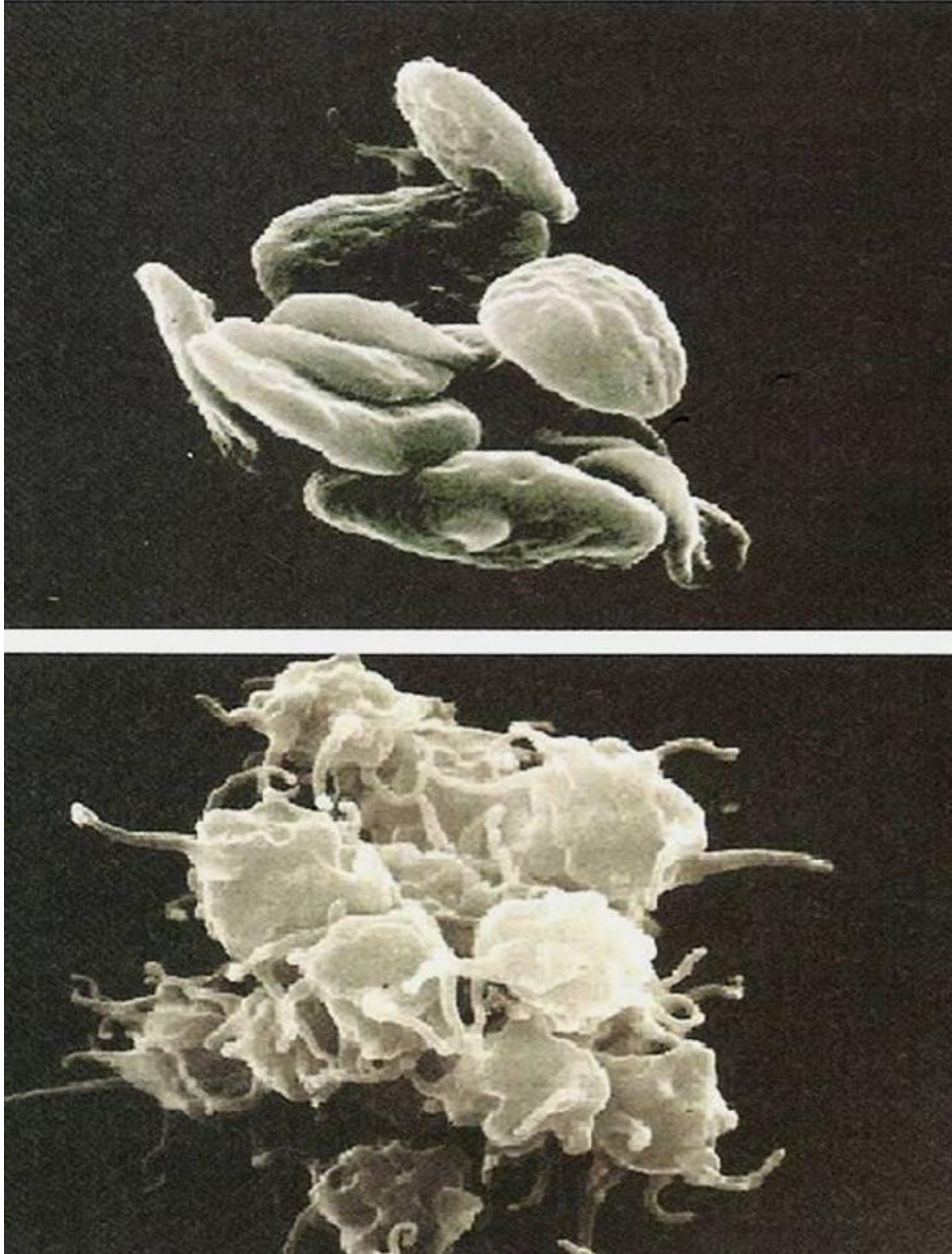
Figure modified after Hämostaseologie für die Praxis: Sicher durch den klinischen Alltag. 2010

Morphology, secretion, adhesion and aggregation

Platelets are the smallest cells in peripheral blood with a diameter of 2.0 to 5.0 μm, a thickness of 0.5 μm and a volume of 6-10 femtoliters.[78,79,80,81] Platelets have a life span of 7-10 days and get then removed from peripheral circulation by the reticuloendothelial system if not previously involved in hemostasis or thrombosis.[82] Platelets derive from their progenitors, the megakaryocytes. Megakaryocytes derive from pluripotent stem cells and undergo multiple DNA replications without cell divisions by the unique process of endo-mitosis.[83] Platelets are rich in secretory granules which are essential for platelet function. 3 types of granules can be differentiated: α–granules, dense granules, and lysosomes with α-granules being the most abundant.[84] The content of α–granules includes both membrane bound proteins that become expressed on the platelet surface and soluble proteins that are released into the extracellular space.[85] These proteins include integrins (e.g. αIIb, α6, β3), immunoglobulin family receptors (e.g. GPVI, Fc receptors, PECAM), leucine-rich repeat family receptors (e.g. GPIb-IX-V complex), tetraspanins (e.g. CD9) and other receptors (e.g. CD36, Glut-3).[86,87,88,89,90] Platelets secrete many mediators involved in blood coagulation. Platelet dense granules contain high concentrations of low molecular weight compounds that potentiate platelet activation (e.g. ADP, serotonin, and calcium). However, α-granules contain large polypeptides like fibrinogen and von Willebrand factor (vWf), that contribute both to primary- and secondary hemostasis.[82] Adhesive receptors like GPIb-IX-V, the major receptor for fibrinogen, integrin αIIbβ3, and the collagen receptor GPVI are found in α-granules and also participate in platelet adhesion.[83,88] Under physiologic conditions, platelets circulate preferentially in close proximity to vascular walls without interacting with endothelial cells. This behavior provides a natural resistance to thrombosis.[91,92] When the continuity of endothelial layer is disrupted and the underlying

subendothelial matrix is exposed, a coordinated series of events are set in motion to seal the defect. Platelets play the primary role in this process.[93] A key initial step in platelet adhesion to the site of injury involves interactions between the GP Ib-IX-V complex and the A1 domain of vWf in the exposed subendothelium.[94,95,96] Endothelial cells serve as a barrier between circulating platelets and different types of collagen localized in the subendothelial matrix.[97] The platelet receptors GPVI and α2β1 mediate the interaction between platelets and collagen. However, these interactions require previous platelet capture through the vWf/GP Ib complex. Both GPVI and α2β1 mediate collagen-induced platelet activation under flow conditions, though the precise sequence of these interactions is not entirely clear yet.[91] However, a variety of inflammatory states may result in platelet adhesion to endothelial cells in the absence of denudation and without evidence of significant alterations in the endothelial integrity. These processes may result from inhibition of the endogenous mechanisms preventing platelet adhesion (e.g. nitric oxide, prostacyclin, ADPase) and/or by inducing endothelial release of molecules that promote platelet adhesion. After initial adhesion of platelets to the site of the injury, platelet aggregation is needed for effective hemostasis. Following adhesion, platelets are activated by a number of agonists such as ADP and collagen that are present at the sites of vascular injury. These agonists activate platelets by binding to specific receptors on the platelet surface discussed earlier. Occupancy of these receptors leads to a series of downstream events that ultimately increases the intracytoplasmatic concentration of calcium ions.[91] Calcium in- and efflux are mediated by diacylglycerol (DAG) and inositol triphosphate (IP_3), respectively. Concentrations of DAG and IP_3 are modulated by a series of G-protein coupled receptors as well as non-receptor tyrosin kinase pathways.[94,98,99] Increased concentrations of free calcium in platelets lead to functional- and structural changes in

platelets. Morphologically, the platelet changes dramatically from a disc to a spiny sphere. This process is called “shape change”.



Electron microscopy of the shape change: (A) resting platelets; (B) activated platelets

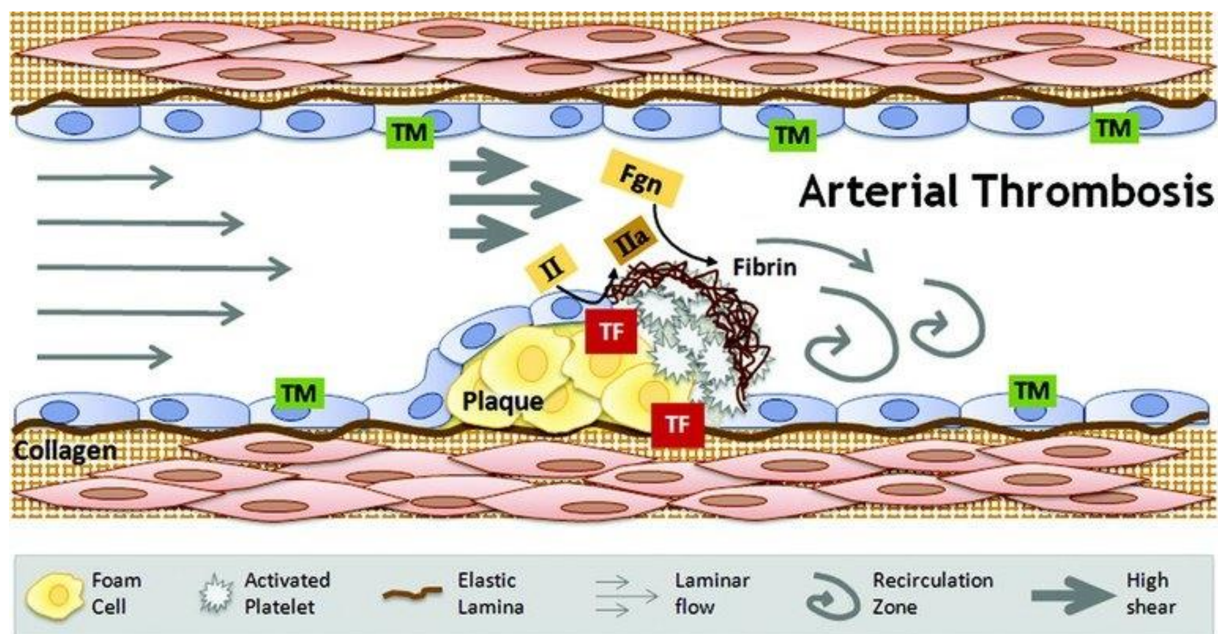
Figure taken from StudyBlue, Lincoln Memorial University.

The "release action" means that the granules in the platelet are centralized and their contents are discharged into the lumen of the open canalicular system, from which they are then released to the exterior.[91] The "shape change" allows the platelets to form aggregates among each other.[91] A main adhesion molecule involved in platelet aggregation is the GP IIb/IIIa complex. GP IIb/IIIa is an integrin receptor present at high density both on the plasma membrane and on α-granules of platelets.[100] This complex is inactive in resting platelets and starts binding soluble plasma fibrinogen after conformational changes, induced by nearly every platelet activator. In turn, ligand binding of GP IIb/IIIa results in conformational changes directed to the cytoplasm.[101,102] Fibrinogen acts as a bridge between two GP IIb/IIIa molecules on adjacent platelets.[94] However, instead of fibrinogen, vWf acts as a bridge molecule between GP IIb/IIIa for platelet aggregation in conditions with high shear conditions, although platelet aggregation under lower shear is mediated by fibrinogen binding to GP IIb/IIIa.[103] Recently, several other molecules have been proposed to mediate platelet aggregation including junctional adhesion molecules (JAMs), signalling lymphocyte adhesion molecules (SLAMs) and CD40 ligand. The relative roles of these mechanisms in platelet aggregation have yet to be clearly defined.[104,105,106]

Development of arterial thrombosis

Consequences of arterial thrombosis such as myocardial infarction are one of the leading causes for death and disability in industrial countries. Usually, an arterial thrombus generates over an underlying atherosclerotic plaque in the high flow- and high shear conditions in the arterial circulation.[107] In general, the thrombus forms over a ruptured plaque or an intact plaque with superficial endothelial erosion.[91] In recent years, the understanding of plaque rupture and subsequent arterial thrombosis has changed. The occurrence of these events is probably affected to a greater extent by

plaque composition than plaque size and degree of stenosis.[108] After plaque rupture, exposition of blood to the released procoagulant materials promotes thrombosis.[91] Under arterial shear stresses, only platelets are capable of adhesion to the damaged vessel wall. Several adhesion molecules and platelet activators are present in the plaque (e.g. collagen and oxidized lipids).[109] However, unlike in venous thrombosis, coagulation factors do not seem to play a major role in arterial thrombosis since they are probably removed due to elevated flow in the arterial system. Atherosclerosis, hypertension, vascular anomalies etc. are considered to be risk factors for arterial thrombosis since these conditions lead to turbulence and altered flow in circulation and therefore facilitate platelet adhesion. Consequently, platelet hyperactivity plays an important role in the pathogenesis of arterial thrombosis.[91]



Arterial thrombosis involves the formation of platelet-rich "white clots" that form after rupture of atherosclerotic plaques and exposure of procoagulant material such as lipid-rich macrophages (foam cells), collagen, tissue factor, and/or endothelial breach, in a high shear environment. TM = thrombomodulin; II = prothrombin; IIa = thrombin; Fgn = fibrinogen; TF = tissue factor.

Figure and text taken from Wolberg AS, et al. Influence of cellular and plasma procoagulant activity on the fibrin network. 2010.

The CXCR4/CXCR7-SDF-1 axis

Chemokines and chemokine receptors

Chemokines are a superfamily of chemoattracting, cytokine-like proteins that bind to- and activate a family of chemokine receptors. Over 50 chemokines have been identified, and they are divided into 4 families (CXC, CX3C, CC, and C) on the basis of the positions of 4 conserved cysteine residues.[110] Chemokine receptors are seven-transmembrane receptors coupled to G-proteins, all with their N-terminus outside the cell surface, three extracellular and three intracellular loops as well as a C-terminus in the cytoplasm. One of the intracellular loops of the chemokine receptors couples with heterotrimeric G-proteins. A ligand binds to the receptor which initiates a cascade of signal transduction events.[111] Most chemokine receptors can bind various ligands. However, some chemokines bind to multiple receptors and some receptors in turn bind multiple chemokines, whereas certain chemokines interact with single receptors and some receptors bind only one chemokine.[112] To date, at least 20 chemokine receptors have been identified. Chemokines and their receptors play important roles in inflammation, infection, tissue injury, allergy, cardiovascular diseases, and malignant tumors.[113]

SDF-1 (CXCL12)

SDF-1 is a CXC chemokine. It was first cloned from a bone-marrow derived stromal stem cell and later recognized as a pre-B-cell growth stimulating factor (PBSF).[110] SDF-1 is expressed in a variety of tissues where it acts as a potent chemoattractant for hematopoetic cells.[114,115] SDF-1 plays an important role in the homing of hematopoetic stem cells to the bone marrow and controlling human- and murine progenitor cell proliferation- and survival.[116,117,118] SDF-1 in- and/or around injured tissues might create a stem cell-attracting environment which possibly results in organ- and tissue repair.[119] SDF-1 is expressed substantially in cases where platelets adhere. Thereafter it causes recruitment of CD34+ cells and affects their proliferation and differentiation to various cell types like macrophages and foam cells or endothelial cells.

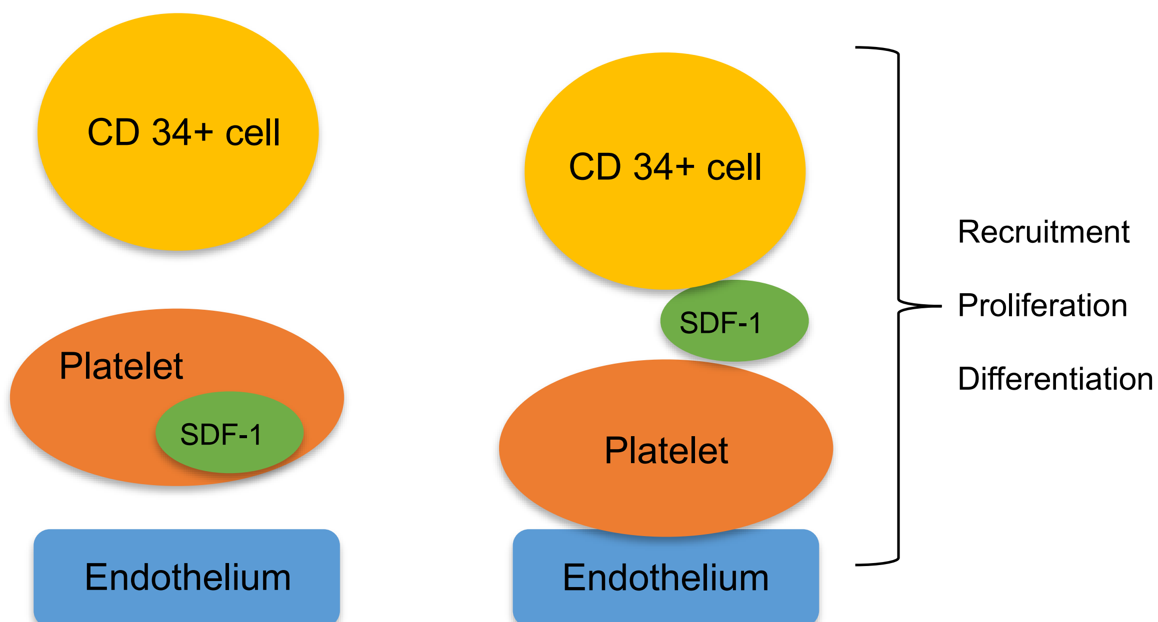


Figure modified after Stellos K, et al. Platelet Aggregates-Induced Human CD34+ Progenitor Cell Proliferation and Differentiation to Macrophages and Foam Cells Is Mediated by Stromal Cell Derived Factor 1 in Vitro. 2010.

In bone marrow, SDF-1 is mainly produced by osteoblasts.[120,121,122] SDF-1 regulates the migration of $CD34^+$ cells. The chemoattractant activity of SDF-1 is significantly impaired when CXCR4 (a SDF-1 receptor) on $CD34^+$ cells is neutralized or blocked.[119] SDF-1 expression is enhanced in endothelial cells expressing hypoxia-inducible factor 1-alpha (HIF-1α).[123] An elevated HIF-1α expression in hypoxic- or damaged tissues increases SDF-1 levels and therefore attraction of CXCR4+ stem cells leading to tissue regeneration.[121] Several experimental studies have shown, that overexpression of SDF-1 in ischemic myocardium leads to in cardioprotection and improved myocardial function after myocardial infarction in vivo.[124,125] It has been recently shown, that SDF-1 is expressed on- and subsequently released by activated platelets.[126] Plasma SDF-1 is decreased in STEMI, compared to patients with stable CAD.[127]

CXCR4

CXCR4 is a highly conserved seven-transmembrane receptor that binds the ligand SDF-1.[110] CXCR4 is expressed in a variety of tissues. In response to SDF-1, CXCR4 can mediate leukocyte- and hematopoetic progenitor cell migration.[128,129,130,131] It has been shown that the SDF-1/CXCR4 axis regulates the homing of human early hematopoietic cells in the bone marrow microenvironment. Mice with “knock-outs'' of CXCR4 genes usually die in utero and display severe hypocellularity of the bone marrow.[127,132] Interestingly, the biological function of CXCR4 seems to change with maturation of cells. Human platelets for example become less responsive to SDF-1 compared to their progenitors.[133]

CXCR7

The existence of an exclusive receptor for SDF-1 has been questioned after the finding, that CXCR4 knock-out mice were still able to bind SDF-1.[134] Additionally, discrepancies between CXCR4 expression and SDF-1 binding affinity in human cancer cell lines were shown.[132] Thus, the presence of another SDF-1 binding receptor was suggested. This receptor was recently identified and named CXCR7.[132,135] Like CXCR4, CXCR7 is a seven-transmembrane receptor.[110] CXCR7 levels are elevated in endothelial cells of certain tumors. Furthermore, CXCR7 is expressed on activated endothelial cells, fetal liver cells, by the placenta and in the vascular endothelium.[132,136,137,138,139] Neutralization of CXCR7 reduces the number of renal multipotent progenitor cells (RMP) in kidneys with acute renal failure. CXCR7 is required for transendothelial migration of RMPs and mediates their adhesion to endothelial cells. In addition CXCR7, but not CXCR4, is responsible for survival of RMPs.[140] In a stroke model in rats, CXCR7 expression is up-regulated in tissue outside the primary ischemic lesion. SDF-1 might protect neurons and non-neuronal cells from ischemic damage via CXCR7.[141] Further, there is some evidence that CXCR7 exclusively mediates endothelial progenitor cell survival.[142] However, the contribution of CXCR7 to SDF-1 mediated effects is to a large extent still unknown.

Aims

There is emerging evidence that platelet surface expression of SDF-1 is enhanced during ischemic events and might play an important role in peripheral homing of stem cells and myocardial repair. Since SDF-1 effects are mediated through CXCR4/CXCR7 we investigated platelet expression of SDF-1, CXCR4 and CXCR7 in patients with coronary artery disease (CAD).

The aims of the thesis were the following:

To show expression of both CXCR4 and CXCR7 on platelets

To assess the surface expression of CXCR4 and CXCR7 on platelets of patients with either stable CAD or ACS

To assess a possible correlation between surface SDF-1 and both surface CXCR4 and CXCR7 on platelets

To investigate an influence of CXCR4 and CXCR7 expression on functional recovery in patients with ACS

Materials and methods

Patient characteristics and blood sampling

From March 2012 till May 2013 we performed a consecutive clinical study including patients suffering from symptomatic coronary artery disease (CAD) at the department of cardiology of the University of Tübingen. We were able to include 215 patients with CAD. 112 individuals presented with stable CAD whereas 103 suffered an acute coronary syndrome (ACS). Prior to percutaneous coronary intervention (PCI) 50ml of blood were drawn via the catheter sheet and analysed within one hour for platelet surface expression of CD42b, SDF-1, CXCR4 and CXCR7 by fluorescence-activated cell sorting (FACS). All subjects gave written informed consent. After initial submission of the manuscript underlying this doctor thesis we were asked to confirm the findings in a healthy control group and therefore did a de-novo investigation of 5 healthy volunteers, 5 patients with stable CAD and another 5 individuals presenting with ACS. Definitions of stable CAD and ACS are provided in the introduction section of this thesis. The study was approved by the institutional ethics committee (270/2011BO1) and complies with the declaration of Helsinki and the good clinical practice guidelines.[143,144,145]

Table 1: Baseline patient characteristics in patients with acute coronary syndrome compared to patients with stable CAD

Characteristics	All (n=215)	Acute Coronary Syndrome (n=103)	Stable CAD (n=112)	p
n male	160	78	82	0.673
n female	55	25	30	0.673
Age years (Mean ± SD)	69 (± 11)	69 (± 11)	70 (± 12)	0.051
CVRF				
Arterial Hypertension	191 (88.8%)	84 (81.6%)	107 (95,5%)	0.006
Hyperlipidaemia	159 (74.0%)	67 (65.0%)	92 (82.1%)	0.332
Diabetes	61 (28.3%)	29 (28.2%)	32 (28.6%)	0.866
Smoking	40 (18.6%)	16 (15.5%)	24 (21.4%)	0.079
Ex-Smoking (>6 months)	40 (18.6%)	19 (18.4%)	21 (18.8%)	0.931
Atrial fibrillation	39 (18.1%)	18 (17.5%)	21 (18.8%)	0.615

LV function (EF%) (Mean ± SD)	50.0 (± 11.2)	49.5 (± 10.2)	50.4 (± 12.0)	0.123
LVEF% normal	79 (36.7%)	29 (28.2%)	50 (44.6%)	0.042
LVEF% mild impairment	44 (20.5%)	26 (25.2%)	18 (16.1%)	
LVEF% moderate impairment	37 (17.2%)	22 (21.4)	15 (13.4%)	
LVEF% severe impairment	32 (14.9%)	16 (15.5%)	16 (14.3%)	
LVEF% unknown	23 (10.7%)	10 (9.7%)	13 (11.6%)	
Renal function (GFR) (Mean ± SD)	73.5 (± 23.9)	73.4 (± 26.8)	73.5 (± 21.2)	0.524
Medication on admission				
Acetyl Salicylic Acid	128 (59.5%)	56 (54.4%)	72 (64.3%)	0.362
Clopidogrel	53 (24.7%)	14 (13.6%)	39 (34.8%)	0.001
Prasugrel	6 (2.8%)	1 (1.0%)	5 (4.5%)	0.142
Ticagrelor	12 (5.6%)	6 (5.8%)	6 (5.4%)	0.776
Oral anticoagulants	26 (12.1%)	9 (8.7%)	17 (15.2%)	0.218

ACE inhibitors	107 (50.0%)	43 (41.7%)	64 (57.1%)	0.066
AT1-receptor antagonists	37 (17.2%)	14 (13.6%)	23 (20.5%)	0.265
Beta blockers	129 (60.5%)	48 (46.6%)	81 (72.3%)	<0.001
Statins	128 (59.5%)	50 (48.5%)	78 (69.6%)	0.005

Platelet surface expression measured by FACS

Platelets in whole blood were analyzed for SDF-1, CXCR4, CXCR7 and the platelet specific marker CD42b. 50ml of blood were drawn through the catheter sheet into a common, sterile 50ml syringe. Immediately, we put this blood into tubes containing citrate-phosphate-dextrose solution with adenine (CPDA). Afterwards, CPDA blood was diluted 1:50 with phosphate buffered saline (PBS, Gibco) and incubated for 30 minutes at room temperature with the following antibodies: CXCR4-PE, CXCR7-PE, SDF-1-CFS and CD42b-FITC (all mouse anti human, R&D, Beckman Coulter). For validation of our results we did respective isotype controls (R&D). After incubation the probes were fixed with 0.5% paraformaldehyde and thereafter analysed by FACS (Becton-Dickinson, Heidelberg, Germany). We further used the platelet activators thrombin receptor agonist peptide (TRAP) and ADP (Sigma-Aldrich) to analyze agonist induced changes in platelet surface expression of the markers of interest.

Immunofluorescence confocal microscopic analysis

This analysis was initially not a part of our investigation. However, after initial submission to the European Heart Journal reviewers asked for another technique for validation of the FACS results. Therefore, we decided to perform immunofluorescence confocal microscopic analysis of CXCR4 and CXCR7 inside- and on the surface of platelets. We fixed platelets in platelet rich plasma (PRP) with 1% paraformaldehyde, applied them to 0.01% poly-L-lysine coated coverslips and then permeabilized platelets with 0.3% Triton X-100. We did not permeabilize platelets for surface expression analysis of the respective antibodies. After blocking with 1% bovine serum albumin-PBS for 1 hour at room temperature, samples were labelled overnight at 4°C with the following primary antibodies: mouse anti-human CXCR4 and rabbit anti-human/mouse CXCR7 (R&D, Abcam). After washing, samples were incubated with corresponding secondary antibodies (Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor 647 donkey anti-mouse IgG, Invitrogen) for 2 hours at room temperature. Finally, the coverslips were mounted with antifade fluorescence mounting medium. The images were taken with a confocal laser scanning microscope by Zeiss (Carl Zeiss Micro Imaging).

Western Blot analysis

Also this analysis was an answer to a reviewer’s question about the validity of the FACS results. We therefore performed western blot analysis to investigate SDF-1, CXCR4 and CXCR7 expression levels in a small subgroup consisting of 5 healthy volunteers, 5 patients with stable CAD and 5 ACS patients. First, we obtained PRP by centrifugation of blood in acid-citrate-dextrose (ACD) buffer at 200g for 20 minutes at room temperature. Afterwards, we washed the PRP in modified Tyrode-HEPES buffer.

After washing, we centrifuged the sample at 900g for another 10 minutes at room temperature. The resulting pellet was then resuspended in Tyrode-HEPES buffer. The carefully prepared platelets were lysed in immunoprecipitation (IP) buffer under resting conditions for 60 min at 4°C. Thereafter, western blot analysis was performed. Washed human platelets were lysed in immunoprecipitation (IP) buffer (15mM Tris-hydrochloride, 155mM NaCl, 1mM EDTA, 0.08mM sodium azide, protease-phosphatase inhibitor cocktail) for 60 min at 4oC. Samples were preheated for 10 min to 95°C after dilution with 2x Lämmli buffer + 5% mercaptoethanol. Processed samples were run on a 8.5% SDS-polyacrylamide gel electrophoresis (PAGE) gel for detection of CXCR4 (43KD), CXCR7 (50KD). Blotting onto a polyvinylidene difluoride membrane (Immibilon, Millipore) was performed using a SemiDry Transfer Cell System (Peqlab). Membranes were incubated overnight at 4°C with respective primary antibodies rabbit polyclonal anti-human CXCR4 (Abcam), rabbit polyclonal anti-human/mouse CXCR7 (Abcam). For detection, corresponding secondary fluorochrome-labeled antibodies and the Odyssey infrared imaging system (LI-COR, Bad Homburg, Germany) were used.

Enzyme-linked immunosorbent assay (ELISA)

Besides measuring platelet surface expression of SDF-1 we investigated circulating SDF-1 levels by a commercially available ELISA kit (R&D) in a subgroup of 160 patients. For storage, plasma probes were initially centrifuged for 15 min at 10 000 g within 30 minutes of collection. Samples were stored at -80°C until analysis.

Follow up of left ventricular function

One main study objective was to investigate functional recovery after ACS. A parameter that can be measured quite easily and gives good information about the myocardial function is the left ventricular ejection fraction (LVEF%). For this part of the study we investigated just ACS patients since ACS often leads to an acute impairment of LVEF%. However, in an emergency situation like ACS, detailed echocardiography is sometimes not possible. Therefore, we decided to investigate LVEF% at baseline via ventriculography during PCI. Before hospital discharge we re-evaluated LVEF% by transthoracic echocardiography (TTE). After three months patients were re-scheduled for following up the course of LVEF% by TTE. LVEF% was assessed using the Simpson's method.[146]

Statistical analysis

We analyzed all our data using SPSS version 21.0 (SPSS Inc., ChicagoIL). Normally distributed data like LVEF% were compared using repeated measures ANOVA. Results are demonstrated as mean values ± standard deviation (SD) in the form of bar diagrams. Non-parametric data, including MFIs were compared using the U-Test by Mann and Whitney. Results are shown as median values with 25th and 75th percentiles and in the form of box plots. Correlations were assessed using Spearman's rank correlation coefficient (ρ) and are presented as scatter plots. Multivariate analysis was performed to show independent associations of CXCR4 and CXCR7 levels after normalization for CD42b receptor count.

Results

Platelets express SDF-1 receptors CXCR4 and CXCR7

The expression of both CXCR4 and CXCR7 on the surface of platelets is one of the major findings of our study. Western blot analysis shows that both CXCR4 and CXCR7 are expressed in human platelets of healthy volunteers, patients with stable CAD and those with ACS (Figure 1A). As evidenced by immunofluorescence confocal microscopy (Figure 1B), we demonstrate that CXCR4 and CXCR7 are expressed both inside the platelet and on its surface. Intracellular analysis showed that CXCR4 was primarily localized towards platelet periphery on the plasma membrane under resting conditions, while CXCR7 was distributed both in the cytoplasm and plasma membrane (Figure 1B). Figure 1C shows platelet surface expression (FACS) of SDF-1, CXCR4 and CXCR7 in the three subgroups under resting condition and after activation with TRAP or ADP, respectively.[147]

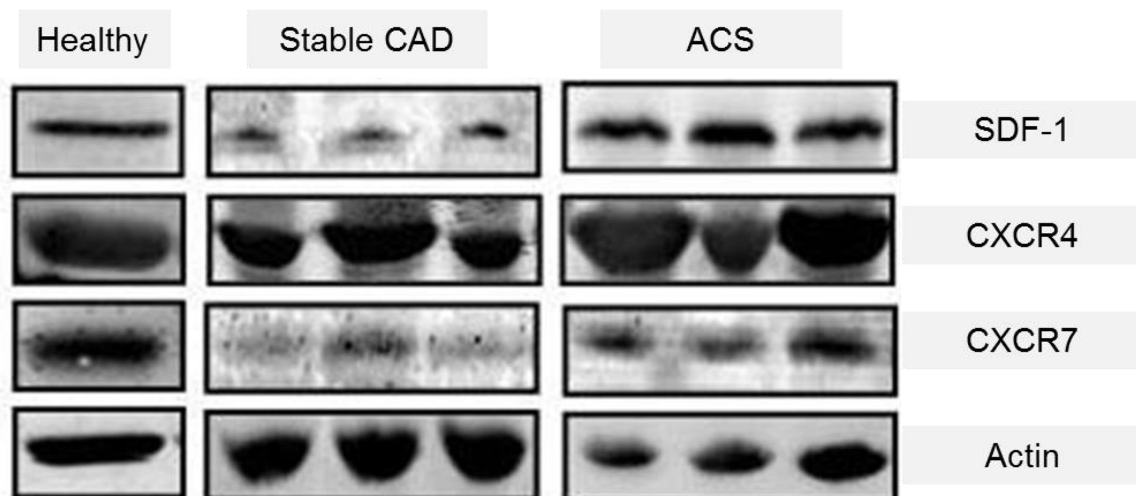


Figure 1A

***Figure 1A** taken from Rath D, 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. 386-394. 2014.*

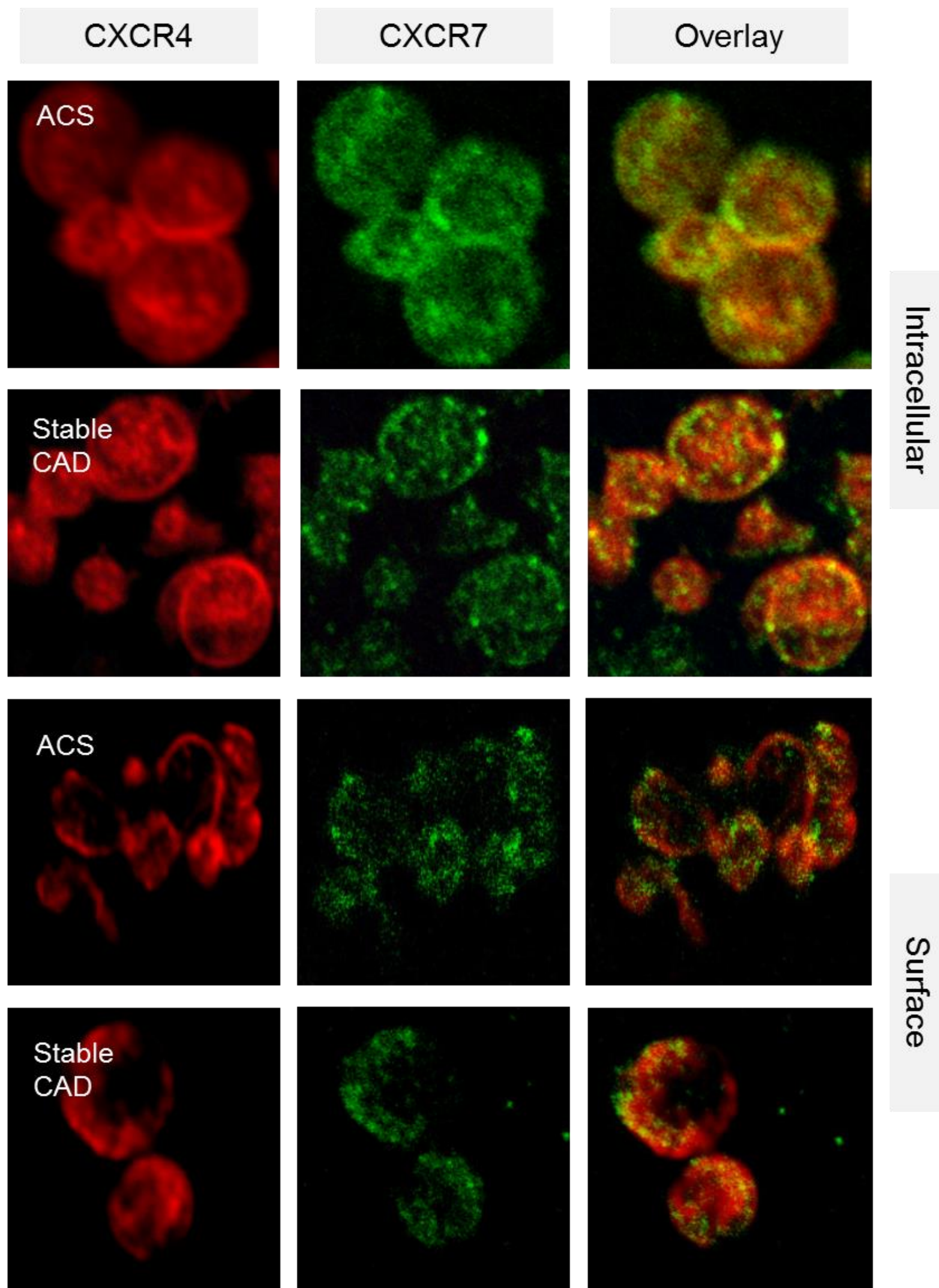


Figure 1B

Figure 1B *taken from Rath D, 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. 386-394. 2014.*

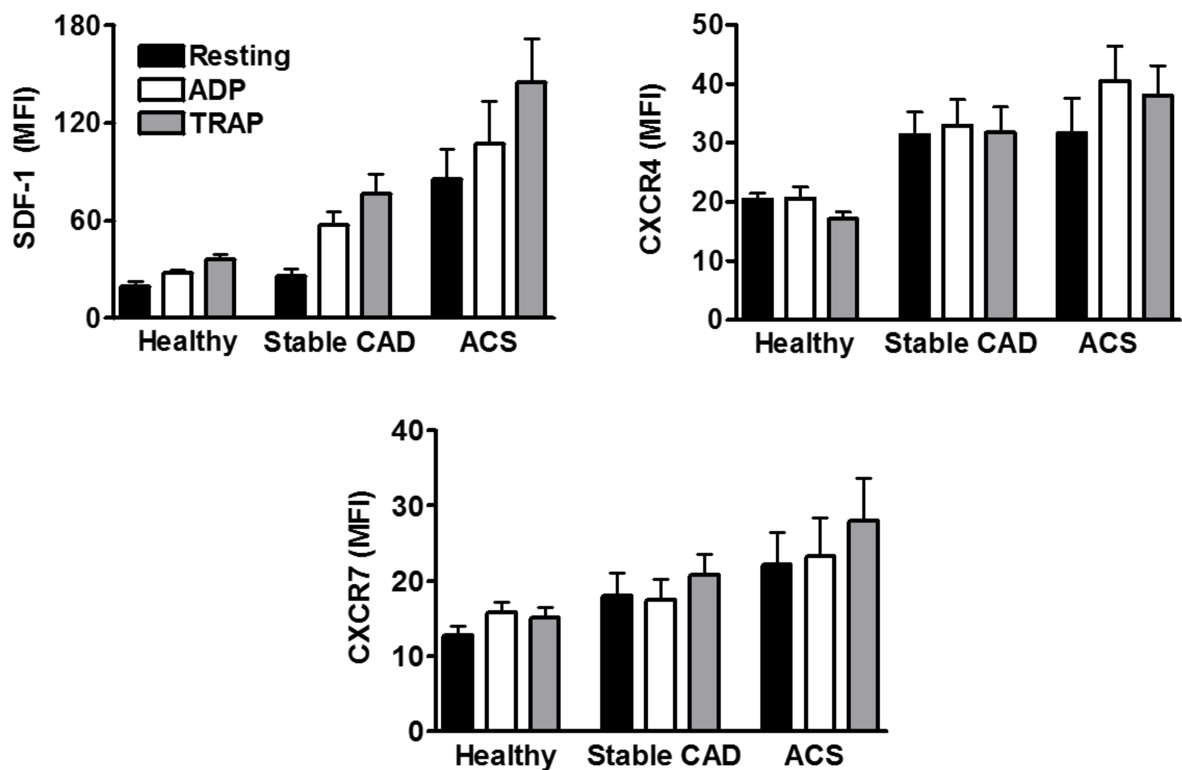


Figure 1C

Figure 1C *taken from Rath D, 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. 386-394. 2014.*

Differential expression of CXCR4 and CXCR7 in patients with ACS compared to stable CAD

Patients' characteristics (age, gender, cardiovascular risk factors, co-medication) of the two subgroups stable CAD and ACS are provided in Table 1. CXCR4 expression was not elevated on the surface of platelets of ACS patients compared to the control group [median MFI 29.0 (25th; 75th percentile 23.5; 40.0) vs. 26.3 (25th; 75th percentile 20.3; 35.4), p=0.122]. In contrast, we could show significantly elevated platelet surface expression of CXCR7 in ACS patients as compared to patients with stable CAD [median MFI 17.8 (25th; 75th percentile 14.6; 27.3) vs. 15.3 (25th; 75th percentile 12.6; 20.6), p=0.004] (Figure 2 A and B).[145] Since ACS consists of unstable angina pectoris, NSTEMI and STEMI we performed a more specific subgroup analysis. There were no statistically significant differences between these three subgroups. We could not find any significant difference CXCR4/-7 platelet surface expression with regard to antiplatelet pre-treatment.

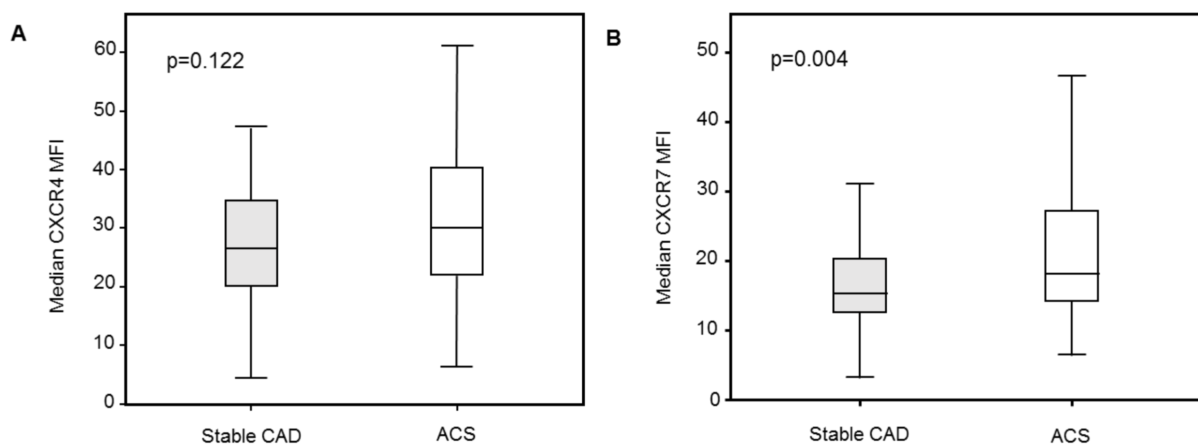


Figure 2A, 2B

Figure 2A *and* ***2B*** *taken from Rath D, 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. 386-394. 2014.*

We found a moderate but significant correlation of CXCR4 and CXCR7 levels on platelet surface (ρ= 0.272, p< 0.001) and of platelet bound SDF-1 and CXCR4 (ρ= 0.273, p< 0.001), respectively (Figure 3A and 3B). Additionally, there was a strong correlation of SDF-1 with CXCR7 expression levels (ρ= 0.454, p< 0.001) (Figure 3C).[145]

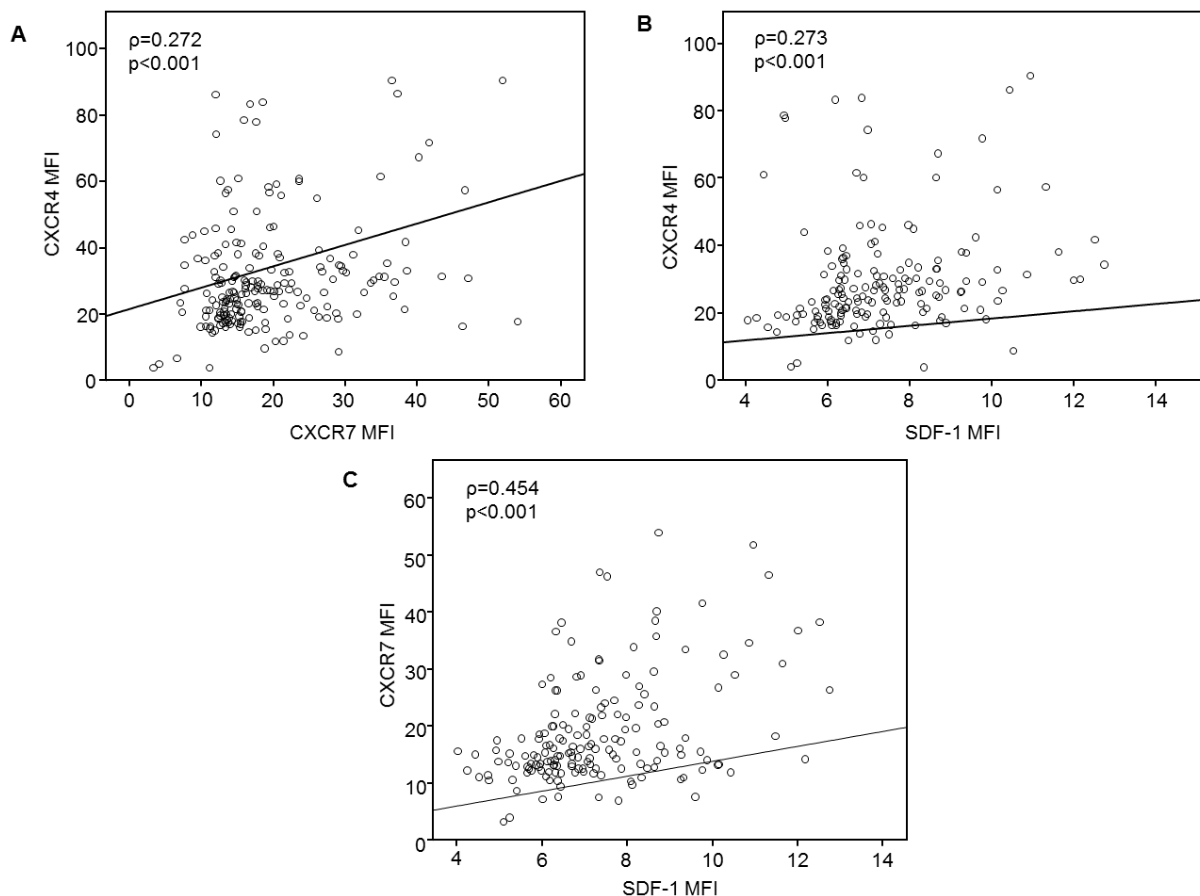


Figure 3A, 3B, 3C

Figure 3C *taken from Rath D, 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. 386-394. 2014.*

Next, we performed univariate analysis of covariance to investigate independent interrelations. We included several cardiovascular risk factors (e.g. hypertension and diabetes mellitus type II), cardiovascular comorbidities (e.g. atrial fibrillation), medication (e.g. ASA and ACE inhibitors) and platelet count in our model. No significant interrelations except for ACS vs. stable CAD on CXCR7 surface expression and beta blockers on CXCR4 surface expression could be shown (Table 2 and 3). Thus, we could demonstrate an independent association of platelet surface expression of CXCR7 but not CXCR4 with ACS. Furthermore, we measured circulating SDF-1 levels in a subgroup of 160 patients. However, we could not show any significant correlations between circulating SDF-1- and platelet CXCR4 and CXCR7 levels.

Table 2: Univariate analysis of covariance with CXCR4 levels as dependent variable and clinical factors as covariates

Variable	Point Estimates (lower bound – upper bound) 95% Confidence Interval	p
CVRF		
Hypertension	-0.16 (-8.81 – 8.50)	0.972

Hyperlipoproteinaemia	-1.06 (-9.09 – 6.97)	0.795
Smoker	1.32 (-5.04 – 7.66)	0.683
Diabetes mellitus type II	0.77 (-4.28 – 5.82)	0.764
Atrial fibrillation	-2.52 (-8.97 – 3.93)	0.442
Medication on admission		
Acetyl Salicylic Acid	3.97 (-1.47 – 9.41)	0.151
Clopidogrel	4.39 (-1.05 – 9.83)	0.113
Oral anticoagulants	-0.33 (-7.67 – 7.01)	0.929
ACE inhibitors	-0.17 (-6.42 – 6.09)	0.958
AT1-receptor antagonists	3.76 (-3.12 – 10.65)	0.282
Beta blockers	-5.35 (-10.24 – -0.46)	0.032
Statins	-3.09 (-11.07 – 4.88)	0.445
Clinical factors		
Age	0.05 (-0.17 – 0.27)	0.651
Gender	0.13 (-4.98 – 5.24)	0.960
LVEF%	0.05 (-0.15 – 0.26)	0.600

GFR	-0.03 (-0.14 – 0.08)	0.612
Platelet count * 1000	0.00 (-0.03 – 0.04)	0.808
Groups		
ACS vs stable CAD	-0.87 (-5.43 – 3.68)	0.706

Table 3: Univariate analysis of covariance with CXCR7 levels as dependent variable and clinical factors as covariates

Variable	Point Estimates (lower bound – upper bound) 95% Confidence Interval	p
CVRF		
Hypertension	2.69 (-5.85 – 11.23)	0.534
Hyperlipoproteinaemia	-4.20 (-12.00 – 3.60)	0.289
Smoker	-1.50 (-7.67 – 4.67)	0.632
Diabetes mellitus type II	1.80 (-3.19 – 6.79)	0.477
Atrial fibrillation	-2.39 (-8.67 – 3.89)	0.453
Medication on admission		

Acetyl Salicylic Acid	-2.79 (-8.21 – 2.63)	0.311
Clopidogrel	0.12 (-5.29 – 5.53)	0.965
Oral anticoagulants	0.21 (-7.18 – 7.60)	0.955
ACE inhibitors	-1.29 (-7.55 – 4.96)	0.684
AT1-receptor antagonists	2.36 (-4.54 – 9.27)	0.500
Beta blockers	-2.45 (-7.34 – 2.44)	0.325
Statins	1.78 (-5.95 – 9.51)	0.694
Clinical factors		
Age	-0.152 (-0.370 – 0.66)	0.171
Gender	2.89 (-2.24 – 8.02)	0.268
LVEF%	0.01 (-0.19 – 0.21)	0.952
GFR	-0.04 (-0.14 – 0.06)	0.453
Platelet count * 1000	-0.01 (-0.03 – 0.02)	0.716
Groups		
ACS vs stable CAD	5.05 (0.58 – 9.52)	0.027

Follow-up of LVEF%

One key finding of our study is, that high platelet CXCR7 is associated with LVEF% recovery after ACS. We found a significant association of CXCR7 but not CXCR4 surface expression on absolute changes in LVEF% at 5 days and after 3 months of follow-up (46.2±10.7, 49.8±9.5, 53.7±9.1; p=0.003 for CXCR7> median vs. 52.1±9.5, 51.5±9.0, 49.9±12.7; p=0.21 for CXCR7≤ median) (Figure 4). We could demonstrate an independent interrelation between CXCR7 expression levels on the platelet surface and LVEF% recovery (Table 4).[145]

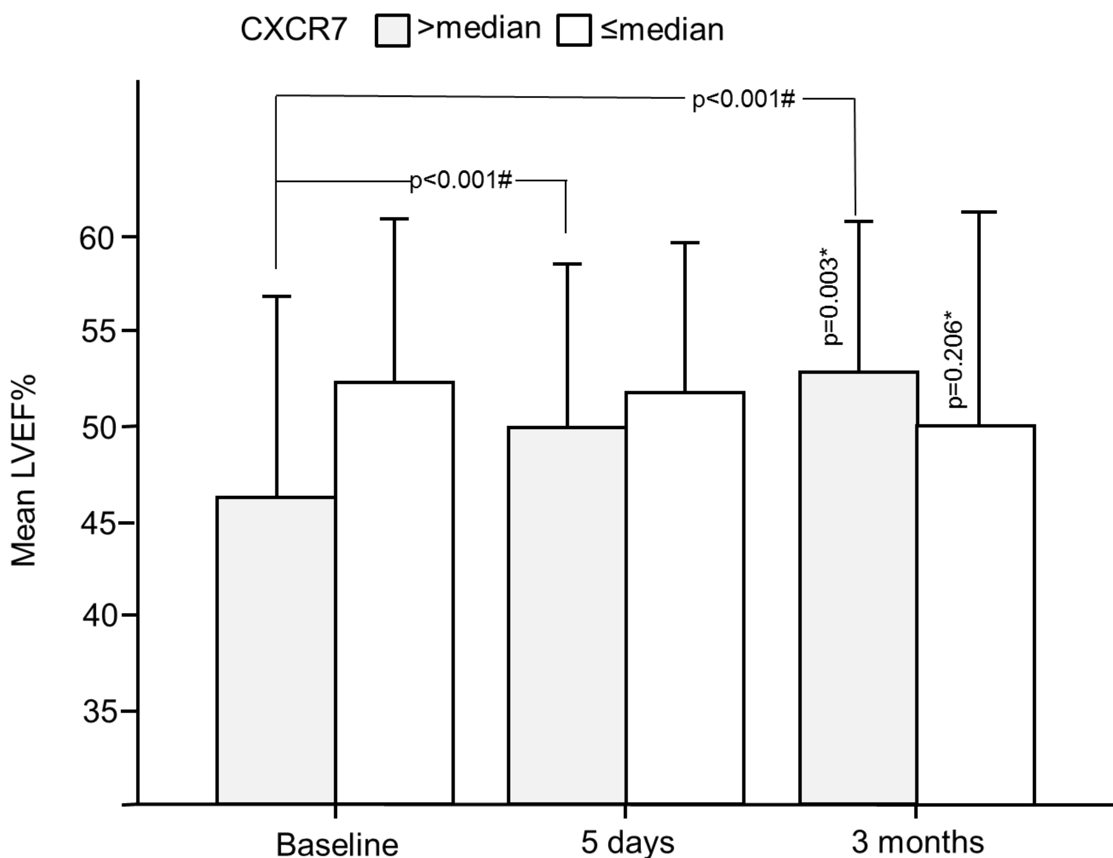


Figure 4

***Figure 4** taken from Rath D, 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. 386-394. 2014.*

Table 4: Univariate analysis of covariance with LVEF% recovery as dependent variable and clinical factors as covariates

Variable	Point Estimates (lower bound – upper bound) 95% Confidence Interval	p
Hypertension	1.07 (-35.79 – 37.94)	0.953
Hyperlipoproteinaemia	-0.7 (-19.11 – 17.71)	0.938
Smoking	30.16 (4.68 – 55.64)	0.022
Diabetes mellitus type II	-7.73 (-30.10 – 14.63)	0.487
Gender	-6.65 (-29.15 – 15.84)	0.551
Troponin I	-0.06 (-0.23 – 0.10)	0.424
CXCR7 >median	26.96 (8.92 – 45.01)	0.005

Discussion

In our study we were able to show that (1) CXCR4 and CXCR7 are constitutively expressed inside- and on the surface of human platelets. (2) CXCR7- but not CXCR4 surface expression is elevated in ACS patients as compared to patients with stable CAD. We found that (3) both CXCR4 and CXCR7 levels correlate with platelet bound SDF-1. Finally, we could demonstrate that (4) high CXCR7 levels are interrelated with LVEF% recovery after ACS.[145]

Platelets are a major source for SDF-1.[124] In the setting of ACS, platelet surface expression levels of SDF-1 are up-regulated. Platelet bound SDF-1 correlates with circulating SDF-1 levels.[148] There is emerging evidence that SDF-1/CXCR4 interactions are vital for the stem cells traffic to damaged tissue *in vitro* and *in vivo*. However, CXCR4 is not the exclusive receptor for SDF-1. Recently, CXCR7 has been identified as another specific receptor for SDF-1, mediating ligand internalization.[136] CXCR4 and CXCR7 interact with each other leading to changes in already existing CXCR4/G-protein complexes, thus impairing CXCR4-promoted calcium signalling and G-protein activation.[149] Both expression and effects of CXCR4 and CXCR7 vary between different cell types. CXCR4 is crucial for SDF-1 mediated progenitor cell migration. On the other hand, CXCR7 seems to be important for progenitor cell adhesion and SDF-1 induced cell survival.[138] Unaffected by its ligand SDF-1, CXCR7 permanently cycles between intracellular compartments and plasma membrane. However, CXCR7 influences SDF-1 concentration and therefore gradients by its ability to scavenge and sequester SDF-1. Altered SDF-1 gradients might lead to diverse signalling by CXCR4.[150,151] Incubation with SDF-1 affects CXCR4 and CXCR7 differentially. While CXCR4 is down-regulated, internalized and degraded[152], CXCR7 experiences a dynamic internalization and time dependent externalization.[153] There

are sparse data about these two SDF-1 receptors on platelets, especially in patients with coronary artery disease. In our study we show for the first time that ACS goes hand in hand with elevated platelet surface expression of CXCR7. We could furthermore demonstrate that both CXCR4 and CXCR7 correlate with platelet surface expression of SDF-1. CXCR4 was elevated by trend, but not significantly in ACS patients compared to patients with stable CAD.[145] Previous studies showed, that SDF-1 incubation hardly affects CXCR7 expression.[154] Thus, there is a possibility that hypoxia-induced enhanced platelet surface expression of CXCR7 prevails in the setting of ACS. Another important finding was the independent association of high CXCR7 levels and LVEF% recovery after ACS. In addition we found an independent association of CXCR7 expression on long-term improvement of LVEF%. We have already shown that platelet surface expression of SDF-1 is associated with LVEF% recovery in patients with acute myocardial infarction.[155] CXCR7 might mediate these effects. When either CXCR4 or CXCR7 are neutralized, hypoxia preconditioned mesenchymal stem cells lose some of their therapeutic potential, pointing out the importance of CXCR4 and CXCR7 for repair mechanisms after ischemic events.[152] CXCR7 blockage can interfere with SDF-1/CXCR4 induced human endothelial progenitor cell (EPC) adhesion to active endothelium and trans-endothelial migration[140]. There exists evidence that the SDF-1/CXCR4/CXCR7 axis influences human progenitor cell survival after organ ischemia.[132,138,140] In our present study we could not find significant confounding by hypercholesterolemia, platelet count or size and atrial fibrillation, which have been shown to possibly influence the SDF-1/CXCR4/CXCR7 axis.[156,157,158]

After investigation of circulating SDF-1 levels in 160 patients with symptomatic CAD, we could neither find a significant correlation with platelet surface expression of

CXCR4 nor CXCR7. This is surprising since there is a significant correlation of platelet surface SDF-1 with its receptors. However, our observation is in line with previous reports demonstrating that circulating SDF-1 levels do not necessarily correlate with CXCR4 expression.[146]

In our present study we demonstrate a possible role of platelet CXCR7 on mediating beneficial SDF-1 driven effects on myocardial regeneration after ACS. Better understanding of the role of platelets and especially platelet derived CXCR4 and CXCR7 in stem cell function, recruitment and tissue regeneration will provide further insight into the myocardial recovery potential of these receptors. Further investigation of CXCR4 and CXCR7 might enable us to develop novel therapeutic strategies for patients with coronary artery disease.

Zusammenfassung

In dieser Studie konnten wir zeigen, dass die SDF-1 Rezeptoren CXCR4 und CXCR7 auf der Thrombozytenoberfläche exprimiert werden. Wenn wir die Oberflächenexpression von CXCR4 und CXCR7 in zwei Patientengruppen (stabile Angina Pectoris vs. akutes Koronarsyndrom) vergleichen, fällt auf, dass die CXCR7 Expression bei Patienten mit akutem Koronarsyndrom deutlich erhöht ist. Dies gilt für CXCR4 nicht. In unserer Studie korrelierte thrombozytäres SDF-1 sowohl mit CXCR4 als auch CXCR7. Abschließend konnten wir einen signifikanten Einfluss von CXCR7 auf die Erholung der LV-Funktion nach ACS zeigen.

Insgesamt wurden 215 Patienten in die Studie eingeschlossen (n=112 stabile Angina Pectoris, n=103 akutes Koronarsyndrom). Mittels Western Blot Analyse, Konfokalmikroskopie und FACS Messungen konnten wir die Expression von CXCR4 und CXCR7 auf Thrombozyten zeigen. Die Oberflächenexpression von CXCR4 und CXCR7 in den beiden Patientenkollektiven wurde mittels FACS Messungen ermittelt. Die LV-Funktion wurde bei Studieneinschluss mittels Lävokardiographie während der Herzkatheteruntersuchung evaluiert. Danach erfolgten echokardiographische Verlaufskontrollen im Rahmen des Krankenhausaufenthaltes sowie nach 3 Monaten.

Die Studienergebnisse deuten an, dass thrombozytäres CXCR7 SDF-1 Effekte vermitteln- und zu einer Erholung der Myokardfunktion nach akutem Koronarsyndrom beitragen könnte. Jedoch muss das regenerative Potential der Thrombozyten sowie insbesondere der Einfluss von CXCR4 und CXCR7 auf Stammzellfunktion sowie Rekrutierung noch besser verstanden werden um schlussendlich neue therapeutische Strategien für Patienten mit ischämischer Herzerkrankung zu entwickeln.

Author contributions

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