Medizinische Universitätsklinik Tübingen

Innere Medizin III – Kardiologie und Angiologie

Deutsches Herzkompetenz Zentrum Tübingen

Platelet-derived PCSK9 is Associated with LDL Metabolism and Modulates Atherothrombotic Mechanisms in Coronary Artery Disease

Inaugural-Dissertation

zur Erlangung des Doktorgrades

der Medizin

der Medizinischen Fakultät

der Ebenhard Karls Universität

zu Tübingen

vorgelegt von

Alvaro Petersen Uribe

2023

Dekan: Professor Dr. rer. nat. Bernd Pichler

1. Berichterstatter: Apl. Prof. Dr. med. Dr. med. univ. Dominik Rath

2. Berichterstatter: Prof. Dr. med. Reimer Riessen

Tag der Disputation: 12. Februar 2024

Content

List of abbreviations

AA……arachidonic acid
ACEi…… angiotensin-converting enzyme inhibitors
ACS …….. acute coronary syndrome
ADP…….. adenosine 5'-diphosphate
ARB…….. angiontensin receptor blockers
Ca2+……..calcium
CABG…….. coronary artery bypass graft
CAD……coronary artery disease
CCS……chronic coronary syndrome
COL……. collagen
CoRP……. collagen related peptide
CRP……..C-reactive protein
ECG……. electrocardiography
HDL……high density lipid cholesterol
LDL……. low density lipid cholesterol
LDLR…….. low density lipid cholesterol receptor
MI ……... myocardial infarction
mRNA…….. messenger ribonucleic acid
NSTEMI…….. non-ST-elevation myocardial infarction
PCSK9…….. proprotein convertase subtilisin/kexin type 9
PRP……platelet rich plasma
STEMI……. ST-elevation myocardial infarction
TRAP…….. thrombin related activated peptide
UA……unstable angina pectoris
vWf……. von Willebrand factor

1. Introduction

1.1 Coronary artery disease

1.1.1 Definition and epidemiology of coronary artery disease

Coronary artery disease (CAD) refers to a progressive damage in the epicardial arteries due to atherosclerotic plaques.[1] Initially understood as a cholesterol storage disease, it is currently accepted that it is inflammatory in nature.[2] These plaques remain for a lengthy period of time stable and silent, and can spontaneously be symptomatic, presenting as stable angina, unstable angina, myocardial infarction (MI) or sudden cardiac death[3], mainly following the plaque's rupture or erosion.[1] Owing to its diverse clinical presentation, CAD has been studied either as acute coronary syndrome (ACS) and chronic coronary syndrome (CCS).[1]

CAD is the leading cause of cardiovascular disease worldwide, which in turn is a major cause for mortality in every region of the world[4], including developing countries.[5] In 2015, an estimate of 442.7 million cases and 17.9 million deaths were attributed to cardiovascular disease.[6] In Germany, in spite of occupying the top position in mortality, incidence and mortality have been falling for several decades.[7]

1.1.2 Risk factors

Since prevention was set as the main strategy, several risk behaviors have been spotted which carry an increased likelihood to develop CAD. Modifiable factors have been extremely attractive for researchers, as a mediation is straightforward. The most prevalent risk factor is hypertension.[1] It can exacerbate atherosclerosis, the underlying cause of CAD.[8] In fact, by managing blood pressure, every 10 mmHg reduction of systolic blood pressure reduces CAD by 17%.[1] Smoking has been increasingly related to a number of diseases; hemodynamics are affected too. The increase in risk is proportional to the number of cigarettes smoked, the duration of the smoking (packs/years) and the increased depth of smoke inhalation.[9] Diabetes mellitus, particularly type 2, is strongly linked to CAD, conferring a two-fold increased risk for CAD.[10] In addition, diabetes is strongly related to dyslipidaemia[11], a risk factor itself as well.[12] Just as smoking, a graded relationship has been proven with high levels of low density lipid cholesterol (LDL) and increasing risk of coronary artery

disease.[13,14] Furthermore, dyslipidaemia accompanies another risk factor called obesity, which also raises the risk for atherosclerotic disease and mortality.[15] As these risk factors are often intertwined, *metabolic syndrome* refers to the cluster of these clinical findings[16], requiring at least three out of the four factors: arterial hypertension, obesity, dyslipidaemia, and insulin resistance (the underlying cause of diabetes mellitus type 2). As it would be expected by the addition of risk factors, metabolic syndrome carries an increased risk for CAD.[16] All these modifiable risk factors share an origin within a person's lifestyle, which certainly contributes to progression of CAD. Additional factors such as chronic kidney disease, hyperuricaemia, homocysteinaemia and stress, can increase the cardiovascular risk further.[9]

Another group of factors are known as the non-modifiable risk factors. These include genetic variants in several systems as lipid metabolism, diabetes likelihood or arterial hypertension propensity.[9] Family history of heart disease is a main factor for disease development.[17] Regardless of sex, age predisposes to a high incidence and prevalence in CAD. Patients >75 years of age have the greatest mortality due to CAD because of the vast extent of comorbidities and the often atypical clinical picture which delays the diagnosis.[1]

1.1.3 Pathophysiology of coronary atherosclerosis

All arteries are subjected to potential atherosclerosis with coronary and cerebral atherosclerosis having a bigger impact on mortality. Major coronary arteries are located on the epicardium and have a diameter of >500 μm.[18] The complex interaction of the risk factors with the endothelial cells and their molecular messengers, as well as inflammation, sustain the formation and progress of the disease.[19] The pathogenesis may be multifactorial, but two aspects are considered as most relevant: deposition of lipids, which are metabolized and oxidized, and ensuing local infiltration of leukocytes.[20] The development of atherosclerotic lesions classically begin with "fatty streaks", a focal thickening of the intima (the most luminal cellular layer of the arterial wall) through accumulation of macrophages containing lipids and extracellular matrix.[21] This might be facilitated by an increased expression of adhesion molecules.

Dyslipidaemia, vasoconstricting hormones, non-enzymatic advanced glycation products and proinflammatory cytokines derived from excess adipose tissue augment the expression of these adhesion molecules as well.[19] The infiltrated phagocytes embedded in the arterial intima, alongside T lymphocytes attracted by adhesion molecules, communicate with endothelial and smooth muscle cells via prostanoids, other arachidonic acid derivates, cytokines and histamine. These mediators attract the latter smooth muscle cells from the tunica media, which proliferate and elaborate an extracellular matrix.

Circulating platelets also play a role in this proinflammatory milieu.[22] A facilitated adherence on an injured endothelial site induces platelets to secrete atherogenic mediators, such as cytokines, growth factors, adhesion molecules and coagulation factors.[23] The perivascular connective tissue will gain metalloproteinases, which exacerbate oxidative stress and inflammation, will also produce lipidic binding sites and will propagate inflammatory response. The growing lesion can merge with other preexisting lesions, forming a lipid rich core with cellular debris of apoptotic cells surrounded by tissue factor, resulting in a high thrombogenic potential.[2] The plaque's bulge towards the lumen accounts for the vascular disruption and stenoses, nonetheless the lesion growth is mainly outward.[19] As a result of the expanding atherosclerotic plaque and flow obstruction, a mismatch occurs between myocardial oxygen requirement and blood supply.[24]

1.1.4 Pathophysiology of the acute coronary syndrome

Coronary artery disease must be divided by the urgency of its presentation into two distinct clinical presentations. Acute CAD, better known as ACS, translates to the sudden flow obstruction, mainly due to thrombosis.[25] Physical disruption of the atherosclerotic plaque accounts for the great amount of coronary thromboses. As the plaque protective cap ruptures, collagen contact triggers platelet activation. Simultaneously, tissue factor in the plaque's core activates the coagulation cascade.[19] The coagulation pathway and the activated platelets continue to stimulate each other, generating thrombin which reinvigorates the amplification mechanism. Natural fibrinolytic systems which are designed to counteract amplification cascades, such as plasmin, have additional obstacles in patients with classical cardiovascular risk factors, as plasminogen activator inhibitor-1 is increased in

diabetes, obesity and finally in hypertension via angiotensin II.[19] Recent studies have carefully reviewed this widely accepted pathophysiological course, as it has been postulated that many plaques can rupture without sparking clinical manifestations. In fact, constant rupture and healing creates progressive luminal obstruction.[25] Consequently, predicting which particular lesion will provoke a clinically-significant vascular thrombosis remains challenging. In addition, the thrombosis-promoting milieu should also be assessed in more detail.[25]

1.1.5 Pathophysiology of the chronic coronary syndrome

Widely known as "stable ischaemic heart disease", CCS refers to recurrent, transient events of thoracic pain secondary to the demand-supply mismatch. CCS is the initial manifestation of CAD in up to 50% of all patients.[26] It is usually results from the obstruction of at least 1 large epicardial artery by atheromatous plaque.[24] Among CCS, other causes of obstruction such as intramural remodeling, vascular rarefaction, myocardial pathology, and perivascular fibrosis are more common than with ACS.[27]

A group of patients with myocardial ischaemia and no evident obstruction has been described and is largely represented by patients without classical risk factors. It has been suggested that these patients might have microvascular damage or autonomic nervous system anomalies.[18]

1.1.6 Diagnosis of CAD

Myocardial ischaemia's clinical equivalent is angina. It is experienced as a substernal heaviness, or discomfort, which may radiate to the jaw, shoulder, back or epigastrium. It typically appears on physical exertion, cold and emotional stress and lasts several minutes, disappearing by rest or nitroglycerin administration.[24] All these factors are required to label angina as *stable* angina. A prolonged duration, its emergence at rest or its persistence after nitroglycerin administration, or a combination of either results in an *unstable* angina. The Canadian Cardiovascular Society (CCS grading of angina is of great aid in differentiating *stable* from *unstable* angina, as the latter is easily defined as a new CCS grade III or IV (Table

1). Despite both resulting from O_2 supply-demand mismatch, unstable angina is a part of ACS spectrum, while non-progressive stable angina signals CCS.

Table 1. Modified Canadian cardiovascular society grading for angina severity (adapted from Campeau, L. 1976)[28]

Class I	Angina occurs with strenuous/rapid/prolonged exertion
Class II	Angina occurs with moderate exertion (walking >2 blocks on ground level, climbing 1 store of stairs at normal pace)
Class III	Angina occurs with mild exertion (walking 1 or 2 blocks on ground level, climbing 1 store of stairs at normal pace)
Class IV	Angina occurs with no level of exertion

Other symptoms such as breathlessness or fatigue are prevalent in CAD, or perhaps even dominant, and are regarded as 'angina equivalents', particularly in elderly patients.[24]

1.1.7 Basic testing

For well over a century, electrocardiography has been the tool of choice for the detection of ischaemia, seen as repolarization anomalies. ACS is basically separated in two scenarios after an ECG: ST-elevation myocardial infarction (STEMI) and non-ST-elevation ACS[1]. Echocardiography studies assist to the diagnosis by informing about the cardiac function and anatomy. Finally, biochemical markers of myocardial injury, as troponins, are required for further differentiation between non-ST-elevation myocardial infarction (NSTEMI) and unstable angina (UA). Serial testing of troponin at presentation and after three hours is extensively encouraged by most current guidelines, as a dynamic rise (with or without signs or symptoms) suffices for the diagnosis of myocardial infarction.[29] Laboratory studies can also aid in the risk stratification of patients.[1] With these tests and the clinical picture, acute myocardial infarction may be diagnosed.[30] More techniques are widely utilized in the stratification of urgency and prognosis in patients suffering from ACS or CCS.

1.1.8 Treatment

Although treatment strategies differ greatly between ACS and CCS, a common ground is shared: antiplatelet therapy. In fact, it is the mainstay treatment of both, as it is in peripheral vascular disease. [31] Even beyond the coronary vascular bed antiplatelet therapy is required in atherosclerotic disease. The reason for this is that a high risk of a future cardiovascular event persists in CAD, as platelet adhesion, activation, and aggregation both initiate and promote atherosclerotic lesions and thrombotic complications.[23] Dual antiplatelet therapy with acetylsalicylic acid, which disables platelet COX-1, inhibiting platelet aggregation irreversibly, and P_2Y_{12} inhibitors, which constrain the adenosine 5'-diphosphate (ADP) receptor, have been found superior for preventing thromboischaemic events when used in combination than as a monotherapy, albeit increasing risk of bleeding.[31]

1.1.9 Treatment of ACS

Ahead of the vascular revascularization therapy, an integral management must be assured. Oxygen must be available to reduce demand and supply mismatch. Its supplementation will ensue only with evidence of low peripheral saturation.[32] Nitrates also decrease oxygen demand, by increasing coronary artery flow. Pain management with morphine is indicated, as it additionally dilates veins, reducing heart rate and blood pressure.[32] Beta blockers, calcium antagonists, inhibitors of the angiotensin-aldosterone system and ranolazine can be all added under qualifying circumstances for a better outcome.[33]

Primary percutaneous coronary intervention is the optimal revascularization technique available for STEMI, as long as it can be provided in a timely matter. When over 120 minutes have elapsed, fibrinolysis may still offer an alternative. Anticoagulant therapy, usually achieved with heparin or low molecular weight heparins, is extensively encouraged in the acute phase of therapy. In addition, dual antiplatelet therapy should be administered and kept for up for a year after ACS.[29]

For patients undergoing an UA or NSTEMI, the main treatment target is to simultaneously provide relief and prevent recurrence of further ischemic events. An urgent coronary

angiography is still advised for clinically unstable patients, with a time frame of up to 24 hours.[34] Low risk patients can be readily managed medically, and revascularized only if ischaemia recurs.

Lipid lowering therapy with statins should be started, or intensified, in all cases. Optimal values to be achieved of LDL have been more rigorous in every guideline actualization, as consistent and ever growing evidence sustains that lower rates of myocardial infarction are reached with intensive therapy.[35] Current objectives lay in a target LDL level <70 mg/dl in high risk patients, and <55 mg/dl for very high risk patients.[35]

1.1.10 Treatment of CCS

General management of CCS intends to reduce symptomatology and improve prognosis, which is achieved by lifestyle modification and medication. These healthier lifestyle improvements are a risk factor counter reduction by including smoking cessation, physical activity, a healthy diet and maintaining a healthy weight.[1] Medical comorbidities acting as additional risk factors should also be identified and treated accordingly.[24] Pharmacological first line therapy are beta-blocking agents, followed by calcium channel antagonists for symptoms, whereas acetylsalicylic acid and statins are added for secondary prevention.[18]

Myocardial revascularization is central to CCS management, but it should be an adjunct therapy to the medical treatment, reserved only for symptomatic relief or prognosis improvement.[1]

1.2 Platelets

Platelets are anucleate, discoid blood cells with numerous functions and a reduced lifespan (7 – 10 days). They are produced primarily in the bone marrow within megakaryocytes by *thrombopoiesis* and are eliminated in the spleen and liver if they have not been consumed to maintain vascular integrity. Platelets are mainly in an inactive state and are activated by thromboinflammatory processes, as they are capable to interact with activated endothelial cells, leukocytes, and coagulation proteins. A heterogeneity in platelet morphology, function, protein expression profile and age has long been described but remains subject of speculation.[36] In this activated state, platelets are capable of altering the expression and

signaling of many active surface receptors and secretory products (CD36, CD41, CD42a, CD42b and CD61).[37]

The normal platelet count is 150,000 to 400,000 per microliter of blood, which accounts for approximately two thirds of total platelets, as the final third is stored in the spleen. Daily platelet production in an adult is about 10^{11}. Although platelets lack a nucleus within their phospholipid bilayer membrane, they remain capable to synthesize new proteins.[38] They additionally carry distinct mitochondria and storage granules, with dense granules and α granules being the major ones. Within the granules a vast array of biologically active molecules are kept: α granules contain coagulation cascade initiators as von Willebrand factor (vWf), fibrinogen, GPIIbIIIa, P-selectin, while dense granules store hemostatically active molecules as ADP, calcium (Ca^{2+}), serotonin, catecholamines and adenosin 5'-triphosphate (ATP).[37]

1.2.1 Hemostasis

When a blood vessel is damaged, subendothelial structures, mainly highly thrombogenic collagen, are exposed to flowing blood. Platelets bind directly to exposed collagen; this initial tethering is called 'adhesion' and it is the initial step to thrombus formation. vWf binds to exposed collagen fibers as well, which slows platelets down and allows stronger bonds to be created between collagen and platelets.[39] Platelets are resultantly activated and form a surface for additional recruitment via transformed receptors and fibrinogen bridges[22], the latter acting as a bridge for platelet aggregation and, hence, thrombosis.[36] By undergoing activation, platelets become spherical and extend long, spiky pseudopods.[40]

In order to aggregate, however, platelets most prevalent (and platelet specific) integrin $\alpha_{IIb}\beta_3$ must undergo transformation from "low affinity" to a "high affinity" state, which enables platelet cross linkage and forms a firm aggregate.[39] In spite of many theories on integrin $\alpha_{IIb}\beta_3$ activation, it is still unclear how various platelet agonists achieve this condition, although it seems increasingly clear that this process involves a phospholipase mediated intracellular Ca^{2+} increase and/or a protein kinase C activation.[39]

Furthermore, tissue factor, stored by macrophages and subendothelial muscle cells, contributes to thrombin generation and thereby increases platelet activation and thrombus formation.[41] During thrombus development, involved platelets are exposed differently to collagen and thrombin, resulting in a heterogeneous platelet response, as platelets in outer shell are less influenced by collagen and thrombin, while inner, densely packed platelets might be more activated.[36]

1.2.2 Platelets and inflammation

The activation of platelets is a common occurrence in a number of inflammatory diseases beyond cardiovascular pathologies, such as in sepsis, inflammatory bowel disease or arthritis.[38]

At the early site of endovascular damage, an increased secretion of vWf could be responsible for the initial platelet recruitment. This process is favored by increased plasmatic cholesterol.[42] Endothelial adhesion by platelets occurs simultaneously to the expression of inflammatory genes, and precedes atherosclerotic invasion by leukocytes.[43]

While platelets are being activated and continue to adhere, they release a wide array of mitogenic and inflammatory substances, as well as coagulation factors, into the local environment. By liberating all these factors, their microenvironment turns proteolytic, chemotactic and adhesive. This naturally supports monocyte recruitment to the site of lesion.[44] In other words, platelets promote inflammation by leukocyte chemoattraction[45], and are themselves a surface provider for leukocyte adhesion.[22] This is achieved not only by the receptor expression of adhesive molecules like P-selectin on their membrane but also through receptor secretion, and via interaction with fibrinogens to generate additional reactive surfaces.[42] Platelets, however, induce inflammatory responses in the endothelium as well via CD40 ligand, which is stored in high amounts within platelets and released instantaneously after activation.[46] This CD40 ligand accounts for smooth muscle cell stimulation as well, eliciting a metalloproteinase response and tissue degradation.[47]

In addition, platelets have a direct impact on the atherosclerotic environment, as one of its secreted chemokines (platelet factor 4) promotes lipoprotein retention, enhances

cholesterol esterification and oxidized LDL uptake.[22] In fact, platelet factor 4 has been found in human atherosclerotic plaques.[48] The existence of multiple mechanisms in which platelets and platelet-derived mediators communicate with inflammatory pathways suggests that inflammatory and haemostatic roles of the platelets overlap.[36]

1.2.3 Platelets lipidomics

Upon platelet activation, significant changes are conducted in membrane lipids, while simultaneously diverse bioactive lipids, essential for hemostasis are formed and released. Ever since mass spectrometry enabled analyzing these key processes, science unraveled "platelet lipidomics" with the task of gaining insight into platelet biology, aggregation, shape alteration, coagulation and degranulation, their hemostasis and regulation as well as exploring how platelets influence endo- and leukocytes.[49]

Platelets host different lipid families, mainly phospholipids, sphingolipids, steroids and prenol lipids. As they become activated, these lipids are substrates of the enzymatically converted bioactive species, including prostaglandines. Their conversion is responsible for platelet structural alteration and degranulation.[49]

An attempt to correlate platelet mean volume, a surrogate of platelet activation, with hypercholesterolaemia, was recently done. Surrounded by a higher amount of cholesterol extracellularly, platelets also displayed greater activation in patients with familiar hypercholesterolaemia.

1.2.4 Additional platelet effects

Platelet adhesion has a pro-angiogenic effect, assisting in blood and lymphatic vessel development. Further, endothelially adhered platelets participate in vascular progenitor cell recruitment.[36] Platelets accomplish this by recruiting bone marrow - derived progenitor cells, and once more, providing adhesion mediated by P – selectin and GPIIb intergrin.[50] Thrombin signaling may additionally enhance angiogenesis.[37]

Moreover, not only vascular progenitor cells can be recruited but also with the help of the chemokine stromal cell-derived factor-1 (SDF-1), an additional pro-angiogenic factor, stem cells can be recruited for myocardial regeneration.[51]

Platelets may have the capacity to store proteins as well. In Alzheimer's disease, a large amount of amyloid precursor protein has been found within platelets. Furthermore, platelets could contribute to the amyloid β accumulation in the brain and its vessels.[52]

1.2.5 Platelets in ACS

Even prior to the acute vessel occlusion, platelets of patients with ACS have an augmented affinity to oxidized LDL, which in turn correlates with platelet activation. Platelets with higher oxidized LDL binding are also more likely to adhere and activate the endothelium, both *in vitro* and *in vivo,* of an injured carotid artery.[53] This effect is maximized in dyslipidaemia, and is mediated by reactive oxygen species.[54]

Plaque rupture is the triggering factor to clot formation and resulting ACS. The clot then occludes the diseased vessel. The likelihood of thrombosis is related to the formation of a platelet plug and its stabilization by insoluble fibrin. The development of a thrombus at the site of plaque rupture, or coronary stenosis, depends greatly on the prothrombotic potential, of which platelets are crucial determinants. Thus, platelets play a dual role in atherothrombosis consisting of aggregation and coagulation.[55]

In presence of strong agonists, a subgroup of platelets assembles coagulation factors, which are required for the efficient formation of thrombin. They are subsequently known as procoagulant platelets and are the link between platelet activation and fibrin recruitment.[55]

Furthermore, it is shown that circulating platelets interact with the vessel wall, and incorporate pathogenic material from the atherosclerotic plaque.[56] Hence, platelets could be carefully analyzed for procoagulant/prothrombotic propensity, potentially carrying information of rupture prone plaques and increased procoagulant propensity.[55] In a nutshell, enhanced activity of circulating platelets increases the number of cardiovascular events, including death, in CAD.[57]

1.2.6 Platelets in CCS

In physiological state, platelets circulate in proximity to endothelial cells, without adhering to them. There is evidence that in subjects with cardiovascular risk factors, platelets are in

a hyper-reactive state. Under pathological endothelial lining, as in a fatty streak, platelets may rigidly attach to the site of lesion.[40]

Several studies have tried to underline this fact: a non-significantly increased ADP responsiveness in patients with angina or history of myocardial infarction has been measured[58], a significant relationship between CAD and platelet aggregation with electrocardiographic evidence of ischaemia has been demonstrated.[59] Moreover, platelets of patients with stable angina pectoris have been found to be more aggregable than those from healthy subjects[60], and in an additional comparison between patients with CCS under acetylsalicylic acid therapy and healthy subjects, low amounts of exercise (Bruce protocol stage III or higher) have demonstrated enhanced platelet aggregability in patients with CAD.[61] Nevertheless, there is plenty of controversy regarding these particular findings.

In CAD, irrespective of CCS or ACS, platelets exhibit a marked propensity to form microaggregates *in vitro* after anticoagulation with citrate, and it's believed to be caused by a high-affinity state of integrin, and do not engage leukocytes.[62]

Moreover, platelets are key mediators in initiation and maintenance of a chronic proinflammatory and prothrombotic milieu, which contribute to vascular inflammation, which in turn underlines atherogenesis and progression.[63]

1.2.7 Medication effect on platelet physiology

Current antiplatelet treatment is aimed to reduce platelet aggregation. Antiplatelet drugs block autocrine release mechanisms rather than receptors of primary agonists.[36]

Acetylsalicylic acid, better known as aspirin, irreversibly inhibits cyclooxygenase. As stated above, this effect lasts throughout the platelets' lifespan. Cyclooxygenase is responsible for thromboxane A2 synthesis, which once formed, usually during intracellular Ca^{2+} influx, diffuses across the plasma and activates other platelets. Its ephemeral lifetime does not exceed 30 seconds, which restricts thromboxane A2 to a reduced, local effect.[39] Cyclooxygenase is however also responsible for synthesis of many other arachidonic acid derivates, such as prostaglandin I_2, an endothelially produced vasodilator and platelet inhibitor. Unlike thromboxane A2, prostaglandin I_2 production is spared by low dose

acetylsalicylic acid. Both effects sustain acetylsalicylic acids 25% risk reduction of thrombotic events in patients with confirmed coronary, peripheral or cerebrovascular disease.[64]

The ADP-receptor is an additional target of drugs. The ADP receptor P_2Y_{12} is a G-protein coupled receptor which stimulation is required for full platelet activation. An irreversible effect is achieved when using slow onset clopidogrel, prodrug prasugrel or reversible ticagrelor.[65]

It must be however noted that although a dual antiplatelet therapy is widely recommended, a residual platelet aggregation remains and is believed to be responsible for the occurrence of ACS after coronary stenting, particularly in patients older than 65 years of age, suffering from diabetes mellitus, reduced left ventricular function and after ACS.[66]

1.3 Proprotein convertase subtilisin/kexin type 9

In 2003, a novel sequence encoding for a proprotein convertase in chromosome one gained attention for the first time, as it was linked to autosomal dominant familiar hypercholesterolaemia, a genetic trait which increases plasmatic cholesterol dramatically, in turn being a risk factor for CAD. This sequence coded for a putative proprotein convertase, belonging to the subtilase family.[67] After being briefly known as NARC-1, it soon would be renamed proprotein convertase subtilisin/kexin type 9 (PCSK9).[68]

There have been nine distinct proprotein convertases isolated in mammals. All these serine proteases have a great resemblance with bacterial subtilisin and yeast kexin.[69,70] The genes, with the exceptions of members 3 and 8 (*FURIN* and *MBTPS1*), are referred to as proprotein convertases subtilisin/kexin types 1–9 (PCSK1 through PCSK9). The first eight members activate secretory proteins like hormones, growth factors, receptors and transcription factors, by cleaving their peptide bond, which irreversibly leads to fragments which can even have distinct or complementary functions.[71]

In humans it is mainly synthesized by the liver and can be released into circulation.[67] It is also produced to a lesser extent in the intestine, endocrine pancreas and brain.[72] PCSK9 undergoes an autocatalytic cleavage between its pro- and catalytic domains in the cell's

endoplasmic reticulum. It is only at that moment where PCSK9 acts as a protease, unlike other proprotein convertases.[71] Once activated, PCSK9 binds to numerous receptors and escorts them to endosomes or lysosomes for degradation.[73]

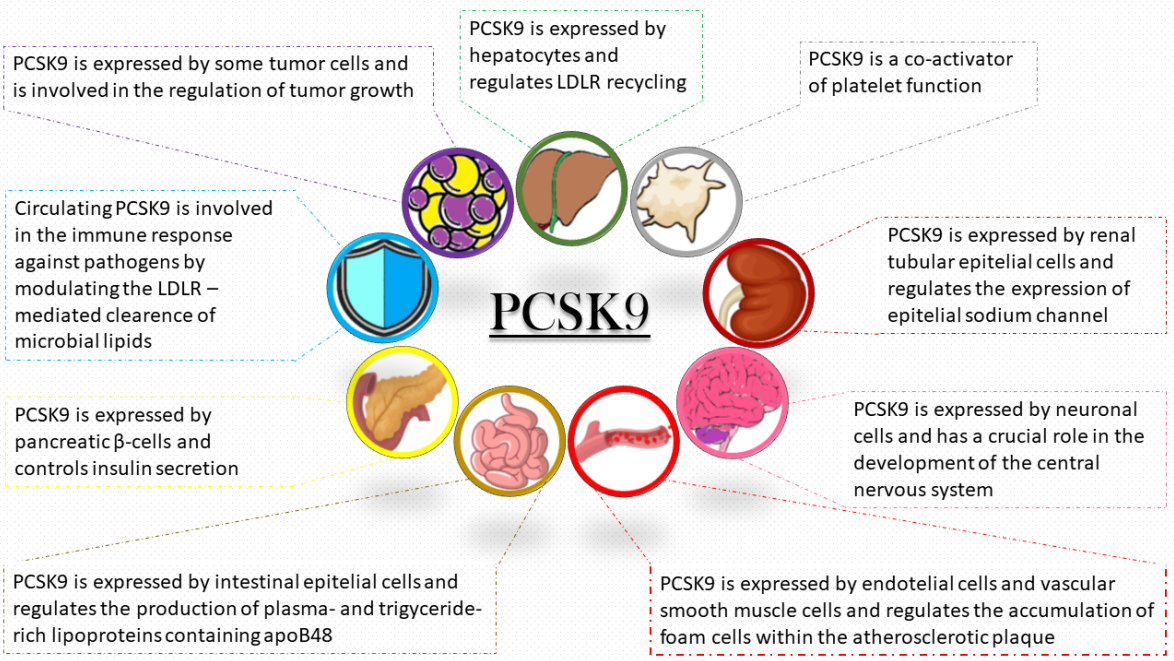

Figure 1. PCSK9 secretion and local effect (adapted from Cesaro, A. et al. 2020).[72]

1.3.1 PCSK9 in cholesterol metabolism

As its first description was made in the search of genes predicting autosomal dominant familiar hypercholesteraemia (currently the third major gene locus to predict this condition), it is unsurprising that PCSK9 plays an important role in cholesterol metabolism. However, this is not true for triglycerides.[68]

PCSK9 promotes the degradation of the LDL receptor. By acting as a chaperone for the internalized LDL-LDL receptor complex and guiding it to a lysosome for premature termination, LDL receptor number decreases.[74] High concentrations of PCSK9 result in a high level of plasma LDL and a shortage of LDL receptors in the liver. Loss of function mutations of PCSK9 are associated with lower LDL levels.[75] In fact, complete loss of PCSK9 seems to have no other additional effect than a profound LDL decrease. This discovery has

raised a lot of interest to pharmaceutically inhibit this protein via monoclonal antibodies as part of the treatment of dyslipidaemia.[71]

Additionally, PCSK9 reduction lowers lipoprotein(a), a highly atherogenic lipoprotein. It is believed that under a higher amount of accessible LDL receptors, lipoprotein(a) may be internalized.[71]

Basic lifestyle modifications may have an impact on PCSK9 levels. Mediterranean diet and exercise reduce circulating PCSK9 levels. In contrast, high-fat and fructose-rich diets increase PCSK9.[76] Counterintuitively, statins upregulate PCSK9 messenger ribonucleic acid (mRNA) levels. This could be the reason for the poor incremental LDL decreases seen with increasing statin doses.[77]

Current guidelines uniformly recommend the use of PCSK9 inhibitors in high risk patients with CAD in addition to statins (with or without ezetimibe), when LDL values cannot be reduced to 70 mg/dl (or 100 mg/dl). The two available monoclonal antibodies are alirocumab and evolocumab, both capable of reducing LDL levels up to 50-60 % and lipoprotein (a) up to 25-30 %. They have been available since 2015 for parenteral administration.[74] The administration of PCSK9 inhibitors additionally reduced the risk of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina by 15 % as a combined endpoint, and up to 20 % when only considering death, myocardial infarction or stroke, during a median of over two years.[78]

1.3.2 PCSK9 in inflammation and sepsis

A substantial change in lipid (and lipoprotein) metabolism occurs in inflammatory states and during infection. In humans with dysfunctional PCSK9, a reduced level of cytokines and a better survival after sepsis has been found, whereas in mutations with excessive PCSK9 production the opposite has been demonstrated. In fact, a positive correlation has been made between PCSK9 and the extent of cardiovascular failure in septic patients. The implication of PCSK9's absence, namely a high LDL receptor proportion, seems to protect from severe sepsis.[79] The monoclonal antibodies used for the treatment of hypercholesterolaemia are promising for the prevention of septic shock.[71]

Recombinant human PCSK9 induces a pro-inflammatory phenotype, as it increases the expression of a number of cytokines such as TNF-α, IL-1β and IL-6, and chemokines in T-helper cells and macrophages.[80]

In animal models, PCSK9 deficiency has even conferred protection against systemic bacterial dissemination, tissue inflammation and organ pathology, particularly in the liver and lungs. PCSK9 overexpression has been noted to exacerbate hypercoagulability and proinflammatory states.[81]

1.3.3 Mechanism of action of PCSK9

The exact mechanisms by which PCSK9 induce LDL receptor degradation remain to be elucidated. To date, it is known that proPCSK9 undergoes autocleavage to be activated. The prodomain remains attached, which prevents PCSK9 from interacting with other substrates and makes it highly selective for transmembrane receptors, particularly LDL. In the absence of PCSK9, the LDL receptor binds to LDL and is endocytosed. The acidic pH of the endosome dissociates the receptor from the substrate and allows the LDL receptor to be recycled while LDL is destroyed in the lysosomes. When LDL receptor is bound to PCSK9, both the LDL and LDL receptor are destroyed with no recycling occurring.[82]

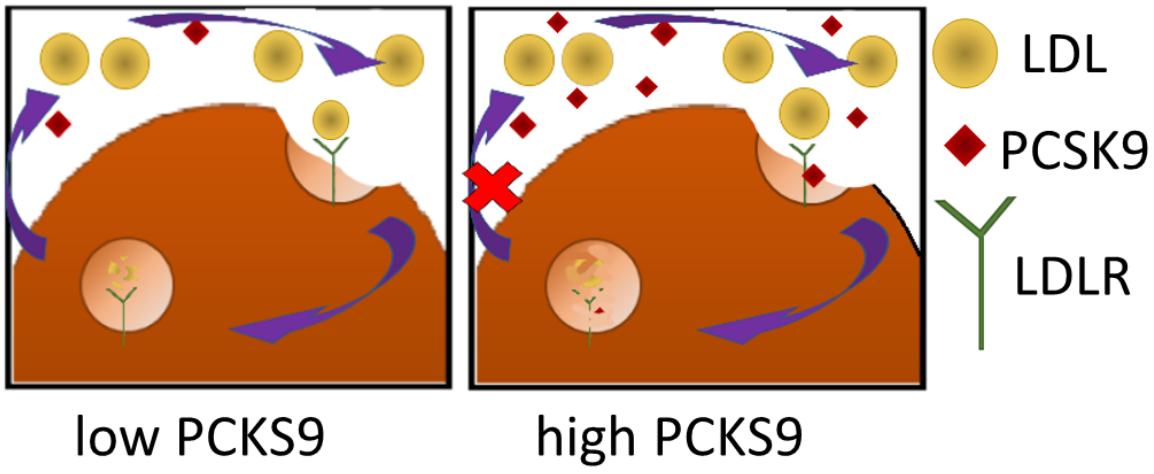

Figure 2. Influence of PCSK9 in cholesterol metabolism (adapted from Seidah, N.G et al. 2017).[71] A schematic representation.

1.3.4 PCSK9 and atherosclerosis

PCSK9 has been isolated within the atherosclerotic plaque. It can be synthesized *de novo* or reach the plaque through the blood stream, as both protein and mRNA of PCSK9 have been found. The presence of PCSK9 suggests that it influences smooth muscle and endothelial cells as well.[83] These proinflammatory effects on macrophages have been observed even at relatively low PCSK9 concentrations.[80]

In addition, macrophage expression of the LDL receptor is influenced by PCSK9.[83] When anti-PCSK9 antibodies have been tested, a reduced macrophage count has been seen in the atherosclerotic plaques, which additionally are more stable and are richer in extracellular matrix.[84]

Several studies point out that PCSK9 may assist in the recruitment of circulating monocytes and neutrophils to the atherosclerotic plaque.[80] However, this should awaits confirmation by large randomized clinical studies.

1.3.5 Association of PCSK9 with platelet reactivity

PCSK9 has furthermore been linked to the activation of thrombogenic pathways. High levels of PCSK9 correlate positively with higher incidence of atherothrombotic events in patients within one year after ACS. Hence, PCSK9 could be a predictor of enhanced platelet activation.[85] In fact, despite treatment with newer P_2Y_{12} inhibitors, this association remains relevant and is independent of serum LDL levels or statin use.[86] It has been reported that the rates of cardiovascular events are lowered by antagonizing PCSK9.[87]

A possible explanation for the association of PCSK9 and platelet reactivity is that high PCSK9 levels impair cholesterol clearance. Oxidized and native LDL are both capable of increasing platelet activation and aggregation.[88] It is clear that oxidized LDL orchestrates the initiation and the progression of atherosclerosis, since it promotes cellularity, macrophage activation and differentiation into foam cells as well as smooth muscle cell proliferation, while also decreasing nitric oxide production.[89] A cholesterol-independent mechanism could additionally enhance this association since a dynamic upregulation of PCSK9 synthesis can be provoked by cardiac ischaemia.[90]

In *in vitro* conditions, human recombinant PCSK9 enhanced platelet aggregation. In this experiment a percentual increase in platelet expression levels of activated GP IIb/IIIa receptor was evident after treatment with PCSK9. In other words, circulating platelets are targeted as well by PCSK9.[91]

1.4 Hypothesis

With platelets being crucial in development and progression of CAD and PCSK9 being linked to atherosclerosis and platelet reactivity a relationship between CAD, platelets and PCSK9 seems undeniable. In the current work, we aimed to explore the possibility of platelets being a carrier of PCSK9 to atherosclerotic sites and we attempt to describe the role of platelet-derived PCSK9 for the ensuing platelet-dependent thrombosis and inflammation in ACS and CCS.

2. Materials and methods

2.1 Type of study and approval of the local ethics committee

The current study represents an observational, non-interventional, monocentric and retrospective clinical study. All 707 consecutively included patients had CAD and provided informed consent, as established by the Declaration of Helsinki's ethical standards.[92] No diagnostic or therapeutic decisions were taken or altered because of the participation in this study. The local ethics committee of the University of Tuebingen's Medical Faculty approved this study and the comprehensive analysis added by the present thesis (141/2018B02, 240/2018B02).

2.2. Definition of study groups, exclusion, and inclusion criteria

CAD patients were firstly studied as a whole. Subsequently, participants subdivided in ACS and CCS were investigated as subgroups. ACS patients included all patients presenting with acute onset of chest pain. ACS patients have been clinically stratified in distinct entities: STEMI, NSTEMI and UA. Briefly, if persistent ST-elevation was found, these patients were labelled as STEMI and were submitted to coronary angiography immediately. For patients with normal ECGs, a further bio-chemistry test (troponin) was undertaken. According to the troponin values, patients were then subclassified in NSTEMI or UA, if troponin remained normal.[93] As all three STEMI, NSTEMI and UA underlie to a similar pathophysiology, we included them in a common ACS group. CCS was defined accordingly to the ESC 2019 guidelines as patients with suspected CAD and stable angina and/or dyspnea, patients with new heart failure onset, patients with stabilized symptoms <1 year after ACS, patients with angina and suspected microvascular disease or asymptomatic subjects with incidental finding of CAD at screening.[1]

2.3 Blood sampling

Blood was drawn from all patients included into this study. Samples were obtained by a trained physician from a peripheral vein. Blood was taken once within 24 hours after catheterization. In CCS patients lacking an indication for catheterization but with previously diagnosed coronary or peripheral vascular disease, blood was drawn at ambulatory presentation.

2.4 Platelet isolation from peripheral blood

Venous blood was drawn and collected in acid-citrate-dextrose-buffer. Platelet-rich plasma (PRP) was collected after centrifugation. After a second centrifugation of the PRP the supernatant was removed. Then Tyrodes-HEPES buffer (HEPES 2.5 mM; NaCl, 150 mM; KCl, 1 mM; NaHCO3, 2.5 mM; NaH2PO4, 0.36 mM; glucose, 6 mM; BSA, 1 mg/ml; pH 7.4) was added.[94,95]

2.5 Whole-blood platelet flow cytometry

Whole blood from citrated tubes was diluted with PCS+Ca^{2+} at 1:50. 80 µl of the diluted blood were added to 1.5 ml preloaded tubes with 5µl anti-CD42b-APC, anti-CD62P-PE, and anti-PCSK9-FITC.

2.6 Immunoblotting

In order to detect PCSK9 western blot analyses were conducted. After centrifugation of the PRP supernatant was taken and the protein concentration was measured with Bradford assay (Biorad). For immunoblotting, proteins were electro-transferred onto a nitrocellulose membrane and blocked with 5% non-fat milk or 5% bovine serum albumin at RT for 1 hour. Then, the membrane was incubated with the Anti-PCSK9 antibodies.[96] These antibodies were obtained as primary antibodies (polyclonal goat IgG, R&D Systems/BioTechne, Minneapolis, USA) and secondary antibodies (IR-Dye 680 RD donkey anti-goat IgG; LICOR, Lincoln, USA). [97]

2.7 *In vitro* monocyte migration assay

Monocyte migration was assessed with a 48-well Boyden chamber. Isolated monocytes were loaded into the upper chamber. In the lower chamber, recombinant human PCSK9, evolucumab (Amgen, Thousand Oaks, USA), or a supernatant obtained from resting or activated platelets were added in different concentrations.

After an incubation of 4 hours, the membrane of the Boyd Chamber was fixed with 100 % ethanol, and consequently stained with May-Gruenwald/Giemsa. The membrane was then placed on glass slides for light microscopy (Nikon Optiphot-2, 20x objective) for image

capturing. The number of migrated monocytes was counted for each well in multiple, randomly selected, microscopic fields.[98]

2.8 Platelet aggregometry

Platelet aggregation was evaluated with the use of an impedance platelet aggregometry (Multiplate Analyzer, F. Hoffmann-La Roche Ltd., Basel, Switzerland). The obtained samples were stored and allowed to warm up at room temperature prior to use.[99] Afterwards, they were treated with different reconstituted agonists: ADP 6.5 µM, arachidonic acid (AA) 484 µM, collagen (COL) 3.2 mg/ml and thrombin related activated peptide (TRAP) 32 µM at the indicated concentrations after prior calibration[100]. Aggregation was measured for 10 minutes with a stir speed of 1000 rpm at 37°C. Maximum platelet aggregation, as well as the area under the curve (AUC), was quantified using the Aggrolink8 software (ChronoLog).[101]

2.9 *Ex vivo* thrombus formation-flow chamber assay

Platelet adhesion and aggregation under flow is an indispensable tool for assessing platelet function. This can readily be achieved with flow chambers, capable of measuring platelet adhesion, platelet aggregation, and coagulation within one experiment. Combining flow chambers and microcapillaries, thrombus formation outside of the circulation has progressed the understanding of rheological processes.[102]

Human whole-blood was incubated with the fluorochrome 3,3'-dihexyloxacarbocyanine iodide (1 mM DiOC6, Sigma Aldrich Co., St. Louis, MO, USA) for 10 min at room temperature. Afterwards 1 ml blood was perfused over a collagen-coated surface (100 µg/ml), through a transparent flow chamber with high (1000 s^{-1}) shear rates. During the perfusion 1 min videos were taken (1 s/frame, Nikon Eclipse Ti2-A, ×20 objective). Subsequently, the chamber was rinsed, and pictures were taken of representative areas (Nikon Eclipse Ti2-A, 20x objective). The covered area was analyzed using the NIS-Elements AR software (Nikon, Japan).[103]

2.10 Monocyte differentiation into macrophages and foam cells

Monocytes and platelets were co-cultured on coated 96-well plates in RPMI-1640 medium for 8 days. For cell culture, IMDM with Glutamax supplemented with 5 % heat-inactivated fetal calf serum, penicillin (100 U/ml)/streptomycin (100 µg/ml), 1 % MEM vitamins, and 1% nonessential amino acids, were used; all purchased from Life Technologies, Inc. (Invitrogen).[104]

We additionally assessed PCSK9 as a chemoattractant by using a modified Boyden chamber. We also added an anti-PCSK9 antibody (evolucumab) (Amgen, Thousand Oaks, USA) to evaluate migration under a PCSK9 blockage.

Post-acquisition image analysis with ImageJ software (National Institutes of Health, Bethesda, MD, USA) was utilized to differentiate and quantify macrophages and foam cells, which characteristically display an increased cell diameter (> 10 µm).

2.11 Immunostaining of atherosclerotic carotid samples

To thoroughly assess the presence of PCSK9 in atherosclerotic lesions, an additional sample of atherosclerotic carotids of mice were procured by carotid endarterectomy in paraffin.

Immunohistochemical staining for PCSK9 (anti-m/rPC9 affinity-purified goat IgG, AF3985, R&D Systems/BioTechne, Minneapolis, MN, USA), CD68 (anti-human mouse monoclonal IgG F1118, Santa Cruz Biotechnologies, Dallas, TX, USA), and CD42b (anti-human mouse monoclonal IgG B0712, Santa Cruz Biotechnologies, Dallas, TX, USA) were performed by using HRP-DAB staining systems (anti-goat kit, CTS008, R&DSystems/BioTechne, Minneapolis, MN, USA, anti-mouse kit, CTS0002, R&D Systems/BioTechne, Minneapolis, MN, USA) according to the manufacturer's instruction.[105]

2.12. Statistics

Patient data was analyzed using the software platform SPSS version 26.0 (SPSS Inc., Chicago IL). Normally distributed variables were presented as mean values ± standard deviation (SD).[106] Correlations were assessed by Spearman's rank correlation coefficient (ρ). General linear models were established using univariate analysis of covariates to show independent associations of platelet count with plasma LDL cholesterol. Variables entered into the model

included age, sex, body mass index, arterial hypertension, diabetes mellitus type II, smoking, statins, and aspirin. When variables followed a non-gaussian distribution, they were presented as medians, with accompanying quartiles. Data of non-normal distribution were tested using the Mann–Whitney U test at 95% CI.[105]

Furthermore, LDL-values were also regrouped as quintiles to reduce the groups sizes and compare them to each other. This was graphically displayed with a box-and-whisker diagram, a non-parametric display fit to show the dispersion amidst all groups.

3. Results

Several studies have shown a relationship between high cholesterol levels and platelet hyperactivity.[107] From March 2017 to February 2020 we recruited 707 consecutive patients. 364 patients (51.5 %) were diagnosed with ACS, and the remaining 343 participants (48.5 %) corresponded to the CCS collective. Baseline characteristics are displayed in Table 2. Mean age was 70 years and less than 30 % of patients were female. Arterial hypertension was highly prevalent and about a third of participants (32.4 %) had a prior diagnosis of diabetes.

Table 2. Baseline characteristics (n=707)

Demographic characteristics	
Age, years (mean ±SD)	70 (±12)
Male, n (%)	500 (70.7)
Body mass index (mean ±SD)	27.5 (±5.1)
Cardiovascular risk factors	
Arterial hypertension, n (%)	630 (89.1)
Hyperlipidaemia, n(%)	459 (64.9)
Diabetes mellitus, n(%)	229 (32.4)
Current smokers, n(%)	126 (17.9)
Ex-smokers >6mo, n(%)	141 (20.0)
Obesity, n(%)	237 (33.5)
Atrial fibrillation, n(%)	167 (23.7)
Prior CABG, n(%)	39 (5.5)
Prior MI,n (%)	153 (21.6)
Chronic kidney disease, n(%)	88 (12.4)
Coronary artery disease	
ACS, n(%)	365 (51.6)
NSTEMI, n(%)	194 (27.4)
STEMI, n(%)	63 (8.9)
Unstable angina, n(%)	108 (15.3)

CCS, n (%)	342 (48.5)
Transthoracic echocardiography	
Ejection fraction %, (mean±SD)	53 (±10)
Medication at blood sampling	
Oral anticoagulation, n(%)	115 (22.1)
ACEi/ARB, n(%)	595 (84.2)
Aldosterone inhibitors, n(%)	160 (22.7)
Diuretics, n(%)	263 (37.4)
Calcium chanel blockers, n(%)	250 (35.6)
Beta blockers, n(%)	489 (69.5)
Statins, n(%)	586 (83.2)
Acetylsalicylic acid, n(%)	503 (71.4)
Clopidogrel, n(%)	263 (37.5)
Ticagrelor, n(%)	160 (22.7)
Prasugrel, n(%)	82 (11.6)
Laboratory parameters	
Leucocytes, 1000/µl (mean ± SD)	8.4 (±2.9)
Platelets, 1000/µl (mean ± SD)	226 (±69)
Creatinin, mg/dl (mean ± SD)	1.2 (±2.2)
Total cholesterol, mg/dl (mean ± SD)	165 (±45)
LDL-cholesterol, mg/dl (mean ± SD)	103 (±42)
HDL-cholesterol, mg/dl (mean ± SD)	47 (±20)
Triglycerides, mg/dl (mean ± SD)	148 (±105)
C-reactive protein, mg/dl (mean ± SD)	1.2 (±3.0)
HB1Ac ,% (mean ± SD)	6.3 (±1.0)

3.1 Platelet count is associated with plasma cholesterol

We found a weak correlation between platelet count and total cholesterol (p≤0.001, Spearman ρ=0.151). This suggests that the higher the platelet count, the higher the cholesterol level in the circulatory system. This was also true for plasma LDL (p≤0.001, ρ=0.156) and plasma high density lipid cholesterol (HDL) (p=0.033, ρ=0.008) but not for plasma triglycerides (p=0.364, ρ=-0.0034). These results are displayed in Figure 3.

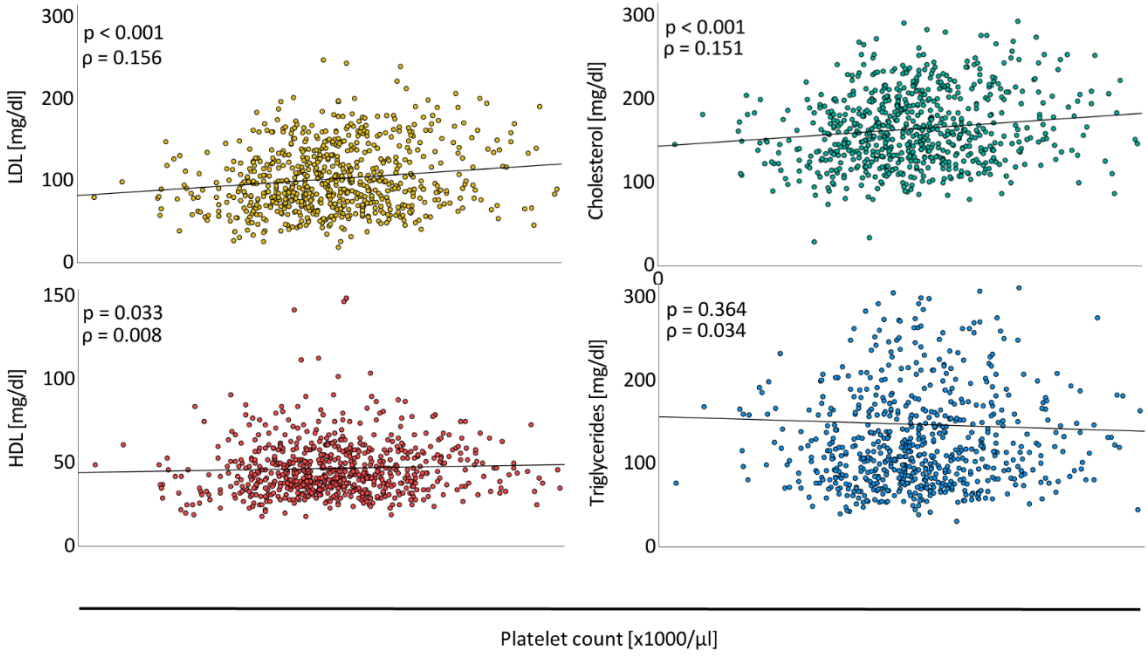

Figure 3. Correlation of platelet count with different plasma lipids

After observing a significant correlation between LDL and platelet count, LDL-values were also regrouped in five equally large quintiles (n = 141) to reduce the groups sizes and compare them to each other. The first quintile contains the lowest LDL values whereas the fifth quintile shows the highest LDL values. Thereafter, we compared the fifth quintile with the other four, and found that the fifth quintile had a higher number of platelets, as seen in Figure 4.

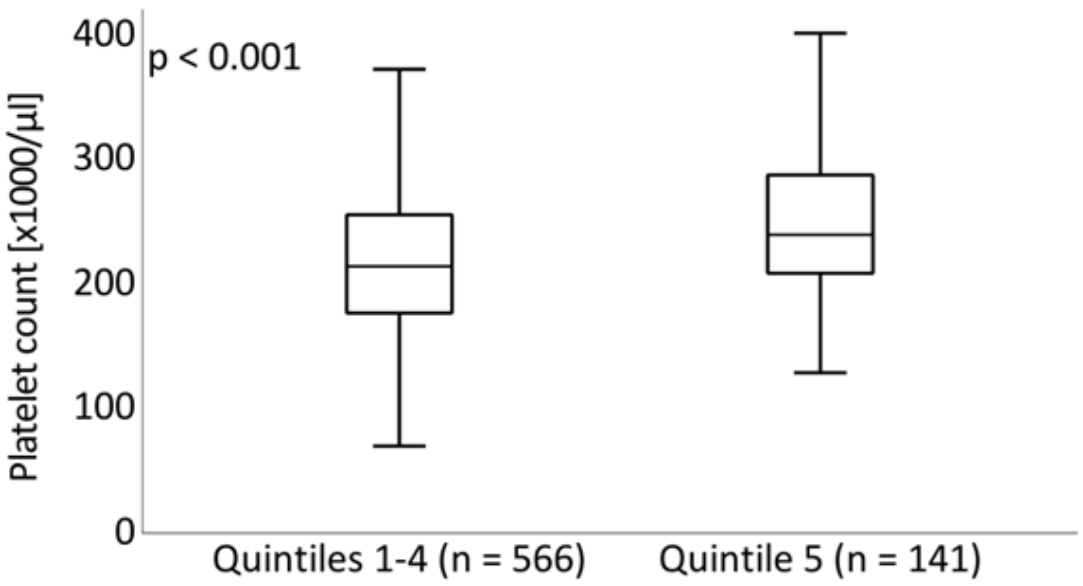

Figure 4. Platelet count stratified according to LDL fifth quintile compared to the remaining LDL quintiles. When subgrouping LDL levels into equally large quintiles, a significant difference was seen in patients with ACS between the fifth quintile and the other quintiles. In patients with CCS, a nonsignificant trend was observed as shown in Figure 5.

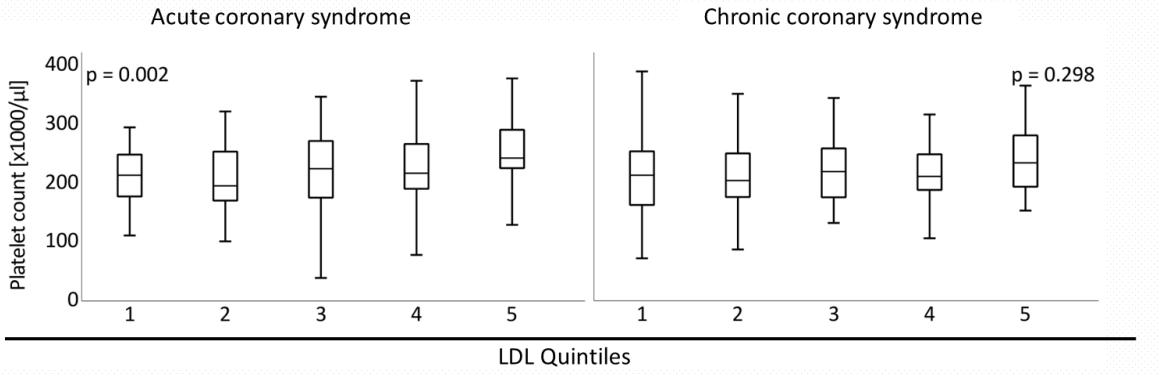

Figure 5. Platelet count stratified according to LDL Quintiles in patients with ACS and CCS

Additionally, platelet count was an independent predictor for LDL plasma levels, as assessed by a univariate analysis of covariance (Table 3). We found that platelet count is independently associated with LDL plasma levels. This hints to a complex correlation between LDL and platelets.

Table 3. Univariate Analysis of Covariance. Dependent variable: LDL

Covariables	*p* value	CI
Platelets (µl*1000)	**0.013**	(0.013 – 0.106)
Age (years)	**<0.001**	(-0.898 – -0.276)
Body mass index (kg/m^2)	0.880	(-0.686 – 0.588)
Sex	**0.010**	(2.227 – 16.315)
Arterial hypertension	0.245	(-16,957– 4.341)
Diabetes mellitus type 2	**0.013**	(-15.334 – -1.838)
Smoking	0.371	(-13.2 – 4.92)
Statins	**<0.001**	(-35.377 – -17.733)
Acetylsalicylic acid	**0.003**	(3.849 – 18.157)

3.2 PCSK9 levels in different subtypes of CAD

We proceeded to explore distinct platelet PCSK9 levels in CCS and ACS subgroups, namely STEMI, NSTEMI and UA patients. Thus, we utilized a subgroup of participants with initial available platelet PCSK9 measurements. This subgroup included 67 patients with CCS, 53 patients with NSTEMI, 13 patients with STEMI and 22 patients with UA. We found, as shown in Figure 6, that platelet PCSK9 levels are relatively homogenously distributed in different atherosclerotic conditions.

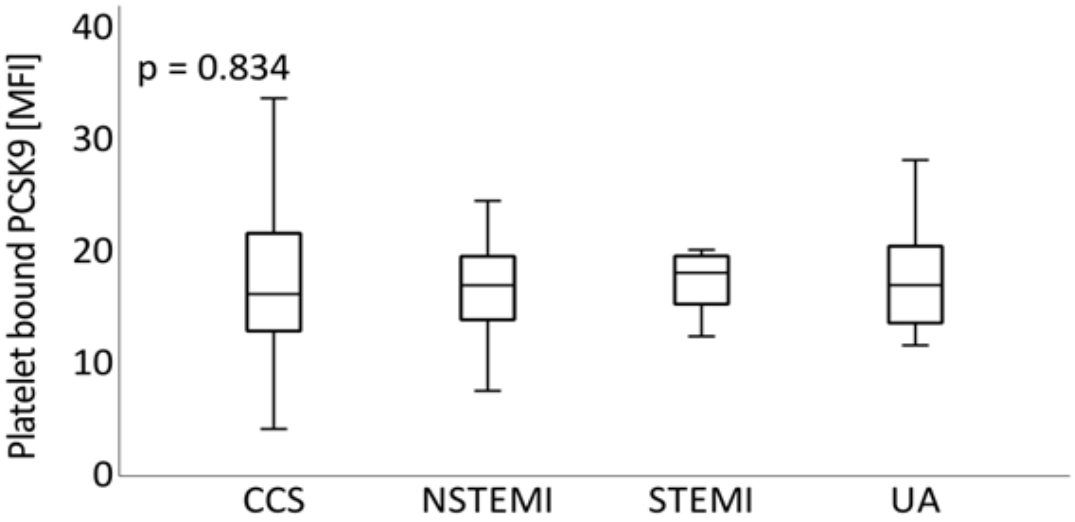

Figure 6. Platelet PCSK9 in different manifestations of atherosclerotic vascular disease

3.3 Platelet count and PCSK9 correlation

Platelet number and platelet PCSK9 were also assessed in a sample of 155 patients. We observed that there is no correlation between platelet number and the amount of platelet bound PCSK9 measured by flow cytometry.

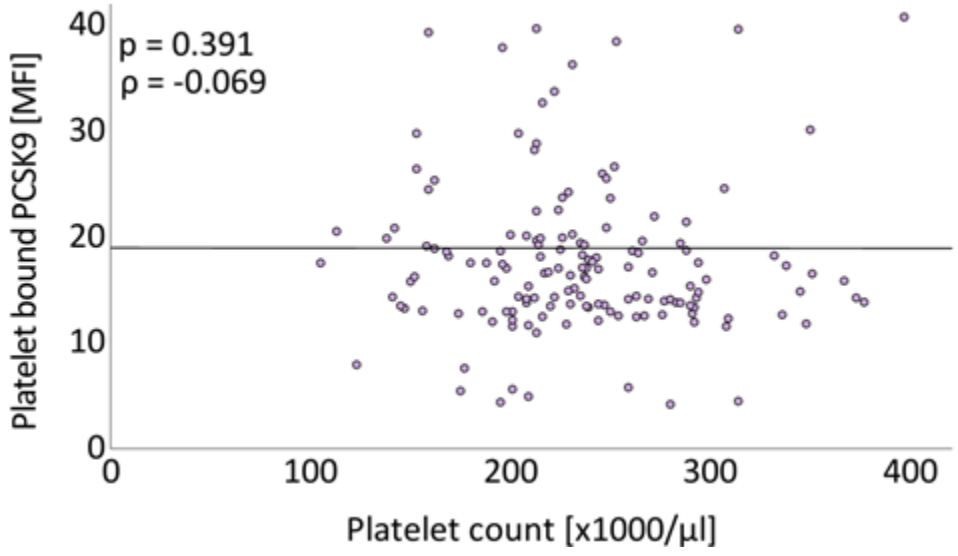

Figure 7. Platelet count correlation with platelet PCSK9

In addition, concerning free plasma PCSK9 and platelet count, available in 126 patients, no correlation could be found among both variables, as demonstrated in Figure 8. After repeating the analysis with ACS (n = 61) and CCS (n = 46) patients (left and right in Figure 9, respectively) no additional differences could be found.

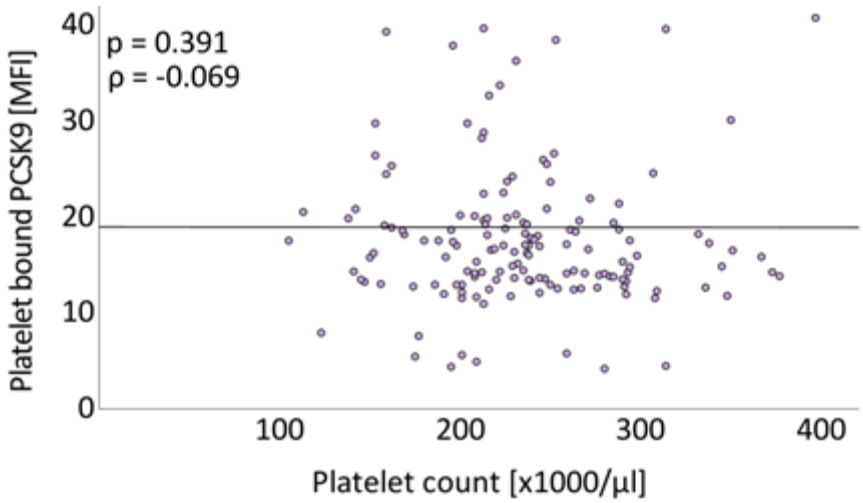

Figure 8. Platelet count correlation with plasma PCSK9

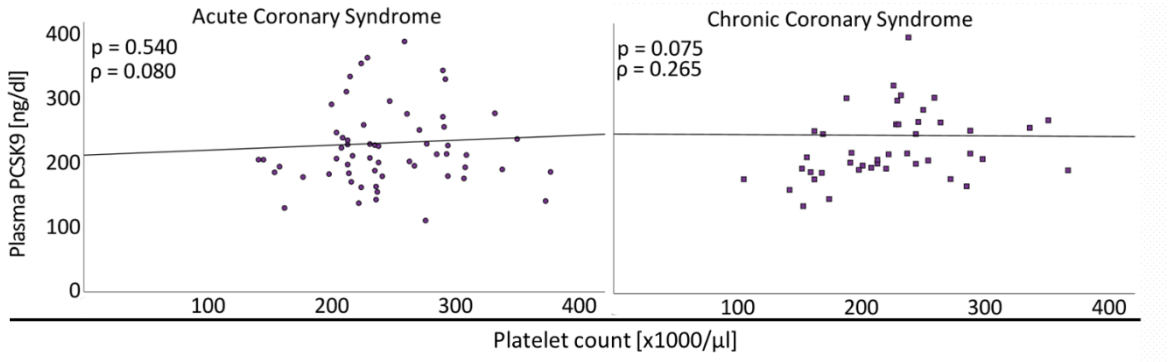

Figure 9. Correlation of platelet count with plasma PCSK9 in ACS and CCS

3.4 LDL influence on platelet hyperreactivity *ex vivo*

After enrichening probes with LDL on the aggregometer, we observed that higher plasma LDL values displayed a weak correlation with *ex vivo* platelet aggregation on platelets stimulated with tetrapropylammonium perruthenate (TRAP) (32 µM) (p=0.002, ρ=0.132)

(n=545). These functional results additionally sustain a relationship of LDL with platelet activity. Platelet aggregometry with LDL enrichment is displayed in Figure 10.

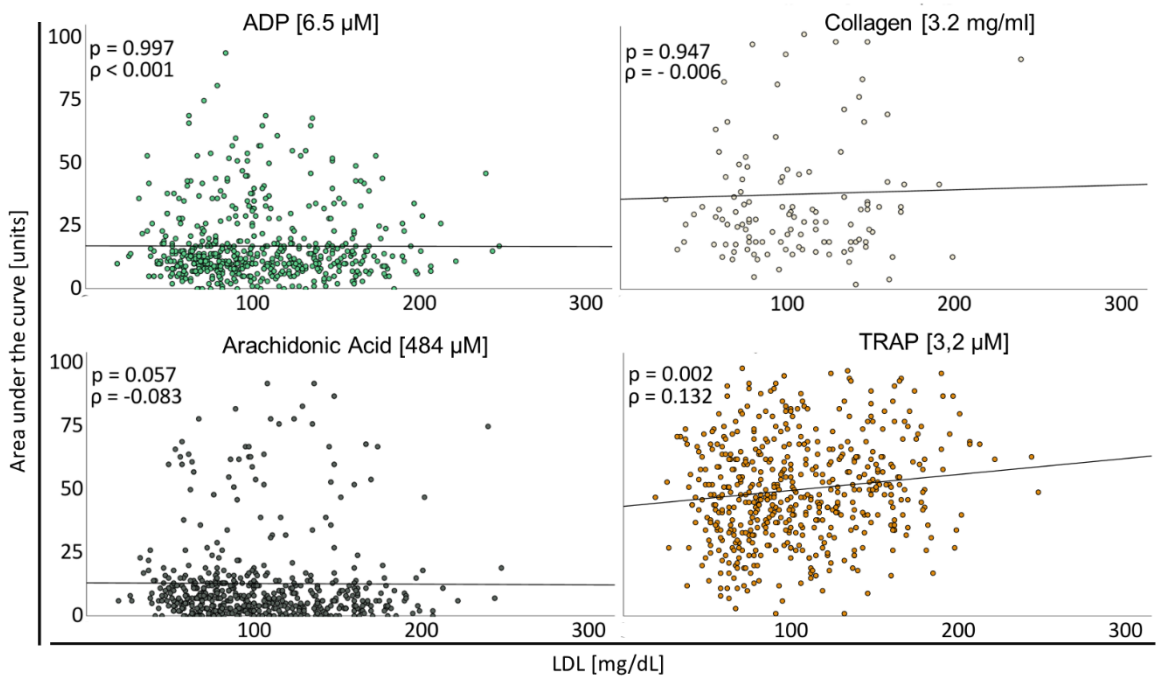

Figure 10. Platelet aggregometry with enriched LDL and distinct aggregation stimulators.

Platelet aggregation tested with adenosine diphosphate (ADP, 6.5 µM) (n=548), arachidonic acid (484 µM) (n=545) or collagen (3.2 mg/mL) (n=119) did not show a statistically significant correlation. However, as it has been previously shown that TRAP is superior ADP to define platelet hyperactivity in platelet aggregometry[108], an LDL induced hyperreactivity *ex vivo* must be considered.

3.5 Platelets produce, host, and secrete PCSK9

It has been recently described that human megakaryocytes express messenger ribonucleic acid (mRNA) for PCSK9, although at low levels. Platelets, however, have not been found to do so. It is believed that platelets receive the mRNA by their precursor.[109] It is to be expected that platelets express and release PCSK9 upon activation, since PCSK9 is a predictor of platelet activation and clinical ischemic events.[85]

Activated platelets display an increased correlation with the amount of platelet bound PCSK9. This was examined by measuring platelet CD62p receptor (P-selectin), a marker of platelet activation, as shown in Figure 11. CD62p, a known thrombotic factor, aids in intracellular signaling and also has adhesive functions.[110] In a sample of patients (n = 154) a significant positive correlation $p<0.001$ was found in non-stimulated and stimulated platelets.

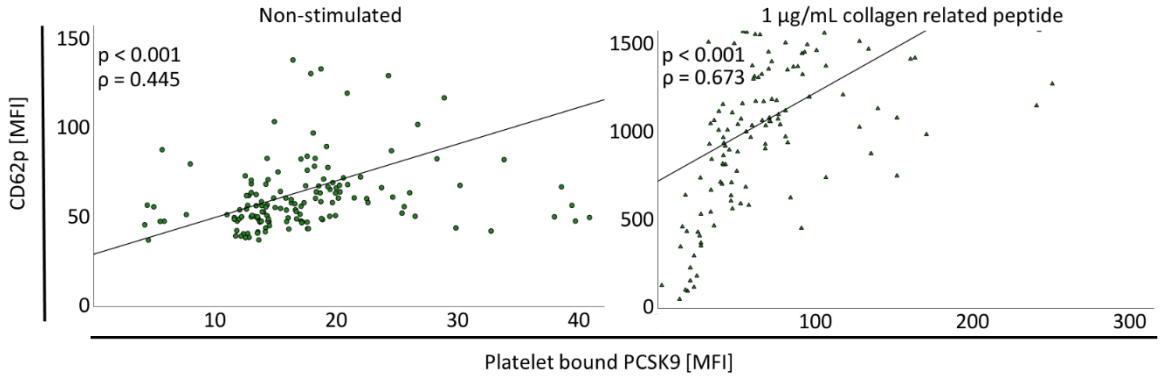

Figure 11. Platelet PCSK9 correlation with platelet surface CD62p, non-stimulated and in previously activated platelets with CoRP

The correlation factor was expectedly even larger when platelets were previously activated with collagen related peptide, as seen in the right panel of Figure 11. Hereafter, we isolated human platelets in high purity to explore their capacity to express and release PCSK9. It is well known that platelets both store and release a number of prothrombotic and proinflammatory regulators of atheroprogression and thrombo-inflammation.[22] We explored platelets capacity to express and release PCKS9 upon activation. For this purpose, a platelet immunoblotting was conducted. After electrophoresis, we found that platelets expressed two PCSK9 immunoreactive bands with molecular mobility of 53 and 62 kDa. In cultivated hepatic human cells (HepG2), a third band of 74 kDa was additionally detected, which represents PCSK9 as an inactive proprotein.[111] The presence of the bands indicates the presence of PCSK9 in human platelets, as seen in Figure 12A. Assessing platelet activation through flow cytometry, we also observed a significant increase in PCSK9 upon activation, as seen in Figure 12B and C.

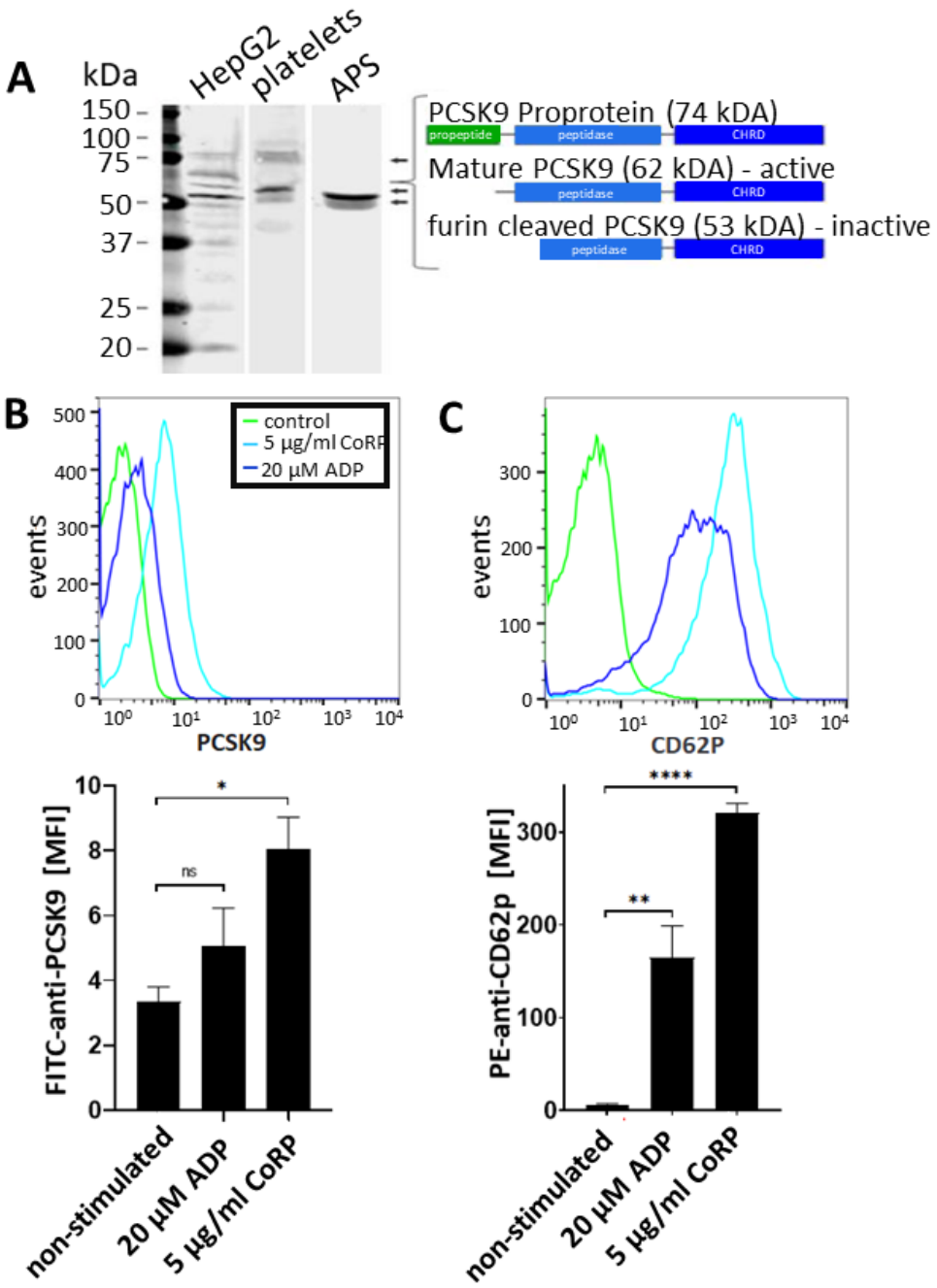

Figure 12. Platelet PCSK9 expression upon activation (Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021)[105]

To further examine PCSK9 thrombogenic role, we assessed platelet aggregation in a PCSK9 lacking milieu. For this purpose, while stimulating platelet aggregation with ADP and CoRP int the aggregometry experiment, monoclonal antibodies against PCSK9 were. Here we could observe a decreased platelet aggregation, as demonstrated in Figure 13.

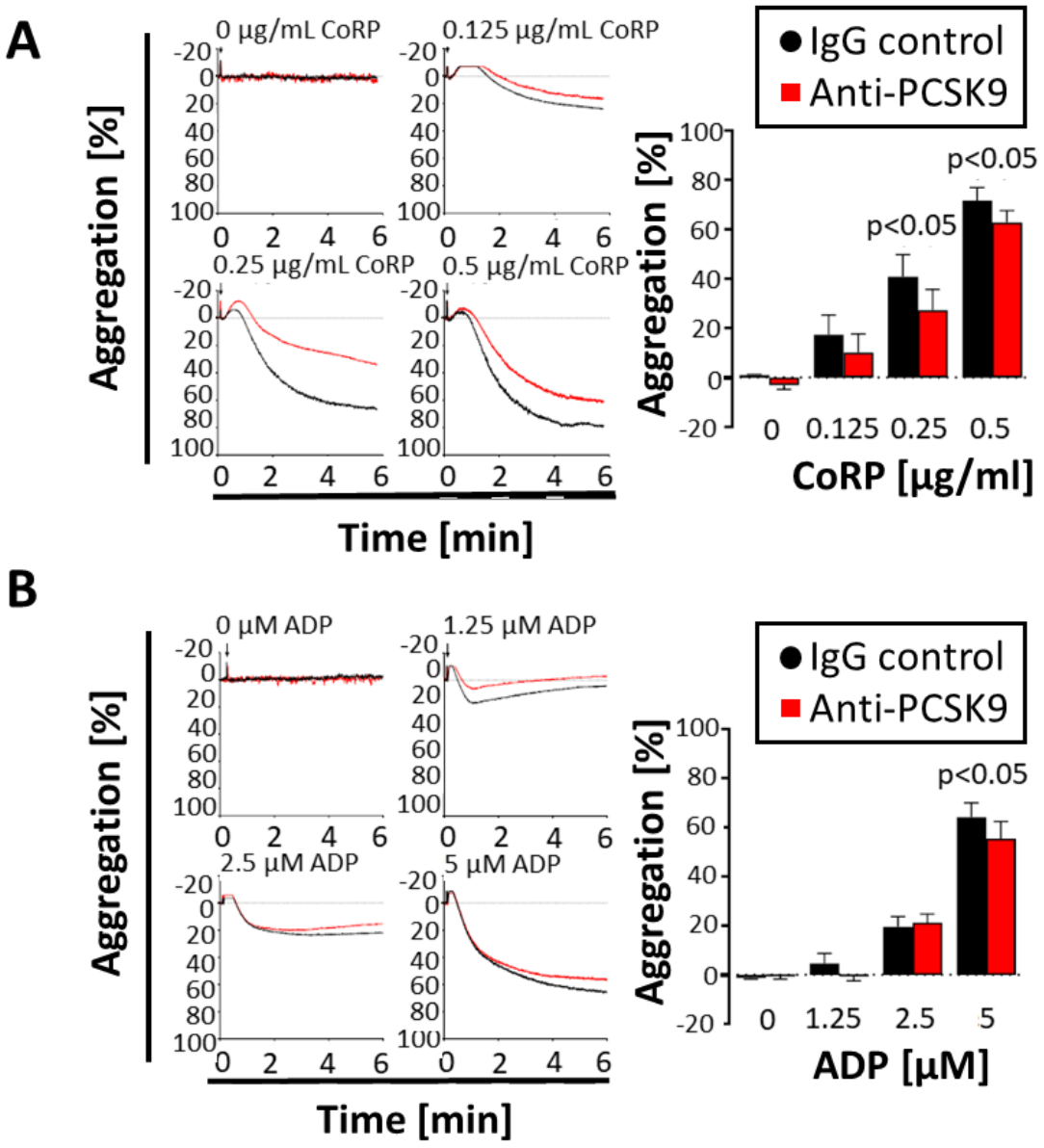

Figure 13. PCSK9 blockage leads to reduced platelet aggregation (Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021)[105]

3.6 Plasma LDL and platelet PCSK9

In a sample of 155 patients, no correlation was found between plasma LDL and platelet bound PCKS9. This is demonstrated in Figure 14.

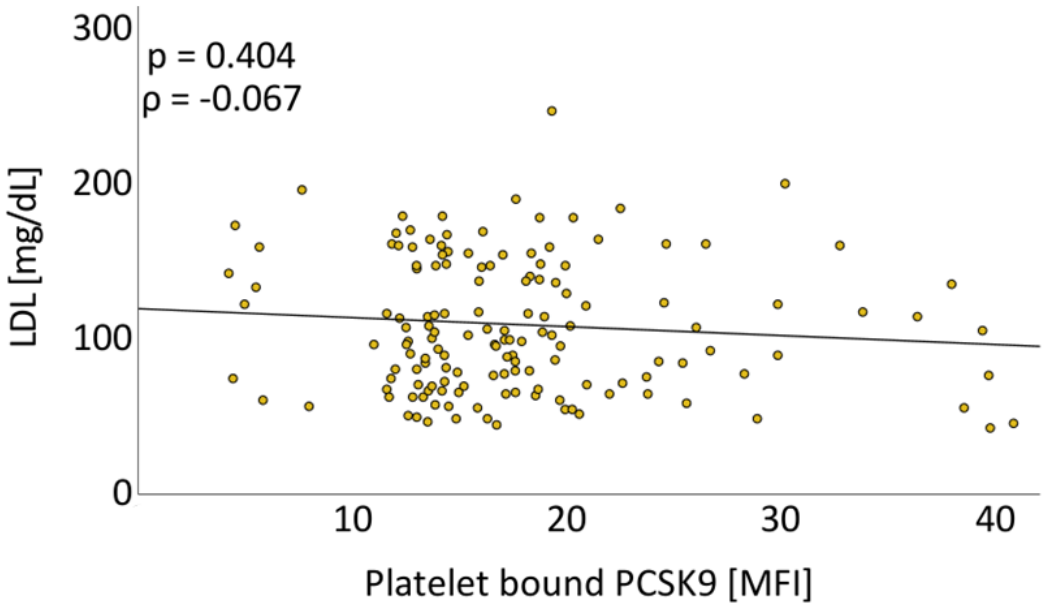

Figure 14. Platelet PCSK9 correlation with plasma LDLIn comparison, when correlating LDL levels with plasma PCSK9 (n = 126), a significant, inverse correlation could be seen, as stated in Figure 15.

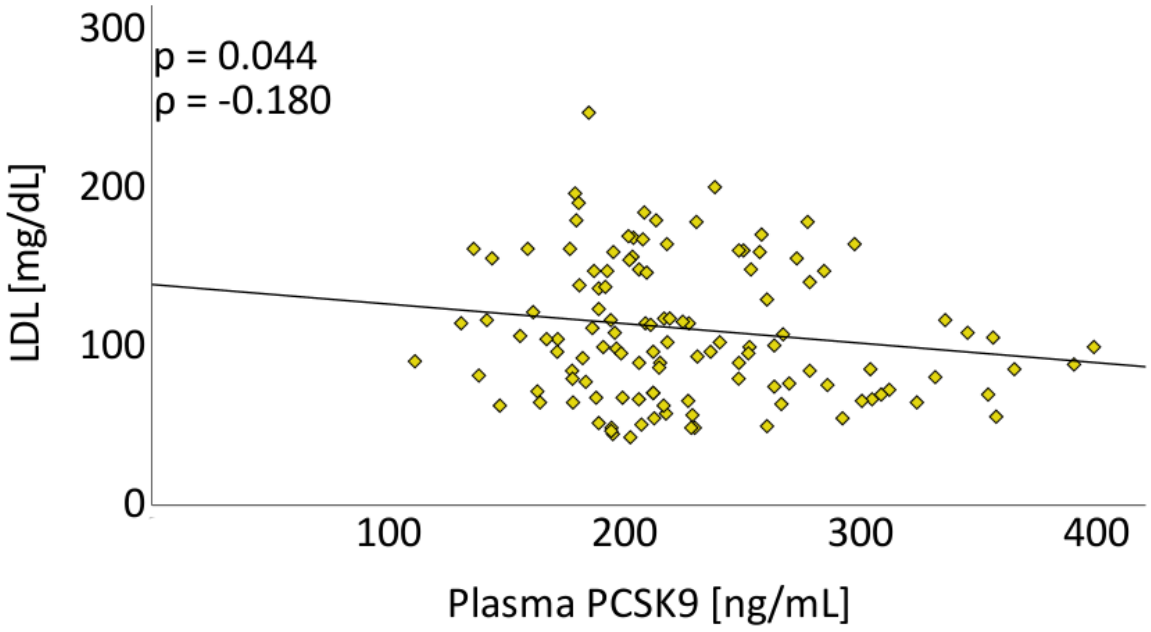

Figure 15. Plasma PCSK9 correlation with plasma LDL

3.7 Platelet PCSK9 correlation with C-reactive protein

Plasma C-reactive protein (CRP) is a hepatic protein and acute phase reactant. As such, CRP is a marker of inflammation. We correlated platelet PCSK9 with C-reactive protein to evaluate PCSK9 in a pro-inflammatory setting. In a sample of 155 participants there was no significant correlation at higher levels of CRP, suggesting that platelet bound PCSK9 is not ubiquitously elevated in inflammatory conditions.

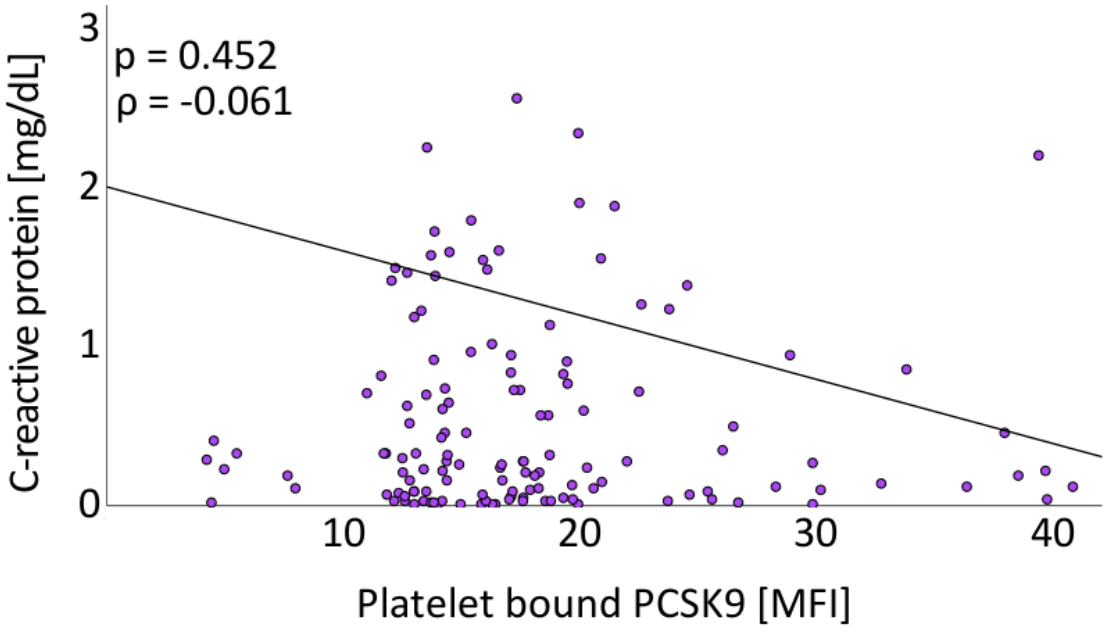

Figure 16. Platelet PCSK9 correlation with CRP

3.8 Platelet PCSK9 correlation with troponin I

Troponin I is a cardiac biomarker of ACS. High values of Troponin I correlate with myocardial ischaemia. Plasma PCSK9 has consistently been studied in patients with ACS.[85] We correlated platelet bound PCSK9 with troponin I plasma levels in a subgroup of 115 participants.

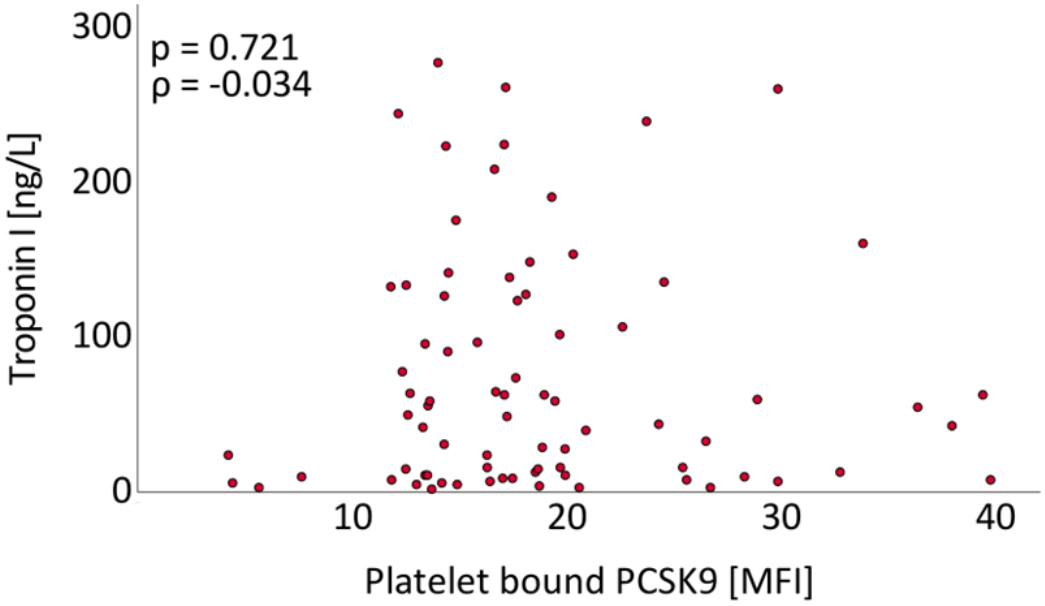

Figure 17. Platelet PCSK9 correlation with troponin I

Creatinin kinase, a further marker of myocardial necrosis, was similarly analyzed in a subgroup of 114 patients and compared with platelet bound PCSK9. As with troponin I, we observed no significant correlation between creatinine kinase and platelet bound PCKS9, as seen in Figure 18.

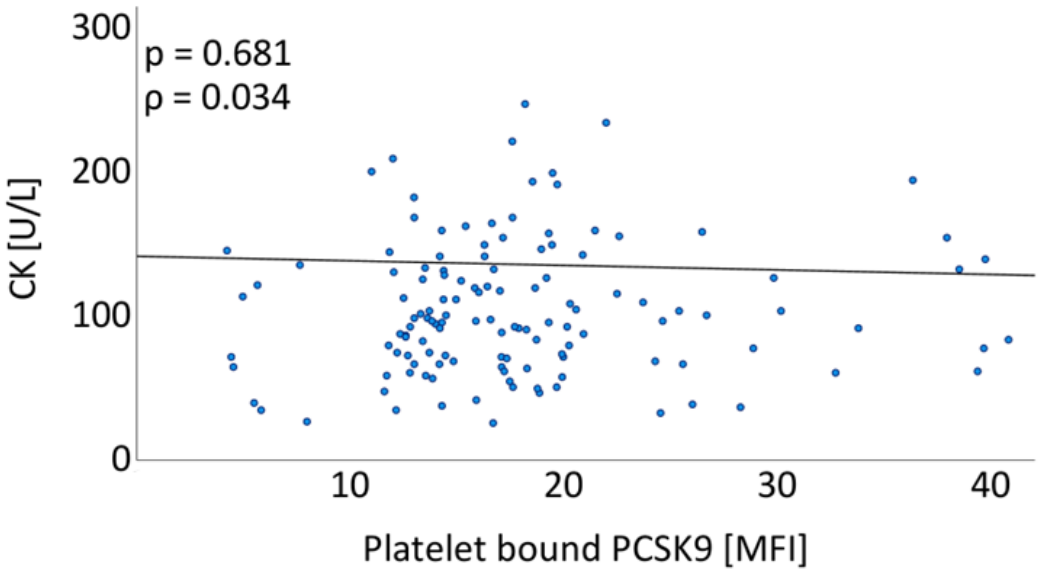

Figure 18. Platelet PCSK9 correlation with creatinine kinase after 48 hours

3.8.1 Gensini Score and platelet PCSK9

Previously, a relationship between high plasma PCSK9 levels and CAD had been described. The Gensini score is a tool to quantify CAD severity angiographically, taking into account the degree of coronary luminal reduction and the localization of the atherosclerotic plaque.[112] By structurally quantifying CAD, high plasma PCSK9 has been correlated with a high Gensini score, which is synonymous with severe CAD.[113] A correlation between platelet bound PCSK9 and the Gensini score in a sample of n = 30 was not significant, as seen in Figure 19. Figure 20 displays a non-significant trend for higher Gensini scores in patients with ACS than with CCS.

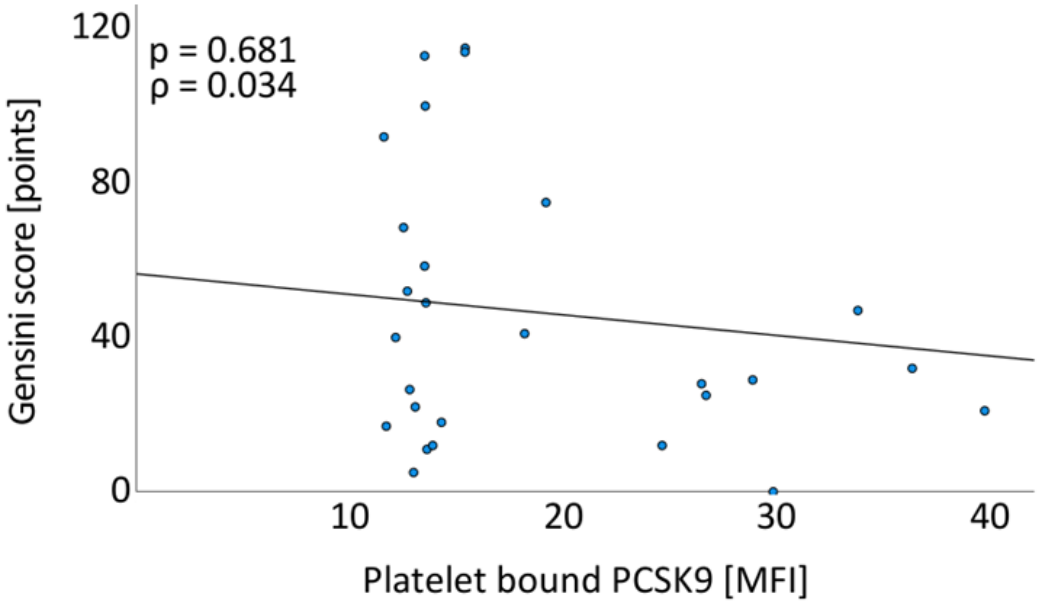

Figure 19. Gensini score correlated with platelet bound PCSK9

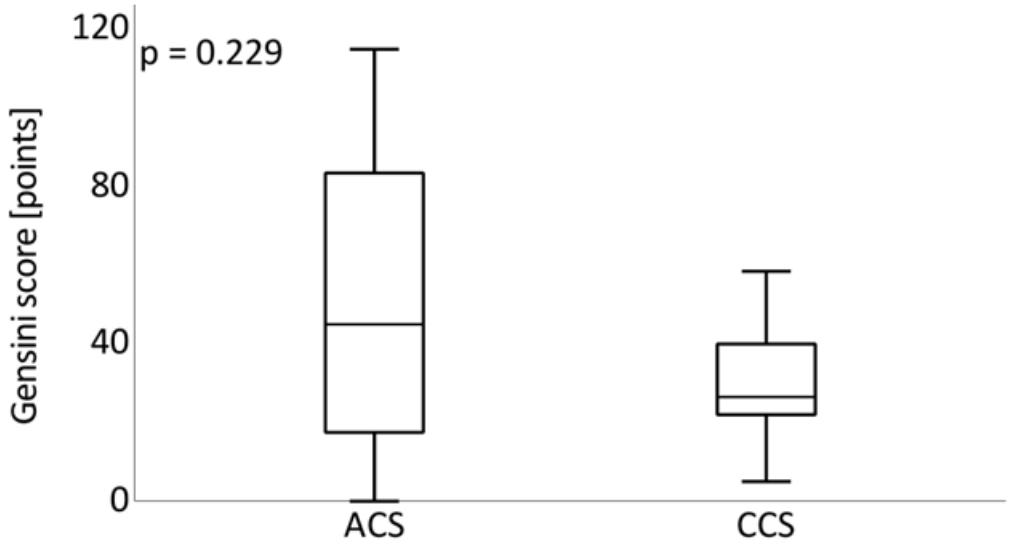

Figure 20. Gensini score stratified according to ACS and CCS

3.9 Plasma PCSK9 and coronary artery disease

No difference could be seen between different CAD phenotypes. Considering the relatively small sample size for this subgroup analysis (n = 94), no significant differences could be shown between ACS and CCS.

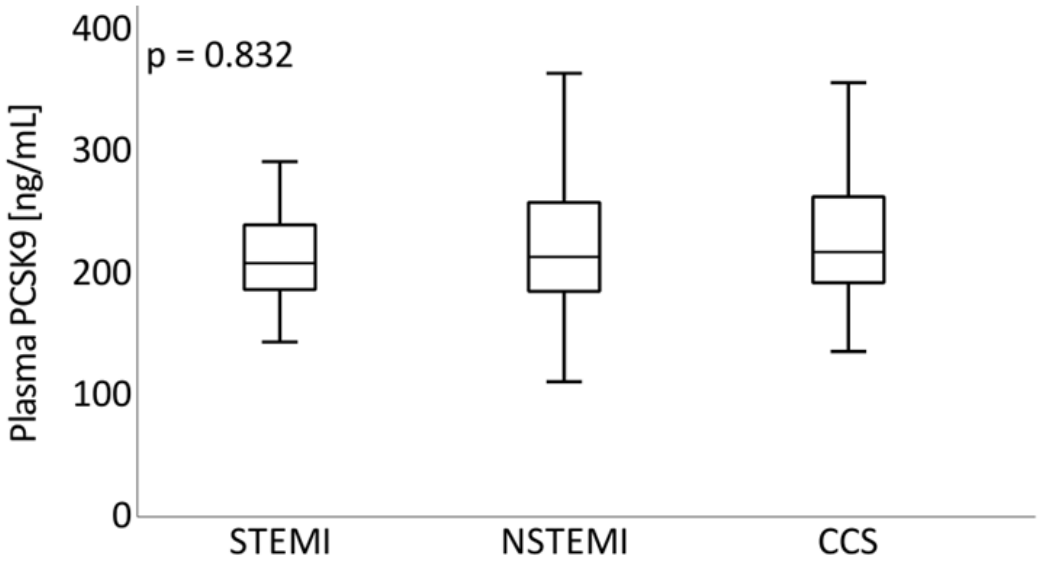

Figure 21. Plasma PCSK9 in different CAD phenotypes

3.10 Plasma PCSK9 and platelet count

Unlike the correlation of platelet number and platelet bound PCSK9, plasma PCSK9 displays a trend towards a positive correlation with platelet count. However, this correlation fails to show statistical significance. This can be seen in Figure 22.

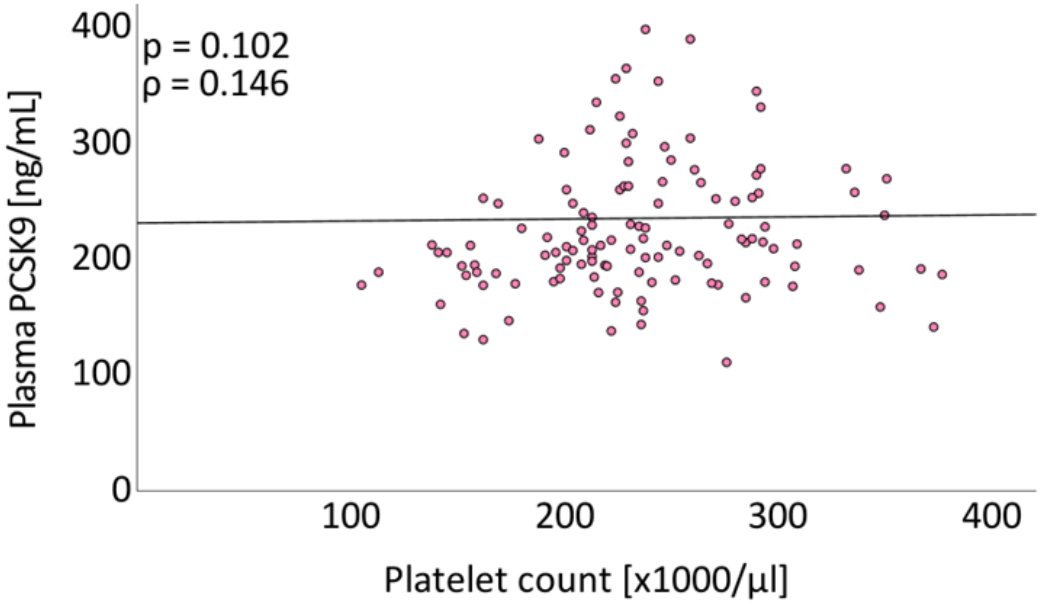

Figure 22. Plasma PCSK9 correlation with platelet count

3.10.1 Plasma PCSK9 and platelet activation

As previously shown in Figure 11, we found a positive correlation between platelet bound PCSK9 and CD62p. Figure 23 displays the correlation of CD62p with free plasma PCSK9 (n = 125).

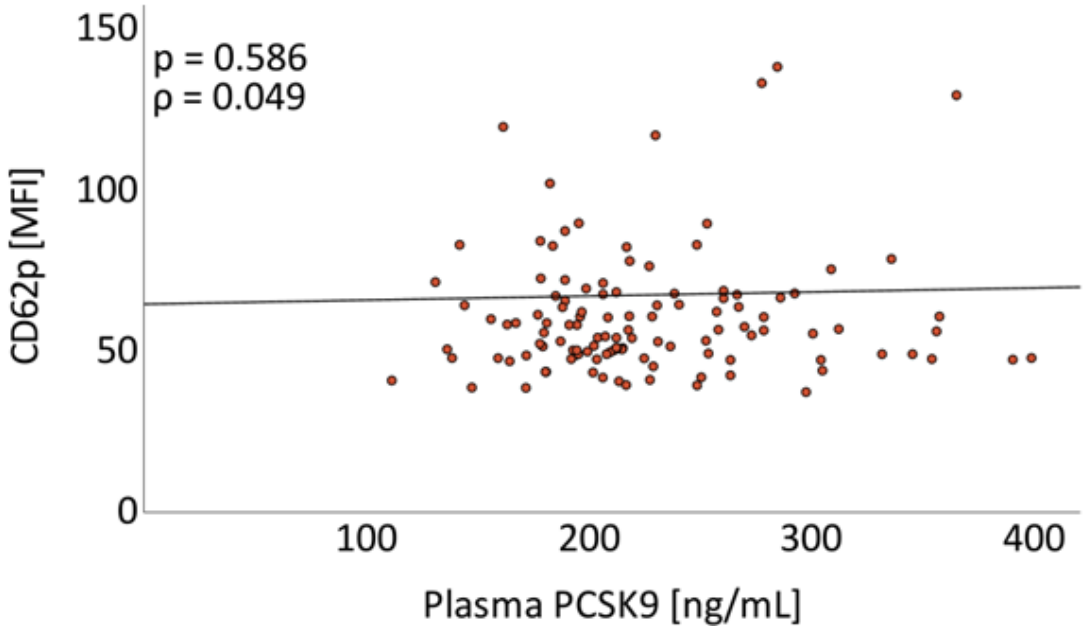

Figure 23. Plasma PCSK9 correlation with platelet CD62p

3.11 Platelet activation and plasma LDL cholesterol

In a subgroup of 154 patients, LDL cholesterol had no significant correlation with CD62p levels, as displayed in the following Figure 24.

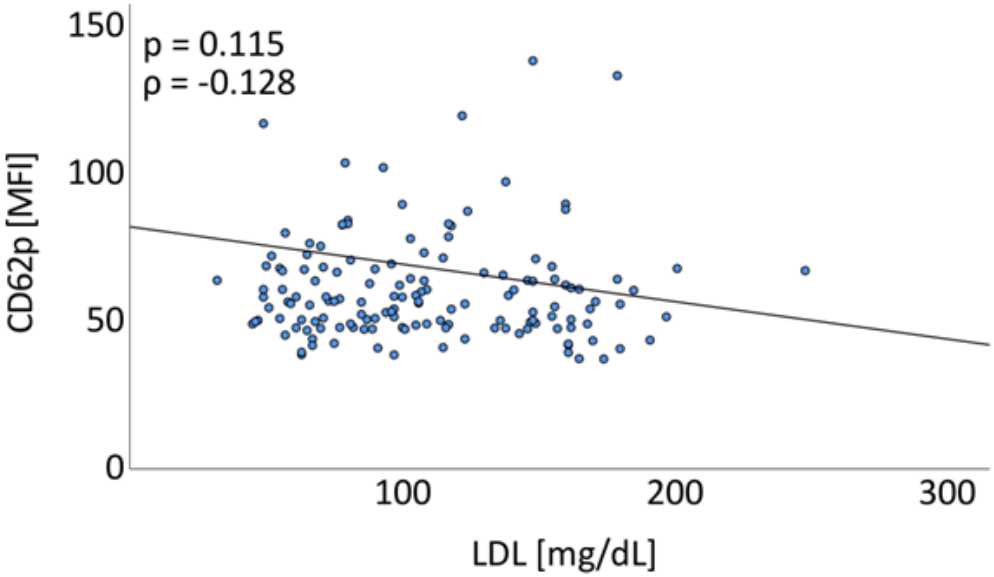

Figure 24. LDL correlation with platelet CD62p

3.12 Plasma PCSK9 and platelet-bound PCSK9

No correlation could be found when comparing platelet-bound PCSK9 to plasma PCSK9. This analysis was performed in three consecutive subgroups, n=40, n=80 and n=128. In either of the groups a trend for a correlation could be seen. Additionally, subgroup analysis was also performed by splitting the collectives in ACS and CCS. Hereto we could neither find a significant correlation.

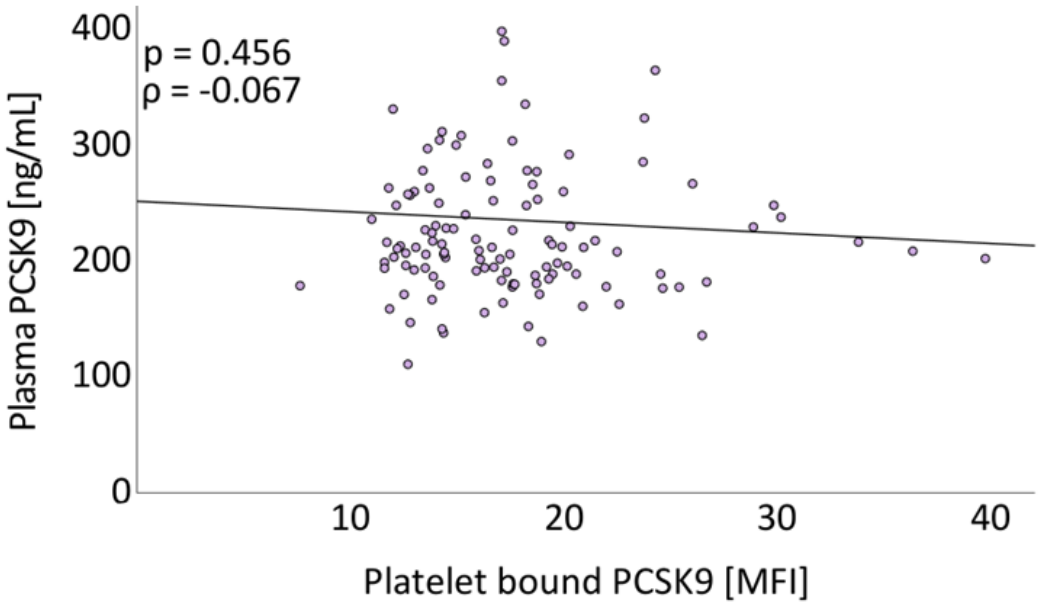

Figure 25. Platelet PCSK9 correlation with plasma PCSK9

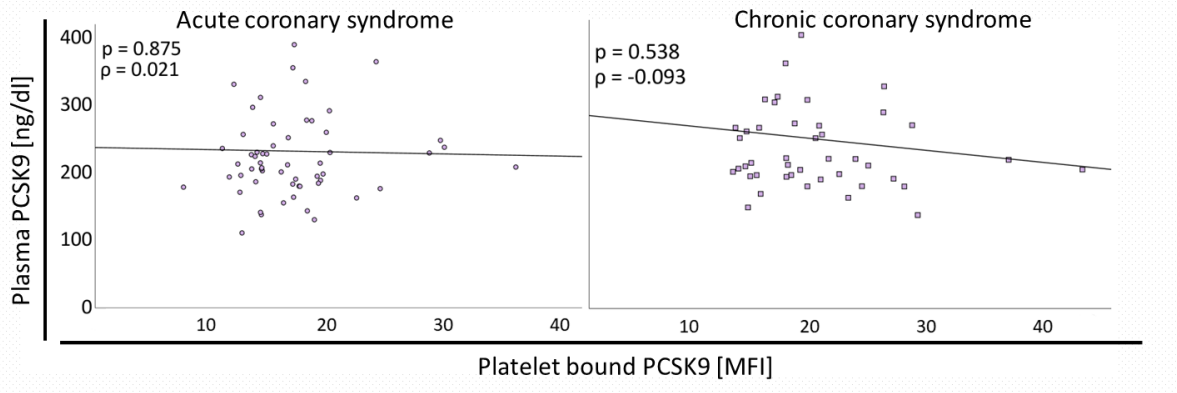

Figure 26. Platelet PCSK9 correlation with plasma PCSK9 in ACS and CCS

3.13 PCSK9 promotes thrombus formation

To further analyze the prothrombotic effect of platelet PCKS9, we evaluated its effects in thrombus formation in a flow chamber. To evaluate PCKS9 role in platelet-dependent thrombus formation, we examined platelets under physiological conditions, i.e. with no added inhibitor (IgG controls), and under PCSK9 deprived settings by adding anti-PCSK9 antibody to the collagen coated flow chamber. Compared to the IgG controls, we saw a reduced onset of thrombus formation in PCSK9-antagonized platelets over time, as shown in Figure 27.

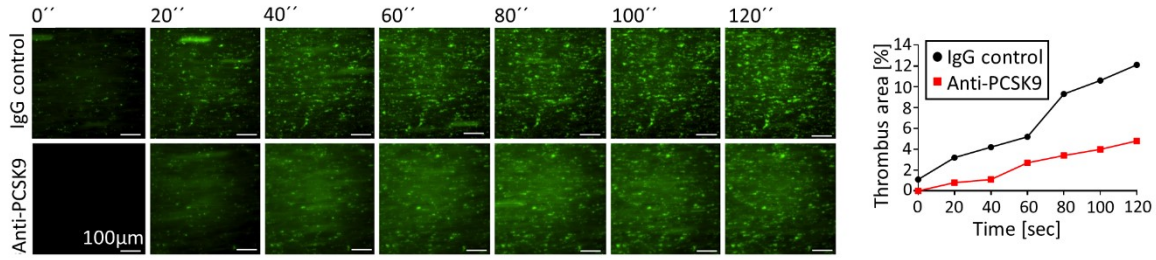

Figure 27. PCSK9 effect on thrombus formation in a flow chamber (adapted from Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021).[105]

By adding anti-PCSK9 antibodies, we observed a reduction in thrombus formation in concentration-dependent manner assessed by the thrombus area.

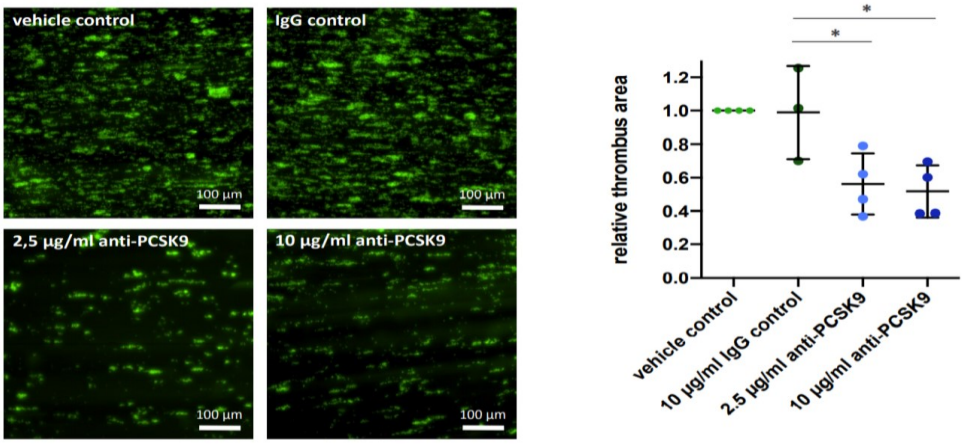

Figure 28. Anti-PCSK9 effect on thrombus formation in a flow chamber (Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021). [105]

3.14 PCSK9 and immunomediatory activity

It has previously been described that platelets are involved differentiation into endothelial cells and foam cells.[104] Additionally, there is increasing evidence of high PCSK9 as risk factor for atherosclerosis, independently from its LDL effects.[72] As atherosclerosis is an inflammatory process, we aimed to test if platelet PCSK9 would explain this proinflamatory change in the vessel. Taking into account that activated platelets are phagocytosed by monocytes[104], we examined the macrophage development in two different settings: a untreated control setting, and a setting with added human recombinant PCSK9.

By adding human recombinant PCSK9, we could observe a significant, concentration dependent increase in number and density of macrophages/foam cells in the cell culture. This indicates that platelet-derived PCSK9 is highly involved in the platelet-dependent differentiation of monocytes into macrophages.

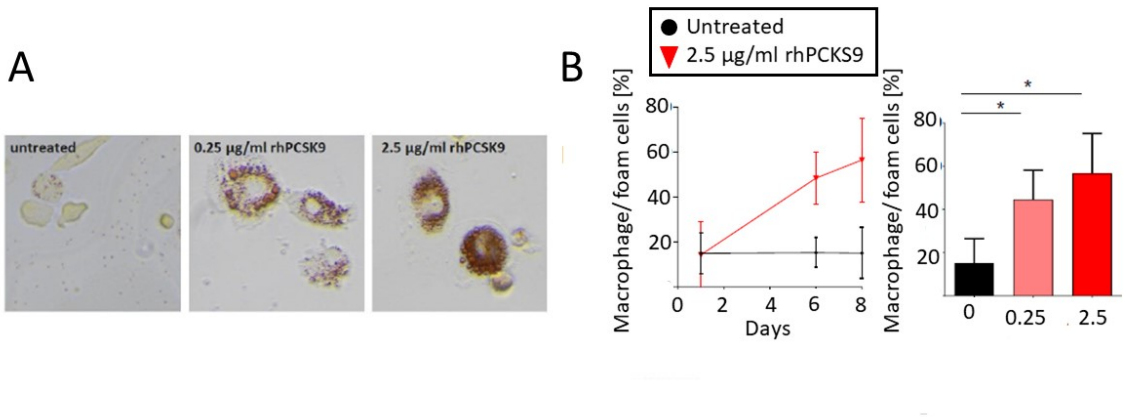

Figure 29. Electronic microscopy and PCSK9 dependent macrophage/foam cell enlargement (Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021)[105]

3.15 PCSK9 induces monocyte migration

PCSK9 proinflammatory's effects are not limited to monocyte differentiation. In mice, PCSK9 may serve as a chemoattractant as it promotes macrophage infiltration in atherosclerotic lesions.[80] In order to test its chemoattractant potential on our patient collective, we evaluated the effect of recombinant human PCSK9 (rhPCSK9) on monocyte

migration with use of a modified Boyden chamber. As displayed in figure 30, we could observe a concentration dependent migration toward PCSK9 rich environment.

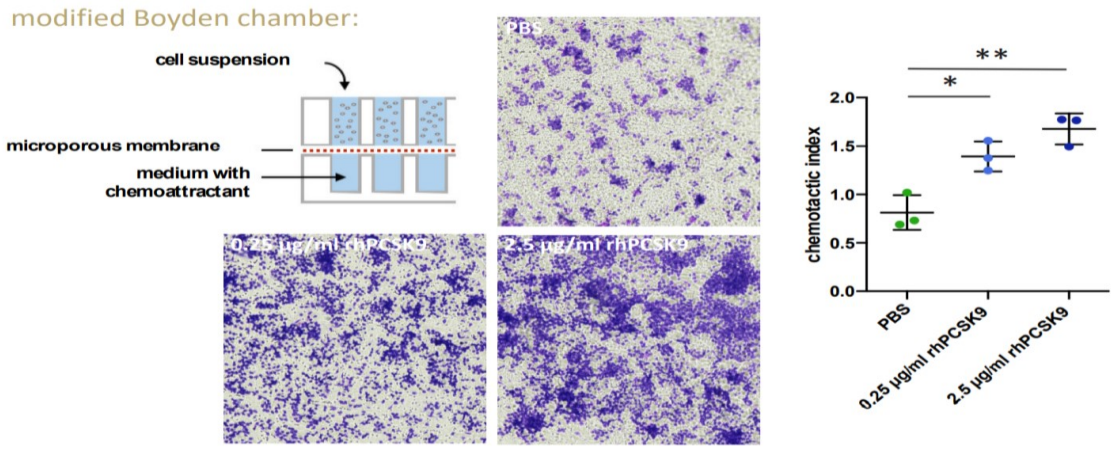

Figure 30. Monocyte migration after PCSK9 enhancement in a modified Boyden chamber(Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021)[105]

Following the same principle, we hypothesized that, if PCSK9 acts as a chemoattractant, its blockage would reverse the effect. Hence, we repeated the test using both recombinant human PCSK9 and evolocumab, which is illustrated in the following Figure 31.

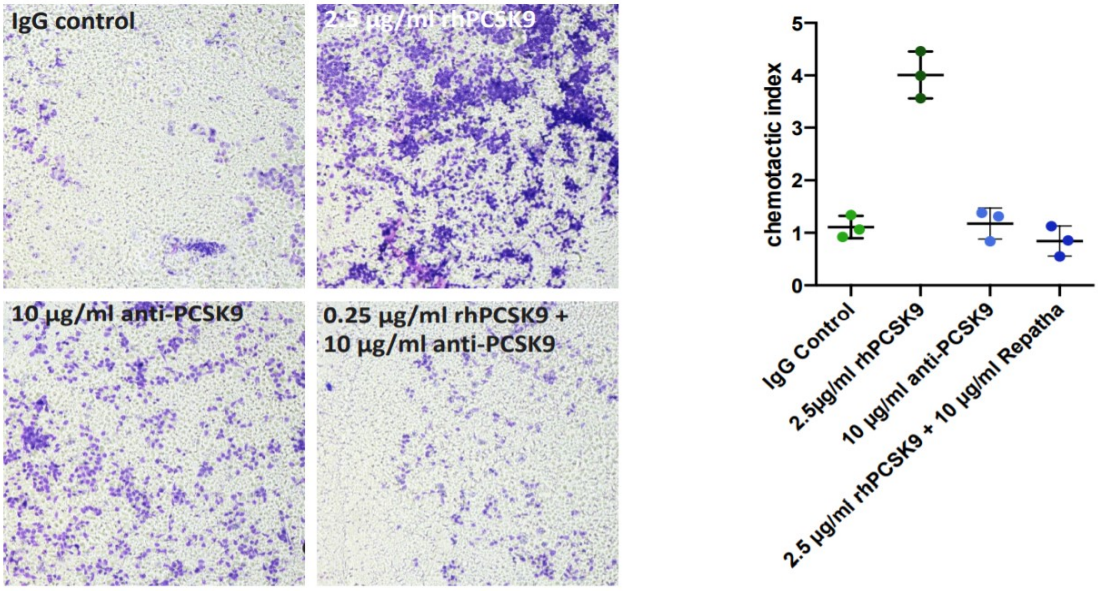

Figure 31. Monocyte migration after anti-PCSK9 antibodies in a modified Boyden chamber (Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021)[105]

4. Discussion

The objective of this study was to study the implications of PCSK9 in platelets, as a potential carrier to atherosclerotic lesions and its implication in platelet dependent thrombosis and inflammation in patients with CAD.

The participation of platelets in LDL-cholesterol levels is likely to be mediated PCSK9. As we have proved, platelets can produce PCSK9 (Figure 12) and release it (Figure 12), particularly during platelet activation (Figure 11). By raising the PCK9 level, it will inhibit hepatic LDL-receptor recycling: as a high amount of PCSK9 is binds to the LDL receptor and triggers endocytosis from the extracellular compartment into primarily liver cells, where a lysosomatic destruction of both of LDL-LDL receptor occur. This will elevate plasmatic LDL levels, which in turn might increase platelet number (Figure 1) and, more importantly, could also explain an increased aggregation of platelets (Figure 10).

Furthermore, in dyslipidaemia LDL has the potential to activate platelets.[89] Activated platelets will secrete their PCSK9. This PCSK9 release will impair the liver to reuse the LDL receptor, implicating that a high LDL value will be maintained. A sustained LDL elevation will then activate platelets, completing the cycle, and keeping it going on. Taking this into account, platelets are a pathophysiological-relevant, and until now unrecognized compartment of PCSK9 and, as a result, mediators of lipid metabolism. In fact, plasma PCSK9 has been correlated with LDL levels,[114] which is why we see an increase in PCSK9 with LDL increase. A therapeutical consequence would be to block PCSK9, which results in recycling of LDL receptor and promotes further clearance of plasma LDL. To further understand this concept, a scheme describing this process is shown in Figure 32.

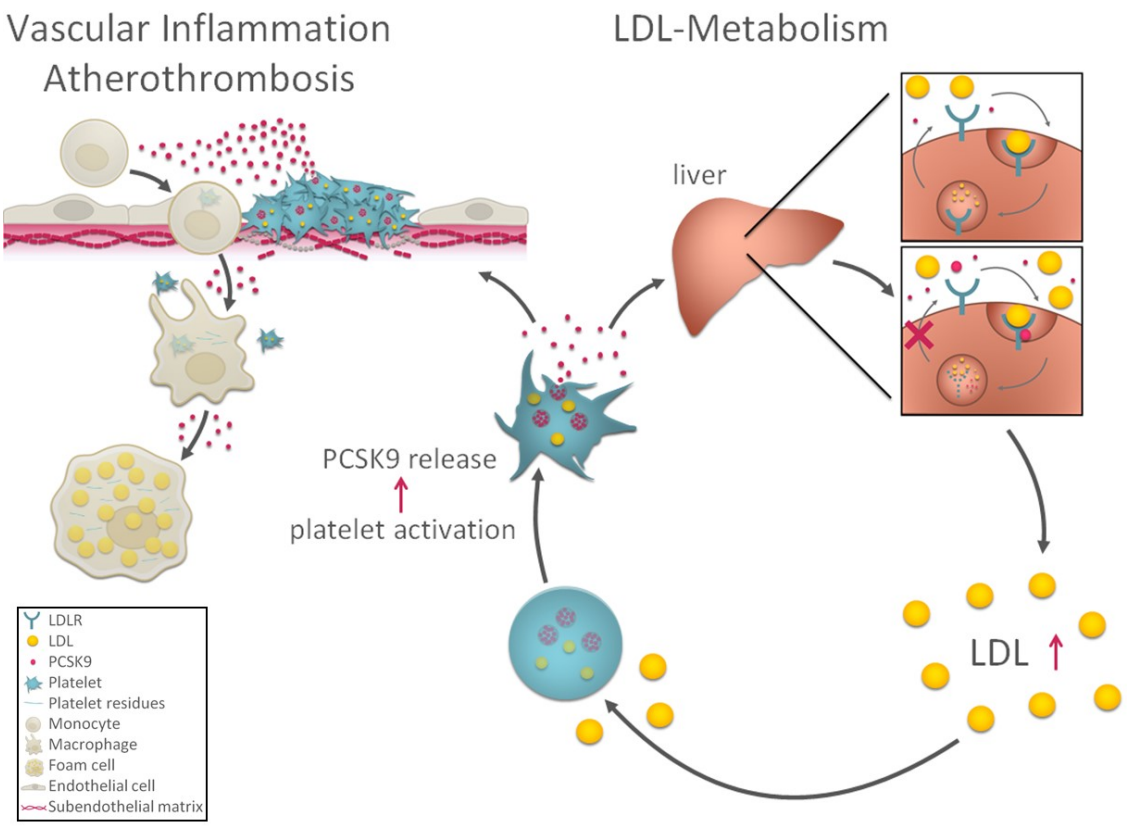

Figure 32. Platelet involvement in PCSK9 levels and LDL metabolism (Petersen-Uribe, Á., Kremser, M. & Rohlfing, A. K. 2021)[105]

As platelets are activated and secrete PCSK9, not only the liver is targeted by the molecule. In the microenvironment were platelets are aggregating, PCSK9 participates in the chemotaxis of leukocytes, and aids in the differentiation of monocytes into foams cells, as it shown in the left part of Figure 32.

A LDL and platelet correlation has also been observed by Fessler et al., who pointed out a positive relationship between platelet count and non-HDL cholesterol.[115] We describe the same correlation (Figures 3 & 4). Plasma PCSK9 and LDL associations have previously been found and significantly modified by lipid-lowering drugs.[116] As seen in Figure 15, where a negative correlation was apparently found between plasma PCSK9 and LDL, this is most likely owed to the fact that over 80% of our participants were on statin therapy (Table 3). A recent meta-analysis highlighted this phenomenon, where statin use was associated with

an increased PCSK9 level.[117] In fact, statins upregulate a transcription factor called sterol regulatory binding protein-2 transcription factor, which itself increases PCSK9 synthesis.[118]

The activation of platelets and PCSK9 secretion additionally promotes atheroprogression, by triggering thrombosis and inflammation. Through the experiments in the flow chamber displayed in Figures 27 and 28, PCSK9 rich environment was shown to have a faster and larger thrombus development, and by antagonizing through anti-PCSK9 antibodies proved to have the contrary effect. In the PCSK9-REACT study, a direct relationship between PCSK9 levels and *ex vivo* platelet reactivity under stimulation with ADP had been described. It was postulated that PCSK9 increases platelet reactivity, which had a significant and independent association, which then could lead to ischemic and thrombotic complications.[85]

Additionally, platelet aggregation was increased under higher platelet bound PCSK9, as found in Figures 11 and 12. And similarly, the addition of anti-PCKS9 antibodies hindered the aggregation (Figure 13). This evidence further suggests that the haemostatic effects of PCSK9 are not only regulated indirectly through LDL/LDL-R but directly as a positive modulator of platelet aggregation.[72] In fact, PCSK9 effects on haemostasis could go beyond platelets. The role of PCSK9 in modulation of coagulation was described. This was found in an animal model under genetically induced PCSK9 deficiency, where it appeared protective against the occurrence of venous thrombosis. As coagulation factor VIII is cleared by LDL receptor, which is upregulated by PCSK9, its inhibition would influence the coagulation cascade.[72]

In further studies in mice, PCSK9 overexpression accentuated a hypercoagulable state during sepsis. In one study wild-type, PCSK9 knockout, and transgenic mice that overexpressed PCSK9 were subjected to sham surgery or cecal ligation and puncture. In wild type and transgenic mice different procoagulant molecules as cfDNA and thrombin–antithrombin (TAT) complexes were found significantly different in comparison to the PCSK9 knockout mice, while all having similar protein C levels.[81]

Indeed, an inhibition of PCSK9 could not only be an effective therapy for plasmatic LDL accumulation,[119] but also for CAD and atherosclerosis altogether. Furthermore, the use of

PCSK9 inhibitors significantly reduced adverse clinical events and mortality in patients with CAD.[120] Finally, in comparison to statins and enteral cholesterol absorption inhibitors, PCSK9 inhibitors are capable of reducing lipoprotein (a) levels, which could further aid to its anti-atherogenic and anti-prothrombotic intervention.[74]

The role of PCSK9 as a chemoattractant and inductor of macrophage transformation was explored (Figures 29-31). We observed a higher rate of monocytes into macrophages differentiation under increasing PCSK9 levels. As we found, activated platelets have an increased PCSK9 bound level (Figure 11), which they rapidly release upon activation, secreting many other cytokines and chemokines: macrophage migration inhibitor factor, C-X-C motif chemokines (e.g. CXCL4, 5, 12, 14), IL-16 and β-thromboglobulin, which have also chemotactic properties on smooth muscle cells, macrophages, monocytes and fibroblasts.[44, 103] These platelet-derived chemokines propagate infiltration of monocytes and modulate thrombo-inflammation.[103] Upon arrival of leucocytes, the platelets induce the differentiation into foam cells and endothelial cells. In this process, $CD34^+$ cells (haematologic progenitor cells) begin phagocytosis, and it has been proven that platelets are internalized into macrophages/foam cells.[104] As platelets can bind to LDL, the uptake of platelets bound with LDL accelerates the transformation into foam cells. In this same study, monocytes which did not internalize platelets with LDL did not turn into foam cells.[104]

Platelets induction of cell type differentiation has been thoroughly documented. As stated, also endothelial cell can be induced by platelet contact, via the platelet-membrane bound SDF-1 with circulating $CD34^+$ cells at sites of vascular injury. This repair mechanism may participate in structural repair in diverse diseased organs.[51]

PCSK9 involvement in immune response goes beyond its chemotactic and transformative effects in cell differentiation. It has been seen in murine sepsis models, that PCSK9 deficiency confers protection against systemic bacterial dissemination, organ pathology, and tissue inflammation.[81] The main PCKS9 effect in downregulating LDL-receptors is responsible for this finding, as polysaccharides from Gram negative bacteria bind plasma lipids to be internalized and are internalized in the hepatocytes by the LDLR.[71]

It has already been established that platelet hyperactivity and circulating PCSK9[114,121] are associated with the intima wall thickness of the carotid artery as they are associated to coronary plaque inflammation.[122] We found a significant amount of PCSK9 in atherosclerotic tissue, areas known for macrophage and platelet reactivity. It can be speculated that PCSK9 has a local effect in atherosclerosis, as our *in vitro* experiments have pointed out.

Recent evidence has also described PCSK9 within atherosclerotic lesions, which has been independently associated of other cardiovascular risk factors, including LDL, lipoprotein (a) and obesity. It has been postulated that PCSK9 has a direct effect on atherogenesis, as it is also secreted by smooth muscle cells and endothelial cells in atherosclerotic lesions. This could enhance the differentiation of macrophages into foam cells in a paracrine way. Furthermore, by upregulating an additional LDL receptor, namely lectin-type oxidized LDL receptor 1, PCSK9 could mediate the uptake of oxidized LDL by macrophages.[72] In our study we were able to identify platelets as an additional PCSK9 compartment. Furthermore, the recent GLAGOV clinical trial found a reduced progression of atheroma volume in patients with coronary artery disease with the use of the PCSK9 inhibitor evolocumab.[123]

The inhibition of PCSK9 gains momentum as an effective and simultaneous safe therapeutic approach for patients with coronary artery disease and refractory hyperlipidemia[120]. An improvement of quality of life in this patient collective is therefore expected, while simultaneous lowering of adverse clinical events and mortality is observed.[124,125]

The interesting fact is that PCSK9 levels, mainly expressed in activated platelets, were not different in our population of patients with ACS and CCS, as shown in Figure 21. This could be explained as follows. For instance, it has been documented that PCKS9 levels are elevated in ischaemic heart disease, regardless of chronicity.[82] Nonetheless, as a correlation between Troponin I and platelet bound-PCKS9 or CK after 48h hours and platelet bound PCKS9 (Figures 17 and 18) did not show any significance, this may be due to a transitory increase, as it has been previously described.[90]

In summary, the main findings of the current work are: 1) a correlation exists between platelets and cholesterol, mainly LDL cholesterol, 2) platelets may participate in cholesterol metabolism, as they can synthesize, carry and secrete PCSK9, particularly at higher concentrations of LDL, 3) platelet PCSK9 additionally influences platelet aggregation and thrombus development, 4) PCSK9 also acts upon monocyte as a chemoattractant, and stimulates their conversion into macrophages/foam cells, and 5) PCSK9 is present in the atherosclerotic lesion.

4.1 Limitations

Our study has several limitations. The studied groups were heterogeneous and had a number of cofounders, as prior medication (particularly statins and antiplatelet agents), which must be considered. All data was taken retrospectively, with clinical follow up not available. As tempting as it is to assume the *in vitro* data reflect aspects of the *in vivo* physiology, conclusive proof is still required.

5.Summary

Modern civilization's sedentary lifestyle has found in CAD an increasing worldwide trend towards morbidity and mortality. Its incidence continues to peak as its risk factors, including hyperlipidemia, have also risen. Recently, a new component in cholesterol metabolism was identified. PCSK 9 proprotein convertase is expressed in many tissues and cell types, and binds to the LDL receptor, reducing the uptake of LDL-cholesterol particles from the extracellular compartment. PCSK9 has a major impact on lipoprotein homeostasis. Inhibition of PCSK9 results in lowered LDL and has shown improved prognosis in patients with coronary artery disease. Recent reports have highlighted additional effects of PCKS9, as a platelet activator and modulator of macrophage function and vascular inflammation. In this study, we characterized the release of PCSK9 from platelets and its consequences for thrombo-inflammation.

We observed that platelet PCSK9 may contribute to atherothrombosis in a collective of CAD patients. This could be through a direct vascular effect, leading to increased aggregation, recruitment of leukocytes, and differentiation into foam cells; as well as indirectly through inhibition of LDL-receptor recycling and increase of plasmatic LDL, which in turn could increase platelet number and facilitate activation, creating a vicious cycle. Hence, it is tempting to assume that the herein-described effects of platelet PCSK9 on thrombosis and thrombo-inflammation might contribute to atherothrombosis.

To summarize, platelets are potential additional and unexpected source of PCKS9 with potential impact on lipoprotein metabolism and further suggest PCSK9 as a thrombo-inflammatory promoter in coronary artery disease. In consequence, PCSK9 should be highlighted as a target in the treatment of vascular inflammation and hypercholesterolemia.

Zusammenfassung

Der bewegungsarme Lebensstil der modernen Gesellschaft hat in der CAD einen weltweit zunehmenden Trend zur Morbidität und Mortalität festgestellt. Die Inzidenz nimmt weiterhin zu, während die Risikofaktoren, einschließlich der Hyperlipidämie, ebenfalls zugenommen haben. Vor kurzem wurde in PCSK9 eine neue Komponente des Cholesterinstoffwechsels identifiziert. Diese Proproteinkonvertase wird in vielen Geweben und Zelltypen exprimiert, sie bindet an den LDL-Rezeptor und reduziert die Aufnahme von LDL-Cholesterinpartikeln aus dem extrazellulären Kompartiment. Dadurch hat PCSK9 einen großen Einfluss auf die Lipoprotein-Homöostase. Die Hemmung von PCSK9 führt zu einer Senkung des LDL-Spiegels und hat die Prognose bei Patienten mit koronarer Herzkrankheit verbessert.

Neue Ergebnisse von verschiedenen Studien haben weitere Wirkungen von PCKS9 als Thrombozytenaktivator und Modulator der Makrophagenfunktion und der Gefäßentzündung aufgezeigt. Wir haben versucht, die Freisetzung von PCSK9 aus Thrombozyten und ihre Auswirkungen auf die Thrombo-Entzündung zu charakterisieren.

Wir haben festgestellt, dass PCSK9 aus Thromboyzten bei einem Kollektiv von KHK-Patienten zur Atherothrombose beitragen kann. Dies könnte durch einen direkten vaskulären Effekt geschehen, der zu erhöhter Aggregation, Rekrutierung von Leukozyten und Differenzierung in Schaumzellen führt, aber auch indirekt durch Hemmung des LDL-Rezeptor-Recyclings und Erhöhung des plasmatischen LDL, was wiederum die Anzahl der Thrombozyten erhöhen und die Aktivierung erleichtern könnte, wodurch ein Teufelskreis entsteht. Daher ist es anzunehmen, dass die hier beschriebenen Auswirkungen von PCSK9 auf Thrombose und Thromboentzündung zur Atherothrombose beitragen könnten.

Zusammenfassend lässt sich sagen, dass Thrombozyten eine potenzielle zusätzliche und unerwartete Quelle von PCKS9 mit potenziellen Auswirkungen auf den Lipoprotein-Stoffwechsel sind, und dass PCSK9 als Thrombo-Entzündungsförderer bei koronarer Herzkrankheit in Frage kommt. Infolgedessen sollte PCSK9 als Ziel bei der Behandlung von vaskuläre Inflammation und Hypercholesterinämie hervorgehoben werden.

6. References

1. Knuuti J, Wijns W, Achenbach S, et al. 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J*. Published online 2020:407-477.

2. Ross R. Atherosclerosis - An inflammatory disease. *N Engl J Med*. Published online 1999:115-126.

3. Álvarez-Álvarez MM, Zanetti D, Carreras-Torres R, Moral P, Athanasiadis G. A survey of sub-Saharan gene flow into the Mediterranean at risk loci for coronary artery disease. *Eur J Hum Genet*. Published online 2017.

4. WHO. Global action plan for the prevention and control of noncommunicable diseases 2013-2020. *World Heal Organ*. Published online 2013.

5. Mendoza-Herrera K, Pedroza-Tobías A, Hernández-Alcaraz C, Ávila-Burgos L, Aguilar-Salinas CA, Barquera S. Attributable burden and expenditure of cardiovascular diseases and associated risk factors in mexico and other selected mega-countries. *Int J Environ Res Public Health*. 2019;16(20):4041.

6. Roth GA, Johnson C, Abajobir A, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol*. Published online 2017.

7. Robert Koch-Institut (Hrsg) (2015) Gesundheit in Deutschland. Gesundheitsberichterstattung des Bundes. Gemeinsam getragen von RKI und Destatis. RKI B. Gesundheit in Deutschland (2015). *Robert Koch-Institut Gesundh Deutschland Gesundheitsberichterstattung des Bundes Gem getragen von RKI und Destatis RKI, Berlin*. Published online 2015.

8. Chobanian A V., Alexander RW. Exacerbation of atherosclerosis by hypertension potential mechanisms and clinical implications. *Arch Intern Med*. Published online 1996:1952-1956.

9. Malakar AK, Choudhury D, Halder B, Paul P, Uddin A, Chakraborty S. A review on

coronary artery disease, its risk factors, and therapeutics. *J Cell Physiol*. Published online 2019:16812-16823.

10. Sarwar N, Gao P, Kondapally Seshasai SR, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *Lancet*. 2010;375(9733):2215-2222.

11. Haffner SM. Diabetes, hyperlipidemia, and coronary artery disease. In: *American Journal of Cardiology*. ; 1999:17F-21F.

12. Ferrières J. Hypercholesterolaemia and coronary artery disease: A silent killer with several faces. *Arch Cardiovasc Dis*. 2019;112(2):75-78.

13. Anderson KM, Castelli WP, Levy D. Cholesterol and Mortality: 30 Years of Follow-up From the Framingham Study. *JAMA J Am Med Assoc*. 1987;257(16):2176-2180.

14. Epstein FH, Blackburn H, Gutzwiller F. Cardiovascular disease epidemiology: A journey from the past into the future. *Circulation*. 1996;93(9):1755-1766.

15. Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and Pathogenesis of Visceral Fat Obesity. *Obes Res*. Published online 1995:187S-194S.

16. Samson SL, Garber AJ. Metabolic syndrome. *Endocrinol Metab Clin North Am*. Published online 2014:1-23.

17. Schildkraut JM, Myers RH, Cupples LA, Kiely DK, Kannel WB. Coronary risk associated with age and sex of parental heart disease in the Framingham Study. *Am J Cardiol*. 1989;64(10):555-559.

18. Ford TJ, Corcoran D, Berry C. Stable coronary syndromes: Pathophysiology, diagnostic advances and therapeutic need. *Heart*. Published online 2018:284-292.

19. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. Published online 2005:3481-3488.

20. Lusis AJ. Atherosclerosis. *Nature*. Published online 2000:233-241.

21. Davies MJ, Woolf N, Rowles PM, Pepper J. Morphology of the endothelium over atherosclerotic plaques in human coronary arteries. *Heart*. 1988;60(6):459-464.

22. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest*. Published online 2005:3378-3384.

23. Borissoff JI, Spronk HMH, Cate H Ten. The hemostatic system as a modulator of atherosclerosis. *N Engl J Med*. Published online 2011:1746-1760.

24. Cassar A, Holmes DR, Rihal CS, Gersh BJ. Chronic coronary artery disease: Diagnosis and management. In: *Mayo Clinic Proceedings*. ; 2009:1130-1146. doi:10.4065/mcp.2009.0391

25. Arbab-Zadeh A, Fuster V. The Myth of the "Vulnerable Plaque." *J Am Coll Cardiol*. 2015;17(1):9-21.

26. Kannel WB, Feinleib M. Natural history of angina pectoris in the Framingham study. Prognosis and survival. *Am J Cardiol*. 1972;29(2):154-163.

27. Mancini M, Petretto E, Kleinert C, et al. Mapping genetic determinants of coronary microvascular remodeling in the spontaneously hypertensive rat. *Basic Res Cardiol*. 2013;108(1):316.

28. Campeau L. Letter: Grading of angina pectoris. *Circulation*. 1976;54(3):522-523.

29. Kotecha T, Rakhit RD. Acute coronary syndromes. *Clin Med*. Published online 2016:s43-s48.

30. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. *Eur Heart J*. 2012;60(16):1581-1598.

31. Gurbel PA, Fox KAA, Tantry US, Ten Cate H, Weitz JI. Combination Antiplatelet and Oral Anticoagulant Therapy in Patients With Coronary and Peripheral Artery Disease: Focus on the COMPASS Trial. *Circulation*. 2019;139(18):2170-2185.

32. Hedayati T, Yadav N, Khanagavi J. Non–ST-Segment Acute Coronary Syndromes. *Cardiol Clin*. Published online 2018:37-52.

33. Kumar A, Cannon CP. Acute coronary syndromes: Diagnosis and management, part I. In: *Mayo Clinic Proceedings*. ; 2009:917-938. 34. National Clinical Guideline Centre. Unstable Angina and NSTEMI: The Early Management of Unstable Angina and Non-ST-Segment-Elevation Myocardial Infarction. *(NICE Clin Guidel No 94)*. Published online 2010.

35. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Eur Heart J*. Published online 2020:111-188.

36. van der Meijden PEJ, Heemskerk JWM. Platelet biology and functions: new concepts and clinical perspectives. *Nat Rev Cardiol*. Published online 2019:166-179.

37. Ghoshal K, Bhattacharyya M. Overview of platelet physiology: Its hemostatic and nonhemostatic role in disease pathogenesis. *Sci World J*. Published online 2014.

38. May AE, Seizer P, Gawaz M. Platelets: Inflammatory firebugs of vascular walls. *Arterioscler Thromb Vasc Biol*. Published online 2008:s5-s10.

39. Thijs T, Nuyttens BP, Deckmyn H, Broos K. Platelet physiology and antiplatelet agents. *Clin Chem Lab Med*. Published online 2010:S3-S13.

40. Willoughby S, Holmes A, Loscalzo J. Platelets and cardiovascular disease. *Eur J Cardiovasc Nurs*. Published online 2002:273-288.

41. Swieringa F, Spronk HMH, Heemskerk JWM, van der Meijden PEJ. Integrating platelet and coagulation activation in fibrin clot formation. *Res Pract Thromb Haemost*. 2018;2(3):450-460.

42. Ruggeri ZM. Platelets in atherothrombosis. *Nat Med*. Published online 2002:1227-1234.

43. Massberg S, Brand K, Grüner S, et al. A critical role of platelet adhesion in the

initiation of atherosclerotic lesion formation. *J Exp Med*. 2002;196(7):887-896.

44. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. *Cardiovasc Res*. Published online 2004:498-511.

45. Ross R, Bowen-Pope DF, Raines EW. Platelets, Macrophages, Endothelium, and Growth Factors Their Effects upon Cells and their Possible Roles in Atherogenesis. *Ann N Y Acad Sci*. 1985;454:254-260.

46. Henn V, Slupsky JR, Gräfe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*. 1998;391(6667):591-594.

47. Sawicki G, Salas E, Murat J, Miszta-Lane H, Radomski MW. Release of gelatinase A during platelet activation mediates aggregation. *Nature*. 1997;386(6625):616-619.

48. Pitsilos S, Hunt J, Mohler ER, et al. Platelet factor 4 localization in carotid atherosclerotic plaques: Correlation with clinical parameters. *Thromb Haemost*. 2003;90(6):1112-1120.

49. O'Donnell VB, Murphy RC, Watson SP. Platelet lipidomics: Modern day perspective on lipid discovery and characterization in platelets. *Circ Res*. 2014;114(7):1185-1203.

50. Massberg S, Konrad I, Schürzinger K, et al. Platelets secrete stromal cell-derived factor 1α and recruit bone marrow-derived progenitor cells to arterial thrombi in vivo. *J Exp Med*. 2006;203(5):1221-1233.

51. Stellos K, Langer H, Daub K, et al. Platelet-derived stromal cell-derived factor-1 regulates adhesion and promotes differentiation of human CD34+ cells to endothelial progenitor cells. *Circulation*. 2008;117(2):206-215.

52. Catricala S, Torti M, Ricevuti G. Alzheimer disease and platelets: How's that relevant. *Immun Ageing*. Published online 2012:20.

53. Stellos K, Sauter R, Fahrleitner M, et al. Binding of oxidized low-density lipoprotein on circulating platelets is increased in patients with acute coronary syndromes and

induces platelet adhesion to vascular wall in vivo-brief report. *Arterioscler Thromb Vasc Biol*. 2012;32(8):2017-2020.

54. Magwenzi S, Woodward C, Wraith KS, et al. Oxidized LDL activates blood platelets through CD36/NOX2-mediated inhibition of the cGMP/protein kinase G signaling cascade. *Blood*. 2015;125(17):2693-2703.

55. Pasalic L, Wang SSY, Chen VMY. Platelets as biomarkers of coronary artery disease. *Semin Thromb Hemost*. 2016;42(3):223-233.

56. Kang JG, Patino WD, Matoba S, Hwang PM. Genomic Analysis of Circulating Cells: A Window Into Atherosclerosis. *Trends Cardiovasc Med*. Published online 2006:163-168.

57. Geisler T, Schaeffeler E, Dippon J, et al. CYP2C19 and nongenetic factors predict poor responsiveness to clopidogrel loading dose after coronary stent implantation. *Pharmacogenomics*. 2008;9(9):1251-1259.

58. Meade TW, Vickers M V., Thompson SG, Stirling Y, Haines AP, Miller GJ. Epidemiological characteristics of platelet aggregability. *Br Med J (Clin Res Ed)*. 1985;290(6466):428-432.

59. Mcfadden EP, Clarke JG, Davies GJ, Kaski JC, Haider AW, Maseri A. Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. *N Engl J Med*. 1991;324(10):648-654.

60. Chirkov YY, Holmes AS, Chirkova LP, Horowitz JD. Nitrate resistance in platelets from patients with stable angina pectoris. *Circulation*. 1999;100(2):129-134.

61. Andreotti F, Lanza GA, Sciahbasi A, et al. Low-grade exercise enhances platelet aggregability in patients with obstructive coronary disease independently of myocardial ischemia. *Am J Cardiol*. 2001;87(1):16-20.

62. Mcbane RD, Karnicki K, Tahirkheli N, Miller RS, Owen WG. Platelet characteristics associated with coronary artery disease. *J Thromb Haemost*. 2003;1(6):1296-1303.

63. Müller KAL, Chatterjee M, Rath D, Geisler T. Platelets, inflammation and anti-inflammatory effects of antiplatelet drugs in ACS and CAD. *Thromb Haemost*. 2015;114(3):498-518.

64. Lièvre M, Cucherat M. Aspirin in the secondary prevention of cardiovascular disease: An update of the APTC meta-analysis. *Fundam Clin Pharmacol*. 2010;24(3):385-391.

65. Cattaneo M, Zighetti ML, Lombardi R, et al. Molecular bases of defective signal transduction in the platelet P2Y12 receptor of a patient with congenital bleeding. *Proc Natl Acad Sci U S A*. 2003;100(4):1978-1983.

66. Geisler T, Graß D, Bigalke B, et al. The residual platelet aggregation after deployment of intracoronary stent (PREDICT) score. *J Thromb Haemost*. 2008;6(1):54-61.

67. Seidah NG, Benjannet S, Wickham L, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): Liver regeneration and neuronal differentiation. *Proc Natl Acad Sci U S A*. 2003;100(3):928-933.

68. Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003;34(2):154-156.

69. Mizuno K, Nakamura T, Ohshima T, Tanaka S, Matsuo H. Yeast KEX2 gene encodes an endopeptidase homologous to subtilisin-like serine proteases. *Biochem Biophys Res Commun*. 1988;156(1):246-254.

70. Julius D, Brake A, Blair L, Kunisawa R, Thorner J. Isolation of the putative structural gene for the lysine-arginine-cleaving endopeptidase required for processing of yeast prepro-α-factor. *Cell*. 1984;37(3):1075-1089.

71. Seidah NG, Abifadel M, Prost S, Boileau C, Prat A. The proprotein convertases in hypercholesterolemia and cardiovascular diseases: Emphasis on proprotein convertase subtilisin/Kexin 9. *Pharmacol Rev*. 2017;69(1):33-52.

72. Cesaro A, Bianconi V, Gragnano F, et al. Beyond cholesterol metabolism: The pleiotropic effects of proprotein convertase subtilisin/kexin type 9 (PCSK9). Genetics, mutations, expression, and perspective for long-term inhibition. *BioFactors*. Published online 2020:367-380.

73. Seidah NG, Prat A. The biology and therapeutic targeting of the proprotein convertases. *Nat Rev Drug Discov*. Published online 2012:367-383.

74. Warden BA, Fazio S, Shapiro MD. The PCSK9 revolution: Current status, controversies, and future directions: The PCSK9 revolution. *Trends Cardiovasc Med*. Published online 2020:179-185.

75. Kotowski IK, Pertsemlidis A, Luke A, et al. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. *Am J Hum Genet*. 2006;78(3):410-422.

76. Cui CJ, Li S, Li JJ. PCSK9 and its modulation. *Clin Chim Acta*. Published online 2015:79-86.

77. Dubuc G, Chamberland A, Wassef H, et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2004;24(8):1454-1459.

78. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376(18):1713-1722.

79. Dos Santos C, Marshall JC. Bridging lipid metabolism and innate host defense. *Sci Transl Med*. Published online 2014:258.

80. Ricci C, Ruscica M, Camera M, et al. PCSK9 induces a pro-inflammatory response in macrophages. *Sci Rep*. 2018;8(1):1-10.

81. Dwivedi DJ, Grin PM, Khan M, et al. Differential expression of PCSK9 modulates infection, inflammation, and coagulation in a murine model of sepsis. *Shock*.

2016;46(6):672-680.

82. Glerup S, Schulz R, Laufs U, Schlüter KD. Physiological and therapeutic regulation of PCSK9 activity in cardiovascular disease. *Basic Res Cardiol*. Published online 2017:32.

83. Ferri N, Tibolla G, Pirillo A, et al. Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by cultured smooth muscle cells reduces macrophages LDLR levels. *Atherosclerosis*. 2012;220(2):381-386.

84. J.W.A. VDH, S. K, E.J. P, et al. Alirocumab, monoclonal antibody to PCSK9, dosedependently decreases atherosclerosis, improves plaque stability and shows additive effects with atorvastatin in APOE∗3Leiden. CETP mice. *Atherosclerosis*. 2014;235(2):E19.

85. Navarese EP, Kolodziejczak M, Winter MP, et al. Association of PCSK9 with platelet reactivity in patients with acute coronary syndrome treated with prasugrel or ticagrelor: The PCSK9-REACT study. *Int J Cardiol*. 2017;227:644-649.

86. J.M. C, R. O, H. G-G, et al. Serum proprotein convertase substilisin/kexin type 9 level is associated with coronary plaque inflammation and cardiovascular outcome independent from serum LDL level. *Circulation*. Published online 2014.

87. Robinson JG, Farnier M, Krempf M, et al. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372(16):1489-1499.

88. Naseem KM, Goodall AH, Bruckdorfer KR. Differential effects of native and oxidatively modified low-density lipoproteins on platelet function. *Platelets*. 1997;8:163-173.

89. Chatterjee M, Rath D, Schlotterbeck J, et al. Regulation of oxidized platelet lipidome: Implications for coronary artery disease. *Eur Heart J*. 2017;38(25):1993-2005.

90. Zhang Y, Liu J, Li S, et al. Proprotein convertase subtilisin/kexin type 9 expression is

transiently up-regulated in the acute period of myocardial infarction in rat. *BMC Cardiovasc Disord*. 2014;14:192.

91. Camera M, Rossetti L, Barbieri SS, et al. PCSK9 as a Positive Modulator of Platelet Activation. *J Am Coll Cardiol*. 2018;71(8):952-954.

92. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - J Am Med Assoc*. Published online 2013.

93. Linden B. ESC guidelines for acute coronary syndromes without ST elevationHamm CW, Bassand JP, Agewall S (2011) ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. The Task Force for the mana. *Br J Card Nurs*. 2011;6(11):547-548.

94. Seizer P, Ungern-Sternberg SNIV, Schönberger T, et al. Extracellular Cyclophilin A Activates Platelets Via EMMPRIN (CD147) and PI3K/Akt Signaling, Which Promotes Platelet Adhesion and Thrombus Formation in Vitro and in Vivo. *Arterioscler Thromb Vasc Biol*. 2015;35(3):655-663.

95. Witte A, Rohlfing AK, Dannenmann B, et al. The chemokine CXCL14 mediates platelet function and migration via direct interaction with CXCR4. *Cardiovasc Res*. 2021;117(3):903-917.

96. Geue S, Aurbach K, Manke MC, et al. Pivotal role of PDK1 in megakaryocyte cytoskeletal dynamics and polarization during platelet biogenesis. *Blood*. 2019;134(21):1847-1858.

97. Kurien BT, Scofield RH. Western blotting. *Methods*. Published online 2006.

98. Witte A, Rohlfing A-K, Dannenmann B, et al. The chemokine CXCL14 mediates platelet function and migration via direct interaction with CXCR4. *Cardiovasc Res*. 2020;117(3):903-917.

99. Kim J, Cho CH, Jung BK, et al. Comparative evaluation of Plateletworks, Multiplate analyzer and Platelet function analyzer-200 in cardiology patients. *Clin Hemorheol*

Microcirc. 2018;70(3):257-265.

100. Münzer P, Walker-Allgaier B, Geue S, et al. PDK1 Determines Collagen-Dependent Platelet Ca 2+ Signaling and Is Critical to Development of Ischemic Stroke in Vivo. *Arterioscler Thromb Vasc Biol*. 2016;36(8):1507-1516.

101. Borst O, Münzer P, Alnaggar N, et al. Inhibitory mechanisms of very low-dose rivaroxaban in non-ST-elevation myocardial infarction. *Blood Adv*. 2018;2(6):715-730.

102. Roest M, Reininger A, Zwaginga JJ, King MR, Heemskerk JWM. Flow chamber-based assays to measure thrombus formation in vitro: Requirements for standardization. *J Thromb Haemost*. Published online 2011.

103. Rohlfing AK, Kolb K, Sigle M, et al. ACKR3 regulates platelet activation and ischemia-reperfusion tissue injury. *Nat Commun*. 2022;13(1).

104. Daub K, Langer H, Seizer P, et al. Platelets induce differentiation of human CD34 + progenitor cells into foam cells and endothelial cells. *FASEB J*. 2006;20(14):2559-2561.

105. Petersen-Uribe Á, Kremser M, Rohlfing AK. Platelet-derived PCSK9 is associated with LDL metabolism and modulates atherothrombotic mechanisms in coronary artery disease. *Int J Mol Sci*. 2021;22(20).

106. Carter RE. A standard error: Distinguishing standard deviation from standard error. *Diabetes*. Published online 2013:e15.

107. Puccetti L, Bruni F, Di Renzo M, et al. Hypercoagulable state in hypercholesterolemic subjects assessed by platelet-dependent thrombin generation: In vitro effect of Cerivastatin. *Eur Rev Med Pharmacol Sci*. 1999;3(5):197-204.

108. Weber M, Gerdsen F, Gutensohn K, Schoder V, Eifrig B, Hossfeld DK. Enhanced platelet aggregation with TRAP-6 and collagen in platelet aggregometry in patients

with venous thromboembolism. *Thromb Res*. 2002;107(6):325-328.

109. Paciullo F, Momi S, Gresele P. PCSK9 in Haemostasis and Thrombosis: Possible Pleiotropic Effects of PCSK9 Inhibitors in Cardiovascular Prevention. *Thromb Haemost*. Published online 2019:359-367.

110. Théorêt JF, Yacoub D, Hachem A, Gillis MA, Merhi Y. P-selectin ligation induces platelet activation and enhances microaggregate and thrombus formation. *Thromb Res*. 2011;128(3):243-250.

111. Schroeder KM, Beyer TP, Hansen RJ, et al. Proteolytic cleavage of antigen extends the durability of an anti-PCSK9 monoclonal antibody. *J Lipid Res*. 2015;56(11):2124-2132.

112. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol*. Published online 1983.

113. Dalgic Y, Abaci O, Kocas C, et al. The relationship between protein convertase subtilisin kexin type-9 levels and extent of coronary artery disease in patients with non-ST-elevation myocardial infarction. *Coron Artery Dis*. 2020;31(1):81-86.

114. Huijgen R, Fouchier SW, Denoun M, et al. Plasma levels of PCSK9 and phenotypic variability in familial hypercholesterolemia. *J Lipid Res*. 2012;53(5):979-983.

115. Fessler MB, Rose K, Zhang Y, Jaramillo R, Zeldin DC. Relationship between serum cholesterol and indices of erythrocytes and platelets in the US population. *J Lipid Res*. 2013;54(11):3177-3188.

116. Macchi C, Banach M, Corsini A, Sirtori CR, Ferri N, Ruscica M. Changes in circulating pro-protein convertase subtilisin/kexin type 9 levels – experimental and clinical approaches with lipid-lowering agents. *Eur J Prev Cardiol*. Published online 2019:930-949.

117. Sahebkar A. Circulating levels of proprotein convertase subtilisin kexin type 9 are elevated by fibrate therapy: A systematic review and meta-analysis of clinical trials.

Cardiol Rev. Published online 2014.

118. Hyun JJ, Lee HS, Kim KS, Kim YK, Yoon D, Sahng WP. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. *J Lipid Res*. 2008;49(2):399-409.

119. Rosenson RS, Hegele RA, Fazio S, Cannon CP. The Evolving Future of PCSK9 Inhibitors. *J Am Coll Cardiol*. Published online 2018.

120. Lloyd-Jones DM, Morris PB, Ballantyne CM, et al. 2017 Focused Update of the 2016 ACC Expert Consensus Decision Pathway on the Role of Non-Statin Therapies for LDL-Cholesterol Lowering in the Management of Atherosclerotic Cardiovascular Disease Risk: A Report of the American College of Cardiology Task Fo. *J Am Coll Cardiol*. 2017;70(14):1785-1822.

121. Nozue T. Lipid lowering therapy and circulating PCSK9 concentration. *J Atheroscler Thromb*. Published online 2017.

122. Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, et al. PCSK9 in relation to coronary plaque inflammation: Results of the ATHEROREMO-IVUS study. *Atherosclerosis*. 2016;248:117-122.

123. Nicholls SJ, Puri R, Anderson T, et al. Effect of Evolocumab on Progression of Coronary Disease in Statin-Treated Patients. *JAMA*. 2016;316(22):2373-2384.

124. Cesaro A, Gragnano F, Fimiani F, et al. Impact of PCSK9 inhibitors on the quality of life of patients at high cardiovascular risk. *Eur J Prev Cardiol*. 2020;27(5):556-558.

125. Schwartz GG, Steg PG, Szarek M, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. *N Engl J Med*. 2018;379(22):2097-2107.

7. Contribution

The concept of the study was designed by Prof. Dr. Meinrad P. Gawaz. I collected the clinical data, performed patient enrollment and blood sampling. I designed and managed the data base. The statistical analysis of the current thesis was performed by me, under supervision of PD Dr. med. univ. Dominik Rath. Platelet isolation from peripheral blood and cytometry were performed by Ms. Nikoletta Fila, while aggregometry and flow chamber assays were performed by cand. Dr. rer. nat. Marcel Kremser. *In vitro* monocyte migration assay, monocyte differentiation and immunostaining of the carotid samples were performed by cand. Dr. rer. nat. Valerie Dicenta. Immunoblotting was performed by cand. Dr. rer. nat. Frederic Emschermann

This thesis includes the data of the original paper. The original publication was drafted by Prof. Dr. Meinrad P. Gawaz. Cand. Dr. rer. nat. Marcel Kremser and myself revised and completed the paper with help of Dr. rer. nat. Anne-Katrin Rohlfing and PD Dr. med. univ. Dominik Rath. All of the authors read and approbed the manuscript.

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere else for any award. Other sources are acknowledged and a reference is appended.

8. Original paper

Petersen-Uribe Á, Kremser M, Rohlfing AK, Castor T, Kolb K, Dicenta V, Emschermann F, Li B, Borst O, Rath D, Müller KAL, Gawaz MP. Platelet-Derived PCSK9 Is Associated with LDL Metabolism and Modulates Atherothrombotic Mechanisms in Coronary Artery Disease. Int J Mol Sci. 2021 Oct 16;22(20):11179.

Acknowledgement

I would like to thank Univ.-Prof. Dr. Meinrad Paul Gawaz, Head of the Department of Cardiology and Angiology at the University of Tübingen, who gave me the opportunity to perform this work under his supervision.

Special thanks to PD Dr. med. univ. Dominik Rath for the excellent supervision of the work, as well as his continuous willingness to discuss and help, who always stood by my side during the data analysis and during the writing down of the study results.

I also would like to thank the members of the research group of Prof. Gawaz: cand. Dr. rer. nat. Marcel Kremser, Dr. rer. nat. Anne-Katrin Rohlfing, cand. Dr. rer. nat. Frederic Emschermann, Dr. sc. hum. Tatsiana Castor, cand. Dr. rer. nat. Kyra Kolb, cand. Dr. rer. nat. Valerie Dicenta and Ms. Nikoletta Fila, for their support and cooperation in data collection and processing.