

Prognostic impact of coronary microvascular function in patients with ischemic heart disease

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“Look beyond what you see”

Rafiki, ”The Lion King”

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ABSTRACT

Background: Ischemic heart disease is the leading cause of death globally. Despite recent advances in interventional and medical therapies, survivors of myocardial infarction are at high risk of recurrent cardiovascular events. In recent years, coronary microvascular function has attracted more attention as the main target for risk stratification and as a possible target for pharmacological intervention as a part of multifaceted treatment of ischemic heart disease. Coronary flow reserve (CFR) is one of the central indices that reflex the status of coronary circulatory function.

Aims: To investigate if transthoracic Doppler echocardiography-CFR (TDE-CFR) can predict significant epicardial coronary artery stenosis (paper I); to investigate the prevalence of reduced CFR in high-risk patients with prior myocardial infarction (paper IV). To investigate the impact of thrombus aspiration (paper II) and pretreatment with P2Y₁₂ inhibitors (paper III) on mortality in patients with STEMI undergoing PCI.

Methods: The SCAAR registry was used for data analysis and patient recruitment. CFR was assessed with TDE-CFR in paper I and IV. Regression modelling was used for statistical analysis of data including propensity score adjusted logistic regression (paper I), instrumental variable analysis (paper II), propensity-score adjusted mixed-effects logistic regression (paper III) and multiple linear regression (paper IV).

Results: TDE-CFR predicts significant coronary artery disease (paper I). Thrombus aspiration was not associated with any effect on mortality or stroke but was associated with decreased risk of stent thrombosis (paper II).

Pretreatment with P2Y₁₂ receptor antagonists was not associated with reduced IRA occlusion at the time of primary PCI or decreased stent thrombosis or improved survival at 30 days (paper III). Impairment of CFR was frequent in a high-risk post infarction population with nearly 40% of patients having CFR<2.5. Incomplete revascularization was the strongest independent predictor of lower CFR (paper IV).

Conclusions: A majority of high-risk patients with previous MI have decreased CFR despite receiving adequate pharmacological treatment as a part of secondary prevention. TDE-CFR is a valuable tool for risk stratification in patients with established ischemic heart disease. High-quality observational studies based on large-scale registries and adequate statistical modelling provide valuable complementary evidence for the external validity of randomized controlled trials.

Keywords: coronary microvascular function, coronary flow reserve, coronary artery disease, acute coronary syndromes, thrombus aspiration, pre-treatment, SCAAR, SWEDEHEART.

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SAMMANFATTNING PÅ SVENSKA

Ischemisk hjärtsjukdom är den vanligaste dödsorsaken i världen. Individer som överlevt en hjärtinfarkt löper stor risk att drabbas av nya hjärthändelser trots dagens avancerade behandlingsformer. Hjärtats mikrocirkulation har under senare år visat sig spela en central roll i utvecklingen av kranskärlssjukdom och värdering av mikrovaskulär funktion har fått allt större intresse vid riskstratifiering och behandling av patienter med kranskärlssjukdom. Coronarflödesreserv (CFR) är ett mått på hjärtats förmåga att reglera blodflödet efter behov. Störd CFR har visats vara associerat med sämre prognos.

Avhandlingens syften var att undersöka om CFR mätt icke-invasivt med ultraljud kan prediktera förekomst av signifikanta stenoser i kranskärlen (delarbete I), att undersöka prevalensen av nedsatt CFR hos högrisk-patienter med känd kranskärlssjukdom (delarbete IV), att undersöka den prognostiska betydelsen av att manuell trombaspation (delarbete II) och förbehandling med blodplättshämmande medicin, P2Y₁₂ hämmare, (delarbete III) hos patienter med ST-höjningsinfarkt som behandlas med ballongdilatation och stentinläggning i hjärtats kranskärl.

Det nationella kvalitetsregistret SWEDEHEART, inkluderande Riks-HIA och SCAAR användes för datainsamling i samtliga delarbeten. CFR mättes med transthorakal ultraljudsteknik i delarbete I och IV.

Vi fann att CFR predikterar förekomst av signifikanta stenoser på kranskärlsröntgen (delarbete I), att varken trombaspation (delarbete II) eller förbehandling med P2Y₁₂ hämmare (delarbete III) leder till minskad mortalitet efter hjärtinfarkt, att nedsatt CFR förekom hos fler än hälften av patienterna i en högrisk-population med känd kranskärlssjukdom (delarbete IV).

Sammanfattningsvis, trots optimal behandling efter hjärtinfarkt har en majoritet av patienterna nedsatt CFR. CFR-mätning med ultraljuds-teknik är ett värdefullt instrument i riskstratifiering och behandling av patienter med kranskärlssjukdom. Observationella studier baserade på patientregister är viktiga komplement för att bekräfta och utvärdera generaliserbarheten av resultaten i randomiserade studier.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. Haraldsson, I. Gan, L. Svedlund, S. Wittfeldt, A. Råmunddal, T. Angerås, O. Albertsson, P. Matejka, G. Omerovic, E.
Non-invasive evaluation of coronary flow reserve with transthoracic Doppler echocardiography predicts the presence of significant stenosis in coronary arteries
International Journal of Cardiology 2014 Sep;176(1):294-7
- II. Angerås, O. Haraldsson, I. Redfors, B. Fröbert, O. Petursson, P. Albertsson, P. Ioanes, D. Odenstedt, J. Olsson, H. Witt, N. Ruck, A. Millgård, J. Nilsson, J. Persson, J. Söderbom, M. Wedel, H. Erlinge, D. James, S. Råmunddal, T. Omerovic, E.
Impact of thrombus aspiration on mortality, stent thrombosis and stroke in patients with ST-elevation myocardial infarction: A report from the Swedish Coronary Angiography and Angioplasty Registry
Journal of American Heart Association 2018 Jan; 7(1): e007680
- III. Redfors, B. Dworeck, C. Haraldsson, I. Angerås, O. Odenstedt, J. Ioanes, D. Petursson, P. Völz, S. Albertsson, A. Persson, J. Koul, S. Erlinge, D. Omerovic, E.
Pretreatment with P2Y₁₂ Receptor Antagonists in ST-Elevation Myocardial Infarction: A Report from the Swedish Coronary Angiography and Angioplasty Registry
Submitted
- IV. Haraldsson, I. Gan, L. Svedlund, S. Torngren, K. Westergren, H. Redfors, B. Lagerström-Fermér, M. Angerås, O. Råmunddal, T. Petursson, P. Odenstedt, J. Albertsson, P. Erlinge, D. Omerovic, E.
PROspective Evaluation of Coronary FLOW Reserve and Molecular Biomarkers in Patients with Established Coronary Artery Disease. The PROFLOW trial: Cross-sectional evaluation of coronary flow reserve.
Manuscript

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ABBREVIATIONS

ACS	Acute coronary syndrome
AMI	Acute myocardial infarction
CAD	Coronary artery disease
CFR	Coronary flow reserve
FFR	Fractional flow reserve
IMR	Index of myocardial resistance
IRA	Infarct related artery
LAD	Left anterior descending artery
LCX	Left circumflex artery
LDL	Low density lipoproteins
MRI	Magnetic resonance imaging
NO	Nitric oxide
PCI	Percutaneous coronary interventions
PET	Positron emission tomography
RCA	Right circumflex artery
SCAAR	Swedish Coronary Angiography and Angioplasty Registry
SPECT	Single photon emission tomography
STEMI	ST-segment elevation myocardial infarction
SWEDEHEART	The Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies
TDE	Transthoracic Doppler Echocardiography

INTRODUCTION

Cardiovascular disease is the leading cause of premature adult mortality worldwide where acute myocardial infarction (AMI) alone stands for the majority of all deaths. Despite highly advanced technologies in revascularization and the most recently available secondary prevention therapies, the risk of recurrent cardiovascular events remains high at 30% in the first 12 month after an acute coronary syndrome^{1, 2}. For long, clinical management has been centered on identification and treatment of focal epicardial obstructive disease. This approach is comprehensible as it is the epicardial part of coronary circulation that can be visualized by coronary angiography and is accessible to revascularization procedures. However, stenting or by-pass grafting of a stenosis does not address fully the underlying pathological mechanisms. Over the last decades, the focus has shifted away from obstructive epicardial coronary atherosclerosis to become centered on coronary microvascular function instead. Since atherosclerosis is associated with microvascular dysfunction which may precede development of atherosclerotic plaques in epicardial vessels, it is reasonable that diagnosis and treatment of ischemic heart disease includes microvasculature and its function.

Coronary circulation

Structure and function of the coronary circulation

The coronary arteries originate from the aortic root and initially wrap around the outer surface of the heart (epicardial arteries) before penetrating into the myocardium (intramural arteries). The epicardial arteries can be visualized on coronary angiogram but constitutes only approximately 5-15% of the total coronary circulation. The remaining part of the coronary circulation, the microvasculature, is beyond what you see (Fig.1).

There are some unique aspects of coronary circulation as compared to skeletal muscle. Energy production in the healthy heart is primarily dependent on oxidative phosphorylation, less than 5% of ATP-production result from glycolytic metabolism. This dependency on oxidative energy production means that any increase in cardiac activity demands an almost instantaneous

parallel increase of oxygen availability. As the heart has to maintain a heart rate of 60-70 beats per minute even at rest, oxygen consumption in the left ventricle during resting conditions is high, about 20-fold that of skeletal muscle. In order to meet this high resting oxygen demand the left ventricle has a high level of oxygen extraction, 70-80%, in contrast to 30-40% oxygen extraction in skeletal muscle. This is also in contrast to the right ventricle which is more similar to skeletal muscle. Oxygen extraction in the heart is facilitated by a high capillary-to-myocyte ratio which enables adequate exchange of oxygen and metabolic waste-products. Since oxygen extraction is high already at rest the primary mean of meeting an increased oxygen demand is to increase coronary flow and consequently coronary flow is strongly correlated with myocardial oxygen consumption³.

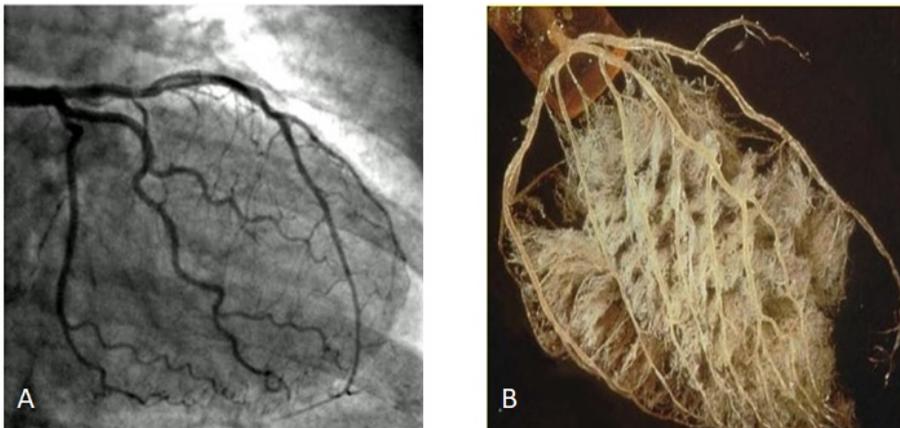


Figure 1. The coronary angiogram reveals the epicardial arteries (A), but those constitute only approximately 5-15% of the total coronary circulation. The remaining part, the microvasculature (B) cannot be visualized.

Regulation of coronary flow

Coronary flow is driven by the pressure difference between the aorta and the capillary bed. Epicardial coronary arteries without obstructive disease have a low vascular resistance and function mainly as conductance vessels. Capillaries and venules are primarily capacitance vessels, holding about 90% of coronary blood volume, and offer very little resistance. Most of coronary vascular resistance is met at the level of the pre-arterioles (epicardial vessels <

500 μm in diameter) and arterioles (intramural vessels $< 200 \mu\text{m}$) which makes this the primary site for regulation of coronary blood flow. By alterations in vascular tone, resistance is changed to modulate blood flow in response to variations in oxygen demand.

There are different main physiological mechanisms controlling vasomotion at different levels of the microcirculation. The vaso-reactivity of pre-arterioles (200-500 μm in diameter) and large arterioles (100-200 μm in diameter) is mainly endothelial-dependent and responds to changes in flow and shear stress. Arterioles ($< 100 \mu\text{m}$ in diameter), on the other hand, are mainly endothelium-independent where medium-sized arterioles (40–100 μm in diameter) are under myogenic control, i.e. stretch receptors located in vascular smooth muscle cells reacts to changes in intraluminal pressure⁴, while small arterioles ($< 40 \mu\text{m}$ in diameter) respond to metabolic activity of the myocardium.

So, in response to an increased metabolic demand the smaller arterioles dilate, which leads to reduced intraluminal pressure with myogenic vasodilation of medium-sized arterioles as a result. The following increase in upstream flow stimulates endothelium-dependent vasodilation at the level of pre-arterioles and arterioles^{5, 6}. Coronary flow is further influenced by circulating neurohormones and autonomic innervation, as well as by extravascular compressive forces, e.g. left ventricle wall hypertrophy and hypertension (Fig.2).

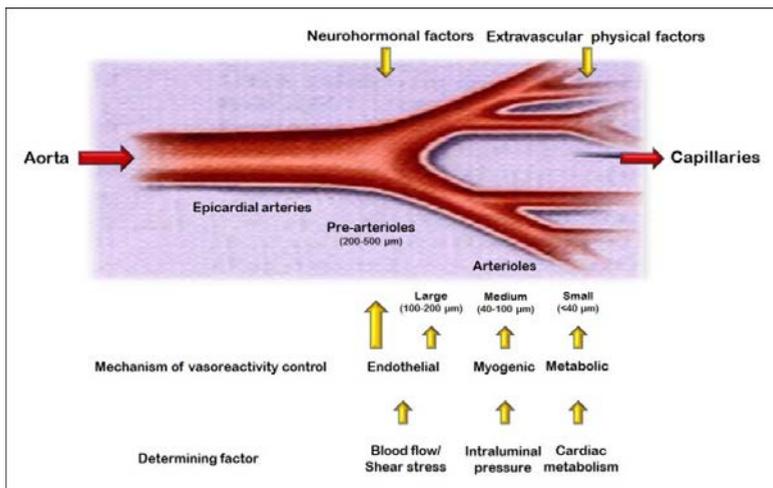


Figure 2. The microcirculation is the primary site for regulation of coronary flow. Different physiological mechanisms control vasomotion at different levels of the microcirculation. Adapted from Herrmann et al., European Heart Journal 2012.

Endothelial function and dysfunction

Since the pioneering work of Furchgott and Zawadzki in the early 1980's the endothelium is recognized as an important multifunctional regulatory organ involved not only in vasomotion but also in maintaining homeostasis⁷⁻¹². Despite being only a thin monolayer structure that separates circulating blood from surrounding tissue, the endothelium is the largest organ in the body as it covers the inner surface of the entire vascular system. Endothelial cells have the ability to respond to physical, chemical and humoral stimuli by production of both agonistic and antagonistic substances that regulate vascular tone, hemostasis, cellular proliferation, adhesion to the luminal surface as well as inflammatory and immune mechanisms in the vascular wall.¹³ Nitric oxide (NO) is a pivotal endothelium-derived substance and mediates endothelium-dependent vasodilation by opposing the effects of endothelium-derived vasoconstrictors such as angiotensin II and endothelin. Nitric oxide also inhibits platelet adherence and aggregation, adhesion/infiltration of leucocytes, and vascular smooth cell proliferation. Furthermore, nitric oxide prevents oxidative modification of low-density lipoproteins (LDL). Thus, the normal healthy endothelium is "quiescent" and maintains a relaxed vascular tone and low levels of oxidative stress, expressing a phenotype with anti-coagulant, anti-thrombotic, anti-proliferative, fibrinolytic as well as anti-inflammatory properties. Conversely, activation of the endothelium leads to morphologic and functional changes characterized by a reduction in NO, prostacyclin, and tissue plasminogen activator, thereby contributing to vasoconstriction mediated by endothelin, serotonin, and thrombin, and the endothelium is turned into a phenotype that is vaso-constrictive, pro-coagulant, pro-thrombotic, proliferative and pro-inflammatory (Fig.3).

Activation of the endothelium is initially a host-defense response triggered by vascular injury or by cytokines but prolonged activation will turn the endothelium into a dysfunctional state prone to development of atherosclerosis. All traditional cardiovascular risk factors including smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, and a family history of premature atherosclerotic disease are associated with endothelial dysfunction¹⁴⁻¹⁹. Endothelial dysfunction has also been associated with obesity, elevated C-reactive protein, and chronic systemic infection^{20, 21}. It is generally accepted that endothelial dysfunction precedes development of atherosclerosis²² and that endothelial function can be used as a risk marker of

atherosclerotic disease²³. However, it is not the disturbed regulatory function in itself that is important. Instead, the functionality of coronary circulation should be seen as a marker of the pro-atherosclerotic properties of the circulatory system.

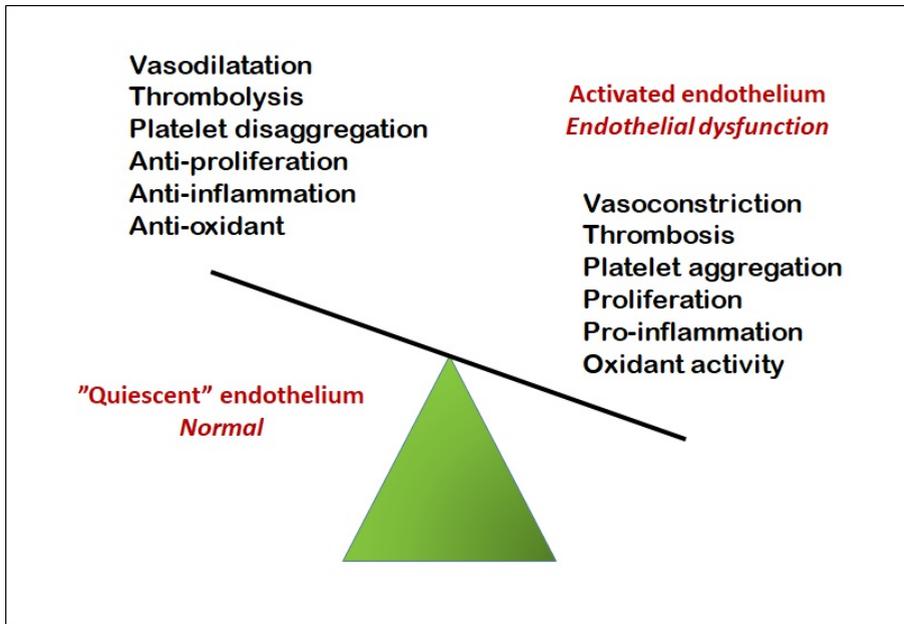


Figure 3. The normal healthy endothelium is “quiescent” and maintains a relaxed vascular tone and low levels of oxidative stress, expressing a phenotype with anti-coagulant, anti-thrombotic, anti-proliferative, fibrinolytic as well as anti-inflammatory properties. The activated endothelium is turned into a dysfunctional state expressing vasoconstrictive, pro-coagulant, pro-thrombotic, proliferative and pro-inflammatory characteristics.

Coronary artery disease

Atherosclerosis

Atherosclerosis is a chronic multifocal immuno-inflammatory disease of the arteries. The atherosclerotic process begins in early teenage years and continues throughout life. It is characterized by accumulation of cholesterol, infiltration of macrophages, proliferation of smooth muscle cells (SMC) and accumulation of connective tissue components in the intima of the vessel wall. The process of atherosclerosis has been reviewed several times^{22, 24} and can be briefly summarized as follows. The earliest event in atherosclerosis is believed to be injury to the endothelium which leads to endothelial activation. The

activated endothelium triggers monocyte adhesion, loosening of endothelial cell junctions and migration of monocytes into the sub-endothelial space where monocytes differentiate to macrophages. The increased endothelial permeability permits LDL to enter the intima where macrophages engulf the LDL-particles by phagocytosis. Lipid-laden macrophages are referred to as “foam cells” and collections of these foam-cells form the initial lesion in atherosclerosis, i.e. fatty streaks. Once in the intima the LDL-particles get oxidized. Macrophages stimulate further oxidation of LDL and oxidized LDL trigger continued recruitment of monocytes into the intima. Oxidized LDL also inhibits nitric oxide synthase with enhanced endothelial dysfunction as a result, and increases generation of reactive oxygen species (ROS) i.e. free radical formation (superoxide, hydrogen peroxide). Oxidative stress increases vascular endothelial permeability and promotes leukocyte adhesion²⁵. Other inflammatory cells, T-cells, are also recruited into the intima. T-cells secrete cytokines that trigger smooth muscle cells to migrate from the media to the intima. Under the influence of growth factors these smooth muscle cells begin to proliferate. Over time the initial lesion in atherosclerosis, the fatty streak, evolves to a sub-endothelial fibrous plaque composed of a lipid core surrounded by smooth muscle cells and connective tissue fibers, characterized by inflammation and high oxidative stress, a fibro-atheroma (Fig.4).

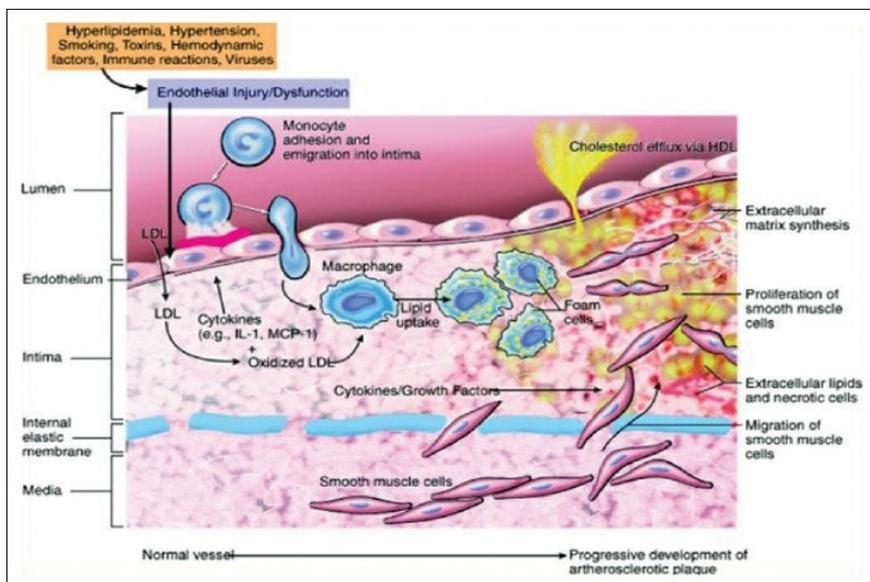


Figure 4. The process of atherosclerotic plaque development. Adapted with permission from *Int J Prev Med*.

As the process advances, the fibrous cap becomes thin and weakened at sites where proteolytic activity dissolves the fibrous tissue. Those thin-cap fibroatheromas are termed vulnerable plaques as they are prone to rupture. Exposure of the thrombogenic interior arterial wall to the circulating blood initiates platelet aggregation and coagulation in the infiltrating and overlying blood with thrombus formation and potential occlusion of the vessel lumen as a consequence.

The process of plaque development throughout life is the same independently of race, sex or geographic location. However, this process of atherosclerosis is dynamic since the presence of risk factors such as tobacco smoking, hypertension, obesity, diabetes mellitus and genetic predisposition, can accelerate the rate of plaque development. Conversely, reducing the cardiovascular risk burden may probably slow down the process since early stage lesions, fatty streaks, may regress and many ruptures of thin fibrous caps remain clinically silent as they have the potential to heal by forming new fibrous tissue.

Clinical manifestations of coronary artery disease

As the fibroatheroma grows, the arterial wall enlarges its caliber trying to avoid reduction of lumen by the plaque. This remodeling of the vessel is stopped when the plaque engages about 40% of the area of the artery. Any further plaque enlargement reduces vessel lumen and when blood flow is insufficient to meet the metabolic demand the plaque has become a hemodynamically significant stenosis, which causes ischemia. This is typically the situation in *stable angina pectoris* where initially a more extensive work load is needed to provoke ischemia and symptoms of angina but with increasing severity of the stenosis lower grades of exertion, or even no physical stress, may elicit symptoms. While a reduction of vessel lumen of 50% is hemodynamically significant at stress, a narrowing of >85% is demanded to elicit ischemia at rest²⁶. *Acute coronary syndromes*(ACS) are predominantly characterized by acute thrombus formation causing partial or total arterial occlusion with subsequent myocardial ischemia and potential death of cardiomyocytes. ACS manifest as *ST-elevation myocardial infarction (STEMI)* in the case of total vessel occlusion and as *non-STEMI* or *unstable angina* in subtotal occlusions (Fig.5).

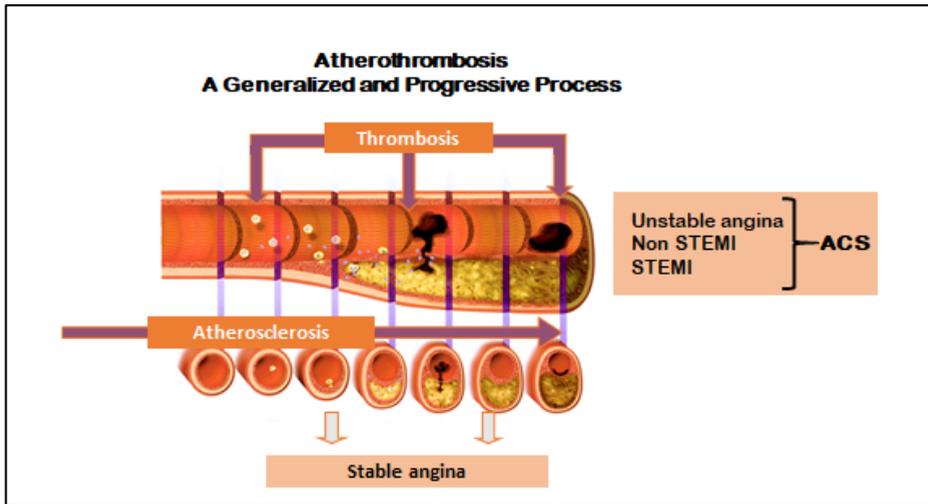


Figure 5. Pathogenesis and clinical manifestations of coronary artery disease. Adapted from Libby P. *Circulation*. 2001.

In post-mortem studies on patients with ACS and sudden death, thrombosis is found to be due to rupture of thin cap fibroatheroma in approximately 50-60% of cases, erosion of the endothelium in 20% and in rare cases, 2%, thrombosis is due to protrusion of a calcified nodule into vessel lumen. Interestingly, in 20-30% of ACS and sudden cardiac death, there is an advanced stenosis but thrombus formation is absent²⁷. Notably, up to one-third of patients with symptoms suggestive of ACS and raised levels of troponins and/or ischemic changes on ECG, have normal or near-normal arteries on coronary angiography^{28, 29}. Likewise, 10-30% of patients referred to elective coronary angiography due to chest pain have coronary arteries without obstructive epicardial disease³⁰. As many as 50-60% of these patients have reduced CFR due to coronary microvascular dysfunction. This condition is termed microvascular *angina (MVA)*³¹⁻³³.

Treatment of coronary artery disease

The primary goal in acute coronary syndromes is to minimize ischemia and myocardial injury due to thrombus formation with subsequent total or sub-total obstruction of the coronary vessel. An urgent reperfusion strategy is needed in the case of STEMI and percutaneous coronary intervention (PCI) is generally the first choice. Pharmacological reperfusion (thrombolysis) is recommended

as an alternative in the case of short duration of symptoms and if transport time to nearest catheterization-laboratory is expected to be long (>120 minutes according to current guidelines). In some settings revascularization with coronary artery by-pass grafting (CABG) is the preferred strategy. Antithrombotic therapy in the acute phase, with aspirin and low-weight molecular heparins, aims at limiting the size of the thrombus in the coronary artery and to prevent continued thrombus organization. In 1967 the mechanism of action of aspirin (acetylsalicylic acid) on platelet activity was discovered³⁴ and later the clinical benefit of aspirin on vascular death, non-fatal MI and stroke has been demonstrated in patients at high risk of serious cardiovascular events^{35, 36}. Addition of a P2Y₁₂-inhibitor to aspirin (i.e. dual antiplatelet therapy) has been shown to reduce acute ischemic complications, recurrent athero-thrombotic events and stent thrombosis after PCI³⁷. There are currently three classes of antiplatelet agents approved for clinical use in patients with ACS or in patients undergoing PCI; cyclooxygenase-1 (COX-1) inhibitors (aspirin), adenosine diphosphate (ADP) P2Y₁₂ receptor antagonists (ticlopidine, clopidogrel, prasugrel, ticagrelor), and glycoprotein IIb/IIIa inhibitors (abciximab, eptifibatide, tirofiban). The use of the first P2Y₁₂-inhibitor, ticlopidine, was limited by frequent side effects, including severe neutropenia and thrombotic thrombocytopenic purpura (TTP), and ticlopidine was soon replaced by clopidogrel since clopidogrel had a better safety profile and a faster onset of action. The benefit of adding clopidogrel to aspirin to prevent recurrent cardiovascular events has been demonstrated in several trials³⁸⁻⁴⁰ and dual antiplatelet therapy with aspirin and clopidogrel has been standard care for more than a decay. Recently, the more potent P2Y₁₂-inhibitors, prasugrel and ticagrelor, have been proven superior compared to clopidogrel in reducing major adverse cardiovascular outcomes although both ticagrelor and prasugrel substantially increase the risk of bleeding^{41, 42}. Long-term treatment of ischemic heart disease aims at preventing future cardiovascular events such as recurrent ACS, arrhythmias, heart failure and sudden cardiac death. Established medical treatment with prognostic value include beta-blockers⁴³, ace-inhibitors(ACE-I)⁴⁴ and statins⁴⁵ on top of dual antiplatelet therapy³⁹. The mechanisms of action for all these therapies are multifaceted and many of them affect microvascular function at some level and are proved to improve endothelial function^{46, 47}.

An important part of secondary prevention consists of lifestyle changes such as smoking cessation, increased physical activity, stress management, dietary changes and participation in rehabilitation programs⁴⁸.

Coronary flow reserve

Coronary flow reserve (CFR) is the ability of the heart to increase coronary flow in response to an increased myocardial oxygen demand and is defined as the ratio of maximum coronary flow to coronary flow at rest. The concept of coronary flow reserve was first established by Gould et al. in 1974²⁶ when they demonstrated a relationship between severity of coronary stenosis and hyperemic coronary flow response. With increasing restriction of a coronary artery, resting flow initially remains unchanged, while maximum coronary flow decreases gradually and a completely abolished flow is found for stenosis >90%. However, coronary anatomy is not the lone determinant of the reserve capacity of coronary flow. CFR is the net result of all the complex mechanisms involved in regulation of coronary blood flow. As a consequence, CFR reflects both endothelial-dependent and -independent microvascular function, as well as flow-limiting epicardial disease and rheological properties of circulating blood. The normal healthy heart can increase coronary blood flow 3-6 times the resting value (Fig.6).

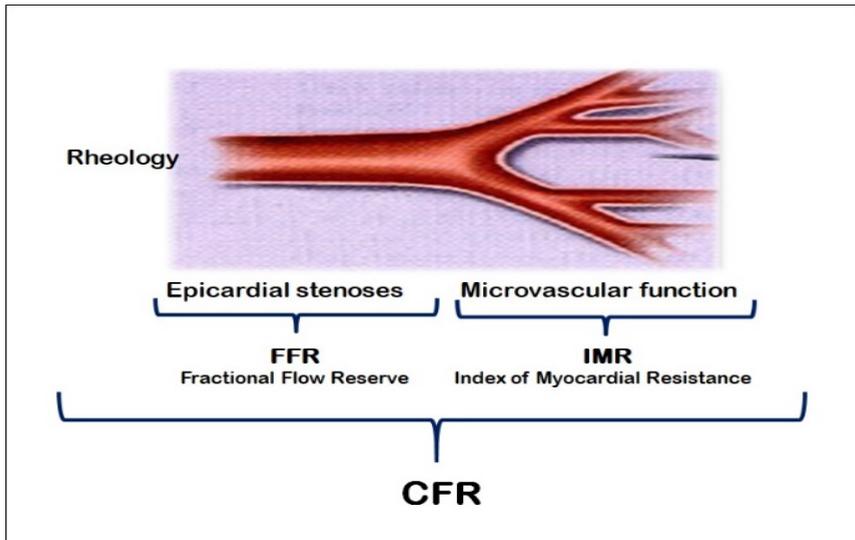


Figure 6. CFR mirrors all complex mechanisms involved in regulation of coronary flow.

Methods to assess coronary flow reserve

Several imaging modalities have been used to assess the functionality of coronary circulation. In most clinical applications, hyperemia is induced pharmacologically, commonly with agents such as adenosine and dipyridamole, and not via an increase in oxygen demand. Adenosine and dipyridamole act mainly by a direct relaxing effect on vascular smooth muscle cells with non-endothelial-dependent vasodilation as the primary effect, followed by a secondary flow-mediated endothelial-dependent response. In some settings cold-pressure test is used to specifically evaluate direct endothelial-dependent coronary vasomotion. Several factors influence measurement of CFR, e.g. the ability to achieve maximal coronary vasodilation, heart rate, myocardial contractility and right atrial pressure. These factors have different impact depending on the method used for evaluation of CFR and must be kept in mind at interpretation of the results. All methods have their technical advantages as well as disadvantages, and also differ when it comes to costs and availability.

The earliest CFR-measurements were invasive with the use of an *intracoronary Doppler flow wire*. Doppler flow wires make it possible to calculate coronary flow velocity reserve (CFVR) by dividing intracoronary mean velocity at pharmacologically induced hyperemia with baseline mean velocity at rest. As long as vessel lumen area is kept constant, which is a reasonable assumption with drugs such as adenosine and dipyridamole, blood velocity is proportional to flow and CFVR can be approximated to CFR^{49, 50}.

Intracoronary Doppler has for long been the gold standard for invasive assessment of CFR but currently *thermodilution-techniques*, with the use of pressure-temperature sensor-tipped guidewires, are more common. Transit times for manual injections of saline at room temperature at rest and during hyperemia induced by intravenous adenosine are used to calculate CFR.^{51, 52} The same pressure guidewire can be used to assess myocardial fractional flow reserve (FFR) and for calculation of the index of myocardial resistance (IMR). FFR is defined as the resting distal coronary pressure to aortic pressure ratio (Pd/Pa) during hyperemia. When coronary resistance is minimized, as it is assumed to be during hyperemia, flow is linearly related to blood pressure and FFR becomes a surrogate measure of flow limitation and an index of coronary stenosis severity and ischemia⁵³. FFR is independent of baseline flow

and heart rate and is easy to perform to the experienced operator. However, a good understanding of the methodology is required to get optimal data and for correct interpretation. In the “DEFER” study it was shown to be safe to defer patients with one-vessel stable CAD and $FFR > 0.75$ ⁵⁴. In the “FAME” trial on patients with multivessel CAD, $FFR < 0.80$ was shown to be a physiological threshold indicating ischemic obstructive coronary disease that could benefit from revascularization⁵⁵. In the “COMPARE ACUTE” trial on patients with STEMI and multivessel disease, Smits et al. showed that FFR-guided revascularization of non-culprit lesions had a 65% reduced risk of MACCE (death from any cause, nonfatal myocardial infarction, revascularization, and cerebrovascular events) at one year (HR 0.35; 95% CI, 0.21-0.58; $P < 0.001$) compared to STEMI-patients with PCI of the infarct-related artery only⁵⁶. Measurement of FFR for functional lesion assessment in CAD is now recommended in guidelines from American College of Cardiology (ACC)/American Heart Association (AHA) and the European Society of Cardiology (ECS)/European Association of Cardio-Thoracic Surgery (EACTS)⁵⁷⁻⁵⁹.

Another rather novel but interesting measure, derived from the pressure-wire, is the index of myocardial resistance (IMR). Coronary microcirculation is the main determinant of myocardial resistance and assessment of myocardial resistance will provide information on microvascular function. Assuming that coronary flow and myocardial flow is equal and that collateral flow is negligible, IMR is the ratio of distal coronary pressure to coronary flow and can be calculated from parameters derived from the CFR-measurement⁶⁰. IMR measurement is reproducible and independent of variations in heart rate, blood pressure and myocardial contractility⁶¹. So far, IMR is not generally used in clinical practice but has been studied in stable CAD and is found to be of prognostic importance in AMI⁶²⁻⁶⁵. Thus, the combined measures of CFR, FFR and IMR give lesion-specific information on epicardial level as well as information on microcirculatory level simultaneously (Fig.6).

Positron Emission Tomography (PET) is considered the non-invasive gold standard for evaluation of quantitative regional blood flows and allows measurement of myocardial perfusion and function at stress and rest. Absolute myocardial blood flow (MBF) in milliliter/minute/gram can be quantified in the same study. This technique also allows calculation of coronary flow reserve (CFR) or myocardial flow reserve (MFR)⁶⁶. Recent studies have shown that

measurements of CFR assessed by PET can provide important prognostic information over semi-quantitative perfusion data⁶⁷. It is an appealing technique through its non-invasive nature but it is expensive and at present only available at a limited number of institutions.

Single Photon Emission Computed Tomography (SPECT), is another commonly used non-invasive technique to assess relative myocardial perfusion but can also get quantitative measurements of myocardial perfusion and perfusion reserve. After intravenous administration of a radionuclide tracer estimates of global and regional myocardial perfusion reserve can be calculated by dividing perfusion values at stress with values at rest. CFR-values estimated by SPECT imaging are well correlated to intravascular Doppler ultrasound-derived CFR⁶⁸ and has also been validated by comparisons with PET imaging⁶⁹.

Cardiovascular magnetic resonance imaging (CMRI) is a technique that has developed rapidly the last fifteen years. CMRI is used to study myocardial structure, cardiac function, macro vascular blood flow, myocardial perfusion and myocardial viability. Key advantages are the non-invasive nature, no ionizing radiation and good image quality. Disadvantages on the other hand, are limited availability, expense and special technical training of those who perform CMRI.

Recent years, evolution of **echocardiography-based techniques** used to assess coronary flow velocities, have been recognized as a valuable tool in evaluation of CFR. Initially, measurements of coronary flow velocities were semi-invasive recorded with a trans-esophageal doppler technique. Development of technological factors, such as second harmonic imaging and high-frequency transducers with better definition of smaller structures and improved resolution imaging of near-field structures, have enabled assessment of CFR with transthoracic Doppler technique. Using pulsed wave Doppler echocardiography under the guidance of color Doppler flow mapping, coronary flow velocity reserve can be measured by dividing mean hyperemic flow velocity to mean flow velocity at rest. Assessment of CFR with transthoracic doppler can be performed in all three coronary arteries e.g. left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCX)^{70, 71}. Validation of feasibility and reproducibility of the transthoracic Doppler technique have been described previously^{58, 59}. Accuracy

of the method has also been validated against invasive techniques⁷⁰. Transthoracic Doppler technique is patient-friendly as it is easy tolerable and associated with low risk, furthermore it is not expensive and easy accessible in all institutions.

Cut-off values of coronary flow reserve

There is not one single defined cut-off value of CFR. Different cut-off values have been proposed for diagnosis of significant epicardial coronary artery disease and for estimation of cardiovascular prognosis. Matsamura et al. measured CFR with transthoracic doppler echocardiography (TDE-CFR) in 138 patients with various cardiovascular risk factors undergoing coronary angiography, and found a best cut-off value of CFR <2.0 for detection of significant LAD stenosis⁷². A similar finding was made by Cortigiani et al who demonstrated a cut-off value of TDE-CFR<1.91 for detecting $\geq 75\%$ stenosis of the left anterior descending artery (LAD) in 2089 patients with known or suspected CAD. This was true in both hypertensive patients (area under the curve 0,86) and in normotensives (area under the curve 0,90)⁷³. Comparable conclusions have been reached by other groups^{70, 74} and a cut-off of CFR<2.0 for detection of significant stenosis has been generally accepted.

However, to predict a favorable cardiovascular prognosis a CFR-value>2.0 is not suitable. Nakanishi et al demonstrated that CFR<2.4 was the best cut-off value in predicting a combination of long-term cardiovascular events, acute coronary syndromes and the development of heart failure (area under the curve=0.82). CFR was an excellent predictor of heart failure development (area under the curve=0.95). For prediction of acute coronary syndromes the area under the curve was 0.67⁷⁵. In this study population TDE-CFR was performed on 272 patients without significant obstructive CAD on angiograms. Similar findings have been made in patients with diabetes where an optimal cut-off value to predict cardiovascular events was 2.5 (area under the curve=0.65). The event rate was significantly higher in patents with CFR<2.5 than in those with CFR>2.5⁷⁶. In conclusion, for detection of significant coronary artery disease a cut-off of CFR<2.0 is accurate while the optimal cut-off value to predict cardiovascular events is CFR< 2.5.

Clinical and prognostic impact of coronary flow reserve

In analogy with dysfunction of the endothelium, impaired CFR is associated with several of the established cardiovascular risk factors, e.g. age^{77, 78}, obesity⁷⁹, hypertension⁸⁰⁻⁸², hyperlipidemia^{83, 84}, diabetes⁸⁵⁻⁸⁷, renal dysfunction⁸⁸, and smoking^{89, 90}. This has been demonstrated in patients without concomitant significant CAD and in this setting impaired CFR reflects microvascular function⁹¹. CFR can be used as an indicator summarizing cardiovascular risk⁹² and has been shown to be a strong predictor of future cardiovascular events including death, MI, stroke, and need for coronary revascularization.

In the “DEBATE” study from 1997, CFR was measured with intra-coronary Doppler post-PCI and was found to have prognostic value in patients with myocardial infarction⁹³. Traditionally long-term prognosis after MI is estimated based on TIMI-flow, age, reduced left ventricular function and infarct size but still accurate identification of high risk individuals in this patient cohort is insufficient. Interestingly, CFR is shown to give additional prognostic information on top of established cardiovascular risk markers in some recent studies. Murthy et al performed positron emission tomography (PET) in patients with known or suspected CAD and found that CFR was an independent predictor of mortality. Risk was also shown to increase with decreasing CFR⁶⁷. In a similar patient cohort, Cortigiani et al evaluated CFR with transthoracic Doppler echocardiography and found a significantly higher 4-year mortality in patients with CFR<2 compared to patients with CFR>2⁹⁴. The findings by the groups of Murthy and Cortigiani, of CFR as an additive prognostic marker, were independent of prevalence of ischemia measured by PET scan or stress-echo respectively. Transthoracic Doppler derived CFR has also been found to add incremental prognostic value above myocardial perfusion scintigraphy in patients with suspected ischemic heart disease⁹⁵.

Other studies have evaluated the prognostic impact of reduced CFR in patients with and without diabetes as well as in patients with renal insufficiency. Both diabetes mellitus and chronic renal dysfunction are in themselves associated with increased cardiovascular risk. Diabetic patients without known CAD had similar rates of cardiac mortality as non-diabetics with known CAD. Conversely, diabetics without known CAD and without CFR-impairment had rates of cardiac death that were comparable to nondiabetics^{96, 97}. Likewise, in patients with chronic renal dysfunction evaluation of CFR improved risk

stratification beyond routine measures of risk such as left ventricular systolic function and severity of myocardial ischemia and infarct size⁹⁸.

Prognostic impact of impaired CFR has also been observed in several other pathological conditions such as non-ischemic cardiomyopathies^{99, 100}, transplant vasculopathy¹⁰¹ as well as in aortic valve disease¹⁰². Thus, there are substantial evidence for the correlation between impaired CFR and cardiovascular risk factors and the prognostic value of CFR on cardiac mortality.

Impact of secondary prevention on coronary flow reserve

Treatment of cardiovascular risk factors may improve CFR. In smokers, improvement of CFR was demonstrated after 1 month of smoking cessation¹⁰³. In patients with hypertension and stable CAD, treatment with angiotensin receptor blockers (ARB) resulted in improved CFR. This improvement preceded the reduction of blood pressure¹⁰⁴. Similar beneficial effects on CFR has been demonstrated after treatment with angiotensin converting enzyme-inhibitors (ACE-I)^{105, 106} and beta-blockers¹⁰⁷ in hypertensive patients. Statin therapy also significantly improved CFR in cardiovascular risk patients with hypertension and average levels of serum cholesterol. The change in CFR correlated with a change in LDL¹⁰⁸. Improvement of CFR has also been demonstrated on patients with multivessel disease treated with statins. In this study increased CFR was delayed compared to the lipid lowering effect of fluvastatin which may be suggestive of a slow recovery of the vaso-dilatory response¹⁰⁹. Further, the improvement of CFR was independent of severity of stenosis and not related to the amount of lipid lowering effect. Treatments in diabetes aim at preventing the metabolic effects of high glucose levels as well as microvascular and macrovascular damage and associated complications. Intense anti-hyperglycemic treatment has been demonstrated to significantly improve CFR in diabetic patients with poor glycemic control¹¹⁰. Physical activity and weight loss are also associated with improvement of CFR¹¹¹⁻¹¹³.

Thrombus aspiration

The goal of reperfusion therapy in STEMI is to limit infarct size by improving blood and oxygen supply to the ischemic myocardium. However, despite optimal restoration of epicardial coronary blood flow with PCI, myocardial reperfusion is still impaired in a substantial number of patients with STEMI^{114, 115}. One possible mechanism is distal micro embolization causing microvascular obstruction¹¹⁶ which is associated with impaired prognosis^{64, 117}. An appealing approach to reduce thrombus burden and embolization to the microvasculature has been mechanical aspiration of thrombus before stenting in STEMI-PCI (Fig.7).

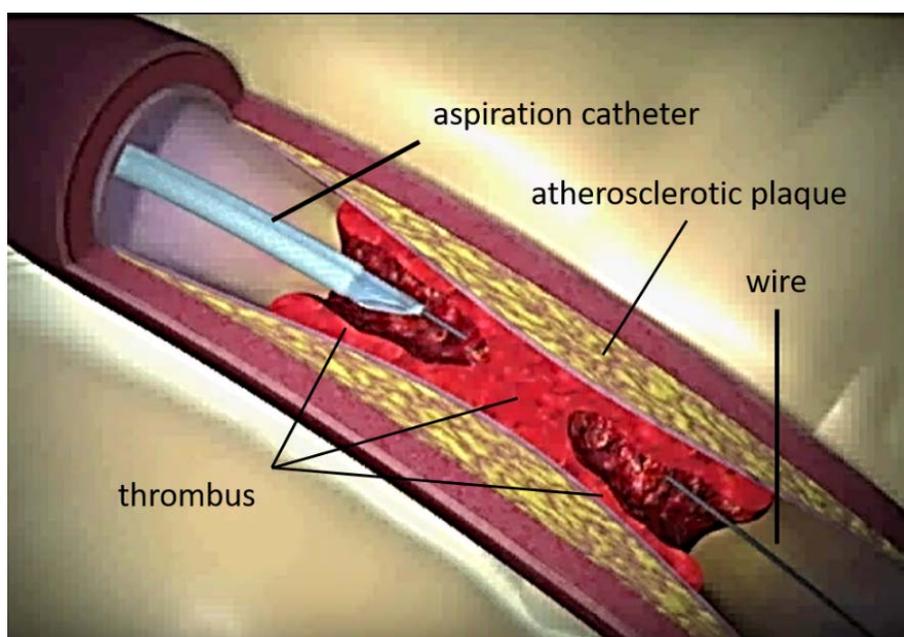


Figure 7. Schematic illustration of manual thrombus aspiration from coronary artery.

Some early, smaller studies demonstrated improved microvascular function, reduced infarct size and increased survival in STEMI-patients treated with thrombectomy before PCI¹¹⁸⁻¹²¹. Though, the beneficial effects of thrombus aspiration on short-term and long-term mortality could not be confirmed in neither of the two recent large randomized controlled trials, TASTE¹²² (Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia) and TOTAL¹²³ (The Trial of Routine Aspiration Thrombectomy With PCI

Versus PCI Alone in Patients With STEMI). The TASTE study demonstrated a trend for reduced risk of stent thrombosis and reinfarction while the TOTAL study found an association between thrombus aspiration and an increased risk of stroke. Still, routine thrombus-aspiration in STEMI-patients is frequently used.

Pretreatment in STEMI

Antiplatelet therapy is a corner-stone in standard-care of ischemic heart disease. Dual antiplatelet inhibition with aspirin and a P2Y₁₂ inhibitor is beneficial in reducing ischemic events even though this may be at the expenses of increased bleeding risk^{39, 41, 42}. However, the timing of administration of P2Y₁₂ inhibitor along with aspirin, remains uncertain. In patients with ACS platelets are more activated and hyper-reactive than in patients with stable coronary artery disease¹²⁴. Particularly in STEMI a prolonged onset of action of P2Y₁₂ inhibitors has been demonstrated compared to onset-times in patents with stable CAD^{125, 126}. In the light of these background facts, a strategy in which P2Y₁₂ inhibitors are administered early to patients with ongoing MI is both logical and attractive. For many years, the European and American guidelines (ESC/ACC/AHA) have recommended pre-hospital administration of P2Y₁₂ inhibitors to patients with ST-elevation myocardial infarction (STEMI) and this strategy has become a common practice^{127, 128}. However, the available evidence regarding the safety and efficacy of this strategy in the setting of primary PCI is conflicting¹²⁸. Most data favoring prehospital administration of P2Y₁₂ inhibitors do not directly address STEMI-population undergoing primary PCI^{40, 129, 130}. Two smaller randomized trials of pretreatment in primary PCI had surrogate endpoints and were negative^{131, 132}. There is only one large randomized trial in patients with STEMI that directly addressed the hypothesis whether prehospital administration of P2Y₁₂ inhibitors results in improved outcomes. In this trial the co-primary endpoints were the proportion of patients without a 70% or greater resolution of ST-segment elevation before percutaneous coronary intervention (PCI) and the proportion of patients without Thrombolysis in Myocardial Infarction flow grade 3 (TIMI-3) in the infarct-related artery at initial angiography. Rates of major adverse cardiovascular events and stent thrombosis at 30 days were secondary endpoints. Beneficial effects could not be demonstrated neither on reperfusion indices nor on clinical outcomes¹³³. So far, no trials investigating

the timing of P2Y₁₂ inhibitors have had enough statistical power to evaluate mortality and complications relevant in the clinical setting.

Statistical considerations in observational studies

Properly planned and executed randomized controlled trials (RCT) are considered the gold standard of evidence based medicine. Random assignment to different treatment (i.e. exposure) makes the groups as equal as possible with respect to all patient characteristics that may have an impact on the outcomes of interest. Accordingly, the act of randomization minimizes the confounding in risk estimation (i.e. causation) by means of statistical models. However, since patients included in RCTs may be—and often are—selected due to specific inclusion and exclusion criteria, many RCTs have limited external validity. That is, patients included in RCTs are usually younger with less comorbidity and at lower risk of mortality (or other adverse events of clinical importance). The study results from such a population cannot be directly extrapolated to the patients that were excluded from the RCTs. Still, the excluded population from RCTs represent a substantial portion of patients who we meet in everyday clinical practice¹³⁴. Within the realm of evidence-based medicine, high-quality observational studies based on large-scale registries and on accurate statistical methodology are valuable complements to RCTs. Such studies may provide important and valuable evidence for the external validity of RCTs that were previously conducted. While large-scale observational studies are an important tool in epidemiology and evidence-based medicine, they have their own limitations. As patients are not randomized to a specific exposure (or treatment) there will often be differences in patient's characteristics between the groups of interests, which makes causation between exposure and outcome harder to prove because of many possible sources of bias many of which have been studied in detail^{135, 136}.

Missing data

Missing data are common in observational studies and may lead to reduced representativeness of the sample. As a consequence, inferences about the population may be biased. It is important to understand the reasons for missing to correctly deal with the remaining data. Values missing completely at random

are not likely to affect the representativeness of the population. But if values are missing systematically, analysis is likely to be biased. There are three main mechanisms of missing. “Missing completely at random” means that missing values occur entirely at random and are independent of observable as well as unobservable variables and analysis of remaining data will be unbiased. However, data are rarely “missing completely at random”. Data are categorized as “missing at random” if there are systematic differences between missing and the observed variables, as long as these differences can be entirely explained by other observed variables. But, if the probability of missing is dependent on the missing value, even after controlling for other variables, then data is “missing not at random”. When data are “missing not at random”, valid inferences require explicit assumptions about the mechanisms that led to the missing data. On the other hand, when missing data can be assumed to be “missing at random” or “missing completely at random”, then standard implementations of multiple imputation methodology can be used and missing data can be handled in a way that is unbiased and statistically valid. Methods dealing with “missing at random”-data are divided into three main classes; likelihood-based approaches, weighted estimation and multiple imputation. The most frequently used method is multiple imputation due to its flexibility especially in the case of multiple missing values¹³⁷⁻¹³⁹. General recommendations on how to report and handle missing data in observational studies are described in the document of “Strengthening the Reporting of Observational Studies in Epidemiology” (STROBE)¹⁴⁰.

Propensity score

In observational studies, factors such as patient characteristics and treatment preferences of the hospital or the individual physician, often influence treatment selection resulting in systematic differences in baseline characteristics between treated and untreated subjects. This has to be accounted for when the effect of treatment on outcomes is estimated. Historically regression models have been used for adjustment of differences in measured baseline characteristics between treatment groups. Another way to handle this is to use methods based on propensity score to reduce or eliminate the effect of confounding. The propensity score was defined by Rosenbaum and Rubin in 1983, as the probability of treatment assignment conditional on observed baseline characteristics¹⁴¹. The propensity score is a balancing score since the

distribution of baseline characteristics will be similar between treated and untreated subjects, conditional on the propensity score. In RCTs, where treatment allocation is random, the propensity score is known and will be 0,50 for each patient, i.e. each patient has 50 percent chance of receiving either treatment, independent of baseline characteristics. In observational studies the propensity score is unknown but can be estimated, usually with logistic regression adjusted for those covariates having most influence on which treatment the patient will be assigned to. Thus, in a set of subjects with the same propensity score, the distribution of observed baseline covariates will be the same in the treated and in the untreated group. There are four different propensity score methods; matching, stratification, weighting and covariate adjustment using the propensity score. Propensity score matching is formation of matched sets of treated and untreated subjects with similar propensity score values. Matching requires a large sample with a large overlap of propensity scores because those observations that cannot be matched will be excluded from the analysis. Stratification is achieved by stratifying subjects into equal-size groups based on their estimated propensity score and then entering the strata as covariates in the equation. In covariate adjustment the estimated propensity score is entered in a regression model as a continuous variable. Weighting is a method where weights, based on the estimated propensity score, are used to make a synthetic sample where the distribution of baseline characteristics is independent of treatment assignment. An important part of any propensity score analysis is balance diagnostics, i.e. examining whether the propensity score model has been adequately specified¹⁴².

Multilevel models

In many observational studies based on data from health care registries, the observations are organized in hierarchical manner. This means that the structure of the data in registries often is such that it contains two or more levels of data (i.e. clusters). In health care registries, patients (first level) may be clustered within the hospital (second level) and hospitals may be clustered within specific regions or different countries (third level). Sometimes the primary unit of observation may be other than the individual patient, for example, implanted coronary stents (first level) may be clustered within patients (second level) while patients may be clustered within hospitals (third level). As a consequence, observations on patients (or other clustered primary

observation unit) will not be independent of each other. Most of the models used for statistical inference assumes independency of individual observations from each other. Patients treated at one hospital are likely to be treated more similar compared to patients at another hospital. This non-independent nature of clustered observations may be appropriately addressed by means of multilevel modelling. Failure to apply proper statistical modelling for clustered data may result in substantially biased risk estimates and both type-1 and type-2 error. In ordinary regression models, data are fitted to the most suitable parameters, i.e. intercept and slope, and the estimated value will be a straight line with a certain intercept and a certain slope. In multilevel models each level will have its own regression line and depending on the nature of data parameters can be fixed, i.e. constant over all groups, or random, i.e. values will be different for each group. Thus, multilevel models can have random intercepts and fixed slopes or vice versa, or both random intercepts as well as random slopes.

Instrumental variable analysis

Confounding in observational studies can be partly handled with multivariate adjusted regression analysis and propensity score methods, as long as confounders are known and measured. Still, the problem with unmeasured and/or unknown confounders causing potential endogeneity remains and makes it difficult to prove causation when estimating the effect of a treatment¹⁴³. Variables are endogenous when they are correlated with other variables and the error term in the regression model. Endogenous correlation may occur when changes in the outcome changes the value of one or more of the covariates (“reverse” causation), when there are variables that affect both the outcome and the independent variables or when there are measurement errors. Instrumental variable procedures can be used to reduce bias in the case of endogenous correlation^{144, 145}. To use instrumental variable analysis, one must identify a naturally varying phenomenon in the observed data, which like the act of randomization in an RCT, predicts the treatment that will be assigned to the individual patient. To become a valid instrument, a variable has to fulfill some necessary criteria. First, it has to be strongly associated with the received treatment. Second, it must not be associated neither directly nor indirectly with the outcome, except through the effect of the treatment itself. The variable with these statistical qualities is called instrumental variable, or instrument.

Commonly, geographic location is used as an instrument e.g. health care regions or different countries. The estimate of an instrumental variable analysis is predominantly determined by subgroups, whose treatment status depends on which category of the instrument the patient belongs to, e.g. patients with the same characteristics receiving different treatments in different geographical regions. The most commonly used technique for instrumental variable analysis is the 2-stage least squares method (2SLS). In the setting of dichotomous exposures and outcomes, 2SLS produces a risk difference estimate¹⁴⁶. This procedure works by sequential application of 2 ordinary least squares regressions in which predicted values of treatment from the first stage are entered into the second stage as a replacement for actual treatment. By replacing the confounded treatment variable with an unconfounded prediction of treatment, the bias due to unmeasured confounding can be avoided.

The SCAAR/SWEDEHEART registries

The Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART) was established in December 2009 after merging of four existing national registries namely the registry of acute cardiac care (RIKS-HIA), the Swedish coronary angiography and angioplasty registry (SCAAR), the Swedish heart surgery registry and the national registry of secondary prevention (SEPHIA). RIKS-HIA was established as a National quality registry in 1995. SCAAR was established in 1999 after a merge of the Swedish Coronary Angiography registry (Acta Coronaria) and the Swedish Coronary Angioplasty registry (SCAP) both initiated in the early 1990s by hospitals which at that time performed coronary angiographies and PCIs. The Swedish heart surgery registry was formed in 1992 and SEPHIA was added to RIKS-HIA in 2005 to register effects of secondary prevention efforts in patients with acute myocardial infarction¹⁴⁷. The Registry for Percutaneous Valve Interventions was incorporated in SWEDEHEART in 2010. SWEDEHEART is a national registry including all patients admitted to hospital due to suspected ACS and patients undergoing coronary or valvular interventions.

Approximately 80,000 cases are enrolled each year: 30,000 with ACS, 40,000 undergoing coronary angiography or angioplasty, 7,000 undergoing heart surgery, and 6,000 who are followed for 12–14 months for secondary

prevention after ACS. The registry is web-based and all data are registered on-line directly by the caregiver and transferred in an encrypted format to a central server. The platform is in direct contact with the Swedish National Population Registry for immediate access to personal data and deaths. The technology is developed and administered by Uppsala Clinical Research Center (UCR). SWEDEHEART, including SCAAR, is independent of commercial funding and is sponsored by Swedish health authorities only.

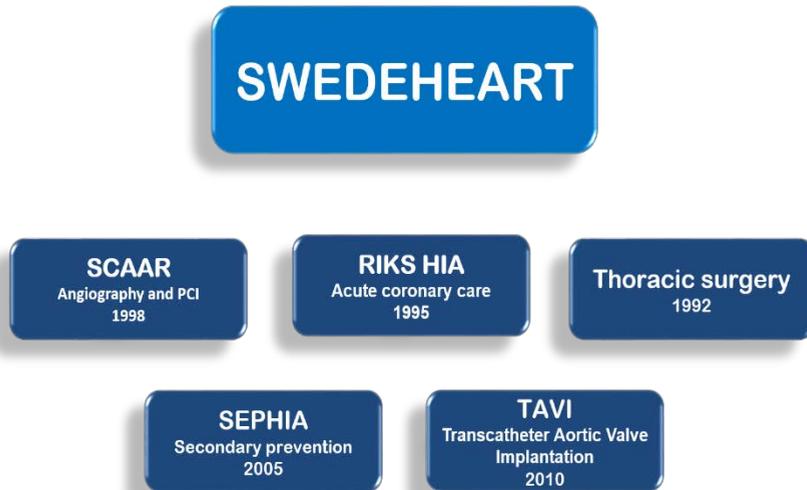


Figure 8 Schematic illustration of organization of the SWEDEHEART registry (The Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies).

For patients admitted to hospital due to symptoms suggestive of ACS, 106 variables are collected prospectively including patient demographics, logistics at admission, risk factors, past medical history, medical treatment prior to admission, electrocardiographic changes, biochemical markers, other clinical features and investigations, medical treatment in hospital, interventions, hospital outcome, discharge diagnoses and discharge-medications.

In SCAAR each angiography procedure is described with ~50 variables and each PCI procedure with ~200 variables including detailed descriptions of angiographic findings, procedures, type of stenosis, type of stent, antithrombotic treatment, and complications. The system presents detailed information about every previously implanted stent anywhere in the country and a question about existence of any form of restenosis or stent thrombosis is

mandatory. The information about clinical characteristics and procedural details is entered into the registry immediately after the procedure by the PCI physician. Data on in-hospital complications, e.g. bleeding and neurological complications, is entered into the registry at discharge, according to local routines at each hospital.

Swedish personal identification numbers together with name, address and hospital are included in the registry. The SWEDEHEART database is merged with the National Cause of Death Register, which includes information about the vital status of all Swedish citizens, and the National Patient Registry, which includes diagnoses at discharge for all hospital stays in Sweden. The merging of the registries is approved by the National Board of Health and Welfare, the Swedish Data Inspection Board, and the ethical committee at Uppsala University. All patients are informed about their participation and follow-up in the registry and have the right to decline inclusion. The registry captures 100% of the patients undergoing angiography, angioplasty or heart surgery and approximately 90% of patients admitted to hospital due to symptoms suggestive of ACS.

AIMS

The specific aims of this thesis were as follows;

I: To investigate if TDE-CFR can predict significant epicardial coronary artery stenosis in patients with symptoms of angina.

II: To investigate the impact of thrombus aspiration on mortality, stent thrombosis and stroke or neurologic complications in patients with STEMI undergoing PCI.

III: To investigate the impact of pre-hospital administration of P2Y₁₂ antagonists on mortality, infarct-related artery occlusion (IRA), stent thrombosis, cardiogenic shock, bleeding, and neurological complications in patients with STEMI undergoing PCI.

IV: To investigate the prevalence of reduced TDE-CFR and to find independent predictors of reduced TDE-CFR in cardiovascular high-risk patients with prior myocardial infarction.

PATIENTS AND METHODS

Data from the prospective SCAAR (Swedish Coronary Angiography and Angioplasty Registry) database were used in all four papers. In paper I and IV non-invasive evaluation of coronary flow reserve (CFR) was performed with transthoracic Doppler echocardiography (TDE-CFR). In paper I we measured CFR in all three coronary arteries, i.e. left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA) while in paper IV CFR-measurements were restricted to LAD only. In short, mean diastolic flow velocity at baseline and during peak hyperemia, induced by intravenous adenosine 140 μ gram/kg/min, was measured by manual tracing of the diastolic Doppler flow signals. CFR was calculated as the ratio between the hyperemic and baseline flow velocity values.

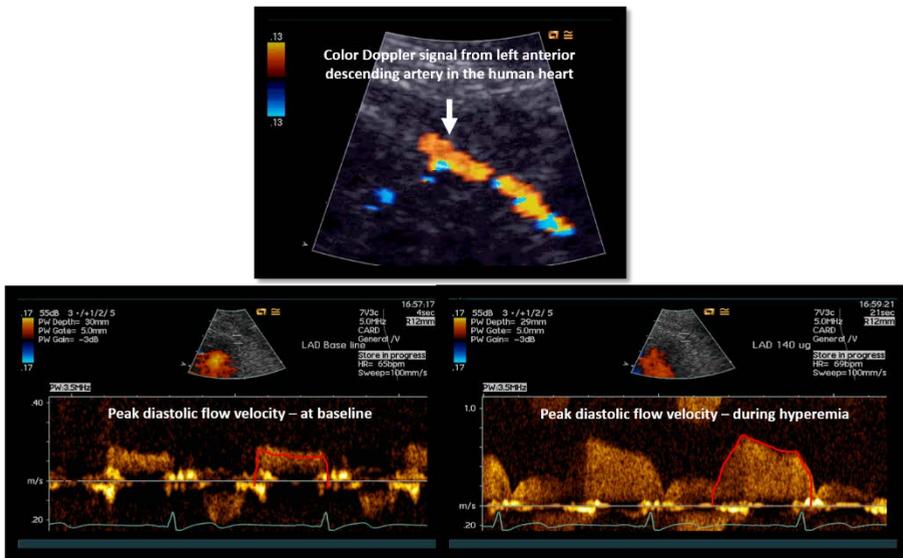


Figure 9. Coronary flow velocity reserve measured with transthoracic Doppler echocardiography. Calculated as mean diastolic flow velocity at hyperemia divided by mean diastolic velocity at rest

Paper I

The study population consisted of patients referred to Sahlgrenska University Hospital in Gothenburg for evaluation with single photon emission tomography (SPECT) due to symptoms of chest pain. Coronary flow velocity reserve, defined as coronary flow velocity at hyperemia induced by intravenous adenosine, divided by coronary flow velocity at rest, was measured in the left anterior descending artery (LAD), circumflex artery (CX) and in the right circumflex artery (RCX) with transthoracic Doppler echocardiography. Coronary angiography was performed on clinical grounds within 180 days before or after measurements of CFR. Data on patient characteristics and angiographic findings were collected from SCAAR, patient charts and from individual angiograms. Significant CAD was defined as a lumen obstruction of >50%.

To examine if CFR can predict the prevalence of significant coronary artery stenosis on coronary angiogram, we used propensity score adjusted multivariable logistic regression for the primary analysis. Missing data were imputed using the multiple imputation chain-equation method with 20 data sets. Several secondary analyses were used to adjust for differences in patient characteristics and for sensitivity analysis.

Paper II

Patients undergoing PCI due to STEMI and registered in SCAAR during the period January 2005 to September 2014, were included. Data from SCAAR were merged with the National Patient Registry. Patients were divided into two groups; patients undergoing primary PCI plus thrombus aspiration and patients undergoing primary PCI alone.

The primary endpoint was mortality at 30 days. The secondary endpoints were mortality at one year, stent thrombosis at 30 days and at one year, and reported in-hospital neurological complication. Stent thrombosis was defined as an acute stent occlusion verified by coronary angiography. Neurological complication was defined as a new neurological deficit during PCI or during in-hospital stay after primary PCI.

Missing data were imputed using the multiple imputation chain-equation method with 20 data sets. Instrumental variable 2SLS regression with 6 administrative health care regions in Sweden based on geographic location as

the treatment-preference instrument was used in our primary model. For sensitivity analysis a treatment-preference instrument was created by dividing hospitals into quintiles based on total number of procedures with thrombus aspiration. Our secondary models were based on unadjusted and propensity score-adjusted multilevel logistic regression. Administrative healthcare regions and individual hospitals were entered into the regression model as random-effects variables since SCAAR is a hierarchical database with clustering of patients within hospitals and regions.

Paper III

Consecutive STEMI-patients who underwent primary PCI (PPCI) in Sweden between January 1th 2005 and November 1th 2016 were included in the study. Patients were stratified by whether or not they were pretreated with P2Y₁₂ antagonists. Demographic and procedural data were based on SCAAR-data and diagnosis according to International Classification of Diseases codes. Vital status and date of death were obtained from the Swedish National Population Registry. SCAAR was merged with the Swedish National Population Registry by the Epidemiologic Center of the Swedish National Board of Health and Welfare according to Swedish personal identification numbers.

The primary endpoint was all-cause death within 30 days. Secondary endpoints were IRA (infarct-related artery) occlusion, stent thrombosis at 30 days, in-hospital bleeding, neurological complications and cardiogenic shock during the index hospitalization.

Missing data were imputed with the multiple imputation chain-equation method with 5 data sets. Associations between P2Y₁₂ pretreatment and the risk of adverse outcomes were investigated with propensity-scores adjusted mixed-effects logistic regression which takes into account clustering of patients within hospitals.

Paper IV

Patients with previous type-1 myocardial infarction (MI) with at least one additional cardiovascular risk factor; age ≥ 65 years, angiographic evidence of multi-vessel coronary artery disease, diabetes mellitus, hypertension, two or more spontaneous MIs, chronic, non-end-stage renal dysfunction, incomplete revascularization, were included. SCAAR was used to screen patients.

Demographic data were collected from SCAAR and from personal visit at the time of CFR-evaluation. CFR in LAD was evaluated non-invasively with transthoracic Doppler echocardiography. Multiple linear regression was used to assess predictors of CFR with the following covariates entered into the model; age, gender, BMI, smoking history, hypertension, diabetes mellitus, hyperlipidemia, renal dysfunction, chronic obstructive pulmonary disease (COPD), peripheral artery disease(PAD), previous stroke, months after MI, additional MI, multi-vessel disease, incomplete revascularization, previous PCI, previous CABG, angina pectoris and NYHA-class.

RESULTS

Paper I

152 patients were included in the study. 38.8% had not significant obstructive CAD on coronary angiogram, 32.9% had single-vessel disease and 28.3% had multi-vessel disease.

In the primary model, with propensity score adjusted multivariable logistic regression, we found that CFR in LAD (adjusted OR 0.61; 95% CI 0.40-0.92, $p=0.018$), LCX (OR 0.40; 95% CI 0.23-0.71, $p=0.002$) and RCA (OR 0.38; 95% CI 0.21-0.72) were independent predictors of significant coronary artery disease. These findings consisted in the secondary models with logistic regression with complete-case analysis as well as after imputation of missing data. Sensitivity analysis based on logistic regression with imputation of missing data on patients who had CFR-measurements performed prior to coronary angiogram ($N=90$), and on patients without previous MI, were also congruent with the results from the primary model. CFR measured in any of the three coronary arteries predicted significant epicardial stenosis independently of the location of the stenosis. There was also a significant correlation between CFR-values from the three different coronary arteries that was independent of stenosis location. In a test for trend CFR was found to decrease significantly with increasing severity of CAD. Definition of severity of CAD was number of arteries with significant stenosis.

Paper II

42 829 consecutive STEMI-patients were included in the study. 29% of these were female. 10660 patients (26% female) were treated with thrombus aspiration. The frequency of thrombus aspiration in the six healthcare regions varied from 18% to 35%. During the study period there was a significant decrease in age- and sex-adjusted 30-days mortality as well as in 1-year mortality with no difference between men and women. Mortality at 30 days was 6.1% in the thrombus aspiration group and 6.3% in the PCI-alone group and at 1 year 9.2% and 9.8% respectively.

In our primary analysis, with 2SLS regression with administrative health care region as treatment preference variable, there was no difference between groups neither in 30-day mortality nor in 1-year mortality. The incidence of stent thrombosis at 30-days in all patients was 0.6%, in the thrombus aspiration group 0.47% and in the PCI-alone group 0,67%. In the primary analysis a significant difference both at 30 days and at 1 year was found but in the landmark analysis after 30 days there was no difference between groups. In hospital-stroke or neurological complications did not differ between groups.

Paper III

44804 consecutive STEMI-patients were included after exclusion of patients who did not receive acetylsalicylic acid before PCI, patients treated with thrombolysis and patients without Swedish identification numbers. 37840 (84.5%) were pretreated with clopidogrel (N=21642, 57.2%), ticagrelor (N=14008, 35.3%) or prasugrel (N=2190, 5.8%). 30387 (67,8%) had an IRA-occlusion. Patients were reported from 30 different hospitals. The two groups, pretreated versus not pretreated, were well balanced after adjustment with propensity score.

At 30 days there was 2488 (5.6%) deaths and 267(0.6%) in stent-thrombosis. The primary analysis with propensity-scores adjusted mixed-effects logistic regression, showed no difference between groups in 30-days mortality (adjusted OR 1.07; 95% CI 0.94-1.22, p=0.313), reduced IRA occlusion (adjusted OR 1.01; 95% CI 0,95-1.08; P=0.635), in stent-thrombosis (adjusted OR 0.99; 95% CI 0.69-1.41, p=0.941), in-hospital bleeding (adjusted OR 1.04; 95% CI 0.89-1.23 p=0.604) or neurological complications (adjusted OR 0.66; 95% CI 0.38-1.30, p=0.129). IRA-occlusion was an independent predictor of 30-day mortality (adjusted OR 1.65; 95% CI 1.48-1.85)

Paper IV

Between July 12th 2013 and December 16th 2015 619 patients were included in the study. Median age was 69.0 years (IQR 64.9-73.3) and 18.4 % were female. Almost one half of the study population (46.0%) had multi-vessel disease and approximately a quarter (23.7%) was incompletely revascularized. The majority of patients were free from symptoms of angina and in NYHA class I. The major part was also on optimal pharmacological standard treatment. The

success-rate for evaluation of CFR in LAD was 98.7% (611 patients). Mean CFR was 2.74 (± 0.79).

A large proportion of patients had CFR <2.5 and almost two thirds had CFR <3.0 . Independent predictors of CFR were age, dyslipidemia, smoking, hypertension, body mass index, incomplete revascularization and treatment with angiotensin receptor blockers in the multiple linear regression model.

DISCUSSION

The main findings of this thesis are as follows.

I. CFR measured with transthoracic Doppler echocardiography is an independent predictor of significant epicardial coronary artery disease.

II. CFR is impaired in a large proportion of patients with high-risk individuals with previous myocardial infarction despite the implementation of the contemporary standard secondary preventive measures.

III. The observational studies based on the SCAAR registry provide external validity for the results from recent randomized trials in which neither thrombus aspiration nor pre-treatment with P2Y₁₂-inhibitors was shown to improve prognosis in patients undergoing PCI due to STEMI.

Coronary flow reserve

In **paper I** we showed that CFR is an independent predictor of significant stenosis on coronary angiogram. Notably, CFR was not only predictive of significant stenosis in the measured artery. Instead, we found that CFR measured in any of the three coronary arteries predicted significant CAD independently of anatomical localization and number of diseased arteries. We also demonstrated a significant correlation between CFR measured in LAD, LCX and RCA respectively, and this was independent of location of lesions. This is well in line with the current view of coronary artery disease (CAD) as a systemic and progressive immuno-inflammatory disease affecting the overall coronary circulation, epicardial as well as microvascular parts. Endothelial dysfunction, and as a consequence microvascular dysfunction, is known to precede the development of atherosclerotic disease²² and the functionality of coronary circulation can be seen as a marker of the pro-atherosclerotic properties of the circulatory system. When CAD eventually manifests with a significant stenosis this is preceded by a disturbance in coronary microvascular function that is not only located at the site of the stenosis, it is a generalized condition engaging the entire coronary circulation. Impaired CFR is proven to be associated with greater plaque burden and a higher prevalence of vulnerable plaques as well as with higher levels of high-sensitivity C-reactive protein,

which indicates an ongoing pro-thrombotic and pro-inflammatory process¹⁴⁸. We also demonstrated that CFR decreased with increasing severity of coronary artery disease. CFR reflects the combined effects of coronary microvascular function, flow-limiting epicardial disease as well as rheological properties of blood and probably other unknown factors involved in regulation of blood flow, and can be used as an indicator of the severity of atherosclerotic disease. Indeed, an inverse dose-response relationship between CFR and prognosis has recently been proven by our group as well as by others^{67, 95}.

In **paper IV** we found impaired CFR in an unexpectedly large proportion of patients with a history of myocardial infarction and with at least one additional cardiovascular risk factor. This was despite the fact that the majority of patients were free from symptoms of angina, in NYHA-class I and on optimal secondary preventive therapy. The strongest independent predictor of CFR was incomplete revascularization. This confirms the findings in recent studies demonstrating the importance of complete revascularization⁵⁶. However, in our study 69% of patients with CFR<2.5 were completely revascularized at the time of the index procedure. In patients without obstructive epicardial disease decreased CFR is associated with increased risk of mortality reflecting the prognostic impact of microvascular dysfunction^{67, 94}. Successful treatment of conventional cardiovascular risk factors improves CFR but apparently this is not enough in a substantial number of high-risk individuals. CFR contains information additive to known cardiovascular risk factors and could be useful in identifying those at extra high-risk in order to individualize targeted treatment and reduce major adverse cardiovascular events.

The success rate in evaluating CFR with transthoracic Doppler technique was high in both paper I and in paper IV. In paper I CFR was measured in all three coronary arteries, e.g. left anterior descending artery, (LAD), left circumflex artery (LCX) and right coronary artery (RCA). CFR from a single artery was equally predictive as the average value from all three arteries. As a consequence, it is reasonable to restrict the examination to LAD since this is technically easier, takes less time and makes it more tolerable to the patient.

Thrombus aspiration in STEMI

In **paper II** we found that treatment with thrombus aspiration was not associated with decreased risk of mortality and in-hospital stroke or neurologic complications and that thrombus aspiration was associated with decreased risk of stent thrombosis. These results are in agreement with the results from two recent large randomized controlled trials, TASTE and TOTAL, and provides important evidence for the external validity of these 2 trials. Our data are derived from SCAAR and all consecutive STEMI patients in Sweden over a period of 9 years, representing unselected patients from everyday practice. Important criticism of both TASTE and TOTAL is the presence of selection bias resulting in limited external validity, a common problem with RCTs. We used treatment preference instrumental variable analysis with 2SLS regression. This method allows for adjustment of both measured and unmeasured confounders. Observational studies based on instrumental variable method mimic the act of randomization in RCT conditional on some central methodological assumptions (i.e. a valid instrument reflecting a naturally occurring randomization process). Stent thrombosis is a feared complication during and after PCI as it is associated with high mortality¹⁴⁹. In our study thrombus aspiration was associated with decreased risk of stent thrombosis at 30 days but not at 1 year. This finding is consistent with the results of TASTE regarding short- and long-term risk of stent thrombosis and supports the evidence for a protective role of thrombus aspiration against acute and subacute stent thrombosis. Similar to TASTE but in contrast to TOTAL, we did not show an increased risk of stroke or neurological complications associated with thrombus aspiration. This could be explained by the low number of events in TOTAL, limiting the statistical power.

Pretreatment in STEMI

At present time, pre-hospital administration of P2Y₁₂ inhibitors is a common practice despite the lack of definite evidence for its benefit. This is reflected in **paper III** where more than 80% of 44804 patients undergoing PCI for STEMI in Sweden between January 2005 and November 2016, received prehospital P2Y₁₂. The ATLANTIC trial is the only larger randomized trial on pre-treatment versus in-hospital treatment with P2Y₁₂ inhibitors in patients undergoing PCI due to STEMI. In ATLANTIC there was no difference

between groups in the composite primary endpoint of ST-segment elevation resolution >70% and TIMI flow III. Similarly, there was no difference in death, myocardial infarction, stroke, urgent revascularization, definite or probable stent thrombosis, patency of infarct related artery or the need for bailout with GP2b/3a receptor antagonists¹³³. Consistent with the findings in the smaller ATLANTIC, we could not demonstrate an association between prehospital P2Y₁₂ -administration and improved survival or higher likelihood of patent infarct related artery, neither a lower risk of stent thrombosis or stroke.

Our large observational study provides evidence for external validity of the smaller ATLANTIC trial. However, the debate about the reason for the lack of beneficial effect of pre-hospital P2Y₁₂ treatment continues. Some clinicians and scientists argue that the difference in time of platelet inhibition in the setting of relatively short reperfusion times in STEMI is simply not long enough to create meaningful differences in clinical outcome. Others argue that impaired gastrointestinal perfusion due to hemodynamic instability or nausea and vomiting, as well as administration of morphine, could cause delayed absorption of oral P2Y₁₂ antagonists. However, our findings are also consistent with the explanation that pretreatment with P2Y₁₂ antagonists in the presence of other prehospital pharmacological interventions (ASA and unfractionated heparin/low-molecular weight heparin) is simply futile. The possibility that pretreatment is futile is directly supported by the results from the ACCOAST trial. ACCOAST trial studied early vs late P2Y₁₂ receptor inhibition with prasugrel in patients with non-STEMI and showed no difference in major adverse cardiovascular events but an increased risk of major bleeding. In the prespecified substudy of ACCOAST trial, the authors evaluated the effect of administration of prasugrel before coronary angiography versus administration at the time for decision to perform PCI. Administration of prasugrel before angiography substantially decreased platelet reactivity at the time of angiography down to 15% of the platelet reactivity measured in patients who were not pretreated. Still, no reduction in the rate of major ischemic events up to 30 days in the pretreated group could be demonstrated. We argue that upstream treatment with P2Y₁₂ receptor antagonists in STEMI should be avoided due to the following reasons. First, no study has convincingly demonstrated superiority with upstream P2Y₁₂ antagonists whereas some studies have shown harm with increased risk of major bleeding. Second, excess early mortality was observed among patients who were treated with upstream ticagrelor in the ATLANTIC trial. Third, inadvertent treatment of

patients who fulfill the criteria for absolute contraindication (e.g. patients with aortic dissection/rupture, intra-abdominal bleeding, subarachnoid hemorrhage) for antiplatelet therapy may result in catastrophic consequences. Inadvertent treatment is particularly problematic in the context of everyday clinical practice as opposite to clinical trials in which indication for initiation of the treatment are usually more thoroughly scrutinized. We argue that in the absence of convincing evidence for a beneficial effect among patients with acute coronary syndromes, accidental upstream administration of a P2Y₁₂ antagonist to any patient with absolute contraindication is inexcusable.

CONCLUSIONS

Paper I:

CFR evaluated with transthoracic doppler echocardiography is predictive of significant coronary artery disease in any of the three coronary arteries.

Paper II:

There was no effect of thrombus aspiration on mortality in this population of 45515 STEMI-patients from the prospective SCAAR database. This study gives external validity to the previous large randomized trials; TOTAL and TASTE. We found that thrombus aspiration may reduce the risk of stent thrombosis but future studies should evaluate if this treatment is cost-effective for prevention of stent thrombosis in the absence of survival benefit.

Paper III:

Pretreatment with P2Y₁₂-inhibitors in patients undergoing PCI due to STEMI is not associated with less mortality, patent IRA, reduced risk of stent-thrombosis, increased risk of in-hospital bleeding or on-hospital neurological complications.

Paper IV:

A majority of patients with previous MI and additional risk factors have decreased CFR despite adequate secondary preventive intervention. Incomplete revascularization is the strongest independent predictor of CFR. CFR is a valuable prognostic tool in stratification of high-risk populations with previous MI.

FUTURE PERSPECTIVES

In the clinical setting

CFR could be used for screening and risk stratifying of both asymptomatic high risk individuals as well as of those with symptoms suggestive of coronary artery disease. CFR could be valuable in individualizing therapies, both interventional and pharmacological, on top of established treatment of coronary artery disease.

CFR-measurements could be implemented in routine echocardiographic procedures, both in outdoor clinic and in the ward, since the procedure is easy to perform for clinicians familiar with echocardiography, and it is an easy accessible and patient friendly method associated with low costs.

In the acute setting interpretation of CFR can be hazardous as it is sensitive to variations in basal flow velocities but in the catheter laboratory the examination can be complemented by assessment of FFR and IMR which are independent of hemodynamic parameters such as blood pressure, heart rate and basal flow.

In general science

CFR could be used as a valid surrogate end-point in interventional studies evaluating the effects of pharmacological and other interventions on cardiovascular morbidity and mortality.

High-quality national registries are valuable tools in research and with adequate statistical modelling they serve as an important complement to randomized trials in providing external validity. Recently, the new concept of registry-based randomized controlled trials (RRCT) has evolved where patients are assigned to a treatment through randomization in a clinical registry. This kind of registry-based trials renders large cohorts in a short time. Data on both included subjects as well as those not included will be complete and costs are low.

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