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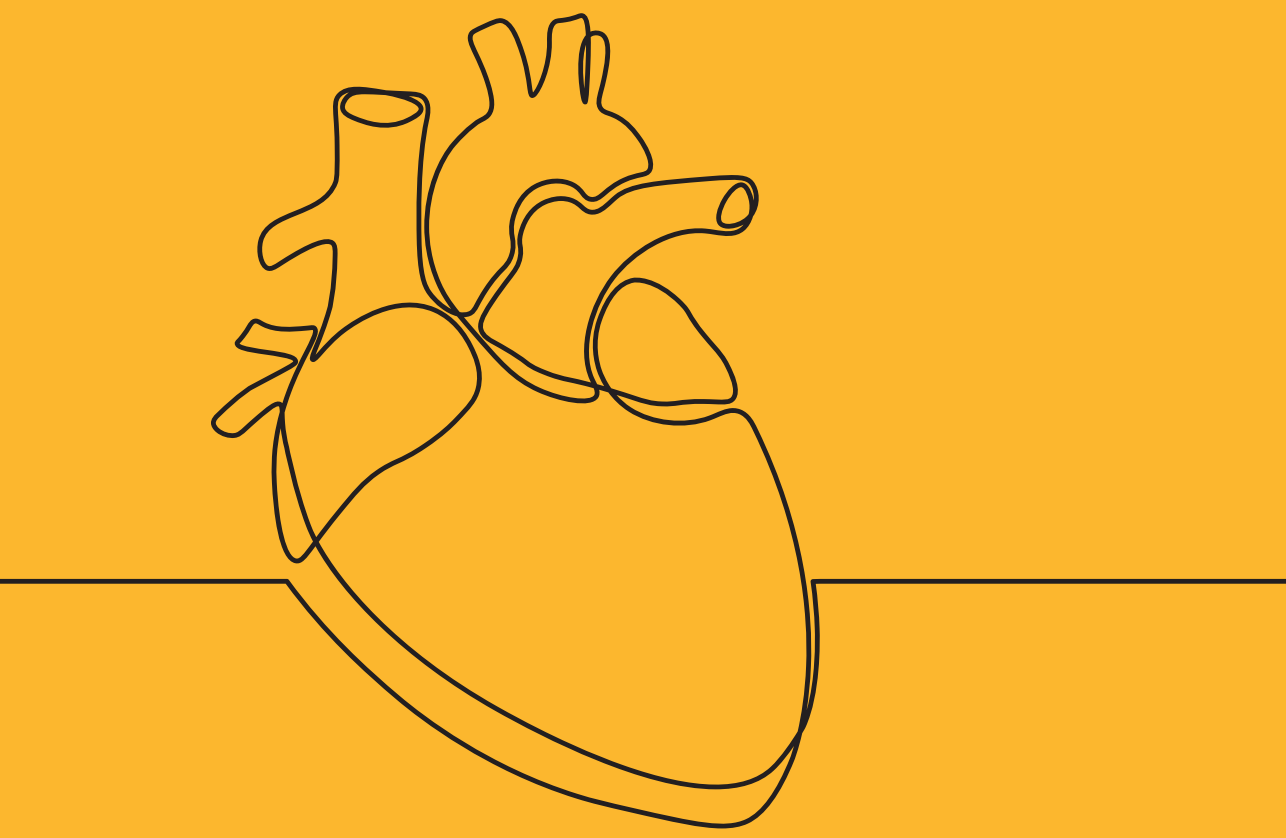

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Colophon
The studies described in this thesis were conducted at the Department of Cardiology of the Leiden University Medical Center, Leiden, The Netherlands

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“Waarheen je ook gaat, ga met je hele hart.”

Confucius, Chinees filosoof

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Chapter 1.

General introduction and thesis outline

General introduction and thesis outline

Ischemic heart disease contributes, with almost 10 million deaths worldwide, for a substantial part to the total deaths in CVD.(1) In Europe, approximately 1.8 million died due to ischemic heart disease, which accounts for 20% of all deaths.(2) Ischemic heart disease is often caused by an acute coronary syndrome (ACS), which is the clinical manifestation of coronary artery disease. ACS can roughly be grouped in non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI). A STEMI is caused by an occlusion of a coronary artery, which occurs when the blood flow is abruptly obstructed by a plaque rupture, erosion or fissuring which results in an obstructing thrombus.(3) This is clinically expressed by ischemic symptoms, and with new ST-segment elevation in 2 contiguous leads and often reciprocal ST-segment depression on the electrocardiogram (ECG). Confirmation of myocardial ischemic injury with elevated cardiac biomarkers is required for the diagnosis STEMI whereas in NSTEMI patients the symptoms of ischaemia is often represented with new or presumed new significant ST-T wave changes or left bundle branch block on 12-lead ECG.(4)

Development of atherosclerosis

Coronary artery disease is caused by the underlying pathological process of atherosclerosis illustrated in figure 1. Atherosclerosis starts early in life and often progresses silently for many years. Hypercholesterolemia is considered one of the main triggers of atherosclerosis. The increase is plasma cholesterol levels results in changes of the endothelial permeability that allow the migration of mainly low-density-lipoprotein-cholesterol (LDL-c) particles into the arterial wall.(3) This triggers an inflammatory reaction. Monocytes adhere to the endothelial cell and migrate in the sub endothelial space.(3) Here, monocytes require macrophage characteristics and incorporate oxidized LDL-c resulting in the formation of plaque-forming foam cells. Fatty streaks appear due to the local accumulation of serum lipoproteins in the vessel wall. Fatty streaks are the precursors of more advanced lesions characterized by the accumulation of lipid-rich necrotic debris and smooth muscle cells (SMCs). Such 'fibrous lesions' typically have a 'fibrous cap'.(5) Vulnerable plaques are characterized by the formation of a necrotic core and by thinning of the fibrous cap. This may result in a rupture of the cap exposing prothrombotic components to platelets and procoagulation factors leading to thrombus formation and clinical events such as N(STEMI).(6) The majority of coronary thrombi are caused by plaque rupture (55-65%), followed by erosions (30-35%), and last frequent from calcified nodules (2-7%)(7)

Treatment

At the beginning of the 20th century, a myocardial infarction (MI) was regarded as a rare condition not compatible with survival. In the first half of the 20th century, early mortality rates were high, around 30%, and for patients surviving the

acute phase, 'treatment' of a MI consisted of 4 weeks of bedrest and pain relief. However, by elucidating the natural history of the MI, physicians set the stage for huge advances that followed. These advances can be summarized into four categories; birth of the Coronary Care Unit (CCU), developments in pre-hospital care, myocardial reperfusion techniques and adjunctive pharmacotherapy.

The CCU is defined as probably the single most important advancement in the treatment of MI. The concept of the CCU was proposed in 1961 by Desmond Julian in *The Lancet*(8) and based on 4 principles. 1; the appreciation of the importance of arrhythmias as the principal cause of early death in MI, 2; the ability to monitor the ECG continuously, 3; the development of closed-chest cardiac resuscitation and 4; the delegation of the treatment of life threatening arrhythmias, to trained nurses in the absence of physicians.(9) Due to the developments of the CCU, early mortality was cut in half to approximately 15%.

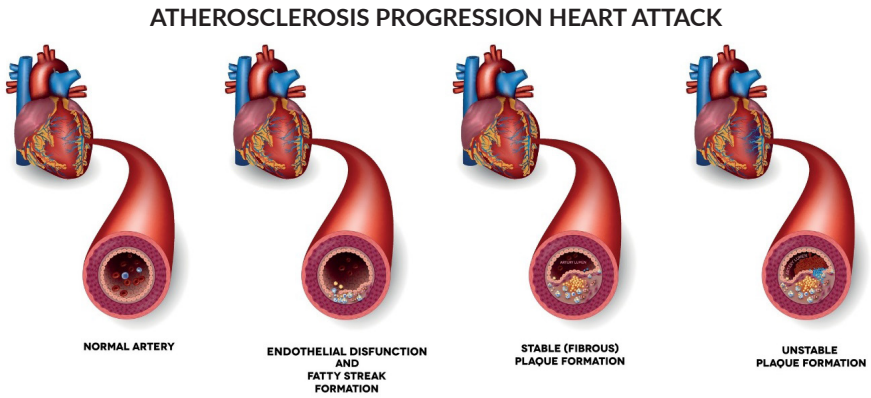

Figure 1. Cross-sectional view of atherosclerosis progression. The left figure shows a normal artery. Due to several stimuli, such as hypertension, smoking and hypercholesterolemia the endothelium begins to dysfunction, which results in an inflammation reaction with the formation of plaque-forming foam cells. Fatty streaks appear due to the local accumulation of serum lipoproteins in the vessel wall. As the atherosclerotic lesions progresses a fibrous cap with a necrotic core develops. When this fibrous cap thinness the plaque becomes unstable and is prone to rupture resulting in an acute occlusion of the artery due to thrombus formation.

Subsequently, it was early recognized that advances in prehospital care were necessary. Most deaths in patients with MI occur before the patient reached the hospital due to life threatening arrhythmias. In 1967, Pantridge designed the concept of a mobile CCU. These ambulances are equipped with ECG monitors, drugs and equipment for defibrillations and resuscitation(10) and have saved many lives. Furthermore, outcomes of patients suffering from MI are depending on the time taken to deliver definitive treatment. Evidence has shown that the

extent of myocardial salvage is greatest if patients are reperfused in the first 3 hours from the onset of symptoms.(11) The biggest delays are seen in the prehospital setting due to patient delay or system delay. Patient delay is the time from the onset of symptoms to contact with the emergency services. System delay is the time between contact with the emergency services to diagnosis and reperfusion treatment.

The use of dilating catheters for the treatment of atherosclerotic vascular disease was first described in 1964 by Charles Dotter and Melvin Judkins.(12) Building on their work, Andreas Gruentzig performed the first successful percutaneous transluminal coronary angioplasty (PTCA) in 1977.(13) However, a common complication of the PTCA was (late) restenosis which occurred in up to 30% of the treated coronary arteries.(14) It was theorized that devices could be placed inside the arteries as scaffolds to keep them open after a successful balloon angioplasty.(13) The first intracoronary stents were successfully deployed in coronary arteries in 1986.(15) Nowadays, primary percutaneous coronary intervention (pPCI) is the golden standard in STEMI patients.

Adjunctive pharmacotherapy after a MI consists of 5 medical therapies, defined as the 'golden five': Aspirin, a P_2Y_{12} inhibitor (e.g. prasugrel or ticagrelor or clopidogrel), ACE-inhibitor, betablocker and a statin. Platelet inhibition remains the core pharmacotherapy component in STEMI patients undergoing pPCI. Aspirin inhibits platelet dependent cyclooxygenase 1 (COX-1) enzyme and consequently preventing synthesis of thromboxane A_2 (TXA_2) which is a powerful promotor of platelet aggregation.(16) Major studies have indicated a central role for aspirin in patients with STEMI.(17-19) Clopidogrel, prasugrel and ticagrelor are P_2Y_{12} inhibitors. P_2Y_{12} receptor plays a key role in the platelet activation process. Adenosine diphosphate (ADP) interacts with the platelet P_2Y_{12} receptor stimulating activation of the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor. In turn, activation of the GPIIb/IIIa results in enhanced platelet degranulation and thromboxane production, driving platelet aggregation.(20) Several trials showed a significant reduction of death using clopidogrel, prasugrel or ticagrelor in ACS patients undergoing PCI.(21-23)

Pump failure secondary to extensive myocardial damages emerged as the principal cause of death in patients surviving the life threatening arrhythmias. Preservation of the left ventricular function is a major predictive factor of prognosis. ACE inhibitors, blocks angiotensin converting enzyme that converts angiotensin I to angiotensin II. Decreased production of angiotensin II enhances natriuresis, lowers blood pressure, and prevents remodelling of smooth muscle and cardiac myocytes. Lowered arterial and venous pressure reduces preload and afterload. Treatment with ACE inhibitors is recommended in patients with systolic LV dysfunction or heart failure, hypertension, or diabetes and should be considered in all STEMI patients.(24, 25)

Beta blocker use decreases oxygen demand due to reduction in heart rate, blood pressure and contractility and can reduce remodelling and can improve left ventricular hemodynamic function.(26-28) The benefit of short-term treatment with oral beta blockers after STEMI is well established, although most of the data come from trials performed in the pre-reperfusion era.(29, 30)

3-hydroxy-3-methyl–glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (usually addressed as "statins") induce an increased expression of LDL receptors (LDL-R) on the surface of the hepatocytes, which determines an increase in the uptake of LDL-C from the blood and a decreased plasma concentration of LDL-C and other apoB-containing lipoproteins, including TG-rich particles.(31, 32) The benefits of statins in secondary prevention have been unequivocally demonstrated.(33) Independent of baseline LDL-c level and baseline cardiovascular risk, a meta-analysis of 26 statin CV outcome trials, showed a 22% risk reduction in CV events per 1mmol/L reduction in LDL-c.(33) Next to the lipid lowering function of statins, they are well-known for their pleiotropic lipid lowering independent effect. Statins positively interfere with critical components of the atherosclerotic process, and have beneficial anti-inflammatory effects on the vascular wall and improve endothelial function.(34, 35) An overview of described pleiotropic mechanisms is provided in figure 2.

The last three decades, mortality rates decreased significantly due to these very successful treatments.(29, 33, 36-40) Current 1-year mortality rates are approximately 10%.(41, 42) In the Netherlands mortality rates have declined progressively since the 70's, with approximately 20.000 deaths to under the 5000 in 2017 (Figure 3).

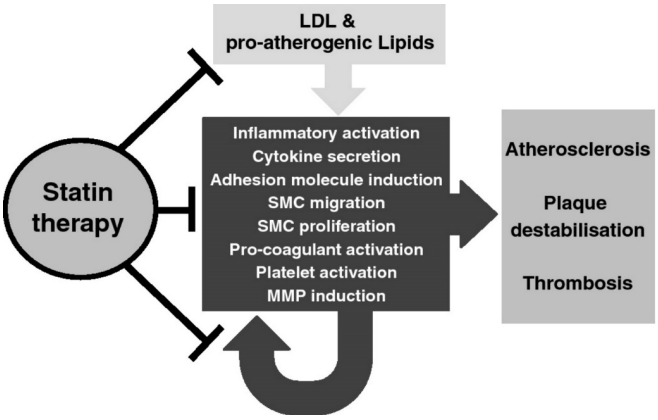

Figure 2. Pleiotropic effects of statins. The reduce hypercholesterolemia and directly attenuate the cellular precesses leading to atherosclerosis. The also break the positive feedback loop operative in the atherosclerotic process. SMC; smooth muscle cell, MMP; matrix metalloproteinase, LDL; low density lipoprotein. Source: *Babelova et al. Anti-atherosclerotic mechanisms of statin therapy*.(35)

Risk stratification and novel biomarkers

Although mortality rates have decreased significantly still a substantial amount of death occur with a wide variability between patients. Therefore it is essential to make an early assessment of short term risk. Readily available parameters in the acute phase before reperfusion have been identified for risk assessment and adjustment. (30) These parameters include older age, hypotension, Kilip class >1, anterior MI, peripheral vascular disease, initial serum creatinine level, elevated cardiac markers, cardiac arrest on admission and ST-segment deviation. All these risk factors are implemented in the GRACE risk score to assess the 6 months risk for death and can guide patient triage and management.(43)

The combination of clinical characteristic in combination with traditional cardiac biomarkers such as cardiac Troponin (cTn) and N-terminal pro-B-type natriuretic peptide (NTproBNP) have shown to improve risk prediction.(44, 45) However current risk prediction models provide only a rough estimate of individual risk. A multimarker strategy that captures a broader spectrum of disease may have added value since it can reveal novel relaease mechanisms and therefore potentioal therepeutic targets. Several biomarkers, such as growth differentiation factor-15 (GDF-15), galectin-3 (GAL-3) and suppression of tumorigenicity 2 (ST-2) have been studied in the recent years.(46) This thesis focusses on GDF-15 which is a systemic stress-responsive member of the transforming growth factor (TGF-β). (47) It is a relatively novel biomarker which is induced in the myocardium after ischemia and reperfusion and released due to heamodynamic stress.(48, 49)

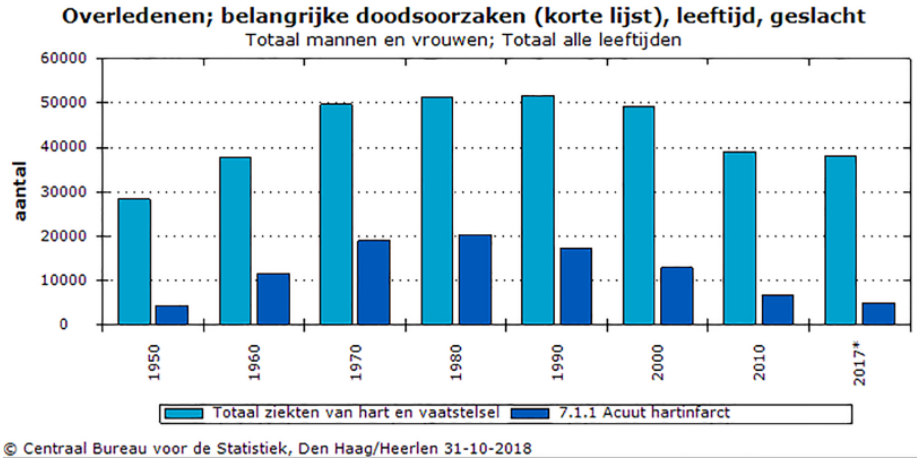

Figure 3. Number of deaths in the period 1950-2017 for cardiovascular disease and acute myocardial infarction separately. *Centraal Bureau voor de Statistiek, Den Haag/Heerlen, The Netherlands*(50)

Objective and outline of this thesis

The first objective of this thesis is to optimize the treatment in patients with STEMI or cardiovascular disease. Secondly, this thesis aims to improve risk stratification and identify high risk populations in STEMI patients. At last, a manuscript about a pre-hospital triage protocol was developed to manage the capacity in the cardiac emergency department.

Recent years have brought a significant amount of new results in the field of atherosclerosis. **Chapter 2** provides a background consisting of the current under-standing of the pathophysiology and treatment of atherosclerotic disease, followed by future perspectives on several novel classes of drugs that target atherosclerosis.

STEMI patients in the LUMC are treated according to the MISSION! MI protocol. Low risk patients are referred to the general practitioner (GP) after one year treatment according to the MISSION! MI protocol. In **chapter 3** the prognosis of STEMI patients referred to the GP after one year is assessed.

The current way to assess the risk of cardiovascular disease (CVD) is to measure conventional lipid and lipoprotein cholesterol fractions. Despite the success of statin treatment residual cardiovascular risk remains high. Therefore **chapter 4** studied the value of extensive serum apolipoprotein (apo) profiling and the risk of adverse events in patients with STEMI.

Identification of high-risk patients is essential for optimal monitoring and initiation of appropriate treatment to reduce risk of events. GDF-15 is a novel biomarker which can on top of other biomarkers improve risk stratification. **Chapter 5** investigated the additive prognostic value of GDF-15 levels at admission in STEMI patients on top of clinical characteristics and known cardiac biomarkers such as cTn and NTproBNP.

The introduction of high sensitivity assays (hs-cTn), which are up to 100 times more sensitive compared to the first-generation assays, permits the accurate determination of very low levels of circulating cardiac troponin. Elevated serum hs-cTn levels, even those within the normal range, are an emerging independent predictor of cardiovascular (CV) mortality in patients with and without CVD. **Chapter 6** sought to characterize the effect (magnitude and time of onset) of atorvastatin (ATOR) or rosuvastatin (ROSU) on hs-cTnI levels within the normal range in patients with stable CVD.

Hypercholesterolemia is a well-known risk factor for developing atherosclerosis and subsequently for the risk of a MI. Moreover, it might also be related to the extent of damaged myocardium in the event of a MI. **Chapter 7** aims to evaluate the association of baseline LDL-c level with infarct size in patients with STEMI after pPCI.

Chapter 8 is about the feasibility and efficacy of a novel pre-hospital triage protocol for use in the cardiac emergency department. Overcrowding in emergency department is a major public health problem and pre-hospital triage can help to allocate patients to the appropriate emergency department and thereby increase quality and efficacy of acute care in hospitals.

The final chapter includes a general summary, conclusions and future perspectives in English and in Dutch.

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Chapter 2.

Pathophysiology and treatment of atherosclerosis
Current view and future perspective on lipoprotein modification treatment

Neth Heart J. 2017 Apr;25(4):231-242

M.C. Bodde,
S.C. Bergheanu,
J.W. Jukema.

Abstract

Recent years have brought a significant amount of new results in the field of atherosclerosis. A better understanding of the role of different lipoprotein particles in the formation of atherosclerotic plaques is now possible. Recent cardiovascular clinical trials have also shed more light upon the efficacy and safety of novel compounds targeting the main pathways of atherosclerosis and its cardiovascular complications.

In this review, we first provide a background consisting of the current understanding of the pathophysiology and treatment of atherosclerotic disease, followed by our future perspectives on several novel classes of drugs that target atherosclerosis. The focus of this update is on the pathophysiology and medical interventions of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and lipoprotein(a) [Lp(a)].

Keywords

Atherosclerosis, hypercholesterolaemia, low-density lipoprotein, cardiovascular disease, statins, proprotein convertase subtilisin/kexin type-9

Atherosclerosis is a chronic condition in which arteries harden through build-up of plaques. Main classical risk factors for atherosclerosis include dyslipoproteinaemia, diabetes, cigarette smoking, hypertension and genetic abnormalities. In this review, we present an update on the pathophysiology of atherosclerosis and related current and possible future medical interventions with a focus on low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and lipoprotein(a) [Lp(a)].

Pathophysiology of atherosclerosis

Hypercholesterolaemia is considered one of the main triggers of atherosclerosis. The increase in plasma cholesterol levels results in changes of the arterial endothelial permeability that allow the migration of lipids, especially LDL-C particles, into the arterial wall. Circulating monocytes adhere to the endothelial cells that express adhesion molecules, such as vascular adhesion molecule-1 (VCAM-1) and selectins, and, consequently, migrate via diapedesis in the subendothelial space (1). Once in the subendothelial space, the monocytes acquire macrophage characteristics and convert into foamy macrophages. LDL particles in the subendothelial space are oxidised and become strong chemoattractants. These processes only enhance the accumulation of massive intracellular cholesterol through the expression of scavenger receptors (A, B1, CD36, CD68, for phosphatidylserine and oxidised LDL) by macrophages, which bind native and modified lipoproteins and anionic phospholipids. The end result is a cascade of vascular modifications (1) described in Table 1. Clinical sequelae of atherosclerosis are vessel narrowing with symptoms (angina pectoris) and acute coronary syndromes due to plaque instability.

The majority of coronary thrombi are caused by plaque rupture (55-65%), followed by erosions (30-35%), and least frequently from calcified nodules (2-7%) (1). Rupture-prone

plaques typically contain a large, soft, lipid-rich necrotic core with a thin (≤ 65 μm) and inflamed fibrous cap. Other common features include expansive remodelling, large plaque size (> 30% of plaque area), plaque haemorrhage, neovascularisation, adventitial inflammation, and 'spotty' calcifications. Vulnerable plaques contain monocytes, macrophages, and T-cells. T-cells promote the vulnerability of plaques through their effects on macrophages (2).

LDL-C, TG and HDL-C emerged as strong independent predictors of atherosclerotic disease after the analysis of the data from the Framingham study. While the role of other parameters is being investigated, TC, LDL-C and HDL-C remain to date the cornerstone in risk estimation for future atherosclerotic events. Low HDL-C has been shown to be a strong independent predictor of premature atherosclerosis(3) and is included in most of the risk estimation

scores. Very high levels of HDL-C, however, have consistently not been found to be associated with atheroprotection. The mechanism by which HDL-C protects against atherosclerosis is still under debate and accumulating evidence strongly suggests that the proportion of dysfunctional HDL versus functional HDL rather than the levels may be of importance.

Hypertriglyceridaemia (HTG) has been shown to be an independent risk factor for cardiovascular disease (CVD). Moreover, high TG levels are often associated with low HDL- C and high levels of small dense LDL particles. The burden of HTG is high, with about one- third of adult individuals having TG levels > 1.7 mmol/l (150 mg/dL) (3).

Lp(a) is a specialised form of LDL and consists of an LDL-like particle and the specific apolipoprotein (apo) A. Elevated Lp(a) is an additional independent risk marker and genetic data made it likely to be causal in the pathophysiology of atherosclerotic vascular disease and aortic stenosis. (4)

Table 1. Vascular modifications in atherosclerotic disease

Vascular modification	Characteristics
Intimal thickening	Layers of SMCs and extracellular matrix More frequent in coronary artery, carotid artery, abdominal aorta, descending aorta, and iliac artery
Fatty streak	Abundant macrophage foam cells mixed with SMCs and proteoglycan-rich intima
Pathologic intimal thickening	Layers of SMCs in proteoglycan-collagen matrix aggregated near the lumen Underlying lipid pool: acellular area rich in hyaluronan and proteoglycans with lipid infiltrates
Fibroatheromas	Acellular necrotic core (cellular debris) Necrotic core is covered by thick fibrous cap: SMCs in proteoglycan-collagen matrix
Vulnerable plaque	'Thin-cap fibroatheroma' Type I collagen, very few/absent SMCs Fibrous cap thickness is ≤ 65 µm
Ruptured plaque	Ruptured fibrous cap Presence of luminal thrombus Larger necrotic core and increased macrophage infiltration of the thin fibrous cap

SMCs: smooth muscle cells.

Lipoprotein modification treatment

Current view

Medication to adequately control lipoprotein levels needs to be initiated when risk reduction through lifestyle modifications such as dietary changes,

stimulation of physical activity and smoking cessation is not sufficient. In secondary prevention, medical therapy is almost invariably needed in addition to lifestyle optimisation.

LDL-C-lowering therapy

HMG-CoA reductase inhibitors (statins)

3-hydroxy-3-methyl–glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (usually addressed as 'statins') induce an increased expression of LDL receptors (LDL-R) on the surface of the hepatocytes, which determines an increase in the uptake of LDL-C from the blood and a decreased plasma concentration of LDL-C and other apo B-containing lipoproteins, including TG-rich particles (3).

Since the 1990's, statin therapy has shown its effect on cardiovascular outcome in several major landmark trials, summarised in Table 2.

Independent of baseline LDL-C level and baseline cardiovascular (CV) risk, meta-analyses concerning up to 27 statin CV outcome trials, showed a 22% risk reduction in CV events per 1 mmol/l reduction in LDL-C (5-7)(Fig. 1).

It is currently known that both the baseline burden of atherosclerotic plaque and the degree of progression on serial evaluation significantly associate with risk of CV events (8,9). The difference in change in percent atheroma volume (PAV) between patients with and without an event can be as low as approximately 0.55% (10).

Not reaching the cholesterol treatment goals and non-compliance are two important causes for statin therapy failure. Although the LDL-C levels obtained in clinical trials are often low, the clinical reality seems different. Vonbank et al (11) showed that in 2 cohorts of high-risk CV patients, one from 1999-2000 and the other one from 2005-2007, only 1.3% and 48.5% of patients, respectively, had the LDL-C <1.8 mmol/l at 2-year follow-up. The fear of possible side effects of statin therapy is an important reason for non-compliance and remains an underestimated problem in clinical practice. One study in high-dose statin patients reported that muscular pain prevented even moderate exertion during everyday activities in 38% of patients, while 4% of patients were confined to bed or unable to work (12). Jukema et al. reviewed available data and concluded that statin use is associated with a small increase in type 2 diabetes mellitus incidence, but no convincing evidence was found for other major adverse effects such as cognitive decline or cancer (13).

Statins are therefore, in general, very efficient drugs that in an overwhelming amount of well conducted clinical trials showed consistent clinical event reductions with a very good safety profile. Nevertheless, side effects of importance may occur making the compound, as in any drug class, sometimes unsuitable for some individual patients.

Table 2. Summary of major clinical trials and programs involving low-density lipoprotein cholesterol lowering treatments

Drug/Target	Clinical trial	Study size	Duration	CV endpoints	Results
Statins					
	4S (44)	4444 patients with CHD	5.4 y	Coronary death	111 in the simvastatin group; 189 in the placebo group; (RR = 0.58, 95% CI: 0.46-0.73).
	WOSCOP (45)	6595 men with hypercholesterolemia	4.9 y	Combined nonfatal MI/coronary death	174 in the pravastatin group; 248 in the placebo group; (RRR = 31%, 95% CI: 17-43%)
	CARE (46)	4159 subjects with high CV risk and normal LDL-C levels	4.9 y	Combined coronary event/nonfatal MI	10.2% in the pravastatin group; 13.2% in the placebo group; (RRR = 24%, 95% CI: 9-36%)
	ASTEROID (47)	349 patients on statin therapy with serial IVUS examinations	2.0 y	IVUS change in PAV	-0.79% (-1.21 to -0.53%) in the rosuvastatin group
	SATURN trial (48)	1039 patients with CAD on intensive statin treatment	2.0 y	IVUS change in PAV	-0.99% (-1.19 to -0.63%) in the atorvastatin group; -1.22% (-1.52 to -0.90%) in the pravastatin group
	REGRESS (9)	885 symptomatic male patients on pravastatin or placebo	2.0 y	Change in lumen diameter	0.10 mm decrease in the placebo group; 0.06 mm decrease in the pravastatin group (p=0.019)
	PROVE-IT TIMI 22 (10)	4162 ACS patients on either intensive or standard statin therapy	2.0 y	Combined death, MI, UAP, revascularization, stroke	22.4% in intensive therapy group; 26.3% in standard statin therapy group; (HR 0.84, 95% CI: 0.74-0.95)
Ezetimibe					
	PRECISE-IVUS (14)	246 patients undergoing PCI on statin alone or statin + ezetimibe	9.9 m	IVUS change in PAV	-1.4% (-3.4 to -0.1%) in the dual lipid lowering group; -0.3% (-1.9 to 0.9%) in the statin monotherapy group
	IMPROVE-IT (15)	18,114 ACS patients on statin + placebo or on statin + ezetimibe	6.0 y	Combined death, MI, UAP, revascularization, stroke	32.7% in simvastatin +ezetimibe group; 34.7% in the simvastatin + placebo group; (HR 0.94, 95% CI: 0.89 to 0.99)

table continues

Drug/Target	Clinical trial	Study size	Duration	CV endpoints	Results
Bile acid sequestrants					
	LRC-CPP (49)	3806 men with hypercholesterolemia on cholestyramine resin or placebo	7.4 y	Combined CAD death/nonfatal acute MI	8.1% in cholestyramine group; 9.8% in the placebo group; (RR 0.81, 90% CI: 0.68 to 0.84)
PCSK-9 inhibitors					
	OSLER (16)	4465 patients on evolocumab + standard therapy or standard therapy alone	11.1 m	%change LDL-C, cardiovascular events	-61% (59% to 63%) LDL-C change in the evolocumab group, 0.95% event-rate in the evolocumab group; 2.18% in the standard therapy group; (HR 0.47, 95% CI 0.28-0.78)
	ODYSSEY LONG TERM (17)	2341 high risk patients receiving in a 2:1 ratio alirocumab or placebo	78 w	%change in LDL-C, combined death, MI, UAP, revascularization, stroke	-61% LDL-C change in the alirocumab group; 0.8% in the placebo group; (p<0.001). 1.7% event-rate in the alirocumab group; 3.3% in the placebo group; (HR 0.52, 95% CI: 0.31 to 0.90)
	GLAGOV (18)	968 presenting for CAG randomized with either evolocumab or placebo	76 w	IVUS change in PAV	-1.0% (-1.8 to -0.64%) in the evolocumab group

CHD, coronary heart disease: CAD, coronary artery disease: MI, myocardial infarction: CV, cardiovascular risk: LDL-C, low-density lipoprotein cholesterol: PAV, percentage atheroma volume: ACS, acute coronary syndrome: PCI, percutaneous coronary intervention: UAP, unstable angina pectoris: CAG, coronary angiography: IVUS, intravascular ultrasonography: y, year: RR, relative risk: HR, hazard ratio: 4S, Scandinavian Simvastatin Survival Study: WOSCOP, West of Scotland Coronary Prevention: CARE, Cholesterol and Recurrent Events: ASTEROID, A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound –Derived Coronary Atheroma Burden: SATURN, The Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin versus Atorvastatin: REGRESS, The Regression Growth Evaluation Statin Study: REVERSAL, Reversal of Atherosclerosis with Aggressive Lipid Lowering: PROVE-IT TIMI 22, pravastatin or atorvastatin evaluation and infection trial-thrombolysis in myocardial infarction: PRECISE-IVUS, Plaque Regression With Cholesterol Absorption Inhibitor or Synthesis Inhibitor Evaluated by Intravascular Ultrasound: IMPROVE-IT, IMProved Reduction of Outcomes: Vytorin Efficacy International Trial: LRC-CPP, Lipid Research Clinics Coronary Primary Prevention: OSLER, open-label study of long-term evaluating against LDL-C: ODYSSEY LONG TERM, Long-term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy: GLAGOV, global assessment of plaque regression with a PCSK-9 antibody as measured by intravascular ultrasound.

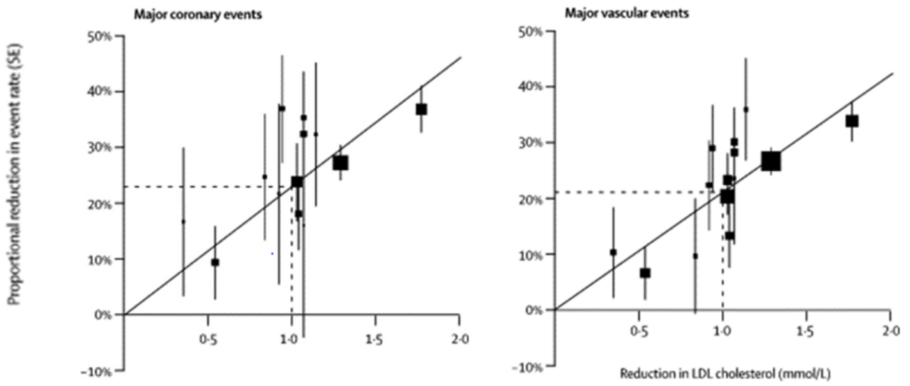

Fig. 1 Relation between proportional reduction in incidence of major coronary events and major vascular events and mean absolute LDL cholesterol reduction at 1 year. Square represents a single trial plotted against mean absolute LDL cholesterol reduction at 1 year, with vertical lines above and below corresponding to one SE of unweighted event rate reduction. Trials are plotted in order of magnitude of difference in LDL cholesterol difference at 1 year. For each outcome, regression line (which is forced to pass through the origin) represents weighted event rate reduction per mmol/l LDL cholesterol reduction. Figure published with permission of the Lancet (owned by Elsevier).

Cholesterol absorption inhibitors

By inhibiting cholesterol absorption, ezetimibe reduces LDL-C. In clinical studies, ezetimibe as monotherapy reduced LDL-C by 15–22% and when combined with a statin it induced an incremental reduction in LDL-C levels of 15–20% (3). No frequent major adverse effects have been reported (3). Results from studies like PRECISE-IVUS (14) and IMPROVE-IT (15) support the use of ezetimibe as second-line therapy in association with statins when the therapeutic goal is not achieved at the maximum tolerated statin dose, in statin-intolerant patients, or in patients with contraindication to statins (3).

Bile acid sequestrants

At the highest dose, cholestyramine, colestipol or the recently developed colesevelam can produce a reduction in LDL-C of 18–25% (3). The use of cholestyramine and colestipol is limited by gastrointestinal adverse effects and major drug interactions with other frequently prescribed drugs. Colesevelam appears to be better tolerated and to have less interaction with other drugs and can be combined with statins. Relatively little hard evidence is available from large clinical trials for this class of drugs.

Proprotein convertase subtilisin/kexin type-9 inhibitors

Inhibitors of proprotein convertase subtilisin/kexin type-9 (PCSK-9) offer the prospect of achieving even lower LDL-C levels than statins in combination with ezetimibe. PCSK-9 binds to LDL-R at the liver and stimulates the absorption and

degradation of these receptors. Through inhibition of PCSK-9, the degradation of LDL-R is prevented thereby improving the absorption by the liver of LDL-C particles, which consequently leads to lower LDL-C plasma concentrations.

In 2015, reports were published from two phase 3 trials that measured the efficacy and safety of evolocumab and alirocumab, two monoclonal antibodies that inhibit PCSK-9 (16, 17). In these trials, the PCSK-9 therapy significantly lowered LDL-C by ≈ 50% and in a preliminary (not powered) analysis reduced the incidence of CV events (Table 3). Other promising results were published from the GLAGOV (18) trial and demonstrated a significant percentage atheroma volume decrease with evolocumab (Table 3). Both evolocumab and alirocumab have been recently approved by the European Medicine Agency and the US Food and Drug Administration for the treatment of elevated plasma LDL-C. The PCSK-9 therapy is suitable in a wide range of patients provided that they express LDL-R, including those with heterozygous and homozygous familial hypercholesterolaemia with residual LDL-R expression (3). Relatively high costs of the compounds and yet the lack of hard outcomes in large randomised controlled trials (RCTs) still limit their use in clinical practice.

The first results of two large RCTs investigating the long-term efficacy and safety of evolocumab (FOURIER trial) and alirocumab (ODYSSEY Outcomes trial) are underway and necessary (19, 20). Recently, the development of another monoclonal PCSK-9 inhibitor, bococizumab, was stopped due to auto-antibodies formation against the compound that significantly reduced the LDL-C-lowering efficacy (The SPIRE program) (21).

TG-lowering therapy

Statins

Statins reduce the plasma concentration of TG-rich particles by inhibiting HMG-CoA reductase. Although recent evidence positions HTG as a CV risk factor, the benefits of lowering elevated TG levels are still modest.

Statins are the first-choice therapy in patients with HTG since they reduce both the CV risk and, in high doses, have a stronger effect on elevated TG levels (up to 27% reduction) (3, 22).

Fibrates

Fibrates are agonists of peroxisome proliferator-activated receptor-α (PPAR-α), acting via transcription factors regulating various steps in lipid and lipoprotein metabolism. Fibrates have good efficacy in lowering fasting TG as well as post-prandial TGs and TG-rich lipoprotein remnant particles, with lowering TG levels up to more than 50% (23). However, results from 5 prospective RCTs and 5 meta-analyses failed to demonstrate superior CV outcomes with fibrates, especially when used on top of statins (3).

Table 3. Trials concerning PCSK-9 inhibition

Clinical trial	Mechanism of action	Molecules	Population	Phase	Endpoint	Expected/ known results
ODYSSEY OUTCOME (19)	PCSK-9 antibodies	Alirocum-ab	18,000 post ACS patients	3	Combined CAD death/ nonfatal acute MI	2017/2018
FOURIER (20)	PCSK-9 antibodies	Evolo-cumab	27,564 high risk patients with LDL-C>1.8mmol/L	3	Combined CAD, death/ nonfatal acute MI	Early 2017
SPIRE 1+2 (21)	PCSK-9 antibodies	Bococi-zumab	28,000 patients on high residual risk	3	Combined death, MI, UAP, revascular-ization, stroke	Terminated due to the emerging clinical profile
ORION (34)	siRNA against PCSK-9	Inclisiran	480 patients with ASCVD or ASCVD-risk equivalents	2	Change in LDL-C from baseline to Day 180	-51%

CAD, coronary artery disease: MI, myocardial infarction: CV, cardiovascular risk: LDL-C, low-density lipoprotein cholesterol: UAP, unstable angina pectoris: ACS, acute coronary syndrome: ASCVD, atherosclerotic cardiovascular disease: PCSK-9, proprotein convertase subtilisin/kexin type-9: siRNA, small interfering RNA: ODYSSEY, Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy: FOURIER, Further cardiovascular OUtcomes Research with PCSK9 Inhibition in subjects with Elevated Risk: SPIRE, Studies of PCSK9 Inhibition and the Reduction of vascular Events: ORION, Trial to Evaluate the Effect of ALN-PCSSC Treatment on Low-density Lipoprotein Cholesterol

n-3 fatty acids

n-3 fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] can lower TG possibly through interaction with PPARs. Although the underlying mechanism is poorly understood n-3 fatty acids can reduce TG levels with up to 45%. A meta-analysis of 20 studies and 63,000 patients found no overall effect of omega-3 fatty acids on composite CV events. n-3 fatty acids appear to be safe and not interact with other therapies (24).

Currently, there are two ongoing phase 3 randomised placebo-controlled clinical trials evaluating the effect of EPA on CV outcomes in 21,000 subjects with elevated serum TG (25, 26). If TG are not controlled by statins or fibrates

n-3 fatty acids may be added to decrease TG further, as these combinations are safe and well tolerated (3).

HDL-C increasing therapy

Even though lifestyle changes may increase HDL-C levels to a certain degree, many patients will also require medication should a robust HDL-C increase be considered necessary. To date, there is no convincing evidence that artificially raising HDL-C leads to an improved CV outcome. However, if HDL-C increasing therapy is considered then the following options are available.

Cholesteryl ester transfer protein (CETP) inhibitors

The inhibition of CETP by small molecule inhibitors represents currently the most efficient pharmacological approach to influence low HDL-C, with an effect of ≥ 100% increase in HDL-C and frequently a reduction of LDL-C levels as well. Despite the impressive HDL-C increase, no effect has been seen yet on CV endpoints, as all the CETP-inhibitors studies (27-29) have failed to demonstrate this thus far.

Torcetrapib was discontinued following a higher mortality in the torcetrapib arm of the ILLUMINATE trial (27), the results of the dalcetrapib trial (Dal-OUTCOMES) showed no clinical impact in acute coronary patients and the ACCELERATE trial of evacetrapib in acute coronary patients on statins was terminated prematurely due to lack of efficacy signals (28, 29).

Of the CETP inhibitors initially developed, only anacetrapib is still active. In mice models it has been reported that anacetrapib attenuates atherosclerosis not by increasing HDL-C but rather by decreasing LDL-C by CETP inhibition and by a CETP independent reduction of plasma PCSK-9 level (30).

The REVEAL study, a very large phase 3 RCT with anacetrapib, is still underway and its results are expected in 2017 (31). This trial will further elucidate whether the additional beneficial effects of anacetrapib on top of a statin can be translated into clinical benefit.

Statin

Statins produce elevations in HDL-C levels between 5-10% (32). It is difficult to extract the amount of effect that HDL-C increase might have in the overall observed CV risk reduction with statins.

Fibrates

Fibrates increase HDL-C in a similar proportion with statins, namely between 5% in long- term trials (especially if type 2 DM patients are included) and up to 15% in short-term studies (23, 33). The FIELD study failed to demonstrate that fenofibrate could significantly lower the CV risk (23).

Future perspectives

LDL-C-lowering therapy

PCSK-9 inhibition (non-monoclonal antibody)

A recent approach in decreasing PCSK-9 levels is the administration of small interfering RNA (siRNA) molecules directed against PCSK-9. The siRNA molecules enable the RNA- induced silencing complex, which cleaves messenger RNA (mRNA) molecules encoding PCSK-9 specifically. The cleaved mRNA is degraded and thus unavailable for protein translation, which results in decreased levels of the PCSK-9 protein. The phase 2 ORION trial showed that one subcutaneous injection of 300 mg inclisiran determined a mean LDL-C reduction of 51% after 6 months (34). Inclisiran was well tolerated with no relevant safety concerns. These results support the start of the phase 3 program. The next step might be the development of a vaccine targeting PCSK-9. Crossey et al. provided in mice and macaques the proof-of-principle evidence that a vaccine targeting PCSK-9 peptide can effectively lower lipid levels and works synergistically with statins (35).

Bempedoic acid

Bempedoic acid is a first-in-class adenosine triphosphate (ATP) Citrate Lyase inhibitor. The mechanism of action involves the inhibition of cholesterol biosynthesis and the up-regulation of LDL-R, which in turn decreases plasma LDL-C levels. A phase 3 clinical trial (CLEAR Harmony) is currently conducted in patients with high CV risk and elevated LDL-C that is not adequately controlled under their current therapy. Almost 2000 subjects will be randomised for bempedoic acid or placebo and will be followed for 52 weeks (36). In continuation of this trial, the CLEAR Outcomes trial will be conducted. This will be an event-driven study of 12,600 patients on either bempedoic acid or placebo with the primary efficacy endpoint of major adverse CV events. The results of this trial will be expected not earlier than 2022.

Peroxisome proliferator-activated receptor delta (PPARδ)

PPARδ is a nuclear receptor that regulates genes involved in lipid storage and transport. MBX-8025 is a selective agonist for PPARδ.

The recently presented partial results from a proof-of-concept phase II trial in patients with homozygous familial hypercholesterolaemia showed that the range of responses to MBX- 8025 was broad, but that MBX-8025 could provide a clinically meaningful reduction in LDL- C for a subset of patients (37).

Other lipoprotein modification targets

Apo A-I mimetics

Apo A-I is the primary functional component of HLD-C and supports the rapid removal of cholesterol from plaque. The MILANO-PILOT study was a proof-of-concept study in which the impact on coronary plaque by MDCO-216 was measured in 120 acute coronary syndrome (ACS) patients using IVUS (38).

MDCO-216 is a complex of dimeric recombinant apolipoprotein A-I Milano and a phospholipid (POPC), and mimics pre-beta HDL. In this study, MDCO-216 did not produce a significant effect on coronary progression. Based on these results further development of the compound was halted. CER-001 is a different engineered pre-beta HDL compound and is currently being tested in a phase 2 clinical trial (CARAT) assessing the nominal change from baseline to follow-up (at 12 weeks) in the PAV in the target coronary artery of ACS patients. Results will be available in early 2017 (39). CSL112 is a plasma-derived apolipoprotein A-I (apo A-I) and was tested in a phase II trial for safety and tolerability. CSL112 was well tolerated and did not significantly alter liver or kidney functions (40). Assessment of the efficacy of CSL112 will be performed in an adequately powered phase 3 clinical trial.

Angiopoietin-like 3 (ANGPTL3)

ANGPTL3 is a protein and main regulator of lipoprotein metabolism. Its function is linked to the inhibition of lipoprotein lipase (LPL) activity. Earlier studies have identified that subjects with ANGPTL3 deficiency have reduced cholesterol and TG levels. Recently, a phase 1/2 study evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics of ANGPTL3-LRx (an antisense inhibitor of ANGPTL3) in healthy volunteers with elevated TG and subjects with familial hypercholesterolaemia. There were no short-term safety concerns and ANGPTL3-LRx induced significant mean reductions in TGs (66%), LDL-C (35%) and total cholesterol (36%). Final results are expected in 2017 (41).

Lipoprotein(a) [Lp(a)]

PCSK-9 inhibitors and nicotinic acid reduce Lp(a) by approximately 30% (16, 17, 42), however, an effect on CV events targeting Lp(a) has not been convincingly shown. A phase 2 clinical trial showed that IONIS-APO(a)Rx, an oligonucleotide targeting Lp(a), induced a lowering of Lp(a) levels of up to 71.6% (43). A phase 1/2a first-in-man trial showed that IONIS-APO(a)-LRx, a ligand-conjugated antisense oligonucleotide designed to be highly and selectively taken up by hepatocytes, induced a lowering of Lp(a) levels of up to 92%. Both antisense oligonucleotides were short-term safe and well tolerated (43).

Plasma Lp(a) is currently not recommended for risk screening in the general population, but measurement should be considered in people with high CV risk or a strong family history of premature atherothrombotic disease (3).

Table 4 provides an overview of the most important ongoing lipoprotein modifying trials and their expected or recently published results.

Table 4. Ongoing trials and future perspective

Target	Clinical trial	Mechanism of action	Molecules	Population	Phase	Endpoint	Results/ expected results
LDL-C							
	CLEAR Harmony (36)	ACL-inhibitor	Bempedoic acid	1950 high CV risk patients	3	Safety, tolerability	2018
	MBX-8025 (37)	Selective PPARδ	MBX-8025	13 patients with HoFH	2	Effect on LDL-C	Full results -early 2017
HDL-C							
	REVEAL (31)	CETP inhibitors	Anacetrapib	30,624 patients with a history of MI stroke or PAD	3	Major coronary events (defined as coronary death, MI or coronary revascularization)	Early 2017
	MILANO-PILOT (38)	Apo A-I mimetics	MDCO-216	120 ACS patients	2	Change in PAV	No significant effect
	CARAT (39)	Apo A-I mimetics	CER-001	301 ACS patients	2	Change in PAV	Early 2017
	AEGIS (40)	Apo A-I mimetics	CSL-112	1258 ACS patients	2b	Safety, tolerability, PK	Well tolerated and safe
Triglycerides							
	IONIS ANGPTL3-LRx (41)	Inhibition of LPL activity	IONIS ANGPTL3-LRx	61 healthy volunteers	1-2	Safety, tolerability, PK/ PD	June 2017
L(p) a							
	IONIS-APO(a)-Rx (43)	Antisense oligonucleotide targeting hepatic	IONIS-APO(a)-LRx	64 participants with high Lp(a) levels	2	%change in Lp(a)	-71.6%
	IONIS-APO(a)-LRx (43)	apo(a) mRNA Ligand-conjugated antisense oligonucleotide	IONIS-APO(a)-LRx,	58 healthy volunteers	1/2	%change in fasting Lp(a)	-92%

LDL-C, low-density lipoprotein cholesterol: ATP, adenosine triphosphate: ACL-inhibitor, ATP-Citrate Lyase inhibitor: PPAR,δ: Peroxisome

proliferator-activated receptor delta: HoFH, homozygous familiar hypercholesterolemia: CV, cardiovascular: ACS, acute coronary syndrome: PAV, percentage atheroma volume: PK, pharmacokinetics: PD, pharmacodynamics: ApoA-I, apolipoprotein A-I : MI, myocardial infarction: PAD, peripheral arterial disease: CETP, Cholesteryl ester transfer protein: LPL, lipoprotein lipase: Lp[a]: lipoprotein (a), mRNA, messenger RNA: MILANO-PILOT, MDCO-216 Infusions Leading to Changes in Atherosclerosis: A Novel Therapy in Development to Improve Cardiovascular Outcomes - Proof of Concept Intravascular Ultrasound (IVUS), Lipids, and Other Surrogate Biomarkers Trial: CARAT, CER-001 Atherosclerosis Regression ACS Trial: AEGIS, The ApoA-I Event Reduction in Ischemic Syndromes I: REVEAL, Randomized EValuation of the Effects of Anacetrapib though Lipid-modification: IONIS ANGPTL3-LRx, IONIS Angiopoietin-like 3-linear RNAx

Conclusions

Lowering LDL-C by statin therapy remains, to date, the cornerstone for the medical prevention and treatment of atherosclerotic disease since it is efficient and generally safe. In high-risk patients with statin intolerance or in high-risk patients who do not obtain the desired LDL-C level with intensive statin treatment, cholesterol absorption inhibitors, especially ezetimibe, should be considered. Bile acid sequestrants, fibrates and niacin are not recommended. Upcoming PCSK-9 inhibitors, whether in the form of monoclonal antibodies or new approaches, appear as potent agents for dyslipoproteinaemia. However, their long- term efficacy and safety still needs to be proven and costs may limit their practical use. HDL- C modulation through CETP inhibition and apo A-I mimetics did not yet provide evidence for better CV outcomes; the REVEAL and CARAT trials will shed light on the future of these drug classes. New classes of molecules targeting ANGPTL3 and Lp(a) have shown promising efficacy and good short-term safety profiles in several early phase trials and these results warrant further development.

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Chapter 3.

Myocardial infarction patients referred to the primary care physician after 1-year treatment according to a guideline-based protocol have a good prognosis

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Abstract

Introduction
ST-segment elevation myocardial infarction (STEMI) patients that could be referred to the general practitioner (GP) can improve patients tailored care. However, the long-term prognosis of patients referred to the GP is unknown. Therefore, the aim of this study was to assess the long-term prognosis of patients referred to the GP after treatment according to a 1-year institutional guideline-based protocol.

Methods
All consecutive patients treated between February 2004 up to May 2013 who completed the 1-year institutional MISSION! Myocardial Infarction (MI) follow-up and who were referred to the GP were evaluated. After 1 year of protocolized monitoring, asymptomatic patients with a left ventricular ejection fraction >45% on echocardiography were referred to the GP. Long-term prognosis was assessed with Kaplan-Meijer curves and Cox proportional hazards analysis was used to identify independent predictors for 5-year all-cause mortality and MACE.

Results
In total, 922 STEMI patients were included in this study. Mean age was 61.6 ± 11.7 years and 74.4% were male. Median follow-up duration after the 1-year MISSION! MI follow-up was 4.55 years (IQR 2.28-5.00). The event-free survival was 93.2%. After multivariable analysis, age, not using an ACE-inhibitor/AT2-antagonist and impaired LV function remained statistically significant predictors for 5-year all-cause mortality. Kaplan-Meijer curves revealed that 80.3% remained event free for MACE after 5-year. Multivariable predictors for MACE were current smoking and a mitral regurgitation grade ≥ 2.

Conclusion
STEMI patients referred to the GP have an excellent prognosis after being treated according to the 1-year institutional MISSION! MI protocol.

Introduction

Due to the implementation of various very successful treatments for ST-segment elevation myocardial infarction (STEMI), such as treatment with primary percutaneously coronary intervention (pPCI), adjunctive antithrombotic therapy and adequate secondary prevention medication,[1-6] the current 1-year and 5-year all-cause mortality rates in STEMI patients decreased the last decades to approximately 10%[7, 8] and 20%,[8, 9] respectively. In an era of growing economic pressure in healthcare, identifying low risk STEMI patients, can improve patient tailored care and could reduce healthcare costs. For example, several studies demonstrated that low risk STEMI patients can be safely discharged within two or three days after admission[10, 11], which resulted in a reduction of healthcare costs[10, 12]. However, to our knowledge, there are no recommendations as to the appropriate duration of follow-up in the outpatient clinic of a cardiologist after STEMI. According to the MISSION! myocardial infarction (MI) protocol[13], after 1-year follow-up, asymptomatic patients with a left ventricular ejection fraction (LVEF) >45% on echocardiography, are referred to the general practitioner (GP). The hypothesis of this study is that these patients can safely be referred to the GP after 1-year MISSION! MI follow-up. As the long-term prognosis of STEMI patients referred to the GP is unknown, the aim of this study was to assess the prognosis of patients referred to the GP after treatment according to the 1-year institutional MISSION! MI protocol in the Leiden University Medical Center.

Methods

Study population

All patients treated with a pPCI for STEMI in the LUMC are included in the prospective MISSION! MI registry.[13] For this current observational retrospective analysis all consecutive patients treated between February 2004 up to May 2013 who, after completion of the 1-year MISSION! MI follow-up were referred to the GP, were evaluated. Patients who died during the first year after their index infarction, or patients who were transferred during admission to another hospital due to logistic reasons were not included in this analysis. Logistic reasons were lack of available space for patients to admit, patient's preference or when patients were transferred back to the referring hospital after the pPCI. STEMI was defined as typical electrocardiographic (ECG) changes (ST-segment elevation ≥ 0.2 mV in ≥ 2 contiguous leads in V_1 through V_3, ≥ 0.1 mV in other leads, or presumed new left bundle branch block) and a typical rise and fall of cardiac biomarkers accompanied with chest pain for at least 30 minutes.[14] Since the data did not contain any identifiers that could be traced back to the individual patient and the data are obtained for patient care, the Dutch Central Committee on Human-Related Research permits the use of anonymous data without prior approval of an institutional review board. This study was

conducted according to the declaration of Helsinki.

Study procedure

The institutional, guideline-based MISSION! MI protocol is a standardized clinical framework which consists of a pre-hospital, in-hospital and an outpatient phase to optimize clinical decision making and treatment up to 1 year after the index event.[13, 15, 16] The MISSION! MI protocol is in accordance with the current STEMI guidelines and was changed when necessary.[15, 16] In the pre-hospital phase a high-quality 12-lead ECG was obtained. If a STEMI was diagnosed, patients were treated by the paramedics with a loading dose of clopidogrel or prasugrel, aspirin, heparin and intravenous glycoprotein IIb/IIIa inhibitors if appropriate. During the in-hospital phase patients were directly transferred to the catheterization laboratory for pPCI according to the current guidelines. If no contraindications existed, β-blockers, angiotensin-converting enzyme (ACE) inhibitors, and statins were administrated within 24 hours of admission. Dual antiplatelet therapy was additionally prescribed, consisting of aspirin 100mg daily for life and prasugrel 10mg daily or clopidogrel 75mg daily for 12 months if appropriate. The outpatient clinic phase consists of 4 clinic visits, where patients were treated in accordance to current guidelines to reach the secondary prevention targets. Furthermore, several functional tests, such as a stress echocardiography and Holter registration, were obtained and if necessary an intervention was performed. An important part of the outpatient clinic was to emphasize the need for drug compliance and was the education on and modification of lifestyle behavior (smoking cessation, healthy diet, exercise and weight management). Patients also participated in a professional cardiac rehabilitation program as part of the routine care where also a dietician, phycological worker and social worker where out at their disposal, as this is associated with better one-year outcome.[13, 17] After 1 year of intensive monitoring patients were, by protocol, referred to the general practitioner if they were asymptomatic with a left ventricular ejection fraction (LVEF) >45%.

Data acquisition/Clinical data

All patients risk factors, clinical features and laboratory measurements are systematically collected for each MISSION! patients in EPD-VISION, using a unique study code. Echocardiographic images were attained from patients at rest in left lateral decubitus position using a commercially available system (Vivid 7 and E9, GE, Healthcare, Horten, Norway). Standard M-mode and 2D (color, pulsed and continuous wave Doppler) images were obtained from the parasternal (long-and short-axis) and apical views (long-axis, 2- and 4-chamber), using 3.5-MHz or M5S transducers, and digitally stored for offline analysis (EchoPac BT13, GE Medical Systems, Horten, Norway). The LVEF, wall motion score index (WMSI) and the grade of mitral regurgitation (MR) were measured according to the current echocardiographic recommendations.[18, 19] Clinical follow-up data were prospectively collected in the electronical patients file by independent clinicians. Data from patients were gathered from either out-

patients chart review or by telephone interview. Information on the vital status was obtained from the Dutch Municipality Records registry. Cause of death was retrieved from the GP.

Study endpoint
The primary endpoint of this study is all-cause mortality. The secondary endpoint is a combined endpoint of coronary revascularization, recurrent myocardial infarction, implantation of an implantable cardiac defibrillator (ICD) or a pacemaker (PM), hospitalization due to heart failure, stroke and death. All these adverse events combined have been defined as major adverse cardiac event (MACE).

Statistical analysis
Data are summarized as means with standard deviation in case of normally distributed or as median with interquartile ranges in case of non-normally distributed data. Categorized data are shown as numbers with percentages. Univariable Cox proportional hazard regression models were used to assess the association of age, gender and pre-specified covariates, that are known associated variables in literature, with all-cause mortality or occurrence of time-dependent adverse events (MACE) in STEMI patients.[1-3, 20-23] Age, gender and other variables significant at $p<0.10$ were entered into a multivariable Cox model to calibrate a combined prognostic index to predict either all-cause mortality or MACE.[24] Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. To classify GP patients into either high or low risk groups based on these Cox regression models, we dichotomized the prognostic index using the median value. Stratified by these two groups, Kaplan-Meier curves were then used to estimate and verify survival expectations (time to either all-cause death or MACE). The log-rank test log-rank test was calculated to compare the cumulative incidences of the endpoints between the 2 groups. All statistical tests were 2-tailed, p-values <0.05 were considered statistically significant. Analyses were performed with SPSS 23.0 statistical analysis software (IBM, Armonk, NY, USA).

Results

Between February 2004 up to May 2013, 2943 patients were admitted to the LUMC and treated with pPCI for STEMI. During the first year after their index infarction 206 (7.0%) patients died and 964 (32.8%) patients did not follow the institutional MISSION! MI protocol for logistical reasons. In total, 1773 (60.2%) patients completed their 1-year follow-up according to the MISSION! MI protocol. Of these patients 851 (48%) received follow-up in the outpatient clinical of a cardiologist according to the MISSION! MI protocol.

Therefore, 922 (52%) patients were referred to the GP and selected for evaluation (Figure 1). Median follow-up duration after the 1-year MISSION! MI follow-up was 4.55 year (IQR 2.28-5.00).

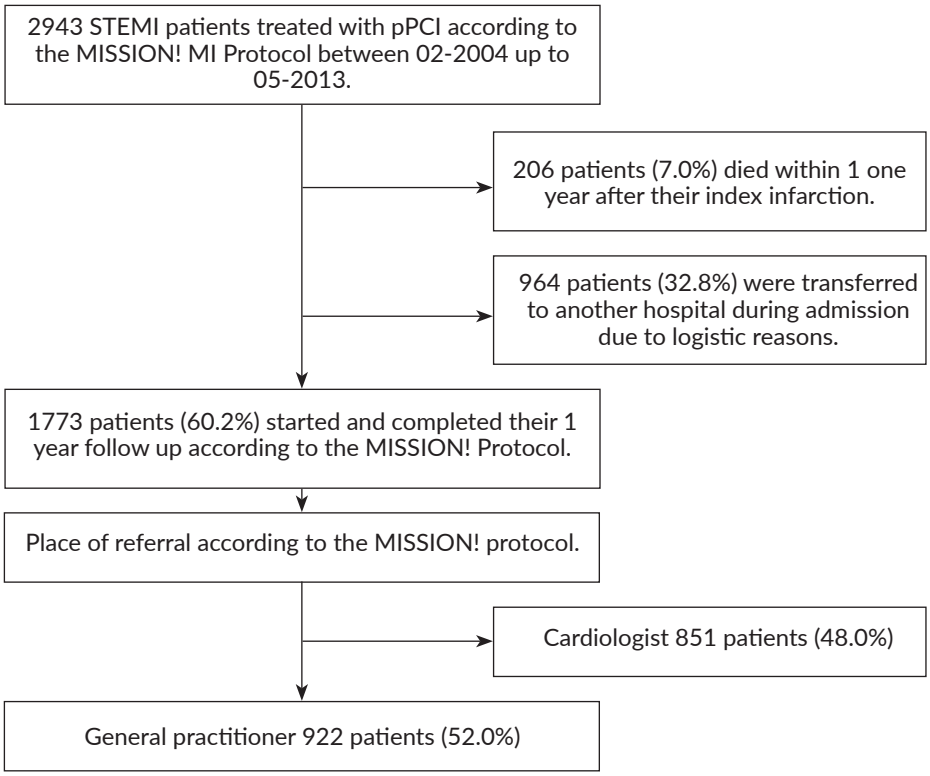

Figure 1. Overview of eligible MISSION! patients. Abreviations: STEMI, ST-elevation myocardial infarction; pPCI, primary percutaneously coronary intervention

Baseline characteristics

Patients characteristics, medication use and laboratory results after 1 year MISSION! MI follow-up are summarized in table 1. Mean age was 61.6 ± 11.7 year and 686 (74.4%) was male gender. The LVEF was in 70 (7.6%) patients below 45%.

Long-term survival analysis

In total, 48 patients deceased after 1 year MISSION! MI follow up. The cause of death was adjudicated as cardiac origin in 6 patients, likely cardiac in 3 patients, non-cardiac in 35 patients, unlikely cardiac in 2 patients and the cause of death was unknown in 2 patients.

The event free survival rate for the primary endpoint was 93.2% in the total GP group. Univariable Cox regression analysis revealed that age, history of a malignancy or stroke, not using a ACE-i/AT2-antagonist or aspirin, an impaired LVEF, a MR grade ≥ 2 and multivessel disease during pPCI were significant predictors for 5-year all-cause mortality. After multivariable analysis, age, not using a ACE-i/AT2-antagonist and an impaired LVEF remained statistically significant predictors for the primary endpoint (Table 2). Stratified by high and low risk GP patients, figure 2 showed that high risk GP patients (n=417) have a significant lower event free survival rate of 88.6% compared to 97.4% in the low risk GP group (n=416) (log rank <0.001).

Table 1. Patients characteristics after 1 year MISSION! follow up

Variable	GP (n=922)
Patient's characteristics	
Age, years	61.6 ± 11.7
Male gender	686 (74.4)
Current smoking	185 (20.1)
Diabetes mellitus	73 (7.9)
History of a malignancy	46 (5.0)
History of cerebrovascular disease	31 (3.3)
Medication use	
Betablocker	824 (89.4)
ACE-inhibitor/AT2-antagonist	877 (95.1)
Statin	887 (96.2)
Aspirin	859 (93.1)
Coumarin	40 (4.3)
Laboratory results	
Total cholesterol (mmol/L)	4.14 ± 0.92
LDL-cholesterol (mmol/L)	2.39 ± 0.75
HDL-cholesterol (mmol/L)	1.34 ± 0.42
Triglycerides (mmol/L)	1.54 ± 0.82
Echocardiographic parameters	
Left ventricular ejection fraction <45%	70 (7.6)
Mitral regurgitation grade ≥ 2	31 (3.4)
Wall motion score index	1.13 (1.00-1.25)
Clinical characteristics	
Number of vessel disease during pPCI >1[a]	451 (48.9)
Complete revascularisation during pPCI	560 (60.7)
Interventions	
Revascularization within 1 year FU	122 (13.2)

Data are expressed as number (%), mean ± standard deviation or median with interquartile range. Abbreviations: GP, general practitioner; ACE, angiotensin converting enzyme; AT, angiotensin; LDL, low density lipoprotein; HDL, high density lipoprotein; [a]A narrowed

coronary artery was defined as a stenosis of ≥ 50% on baseline coronary angiogram; FU, follow-up; pPCI, primary percutaneously coronary intervention

Table 2. Univariable and multivariable Cox proportional hazard regression analysis to identify independent predictors of 5-year all-cause mortality

Parameter	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, y	1.085 (1.056-1.115)	<0.001	1.071 (1.040-1.108)	<0.001
Male gender	0.973 (0.506-1.870)	0.935	1.441 (0.678-3.064)	0.342
Current smoker	1.446 (0.757-2.764)	0.264		
Diabetes mellitus	1.725 (0.733-4.057)	0.212		
Comorbidities				
History of malignancy	2.812 (1.195-6.615)	0.018	1.896 (0.704-5.104)	0.205
History of cerebrovascular disease	3.359 (1.330-8.480)	0.010	1.077 (0.388-2.987)	0.887
Current medication use				
Betablocker	0.493 (0.230-1.054)	0.065	0.498 (0.221-1.124)	0.093
ACE-inhibitor/AT2-antagonist	0.301 (0.119-0.760)	0.011	0.294 (0.110-0.788)	0.015
Statin	0.627 (0.152-2.586)	0.519		
Aspirin	0.424 (0.180-0.998)	0.049	0.831 (0.327-2.116)	0.698
Coumarin	2.002 (0.718-5.584)	0.185		
Echocardiographic parameters				
Left ventricular ejection fraction <45%	3.088 (1.493-6.388)	0.002	2.807 (1.298-6.071)	0.009
Mitral regurgitation grade ≥ 2	3.712 (1.465-9.406)	0.006	1.747 (0.642-4.755)	0.275
Wall motion score index	1.655 (0.638-4.349)	0.307		
Clinical characteristics				
Number of vessel disease during pPCI >1[a]	2.043 (1.143-3.797)	0.017	1.540 (0.676-3.512)	0.304
Complete revascularisation during pPCI	0.585 (0.330-1.036)	0.066	1.041 (0.482-2.251)	0.918
Intervention				
Revascularization within 1 year FU	1.302 (0.610-2.782)	0.501		

Data are expressed as hazard ratios with 95% confidence interval

Abbreviations: CHD, cardiac heart disease; ACE, angiotensin converting enzyme; AT, angiotensin; pPCI, primary percutaneously coronary intervention; FU, follow up

Table 3. Univariable and multivariable Cox proportional hazard regression analysis to identify independent predictors of 5-year MACE

Parameter	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, y	1.016 (1.002-1.030)	0.029	1.008 (0.991-1.026)	0.370
Male gender	1.179 (0.797-1.745)	0.409	1.374 (0.862-2.189)	0.181
Current smoker	1.460 (1.010-2.109)	0.044	1.788 (1.190-2.687)	0.005
Diabetes mellitus	1.739 (0.683-4.432)	0.246		
Comorbidities				
History of malignancy	1.778 (0.985-3.210)	0.056	1.534 (0.765-3.074)	0.228
History of cerebrovascular disease	1.788 (0.911-3.510)	0.091	1.362 (0.639-2.902)	0.424
Current medication use				
Betablocker	0.830 (0.49441.396)	0.483		
ACE-inhibitor/AT2-antagonist	0.659 (0.323-1.345)	0.252		
Statin	1.369 (0.436-4.296)	0.590		
Aspirin	0.529 (0.310-0.904)	0.020	0.381 (0.093-1.557)	0.179
Coumarin	1.757 (0.950-3.249)	0.073	0.562 (0.117-1.269)	0.471
Echocardiographic parameters				
Left ventricular ejection fraction <45%	1.987 (1.226-3.221)	0.005	1.649 (0.936-2.907)	0.083
Mitral regurgitation grade ≥ 2	2.759 (1.488-5.115)	0.001	2.463 (1.247-4.867)	0.009
Wall motion score index	0.870 (0.473-1.600)	0.654		
Clinical characteristics				
Number of vessel disease during pPCI >1	1.666 (1.194-2.325)	0.003	1.321 (0.794-2.197)	0.284
Complete revascularisation during pPCI	0.665 (0.478-0.926)	0.016	0.802 (0.490-1.314)	0.381
Intervention				
Revascularization within 1 year FU	1.074 (0.677-1.704)	0.763		

Data are expressed as hazard ratios with 95% confidence interval

Abbreviations: CHD, cardiac heart disease; ACE, angiotensin converting enzyme; AT, angiotensin; pPCI, primary percutaneously coronary intervention; FU, follow up

Long-term MACE analysis

In total, 147 reached the secondary endpoint. A recurrent myocardial infarction occurred in 36 patients, in 51 cases a patient was revascularized, 42 patients died, 15 patients had a cerebrovascular event, in 2 patients an ICD or PM was implanted and 1 patient was admitted for heart failure. In total, 80.2% remained event free after 5 years for the secondary endpoint. Table 3 demonstrates the univariable and multivariable predictors for MACE within 5 years. Patients with an unfavorable outcome according to the univariate Cox regression analysis were patients with an older age, current smoking, not using aspirin, a lower LVEF, a MR grade ≥ 2, multivessel disease during pPCI. Patients who underwent complete revascularization during pPCI had a favorable outcome in the univariate analysis. Current smoking and a MR grade ≥ 2 remained significant predictors for MACE after multivariable Cox regression analysis. Figure 3 showed the Kaplan-Meijer curves of the patients stratified by high and low risk GP patients. High risk GP patients (n=387) reached the secondary endpoint in 73.8% of the cases, compared to 88.3% in the low risk GP group (n=387) (log-rank <0.001).

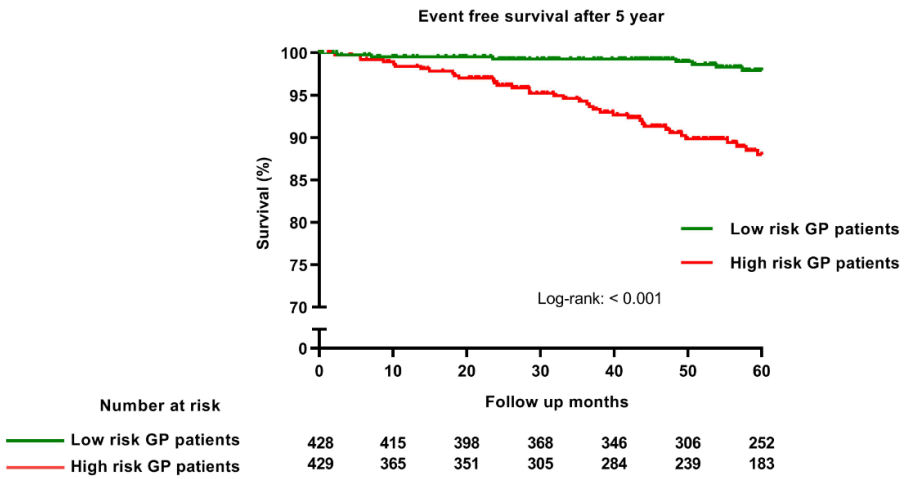

Figure 2. Kaplan-Meier analysis to evaluate the event free survival of experiencing the primary endpoint of 5-year all-cause mortality, stratified by high and low risk GP patients

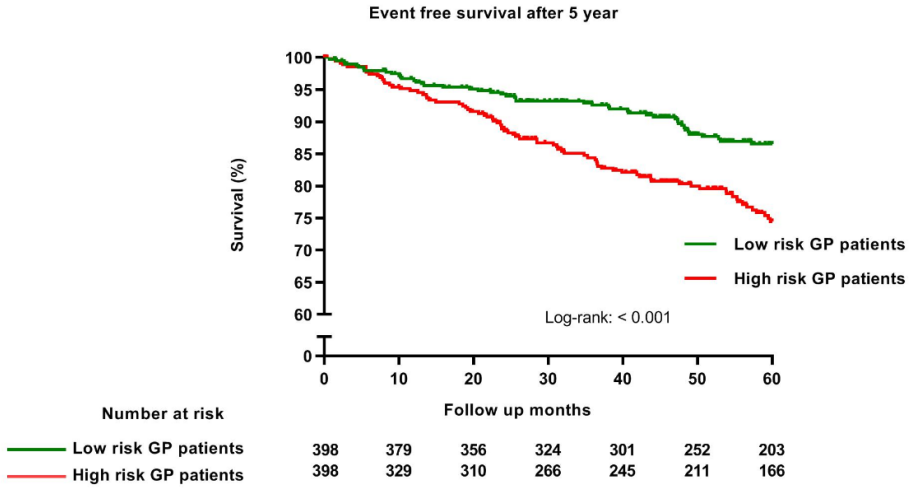

Figure 3. Kaplan-Meier analysis to evaluate the event free survival of experiencing the secondary endpoint of MACE, stratified by high and low risk GP patients

Discussion

In the present study, it is demonstrated that STEMI patients treated with pPCI and referred to the GP after being treated according to the 1-year MISSION! MI protocol have an excellent prognosis with a 5-year event free survival rate for all-cause mortality of 93.2% after 1 year MISSION! MI follow-up. Furthermore, 4 out of 5 patients remained event-free for MACE after they were referred to the GP. In an era of growing economic pressure in healthcare, identifying low risk STEMI patients, can improve patient tailored care and could reduce healthcare costs. Since there are no recommendations in international guidelines for the appropriate duration of follow-up in the outpatient clinic after a STEMI, this 1-year period might be applied in future guidelines.

The decision whether STEMI patients can be referred to the GP in the MISSION! MI protocol is mainly based on the LVEF measured after 1-year MISSION! MI follow-up. An impaired LV function in STEMI patients is strongly associated with worse outcome,[21, 22] and an EF of 45% seems to be a good discriminator between high and low risk patients.[21, 25]

There are several reasons in this study that support the idea that patients can be referred to the GP after 1-year MISSION! MI follow-up. First, in line with other large registry studies,[7-9] this study shows that after the first year after STEMI, the yearly risk of death decreases. In the current analysis, the annual mortality rate was slightly more than 1% after 1 year MISSION! MI follow-up. Secondly, cardiac death was observed in a very small number of patients. In the majority of the patients, 37 out of 48 patients, the cause of death was of non-cardiac origin,

mainly malignancies and pulmonary diseases, which is in line with results found by Pedersen et al.[8] Furthermore, according to the Central Bureau of Statistics (CBS) in the Netherlands the chance to survive for the next 5-year for a 61-year old healthy individual, which is the average age of the cohort referred to the GP, is 95.8%.[26] This is only slightly better than the observed risk of STEMI patients referred to the GP.

All patients in this study were treated according to the institutional MISSION! MI protocol. Other studies confirmed earlier that the extent of guideline implementation is associated with improved outcome.[27-29] The MISSION! MI protocol contains one structured patients-centered framework with a pre-hospital, in-hospital and an outpatient phase. An important part of the outpatient phase is to emphasize the need for drug compliance and is the education on and modification of lifestyle behavior. For example, a high percentage of patients still used their medication, prescribed during admission, after 1 year follow-up. Several studies indicated the importance of medication adherence that prevent CVD in patients with an AMI [30] which is associated with positive health outcomes.[30] Another possible explanation for the low event rate is the well-organized primary care in the Netherlands. In the region 'Zuid-Holland Noord' which also covers the Leiden region, the GP's use a uniform CVRM care program for all patients with CVD.[31] In this program, patients routine follow-up is performed by nurse specialists in primary care who monitor patients risk factors and adjust if necessary.

Several risk factors for a worse outcome were identified during this study. As this uncontrolled, observational study reflects the situation in the daily practice minor protocol deviations can be expected. A small percentage of patients referred to the GP had an LVEF < 45% or a mitral regurgitation grade ≥ 2. These patients were at higher risk of developing an adverse event. These results emphasize the importance that these patients stay in the outpatient clinic of a cardiologist where closer follow-up is available and where, for example additional treatment such as heart failure medication can get started when indicated or potentially the need for cardiac resynchronization therapy (CRT), ICD implantation or left ventricle reconstruction can be considered. Furthermore, current smoking and not using an ACE-inhibitor were identified as risk factors for developing an adverse event, which most likely reflects a surrogate marker for overall healthy behavior.[30] Before these patients are referred to the GP, addressing these issues to the GP are of importance.

There are several limitations that should be pointed out. First, since this is a retrospective observational single center study, with patients treated according to the MISSION! MI protocol, it is difficult to expand these results to other hospitals or countries. Secondly, this study may have introduced bias since a substantial part of the patients was referred to the referring hospital after treatment with a pPCI. However, these patients were not referred due to

medical reasons but due to logistic reasons such as lack of available space for patients to admit or patient's preference. So, a random cohort of patients was referred to the referring hospital thereby preventing selection bias. Thirdly, next to the LVEF, the presence of symptoms was a criterium to keep patients in the outpatient clinic of the cardiologist. In this study, no detailed information about patients' symptoms was available. Although it is not unquestionable, it is unlikely that there is a large proportion of patients with serious complaints referred to the GP since the number of adverse events in the GP group in the first year after referral was 4.4%. At last, this project did not focus on the 50% of the patients who stayed in the outpatient clinic of the cardiologist. Perhaps, amongst this group, there are patients who should considered to be low risk as well and could be referred to the GP. Future research is needed to optimize and identify all the patients eligible for referral to the GP after being treated according to the 1-year MISSION! MI protocol and should evaluate the possibilities the refer stable patients after a STEMI within one year to the GP as is already suggested in 2005 by Boomsma et al.[32]

In conclusion, STEMI patients referred to the GP after 1 year MISSION! MI follow-up have an excellent prognosis for 5-year survival and have a low risk for MACE. Patients with an impaired LV function or a mitral regurgitation grade ≥ 2 should be considered as higher risk patients and should stay in the outpatient clinic of a cardiologist.

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Chapter 4.

Apolipoprotein A1, B and apoB/apoA1 ratio are associated with first ST-segment elevation myocardial infarction but not with recurrent events during long term follow-up

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Abstract

Introduction
The current way to assess the risk of cardiovascular disease (CVD) is to measure conventional lipid and lipoprotein cholesterol fractions. Despite the success of statin treatment residual cardiovascular risk remains high. Therefore, the value of extensive serum apolipoprotein (apo) profiling to assess the risk of ST-segment elevation myocardial infarction (STEMI) and of major adverse cardiac events (MACE) in patients with STEMI was investigated in a case control design.

Methods and Results
Serum apo levels were measured using liquid chromatography and mass spectrometry in 299 healthy individuals and 220 patients with STEMI. First, the association of apo profiles in baseline samples with risk of STEMI was examined, and secondly the association of apo profiles at baseline with risk of recurrent MACE in patients with STEMI in a longitudinal study design was studied. High baseline (>1.25g/L) apoA1 levels were associated with a decreased risk of STEMI (odds ratio (OR) 0.17; 95% CI: 0.11-0.26) whereas high apoB (>1.00 g/L) levels (OR 2.17; 95% CI: 1.40-3.36) and apoB/apoA1 ratio (OR per 1 SD (OR/SD): 2.16; 95% CI: 1.76-2.65) were associated with an increased risk. Very-low-density-lipoprotein (VLDL) -associated apos gave conflicting results. Neither conventional lipid levels nor apo levels were associated with MACE in the STEMI group.

Conclusion
In conclusion, apoA1, apoB and apoB/apoA1 were strongly associated with risk of STEMI. No clear relation between VLDL-associated apos and the risk of STEMI was found. Neither baseline serum apos nor lipids predicted MACE in statin-treated patients during long-term follow-up after a first STEMI.

Introduction

Identification of patients at high risk for developing ST-segment elevation myocardial infarction (STEMI) is essential. Dyslipidemia in these patients is one of the factors that impair their long-term clinical outcome.(1-4) The current way to assess risk of cardiovascular disease (CVD) and its complications such as acute myocardial infarction (AMI), is, amongst others, quantification of total cholesterol (TC), LDL-cholesterol (LDLc), HDL-cholesterol (HDLc), and triglycerides (TG) concentrations in serum. Notwithstanding the success of statin treatment for reaching treatment goals, the residual cardiovascular risk being about 70% remains remarkably high.(5-7) The R3I initiative aims to explain this residual cardiovascular risk by looking for improved diagnostic, prognostic and therapeutic biomarkers, beyond traditional risk factors.(8)

The measurement of functional and structural protein components of lipoproteins, *i.e.* apolipoprotein (apos), is suggested to have additional value for coronary artery disease (CAD) risk assessment.(9-15) It has been suggested that apoB is a better marker of CAD risk than LDLc (9-11) and superior to non-HDLc. (14, 16) Furthermore, investigators from the INTERHEART study have shown that the apoB/apoA1 ratio was a better risk marker of AMI than the TC/HDLc ratio.(13) Recently, Pechlaner et al. presented in the Bruneck study new data about the relation of very-low-density-lipoprotein (VLDL) -associated apos, *i.e.* apoCII, apoCIII and apoE, with incident CVD. These apos were found to be strong predictors of CVD.(17) The association of apoCIII with incident CVD was further corroborated by Van Capelleveen et al.(18) in a nested case-control study of the EPIC-Norfolk cohort. As residual cardiovascular risk remains high(8) even after successful treatment of traditional risk factors, a case-control study with long-term follow-up of patients with STEMI was executed to evaluate the value of extensive serum apo profiling for (1) prediction of STEMI, and for (2) prediction of recurrent major adverse cardiac events (MACE) in patients with STEMI. The aim of this study was to focus on quantifying serum apoA1, apoB, apoCI, apoCII, apoCIII and apoE, beyond serum lipids and lipoprotein cholesterol fractions. A previously developed method for quantitative serum apo profiling using liquid chromatography (LC) and mass spectrometry (MS), which has proven to be highly accurate and in concordance with quality requirements for medical tests, independent of the presence of hypertriglyceridemia, was used.(19)

Methods

Study design

In the current study, the lipid and apo profiles in patients with STEMI from the MISSION! Intervention Trial (20) were compared with those of random digit dialing (RDD) controls from the Dutch Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, a population

based study on risk factors for venous thrombosis.(21) Secondly, in patients with STEMI, the risk of recurrent MACE in a longitudinal study design was evaluated.

Study participants
Patients with first STEMI admitted to the Leiden University Medical Center between February 2004 and October 2006 and included in the MISSION! Intervention Trial were included as cases. The study cohort consisted of 297 consecutive STEMI patients treated with primary percutaneous coronary intervention (pPCI). STEMI was defined as ongoing chest pain (>30 min), accompanied with ST-elevation (≥0.2 mV in ≥2 leads in V1-V3 or ≥0.1 mV in other leads) or presumed new left bundle branch block and a typical rise of high-sensitivity cardiac troponin-T (hs-cTnT). In case of out-of-hospital cardiac arrest, only patients with return of spontaneous circulation at the moment of arrival at the catheterization laboratory were included. Patients with prior AMI (n=11), prior PCI (n=3), and/or prior coronary artery bypass grafting (CABG) (n=1) were excluded. A total of 5 patients had no available frozen serum samples for quantitative serum apo profiling. Since in the MEGA study only controls were included who were <70 years, MISSION patients who were >70 years were excluded in this logistic regression analysis (n=57). So, in total 220 cases were included. During the study, all patients were treated according to the institutional MISSION! protocol, (22) based on guidelines of the European Society of Cardiology, American College of Cardiology, and the American Heart Association.(23, 24) The MISSION! protocol contains a standardized pre-hospital, in-hospital and outpatient clinical framework to optimize treatment. One of the in-hospital MISSION! performance indicators were to administrate statin therapy (rosuvastatin 10mg) within 24 hours after admission. In the outpatient phase patients visited the outpatient clinic 4 times during the first year after STEMI. LDLc treatment goals at that time were <2.5 mmol/L according to the guidelines at the time. Based on patients LDLc levels statin therapy was adjusted accordingly to reach the treatment goals.

Controls were individuals who participated in the control arm of the MEGA study.[19] This case control study included 4956 consecutive patients aged 18 to 70 years with a first deep vein thrombosis or pulmonary embolism, between March 1999 and September 2004. Partners of patients and individuals identified by RDD were asked to participate as controls. In total, 6,297 controls (3,297 partners and 3,000 by random digit dialing) were included.

Of 3,000 enrolled RDD controls, a random sample of 300 individuals was drawn for apo profile analysis and an extensive questionnaire including a list of potential risk factors for CVD. One RDD control was excluded because of a technical failure. No other exclusion criteria were applied to RDD controls.

Data collection
Data of each MISSION! patient are systematically collected in the electronic patient file (EPD-VISION, Leiden) using a unique identification number. Of interest for the present analysis are the items on age, sex and statin use at time of blood draw. Body mass index (BMI) was calculated by dividing weight (in kg) by height squared (m^2). A BMI between 18.5 and 25 kg/m^2 was defined as normal, between 25 and 30 kg/m^2 as overweight, and ≥30 kg/m^2 as obese. Patients using statins at the time of blood withdrawal were regarded as statin users.

Follow- up in the MISSION! study
Information on all-cause mortality was obtained from the Dutch Municipality Records registry. Cause of death was retrieved from general practitioners. Clinical follow-up data was collected during the 30 days, 3, 6 and 12 months outpatient clinic visits. Follow-up data on serious adverse events including myocardial infarction, revascularisation and stroke was obtained by telephone interviews at 2, 5 and 10 years after admission.

MACE was defined as the combined endpoint of 10-year clinical outcome, including death, AMI, revascularisation (PCI or CABG) and stroke.

Blood collection and laboratory analysis
In the MISSION Intervention Trial, baseline blood samples were obtained at presentation (immediately before the PCI procedure was performed). The median time between onset of symptoms and blood withdrawal was 180 (IQR 120-252) min. Standard lab included non-fasting TC, HDLc, and TG, which were analysed directly. These levels enabled calculation of LDLc. An extra serum sample was coagulated for at least 60 min before centrifugation at 1500xg for 10 min at a temperature below 18°C. Sera were pipetted into 1.1 mL Micronic tubes. Within 2 h after vena puncture, the serum samples were frozen in a -70/-80°C freezer. To determine the apo profile, a mass spectrometric method was developed for multiplexed quantification of six serum apos (apoA1, apoB, apoCI, apoCII, apoCIII, and apoE), including apoE phenotyping (19). In contrast to classical HDLc and LDLc tests and lipoprotein particle counting methods, the quantitative proteomics test allows adequate quantification of unequivocally characterized apos with an analytical performance that meets test requirements derived from biological variation. Apo quantification by liquid chromatography (LC) – mass spectrometry (MS/MS) starts with solubilisation and denaturation of serum proteins before enzymatic digestion that generates signature peptides for the intact serum proteins. The peptides in the serum digest are separated by LC and detected by tandem MS/MS.

In MEGA controls, TC, TG, HDLc, apoA1 and apoB were measured on stored (-80 °C) fasting serum samples. In 2015 apoA1 and apoB were measured with immunoturbidimetric tests on routine clinical chemistry analysers. In 2017 the complete apo profile (apoA1, apoB, apoCI, apoCII, apoCIII and apoE, plus apoE

phenotyping) was measured in the same stored fasting serum samples that were thawed twice. Since apoA1 and apoB were measured twice, *i.e.* both in once and twice thawed sera, Bland-Altman plots and scatter plots were used to evaluate potential systematic error due to freeze-thawing once or twice. Results showed a mean difference of 0.09 g/L (95% CI -0.12 to 0.30 g/L) for apoA1 and 0.08 g/L (95% CI -0.08 to 0.23 g/L) for apoB, and r^2 of 0.89 and 0.93, respectively. Since no systematic error appeared to be present, it was considered likely that freezing and thawing serum samples did not influence levels of apo profiles in MEGA.

Frozen storage of the serum samples was ensured by continuous, on-line temperature registration of the freezers.

TC and TG were measured by a colorimetric method (CHOD-PAP for TC and GPO-PAP for TG) on a Modular P analyser (Roche Diagnostics, Indianapolis, IN). HDLc was measured by a direct method based on the Kyowa Medex reaction principle using polyethylene glycol-modified enzymes (Roche Diagnostics, Indianapolis, IN). LDLc levels were calculated using the Friedewald formula [LDLc = TC - HDLc - (TG/2.2) for mmol/L]. If TG exceeded 4.52 mmol/L, LDLc was not calculated. In the MEGA study the apo profile was determined similarly as in the MISSION! cohort. Remnant cholesterol concentration was calculated by TC - LDLc – HDLc.

Cut off points for lipid and apo profiles

The definitions of Nordestgaard et al. for abnormal lipid or apo levels in non-fasting state were used: TC 5.0 mmol/L, LDLc 3.0 mmol/L, HDLc 1.0 mmol/L, TG 2.0 mmol/L, remnant cholesterol 0.9 mmol/L, non-HDLc 3.9 mmol/L, apoA1 1.25 g/L, and apoB 1.0 g/L.(25) Since these cut-off points were not known for apoCI, apoCII, apoCIII and apoE, quartile cut-off points of both conventional lipids and apos were used. One prior study analysed new apo profiles by a per standard deviation (SD) increase in controls,(17) which was also performed in the current study to evaluate whether this would yield similar results.

Statistical analysis

Continuous variables with a normal distribution are presented as mean ± standard deviation (SD). Not-normally distributed data are presented as medians and interquartile range (IQR). The Mann-Whitney U-test was used to test differences between two groups of not-normally distributed data. Lipid/apo profiles were log-transformed if not normally distributed. Categorical variables are expressed as numbers and percentages.

To study determinants of conventional lipid and apo profiles in the general population, simple and multiple linear regression analyses were performed in the group of healthy individuals. To determine the association of various lipid and apo profiles with (1) risk of STEMI, and (2) risk of MACE in patients with STEMI, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated,

and were adjusted for age, sex, and statin use by logistic regression methods. Model 1 in table 3 is defined as the OR for the risk of STEMI adjusted for age, sex and statin use. Model 1 – statin users is the same model but with statin users excluded.

Duration of follow-up was counted from time of first STEMI to end of follow-up, defined as 10-year follow-up, the date of a MACE, or lost to follow-up, whichever occurred first. Incidence rates of MACE or death were estimated as the number of events over the accumulated follow-up time. Cox-proportional hazards models were used to evaluate risks between groups, and were progressively adjusted for age, sex, and statin use. In all regression analyses, a preplanned sensitivity analysis was performed in which statin users were excluded at the time of blood draw (n=40), as statin use do affect conventional lipid levels and apo profiles. To evaluate changes over time in lipid levels within patients a paired T-test or a Wilcoxon signed rank test was performed when appropriate. All statistical tests were 2-tailed, p-values <0.05 were considered statistically significant. All statistical analyses were performed with SPSS for Windows, version 24.0 (SPSS Inc., IBM, Armonk, NY).

Results

In total, 220 STEMI patients and 299 control subjects were eligible for this study. As expected, patients in the STEMI group were older (mean age 55.0 ± 9.37 y) than RDD controls (mean age 47.5 ± 13.1 y) in the control group. Patients with STEMI were more often men as compared with the control group (78.6% and 46.2%, respectively). Overall, the STEMI group had a lipid profile consistent with an increased cardiovascular risk. Whereas TC and LDLc levels did not differ between the two groups, HDLc was significantly lower and TG and remnant cholesterol were significantly higher in the STEMI group. ApoA1 was significantly lower in the STEMI group than in the control group (1.24 ± 0.24 g/L vs 1.53 ± 0.31 g/L, respectively, p<0.001) and apoB was significantly higher in the STEMI group than in the control group (1.18 ± 0.25 g/L vs 1.08 ± 0.29 g/L, respectively, p<0.001). All baseline characteristics are summarized in Table 1.

Table 2 shows the association of conventional lipids and apos with cardiovascular risk factors (age, gender, smoking, obesity and statin use) in control subjects. In these individuals, older age was correlated with significantly higher levels of almost all lipid and apo levels. Female controls had a slightly more favorable apo profile with a significantly higher apoA1 and lower apoB, than male controls. Furthermore, smoking and obesity were associated with significantly higher levels of apoB, but not with significantly higher LDLc levels.

Table 3 demonstrates the OR of STEMI for various lipid and apo profiles. These are shown as high versus low levels, divided per quartile and per 1-SD increase.

ORs were adjusted for age, gender, and statin use (model 1). Figure 1 illustrates the association of risk of STEMI per 1-SD increase for each lipid or lipoprotein, in which statin users are excluded (Model 1 – statin users). TC and LDLc showed no association with the risk of STEMI. The adjusted OR for high HDLc was 0.51 (95% CI 0.32-0.82) which was similar in the group without statin users. High remnant cholesterol levels were significantly associated with risk of STEMI with an OR of 1.61 (95% CI 1.03-2.51). The OR per SD increase (0.36 mmol/L) was 1.21 (95% CI 1.03-2.42).

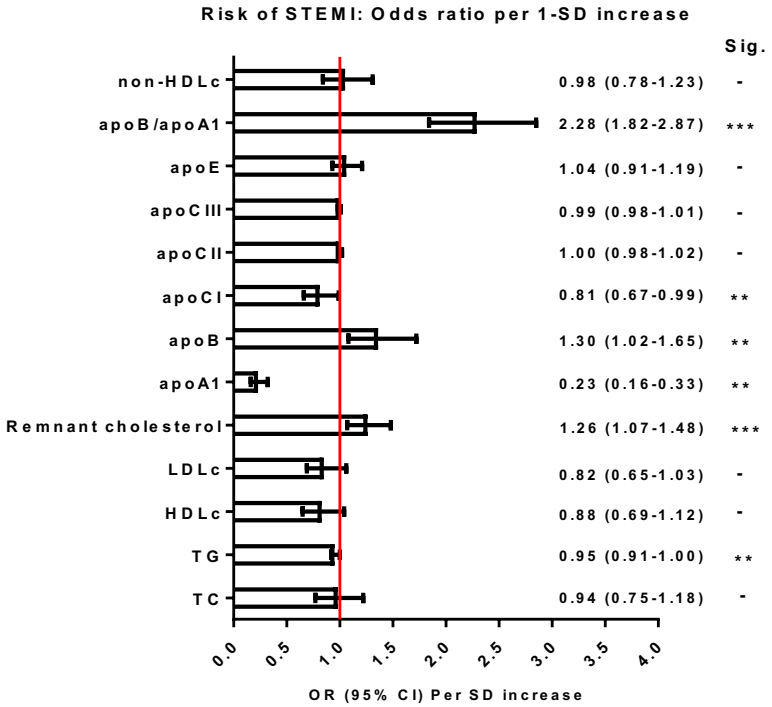

Fig 1 Odds ratio for a 1-SD higher (95% CI) risk of STEMI for each individual lipid marker. The risk was adjusted for age and sex. Statin users were excluded. Significance: ***p<0.001, **p<0.05, -p>0.05

1 SD corresponds to: TC, 1.10 mmol/L; TG, 0.81 mmol/L; HDLc, 0.40 mmol/L; LDLc, 1.00 mmol/L; remnant cholesterol, 0.36 mmol/L; apoA1, 0.31 g/L; apoB, 0.29 g/L; apoCI, 5.33 mg/L; apoCII, 27.64 mg/L; apoCIII, 37.12 mg/L; apoE, 13.04 mg/L; apoB/apoA1, 0.25; non-HDLc, 1.13 mmol/L. Apo = apolipoprotein; OR = Odds Ratio; STEMI = ST-segment elevation myocardial infarction; SD = Standard deviation; TC = Total cholesterol; TG = Triglycerides; HDLc = high-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol; Apo = apolipoprotein

Table 1. Baseline characteristics table.

	Patients (n=220)	Controls (n=299)	p-value
Mean age, y (SD)	55.0 (9.37)	47.5 (13.1)	<0.001
Men, n (%)	173 (78.6)	138 (46.2)	<0.001
Statin use at blood draw, n (%)	18 (8.2)	22 (7.4)	0.717
Mean BMI, kg/m^2 (SD)	26.63 (3.98)	25.32 (4.24)	<0.001
Normal weight, n (%)	87 (40.1)	153 (52.4)	0.016
Overweight, n (%)	91 (41.9)	104 (35.6)	0.028
Obesity, n (%)	39 (18.0)	35 (12.0)	0.012
Smoking history, n (%)	139 (63.2)	174 (58.8)	0.312
Cholesterol, mmol/L, mean (SD)	5.63 (1.09)	5.54 (1.10)	0.333
Triglycerides, mmol/L, median (IQR)	1.53 (1.01-2.22)	1.23 (0.97-1.88)	0.002
HDLc, mmol/L, mean (SD)	1.27 (0.36)	1.37 (0.40)	0.004
LDLc, mmol/L, mean (SD)	3.48 (0.94)	3.49 (1.00)	0.912
Remnant cholesterol, mmol/L, mean (SD)	0.88 (0.72)	0.68 (0.36)	<0.001
ApoA1, g/L, mean (SD)	1.24 (0.24)	1.53 (0.31)	0.001
ApoB, g/L, mean (SD)	1.18 (0.25)	1.08 (0.29)	<0.001
ApoCI, mg/L, mean (SD)	20.03 (7.82)	21.38 (5.33)	0.020
ApoCII, mg/L, median (IQR)	36.10 (20.99-58.71)	34.32 (19.38 -55.24)	0.440
ApoCIII, mg/L, median (IQR)	86.89 (65.48-117.08)	97.33 (77.56-119.90)	0.002
ApoE, mg/L, mean (SD)	33.15 (26.6)	30.71 (13.04)	0.170
Ratio apoB/apoA1, mean (SD)	1.00 (0.29)	0.74 (0.25)	0.001
Non-HDLc, mmol/L, mean (SD)	4.36 (1.07)	4.17 (1.13)	0.050

Categorical variables expressed by number (%)

Numerical variables expressed by mean (SD) or median (IQR)

Comparisons between groups were made using chi-square test for categorical variables and independent T-test or Mann-Whitney U test for continuous variables. Abbreviations: BMI, Body mass index; HDLc, High density lipoprotein-cholesterol; LDLc, Low density lipoprotein-cholesterol; Apo, Apolipoprotein.

High apoA1 levels were strongly associated with risk of STEMI with an OR of 0.17 (95% CI 0.11-0.26). Per SD increase of apoA1 (0.31 g/L) the OR was 0.24 (95% CI 0.18-0.33). These results were similar in the group without statin users. High apoB levels were also associated with risk of STEMI with an OR of 2.17 (95% CI 1.40-3.36). This effect appeared to be even stronger in the group without statin users (OR 2.46; 95%CI 1.53-3.95). Per SD increase of apoB (0.29 g/L), the OR of STEMI was 1.27 (95% CI 1.02-1.57) in the total group, and 1.36 (95% CI 1.08-1.72) in the group without statin users. High baseline apoB/apoA1 ratios were also associated with STEMI risk. The OR per SD increase (0.25) was 2.16 (95% CI 1.76-2.65) in the total group and 2.29 (95% CI 1.84-2.85) in the group without statin users.

Table 2. Association of lipid and apo profiles with cardiovascular risk factors in control subjects.

	Age		Sex		Smoking history		Obesity		Statin use	
	< 50 y	> 50 y	Men	Women	No	Yes	No	Yes	No	Yes
Cholesterol										
Mean level, mmol/L	5.22	5.93	5.59	5.50	5.34	5.68	5.54	5.64	5.56	5.22
Mean difference (95% CI)	Ref.	0.71 (0.47 to 0.95)***	Ref.	-0.09 (-0.34 to 0.17)¯	Ref.	0.33 (0.08 to 0.59)**	Ref.	0.11 (-0.28 to 0.50)¯	Ref.	-0.34 (-0.82 to 0.14)¯
Mean difference (95% CI)*	Ref.	0.77 (0.52 to 1.02)***	Ref.	-0.05 (-0.29 to 0.19)¯	Ref.	0.22 (-0.03 to 0.47)¯	Ref.	0.13 (-0.24 to 0.50)¯	Ref.	-0.69 (-1.18 to -0.20)**
Mean difference(95% CI)†	Ref.	0.79 (0.54 to 1.04)***	Ref.	-0.08 (-0.33 to 0.16)¯	Ref.	0.20 (-0.05 to 0.48)¯	Ref.	0.14 (-0.25 to -0.52)¯	NA	
Triglycerides, mmol/L										
Mean level, mmol/L	1.41	1.63	1.60	1.44	1.37	1.60	1.49	1.68	1.50	1.70
Mean difference (95% CI)	Ref.	0.21 (0.03 to 0.40)**	Ref.	-0.15 (-0.34 to 0.03)¯	Ref.	0.23 (0.04 to 0.42)**	Ref.	0.19 (-0.10 to 0.48)¯	Ref.	0.19 (-0.16 to 0.55)¯
Mean difference (95% CI)*	Ref.	0.14 (-0.06 to 0.33)¯	Ref.	-0.13 (-0.32 to 0.06)¯	Ref.	0.21 (0.02 to 0.40)**	Ref.	0.21 (-0.08 to 0.49)¯	Ref.	0.12 (-0.26 to 0.051)¯
Mean difference(95% CI)†	Ref.	0.15 (-0.05 to 0.35)¯	Ref.	-0.14 (-0.34 to 0.06)¯	Ref.	0.17 (-0.03 to 0.38)¯	Ref.	0.23 (-0.08 to 0.54)¯	NA	
HDLc, mmol/L										
Mean level, mmol/L	1.34	1.41	1.23	1.49	1.42	1.34	1.39	1.22	1.37	1.26
Mean difference (95% CI)	Ref.	0.07 (-0.02 to 0.16)¯	Ref.	0.26 (0.17 to 0.35)***	Ref.	-0.08 (-0.17 to 0.02)¯	Ref.	-0.17 (-0.31 to -0.03)*	Ref.	-0.09 (-0.26-0.08)¯
Mean difference (95% CI)*	Ref.	0.13 (0.04 to 0.22)**	Ref.	0.27 (0.18 to 0.35)***	Ref.	-0.07 (-0.16 to 0.02)¯	Ref.	-0.20 (-0.34 to -0.07)*	Ref.	-0.09 (-0.26 to 0.09)¯
Mean difference(95% CI)†	Ref.	0.12 (0.03 to 0.22)**	Ref.	0.26 (0.17 to 0.35)***	Ref.	-0.05 (-0.15 to 0.04)¯	Ref.	-0.19 (-0.33 to -0.05)*	NA	
LDLc, mmol/L										
Mean level, mmol/L	3.24	3.79	3.64	3.36	3.31	3.61	3.47	3.67	3.51	3.18
Mean difference (95% CI)	Ref.	0.55 (0.33 to 0.76)***	Ref.	-0.27 (-0.50 to -0.05)**	Ref.	0.30 (0.07 to 0.53)**	Ref.	0.19 (-0.16 to 0.55)¯	Ref.	-0.34 (-0.77 to 0.10)¯
Mean difference (95% CI)*	Ref.	0.58 (0.35 to 0.81)***	Ref.	-0.26 (-0.48 to -0.04)**	Ref.	0.20 (-0.03 to 0.42)¯	Ref.	0.24 (-0.10 to 0.58)¯	Ref.	-0.66 (-1.11 to -0.22)¯

table continues

	Age		Sex		Smoking history		Obesity		Statin use	
	< 50 y	> 50 y	Men	Women	No	Yes	No	Yes	No	Yes
Mean difference(95% CI)†	Ref.	0.59 (0.37 to 0.82)***	Ref.	-0.28 (-0.50 to -0.06)**	Ref.	0.18 (-0.05 to 0.40)¯	Ref.	0.22 (-0.13 to 0.58)¯	NA	
Remnant cholesterol										
Mean level, mmol/L	0.64	0.73	0.72	0.65	0.62	0.72	0.67	0.76	0.68	0.76
Mean difference (95% CI)	Ref	0.10 (0.01 to 0.18)**	Ref	0.07 (-0.15 to 0.01)¯	Ref	0.10 (0.02 to 0.19)**	Ref	0.09 (-0.04 to 0.22)¯	Ref	0.09 (-0.07 to 0.25)¯
Mean difference (95% CI)*	Ref.	0.06 (-0.03 to 0.15)¯	Ref.	-0.06 (-0.14 to 0.03)¯	Ref.	0.09 (0.01 to 0.18)**	Ref.	0.09 (-0.04 to 0.22)¯	Ref.	0.06 (-0.12 to 0.23)¯
Mean difference(95% CI)†	Ref.	0.07 (-0.02 to 0.16)¯	Ref.	-0.06 (-0.15 to 0.03)¯	Ref.	0.08 (-0.01 to 0.17)¯	Ref.	0.10 (-0.04 to 0.25)¯	NA	
Apo A1, g/L										
Mean level	1.50	1.58	1.42	1.63	1.56	1.52	1.54	1.49	1.54	1.48
Mean difference (95% CI)	Ref.	0.08 (0.01 to 0.15)**	Ref.	0.21 (0.14 to 0.28)***	Ref.	-0.04 (-0.11 to 0.03)¯	Ref.	0.05 (-0.16 to 0.06)¯	Ref.	-0.06 (-0.19 to 0.08)¯
Mean difference (95% CI)*	Ref.	0.12 (0.05 to 0.19)***	Ref.	0.21 (0.14 to 0.28)***	Ref.	-0.04 (-0.11 to 0.03)¯	Ref.	-0.07 (-0.17 to 0.03)¯	Ref.	-0.07 (-0.20 to 0.07)¯
Mean difference(95% CI)†	Ref.	0.12 (0.05 to 0.19)***	Ref.	0.20 (0.13 to 0.27)***	Ref.	-0.02 (-0.10 to 0.05)¯	Ref.	-0.06 (-0.17 to 0.05)¯	NA	
Apo B, g/L										
Mean level	1.02	1.16	1.14	1.04	1.02	1.13	1.07	1.19	1.09	1.06
Mean difference (95% CI)	Ref.	0.14 (0.08 to 0.21)***	Ref.	-0.09 (-0.16 to -0.03)**	Ref.	0.11 (0.04 to 0.17)**	Ref.	0.12 (0.01 to 0.22)**	Ref.	-0.02 (-0.15 to 0.10)¯
Mean difference (95% CI)*	Ref.	0.13 (0.07 to 0.20)***	Ref.	-0.09 (-0.15 to -0.03)**	Ref.	0.08 (0.02 to 0.15)**	Ref.	0.13 (0.03 to 0.23)**	Ref.	-0.11 (-0.24 to 0.02)¯
Mean difference(95% CI)†	Ref.	0.14 (0.07 to 0.21)***	Ref.	-0.10 (-0.16 to -0.03)**	Ref.	0.12 (0.02 to 0.23)**	Ref.	0.07 (0.00 to 0.14)**	NA	
Apo C-I, mg/L										
Mean level	20.23	22.77	21.30	21.44	20.80	21.78	21.35	21.40	21.37	21.53
Mean difference (95% CI)	Ref.	2.54 (1.35 to 3.72)***	Ref.	0.14 (-1.08 to 1.36)¯	Ref.	0.98 (-0.27 to 2.22)¯	Ref.	0.05 (-1.85 to 1.96)¯	Ref.	0.16 (-2.17 to 2.49)¯
Mean difference (95% CI)*	Ref.	2.65 (1.36 to 3.93)***	Ref.	0.26 (-0.98 to 1.49)¯	Ref.	0.52 (-0.74 to 1.78)¯	Ref.	-0.04 (-1.92 to 1.85)¯	Ref.	-1.02 (-3.51 to 1.48)¯
Mean difference(95% CI)†	Ref.	2.70 (1.40 to 4.01)***	Ref.	0.00 (-1.28 to 1.28)¯	Ref.	0.12 (-1.90 to 2.13)¯	Ref.	0.61 (-0.70 to 1.92)¯	NA	

table continues

	Age		Sex		Smoking history		Obesity		Statin use	
	< 50 y	> 50 y	Men	Women	No	Yes	No	Yes	No	Yes
Apo C-II, mg/L										
Mean level	31.71	49.85	45.52	35.08	35.98	42.13	38.65	47.04	38.80	53.68
Mean difference (95% CI)	Ref.	18.14 (12.15 to 24.12)***	Ref.	-10.44 (-16.65 to -4.23)***	Ref.	6.16 (-0.23 to 12.54)¯	Ref.	8.39 (-1.38 to 18.16)¯	Ref.	15.88 (2.92 to 26.83)**
Mean difference (95% CI)*	Ref.	15.31 (8.99 to 21.63)***	Ref.	-9.19 (-15.27 to -3.10)**	Ref.	2.80 (-3.42 to 9.01)¯	Ref.	7.78 (-1.51 to 17.07)¯	Ref.	5.02 (-7.28 to 17.33)¯
Mean difference(95% CI)†	Ref.	15.39 (8.94 to 21.84)***	Ref.	-9.89 (-16.22 to -3.56)**	Ref.	9.54 (-0.42 to 19.49)¯	Ref.	2.41 (-4.07 to 8.88)¯	NA	
Apo C-III, mg/L										
Mean level	95.77	113.07	102.77	104.28	99.73	105.93	103.20	107.10	102.51	117.17
Mean difference (95% CI)	Ref.	17.30 (9.03 to 25.57)**	Ref.	1.51 (-6.98 to 10.00)¯	Ref.	6.20 (-2.41 to 14.82)¯	Ref.	3.89 (-9.37 to 17.16)¯	Ref.	14.67 (-1.45 to 30.79)¯
Mean difference (95% CI)*	Ref.	14.90 (5.98 to 23.82)**	Ref.	3.80 (-4.79 to 12.38)¯	Ref.	4.69 (-4.08 to 13.45)¯	Ref.	2.42 (-10.67 to 15.52)¯	Ref.	9.02 (-8.33 to 26.40)¯
Mean difference(95% CI)†	Ref.	14.56 (5.36 to 23.77)**	Ref.	3.23 (-5.81 to 12.26)¯	Ref.	4.68 (-9.53 to 18.89)¯	Ref.	5.61 (-3.62 to 14.89)¯	NA	
Apo E, mg/L										
Mean level	27.91	34.12	30.46	30.93	30.40	30.74	30.37	32.20	30.69	30.06
Mean difference (95% CI)	Ref.	6.21 (3.31 to 9.11)***	Ref.	0.46 (-2.52 to 3.44)¯	Ref.	0.37 (-2.66 to 3.40)¯	Ref.	1.87 (-2.59 to 6.32)¯	Ref.	0.37 (-5.32 to 6.06)¯
Mean difference (95% CI)*	Ref.	5.96 (2.96 to 8.95)***	Ref.	0.46 (-2.42 to 3.35)¯	Ref.	-0.65 (-3.60 to 2.29)¯	Ref.	1.60 (-2.80 to 6.00)¯	Ref.	-2.47 (-8.30 to 3.36)¯
Mean difference(95% CI)†	Ref.	5.71 (2.75 to 8.68)***	Ref.	-0.28 (-3.18 to 2.63)¯	Ref.	2.93 (-1.65 to 7.50)¯	Ref.	-0.10 (-3.07 to 2.87)¯	NA	
Ratio ApoB/ApoA1										
Mean level, mmol/L	0.71	0.76	0.82	0.67	0.68	0.78	0.73	0.82	0.74	0.74
Mean difference (95% CI)	Ref.	0.05 (-0.01 to 0.11)¯	Ref.	-0.15 (-0.21 to -0.10)***	Ref.	0.10 (0.04 to 0.16)***	Ref.	0.09 (0.00 to 0.18)**	Ref.	0.06 (-0.11 to 0.12)¯
Mean difference (95% CI)*	Ref.	0.03 (-0.03 to 0.08)¯	Ref.	-0.15 (-0.21 to -0.09)***	Ref.	0.09 (0.03 to 0.14)**	Ref.	0.11 (0.03 to 0.20)**	Ref.	-0.04 (-0.15 to 0.08)¯
Mean difference(95% CI)†	Ref.	0.03 (-0.03 to 0.09)¯	Ref.	-0.15 (-0.21 to -0.09)***	Ref.	0.07 (0.01 to 0.13)**	Ref.	0.11 (0.02 to 0.20)**	NA	
Non HDLc, mmol/L										

table continues

	Age		Sex		Smoking history		Obesity		Statin use	
	< 50 y	> 50 y	Men	Women	No	Yes	No	Yes	No	Yes
Mean level, mmol/L	3.88	4.52	4.36	4.01	3.93	4.33	4.14	4.42	4.19	3.94
Mean difference (95% CI)	Ref.	0.64 (0.39 to 0.89)***	Ref.	-0.35 (-0.60 to -0.09)**	Ref.	0.41 (0.15 to 0.67)**	Ref.	0.28 (-0.12 to 0.68)¯	Ref.	-0.25 (-0.74 to 0.24)¯
Mean difference (95% CI)*	Ref.	0.64 (0.38 to 0.90)***	Ref.	-0.32 (-0.57 to -0.07)**	Ref.	0.29 (0.04 to 0.55)**	Ref.	0.33 (-0.05 to 0.71)¯	Ref.	-0.61 (-1.11 to -0.10)**
Mean difference(95% CI)†	Ref.	0.66 (0.40 to 0.92)***	Ref.	-0.34 (-0.60 to -0.09)**	Ref.	0.25 (-0.01 to 0.52)¯	Ref.	0.33 (-0.08 to 0.73)¯	NA	

** Multivariate adjusted for age, sex, smoking status, obesity and statin use*

† Multivariate adjusted for age, sex, smoking status and obesity, statin users excluded

*Significance: ***P≤0.001, **P≤0.05,* ¯P>0.05 *Ref. denotes reference; NA, not applicable*

Table 3. Risk of STEMI according to various levels of lipoproteins.

					Univariate analysis		Model 1		Model 1-statin users	
		Level	Cases	Controls	Odds ratio	(95%)	Adjusted odds ratio †	(95% CI)	Adjusted odds ratio#	(95% CI)
Total-c, mmol/L										
High		>5.0	159 (72)	202 (68)	1.25¯	(0.85-1.83)	0.88¯	(0.56-1.38)	0.85¯	(0.52-1.38)
Quartiles	1	<4.82	45 (21)	75 (25)	1	(reference)	1	(reference)	1	(reference)
	2	4.82-5.52	65 (30)	74 (25)	1.46¯	(0.89-2.41)	1.23¯	(0.69-2.17)	1.29¯	(0.69-2.40)
	3	5.52-6.20	49 (22)	75 (25)	1.09¯	(0.65-1.82)	0.74¯	(0.41-1.35)	0.81¯	(0.43-1.52)
	4	>6.20	61 (28)	75 (25)	1.36¯	(0.82-2.24)	0.90¯	(0.54-1.73)	1.07¯	(0.57-2.01)
Per SD increase		1.10			1.09 ¯	(0.92-1.30)	0.94¯	(0.7-1.16)	0.98¯	(0.79-1.22)
Triglycerides, mmol/L										
High		>2.0	65 (30)	60 (20)	1.67**	(1.11-2.05)	1.42¯	(0.92-2.21)	1.60**	(1.00-2.56)
Quartiles	1	<0.97	44 (20)	70 (23)	1	(reference)	1	(reference)	1	(reference)

table continues

					Univariate analysis		Model 1		Model 1-statin users	
		Level	Cases	Controls	Odds ratio	(95%)	Adjusted odds ratio †	(95% CI)	Adjusted odds ratio#	(95% CI)
	2	0.97-1.23	34 (16)	79 (26)	0.69⁻	(0.40-1.19)	0.59⁻	(0.32-1.09)	0.53⁻	(0.27-1.01)
	3	1.23-1.88	64 (29)	75 (25)	1.36⁻	(0.82-2.25)	0.93⁻	(0.53-1.63)	0.99⁻	(0.54-1.79)
	4	>1.88	78 (36)	75 (25)	1.66⁻	(1.01-2.71)	1.13⁻	(0.66-1.95)	1.21⁻	(0.68-2.16)
Per ln SD increase		0.81			0.93***	(0.90-0.97)	0.95**	(0.91-0.99)	0.94**	(0.91-0.99)
HDLc, mmol/L										
High		>1.0	159 (72)	257 (86)	0.43***	0.27 -0.66	0.51**	(0.32-0.82)	0.47**	(0.28-0.78)
Quartiles	1	<1.10	72 (33)	74 (25)	1	(reference)	1	(reference)	1	(reference)
	2	1.10-1.29	50 (23)	74 (25)	0.69-	(0.43-1.13)	0.72⁻	(0.42-1.22)	0.77⁻	(0.45-1.35)
	3	1.29-1.56	52 (24)	73 (24)	0.73-	(0.45-1.19)	0.88⁻	(0.51-1.51)	0.84⁻	(0.47-1.48)
	4	>1.56	46 (21)	78 (26)	0.61**	(0.37-0.99)	0.81⁻	(0.46-1.43)	0.73⁻	(0.40-1.33)
Per SD increase		0.40			0.76**	(0.63-0.92)	0.85⁻	(0.67-1.06)	0.83⁻	(0.65-1.04)
LDLc, mmol/L										
High		>3.0	156 (71)	196 (66)	1.28⁻	(0.88-1.87	0.86⁻	(0.55-1.34)	0.89⁻	(0.55-1.43)
Quartiles	1	<2.77	47 (21)	74 (25)	1	(reference)	1	(reference)	1	(reference)
	2	2.77-3.47	56 (26)	76 (25)	1.16⁻	0.70-1.92)	0.85⁻	(0.47-1.53)	0.89⁻	(0.47-1.67)
	3	3.47-4.12	71 (32)	74 (25)	1.51⁻	(0.93-2.47)	1.05⁻	(0.59-1.86)	1.08⁻	(0.59-2.01)
	4	>4.12	46 (21)	75 (25)	0.97⁻	(0.58-1.62)	0.58⁻	0.32-1.08)	0.63⁻	(0.33-1.20)
Per SD increase		1.00			0.99⁻	(0.83-1.19)	0.82⁻	(0.66-1.01)	0.85⁻	(0.69-1.06)
Remnant cholesterol, mmol/L										
High		0.9	75 (34)	60 (20)	2.06***	(1.39-3.07)	1.72**	(1.12-2.65)	1.92**	(1.22-3.05)
Quartiles	1	<0.44	44 (20)	75 (25)	1	(reference)	1	Reference	1	Reference
	2	0.44-0.56	33 (15)	74 (25)	0.76⁻	(0.44-1.32)	0.67⁻	(0.36-1.24)	0.62⁻	(0.33-1.19)
	3	0.56-0.85	63 (29)	76 (25)	1.41⁻	(0.86-2.33)	0.98⁻	(0.56-1.71)	1.05⁻	(0.59-1.89)
	4	0.85	80 (36)	74 (25)	1.84⁻	(1.13-3.00)	1.28⁻	(0.75-2.19)	1.37⁻	(0.78-2.42)
Per SD increase		0.36			1.35***	(1.17-1.57)	1.25**	(1.07-1.45)	1.26**	(1.07-1.48)

table continues

					Univariate analysis		Model 1		Model 1-statin users	
		Level	Cases	Controls	Odds ratio	(95%)	Adjusted odds ratio †	(95% CI)	Adjusted odds ratio#	(95% CI)
Apo A1, g/L										
High		>1.25	96 (42)	246 (82)	0.17***	(0.11-0.25)	0.17***	(0.11-0.26)	0.15	(0.09-0.25)
Quartiles	1	<1.31	153 (70)	75 (25)	1	(reference)	1	(reference)	1	(reference)
	2	1.31-1.48	30 (14)	73 (24)	0.20***	(0.12-0.33)	0.18***	(0.10-0.32)	0.15***	(0.09-0.24)
	3	1.48-1.72	28 (13)	74 (25)	0.19***	(0.11-0.31)	0.15***	(0.08-0.27)	0.15***	(0.08-0.27)
	4	>1.72	9 (4)	77 (26)	0.06***	(0.03-0.12)	0.06***	(0.02-0.13)	0.05***	(0.02-0.12)
Per SD increase		0.31			0.26***	(0.20-0.34)	0.24***	(0.18-0.33)	0.23***	(0.16-0.32)
Apo B, g/L										
High		>1.0	172 (78)	168 (56)	2.79***	(1.89-4.14)	2.17***	(1.40-3.36)	2.46***	(1.53-3.95)
Quartiles	1	<0.87	17 (8)	74 (25)	1	(reference)	1	(reference)	1	(reference)
	2	0.87-1.04	43 (20)	72 (24)	2.60**	(1.36-4.97)	1.82⁻	(0.89-3.72)	3.05**	(1.29-7.23)
	3	1.04-1.26	74 (34)	77 (26)	4.18***	(2.26-7.75)	2.89**	(1.47-5.68)	4.67**	(2.06-10.63)
	4	>1.26	86 (39)	76 (25)	4.93***	(2.67-9.07)	3.04**	(1.55-5.95)	5.11***	(2.27-11.53)
Per SD increase		0.29			1.48***	(1.22-1.80)	1.27**	(1.02-1.57)	1.36**	(1.08-1.72)
Apo C-I, mg/L										
Quartiles	1	<17.40	85 (39)	74 (25)	1	(reference)	1	(reference)	1	(reference)
	2	17.40-20.80	54 (25)	75(25)	0.63⁻	(0.39-1.00)	0.50**	(0.29-0.85)	0.48**	(0.27-0.85)
	3	20.80-24.74	44 (20)	76 (25)	0.50**	(0.31-0.82)	0.35***	(0.20-0.60)	0.40**	(0.22-0.71)
	4	>24.74	37 (17)	74 (25)	0.44***	(0.26-0.72)	0.32***	(0.18-0.57)	0.37***	(0.20-0.68)
Per SD increase		5.33			0.82**	(0.69-0.97)	0.76**	(0.62-0.92)	0.81**	(0.66-0.99)
Apo C-II, mg/L										
Quartiles	1	<19.38	47 (21)	74 (25)	1	(reference)	1	(reference)	1	(reference)
	2	19.38-34.32	58 (26)	75 (25)	1.22⁻	(0.74-2.01)	0.83⁻	(0.46-1.47)	0.90⁻	(0.50-1.65)
	3	34.32-55.24	52 (24)	76 (25)	1.08⁻	(0.65-1.79)	0.62⁻	0.35-1.12)	0.73⁻	(0.39-1.35)
	4	>55.24	63 (29)	74 (25)	1.34⁻	(0.82-2.20)	0.64⁻	(0.36-1.15)	0.80⁻	(0.43-1.48)

table continues

					Univariate analysis		Model 1		Model 1-statin users	
		Level	Cases	Controls	Odds ratio	(95%)	Adjusted odds ratio †	(95% CI)	Adjusted odds ratio#	(95% CI)
Per ln SD increase		27.64			1.01⁻	(1.00-1.03)	1.00⁻	(0.98-1.02)	1.00⁻	(0.99-1.02)
Apo C-III, mg/L										
Quartiles	1	<77.56	81 (37)	74 (25)	1	(reference)	1	(reference)	1	(reference)
	2	77.56-97.33	51 (23)	75 (25)	0.62**	(0.39-1.00)	0.54***	(0.32-0.93)	0.53**	(0.30-0.94)
	3	97.33-119.90	40 (18)	76 (25)	0.48**	(0.29-0.79)	0.32***	(0.18-0.56)	0.37***	(0.21-0.67)
	4	>119.90	48 (22)	74 (25)	0.59**	(0.37-0.96)	0.41**	(0.24-0.72)	0.51**	(0.28-0.91)
Per ln SD increase		37.12			1.00⁻	(0.98-1.01)	0.99⁻	(0.98-1.01)	1.00⁻	(0.98-1.01)
Apo E, mg/L										
Quartiles	1	<22.14	45 (21)	75 (25)	1	(reference)	1	(reference)	1	(reference)
	2	22.14-28.19	61 (28)	75 (25)	1.36⁻	(0.82-2.24)	1.10⁻	(0.62-1.95)	1.26⁻	(0.68-2.32)
	3	28.19-36.55	62 (28)	74 (25)	1.40⁻	(0.85-2.30)	0.98⁻	(0.55-1.73)	1.02⁻	(0.55-1.88)
	4	>36.55	52 (24)	75 (25)	1.16⁻	(0.69-1.93)	0.86⁻	(0.48-1.54)	0.99⁻	(0.53-1.86)
Per SD increase		13.04			1.09⁻	(0.95-1.26)	1.04⁻	(0.93-1.18)	1.06⁻	(0.93-1.21)
Ratio ApoB/ApoA1										
Quartiles	1	<0.53	5 (2)	74 (25)	1	(reference)	1	(reference)	1	(reference)
	2	0.53-0.70	27 (12)	78 (26)	5.12***	(1.87-14.01)	3.52**	(1.24-10.03)	2.88⁻	(0.89-9.30)
	3	0.70-0.88	50 (23)	70 (23)	10.57***	(3.99-28.05)	6.99***	(2.52-19.39)	7.21***	(2.33-22.34)
	4	>0.88	138 (63)	77 (26)	26.53***	(10.28-68.42)	16.20***	(5.97-43.91)	16.98***	(5.67-50.85)
Per SD increase		0.25			2.39***	(1.98-2.89)	2.16***	(1.76-2.65)	2.29***	(1.84-2.85)
Non HDLc, mmol/L										
Quartiles	1	<3.32	33 (15)	73 (24)	1	(reference)	1	(reference)	1	(reference)
	2	3.32-4.07	47 (21)	77 (26)	1.35⁻	(0.78-2.34)	0.92⁻	(0.49-1.72)	0.75⁻	(0.56-2.24)
	3	4.07-4.96	85 (39)	74 (25)	2.54***	(1.52-4.26)	1.74⁻	(0.96-3.14)	1.94**	(1.01-3.72)
	4	>4.96	55 (25)	75 (25)	1.62⁻	(0.95-2.78)	1.04⁻	(0.54-1.86)	1.21⁻	(0.62-2.37)
Per SD increase		1.13			1.20⁻	(1.00-1.43)	1.00⁻	(0.81-1.22)	1.05⁻	(0.84-1.31)

†Model 1; Adjusted for age, sex and statin use

#Model 1-statin users; Adjusted for age and sex, statin users excluded

*Significance: ***P≤0.001; **P≤0.05;* ⁻P>0.05

Table 4. Risk of MACE according to various levels of lipoproteins

							Univariate analysis		Model 1		Model 1-statin users	
		No. at risk	Obs. years	Events	Incidence rate*	(95% CI)	Hazard ratio	(95% CI)	Hazard ratio†	(95% CI)	Hazard ratio#	(95% CI)
Total-c, mmol/L												
High		159	1113	55	4.94	(3.72-6.43)	0.73¯	(0.46-1.15)	0.73¯	(0.45-1.18)	0.75¯	(0.45-1.25)
Quartile	1	45	302	19	6.29	(3.79-9.82)	1	(reference)	1	(reference)	1	(reference)
	2	65	441	23	5.22	(3.31-7.83)	0.80¯	(0.43-1.48)	0.81¯	(0.42-1.53)	0.76¯	(0.38-1.53)
	3	49	341	20	5.87	(3.58-9.06)	0.94¯	(0.50-1.77)	0.99¯	(0.52-1.92)	1.01¯	(0.51-2.00)
	4	61	415	21	5.06	(3.13-7.74	0.82¯	(0.44-1.53)	0.85¯	(0.44-1.64)	0.82¯	(0.41-1.65)
Per SD increase		1.10					0.99¯	(0.78-1.25)	1.02¯	(0.79-1.30)	1.08¯	(0.83-1.39)
Triglycerides												
High		65	428	25	5.84	(3.78-8.62)	1.10¯	(0.69-1.75)	1.13¯	(0.70-1.81)	1.11¯	(0.68-1.83)
Quartile	1	44	291	18	6.19	(3.67-9.78)	1	(references)	1	(reference)	1	(reference)
	2	34	242	15	6.20	(3.47-10.22)	1.04¯	(0.52-2.09)	1.06¯	(0.53-2.15)	0.93¯	(0.43-1.98)
	3	64	429	22	5.13	(3.21-7.76)	0.89¯	(0.47-1.68)	0.89¯	(0.47-1.70)	0.79¯	(0.40-1.54)
	4	78	538	28	5.20	(3.46-7.52)	0.91¯	(0.50-1.66)	0.94¯	(0.51-1.73)	0.87¯	(0.46-1.64)
Per ln SD increase		0.81					1.01¯	0.97-1.04)	1.00¯	(0.96-1.04)	1.00¯	(0.96-1.04)
HDLc, mmol/L												
High		159	1118	56	5.01	(3.78-6.50)	0.72¯	(0.46-1.15)	0.70¯	(0.44-1.11)	0.68¯	(0.42-1.12)
Quartile	1	72	462	30	6.49	(4.38-9.27)	1	(references)	1	(reference)	1	(reference)
	2	50	378	14	3.70	(2.02-6.21)	0.56¯	(0.29-1.07)	0.56¯	(0.29-1.08)	0.53¯	(0.27-1.04)
	3	52	309	25	8.09	(5.24-11.94)	1.21¯	(0.71-2.06)	1.18¯	(0.69-2.02)	1.09¯	(0.61-1.93)
	4	46	350	14	4.00	(2.19-6.71)	0.66¯	(0.35-1.24)	0.57¯	(0.29-1.14)	0.60¯	(0.29-1.23)
Per SD increase		0.40					0.95¯	(0.75-1.22)	0.92¯	(0.71-1.20)	0.94¯	(0.71-1.24)

table continues

							Univariate analysis		Model 1		Model 1-statin users	
		No. at risk	Obs. years	Events	Incidence rate*	(95% CI)	Hazard ratio	(95% CI)	Hazard ratio†	(95% CI)	Hazard ratio#	(95% CI)
LDLc, mmol/L												
High		156	57	1083	5.26	(3.99-6.92)	0.83¯	(0.52-1.33)	0.90¯	(0.54-1.48)	0.84¯	(0.50-1.41)
Quartile	1	72	295	21	7.12	(4.41-10.88)	1	(references)	1	(reference)	1	(reference)
	2	50	414	20	4.83	(2.95-7.46)	0.68¯	(0.37-1.26)	0.76¯	(0.40-1.45)	0.65¯	(0.33-1.28)
	3	52	491	24	4.89	(3.13-7.27	0.65¯	(0.36-1.18)	0.69¯	(0.37-1.30)	0.64¯	(0.34-1.23)
	4	46	300	18	6.00	(3.56-9.48)	0.81¯	(0.43-1.52)	0.89¯	(0.46-1.73)	0.77¯	(0.39-1.54)
Per SD increase		1.00					0.96¯	(0.75-1.22)	0.99¯	(0.76-1.28)	0.92¯	(0.75-1.29)
Remnant cholesterol, mmol/L												
High		75 (34)	507	28	5.53	(3.67-7.98)	1.02¯	(0.65-1.61)	1.05	(0.66-1.66)	1.07¯	(0.66-1.73)
	1	44 (20)	291	18	6.19	(3.67-9.78)	1	(reference)	1	(reference)	1	(reference)
	2	33 (15)	232	15	6.47	(3.62-10.66)	1.08¯	(0.54-.2.17)	1.11¯	(0.55-2.25)	0.92¯	(0.43-1.98)
	3	63 (29)	419	22	9.48	(5.94-14.38)	0.91¯	(0.48-1.72)	0.91¯	(0.48-1.74)	0.80¯	(0.41-1.57)
	4	80 (36)	557	28	5.03	(3.34-7.27)	0.88¯	(0.48-1.60)	0.91¯	(0.49-1.67)	0.86¯	(0.46-1.61
Per SD increase		0.36					1.06¯	(0.95-1.19)	1.04¯	(0.93-1.16)	1.07¯	(0.94-1.21)
Apo A1, g/L												
High		96	651	36	5.53	(3.87-7.66)	1.05¯	(0.68-1.63)	1.01¯	(0.63-1.61)	0.93¯	(0.56-1.55)
Quartile	1	153	1034	61	5.60	(4.51-7.58)	1	(references)	1	(reference)	1	(reference)
	2	30	165	13	7.88	(4.20-13.47)	1.36¯	(0.74-2.47)	1.25¯	(0.67-2.36)	1.11¯	(0.54-2.27)
	3	28	235	6	2.55	(0.94-5.56)	0.48¯	(0.21-1.11)	0.42¯	(0.18-1.02)	0.44¯	(0.18-1.06)
	4	9	65	3	4.62	(0.95-13.49)	0.82¯	(0.29-2.63)	0.70¯	(0.21-2.30)	0.82¯	(0.25-2.72)
Per SD increase		0.31					0.84¯	(0.63-1.13)	0.80¯	(0.59-1.10)	0.78¯	(0.56-1.09)
Apo B, g/L												
High		172	1176	60	5.10	(3.89-6.57)	0.71¯	(0.44-1.16)	0.77¯	(0.46-1.31)	0.67¯	(0.40-1.15)
Quartile	1	17	116	7	6.03	(2.43-12.43)	1	(references)	1	(reference)	1	(reference)

table continues

							Univariate analysis		Model 1		Model 1-statin users	
		No. at risk	Obs. years	Events	Incidence rate*	(95% CI)	Hazard ratio	(95% CI)	Hazard ratio†	(95% CI)	Hazard ratio#	(95% CI)
	2	43	275	21	7.64	(4.73-11.67)	1.17	(0.50-2.78)	1.24	(0.48-3.19)	0.97	(0.33-2.90)
	3	74	537	24	4.47	(2.86-6.65)	0.75	(0.32-1.73)	0.84	(0.33-2.15)	0.64	(0.22-1.87)
	4	86	571	31	5.43	(3.69-7.71)	0.89	(0.39-2.02)	0.99	(0.39-2.53)	0.78	(0.27-2.23)
Per SD increase		0.29					0.91	(0.70-1.19)	0.94	(0.71-1.25)	0.93	(0.69-1.25)
Apo C-I, mg/L												
Quartile	1	85	544	37	6.80	(4.79-9.37)	1	(references)	1	(reference)	1	(reference)
	2	54	406	17	4.19	(2.44-6.70)	0.65	(0.37-1.16)	0.66	(0.37-1.18)	0.64	(0.34-1.20)
	3	44	294	17	5.78	(3.37-9.26)	0.91	(0.51-1.61)	0.89	(0.49-1.62)	0.86	(0.48-1.60)
	4	37	256	12	4.69	(2.42-8.19)	0.73	(0.38-1.40)	0.76	(0.39-1.50)	0.73	(0.37-1.49)
Per SD increase		5.33					1.03	(0.86-1.22)	1.04	(0.88-1.22)	1.04	(0.88-1.23)
Apo C-II, mg/L												
Quartile	1	47	322	19	5.90	(3.55-9.21)	1	(references)	1	(reference)	1	(reference)
	2	58	379	26	6.86	(4.48-10.05)	1.32	(0.68-2.25)	1.34	(0.72-2.49)	1.46	(0.75-2.86)
	3	52	375	16	4.27	(2.44-6.93)	0.78	(0.40-1.52)	0.83	(0.42-1.64)	0.89	(0.43-1.85)
	4	63	423	22	5.20	(3.26-7.87)	0.96	(0.51-1.78)	0.98	(0.51-1.86)	1.05	(0.52-2.12)
Per ln SD increase		27.64					1.01	(0.99-1.02)	1.01	(0.99-1.02)	1.01	(0.99-1.02)
Apo C-III, mg/L												
Quartile	1	81	509	37	7.27	(5.12-10.02)	1	(references)	1	(reference)	1	(reference)
	2	51	392	14	3.57	(1.95-5.99)	0.55	(0.30-1.02	0.55	(0.30-1.02)	0.53	(0.27-1.03)
	3	40	281	15	5.34	(2.99-8.80)	0.79	(0.43-1.44)	0.81	(0.44-1.50)	0.75	(0.39-1.42)
	4	48	317	17	5.36	(3.12-8.59	0.81	(0.45-1.44)	0.79	(0.43-1.44)	0.77	(0.41-1.44)
Per SD increase		37.12					1.01	(1.00-1.02)	1.01	(1.00-1.02)	1.01	(1.00-1.02)
Apo E, mg/L												
Quartile	1	45	309	18	5.83	(3.45-9.21)	1	(references)	1	(reference)	1	(reference)
	2	61	367	28	7.63	(5.07-11.03)	1.33	(0.73-2.44)	1.49	(0.78-2.86)	1.28	(0.65-2.53)

table continues

							Univariate analysis		Model 1		Model 1-statin users	
		No. at risk	Obs. years	Events	Incidence rate*	(95% CI)	Hazard ratio	(95% CI)	Hazard ratio†	(95% CI)	Hazard ratio#	(95% CI)
	3	62	479	18	3.76	(2.23-5.94)	0.70¯	(0.36-1.36)	0.75¯	(0.38-1.49)	0.81¯	(0.39-1.66)
	4	52	344	19	5.52	(3.33-8.63)	0.99¯	(0.51-1.90)	1.13¯	(0.57-2.26)	0.96¯	(0.46-1.99)
Per SD increase		13.04					1.07¯	(0.98-1.16)	1.07¯	(0.99-1.16)	1.07¯	(0.99-1.16)
Ratio ApoB/ApoA1												
Quartile	1	5	28	4	1.14	(0.31-2.93)	1	(references)	1	(reference)	1	(reference)
	2	27	186	10	5.38	(2.58-9.89)	0.44¯	(0.14-1.40)	0.43¯	(0.13-1.41)	0.55¯	(0.15-2.01)
	3	50	337	20	5.93	(3.63-9.17)	0.46¯	(0.16-1.36)	0.43¯	(0.15-1.33)	0.41¯	(0.12-1.44)
	4	138	948	49	5.17	(3.82-6.83	0.40¯	(0.14-1.11)	0.41¯	(0.14-1.18)	0.45¯	(0.14-1.48)
Per SD increase		0.25					1.05¯	(0.86-1.27)	1.08¯	(0.88-1.33)	1.07¯	(0.86-1.32)
Non HDLc, mmol/L												
Quartile	1	33	207	15	7.25	(4.01-11.95)	1	(references)	1	(reference)	1	(reference)
	2	47	341	17	4.99	(2.90-7.98)	0.68¯	(0.34-1.38)	0.70¯	(0.32-1.51)	0.53¯	(0.23-1.12)
	3	85	601	29	4.86	(3.23-6.93	0.68¯	(0.36-1.27)	0.71¯	(0.37-1.39)	0.65¯	(0.32-1.32)
	4	55	350	22	6.29	(3.94-9.52)	0.87¯	(0.45-1.68)	0.92¯	(0.45-1.89)	0.78¯	(0.38-1.62)
Per SD increase		1.13					1.00¯	(0.78-1.28)	1.05¯	(0.81-1.35)	1.05¯	(0.81-1.37)

**Model 1; Adjusted for age, sex and statin use*

†Model 1-statin users; Adjusted for age and sex, statin users excluded

Significance: ¯P>0.05

High apoCI levels were associated with risk of STEMI with an OR of 0.32 (95% CI 0.18-0.57) of the highest quartile versus the lowest quartile. Per SD increase of apoCI (5.33 mg/L), the OR was 0.76 (95% CI 0.62-0.93). The VLDL-associated apos gave conflicting results. No association of apoCII and apoE with the risk of STEMI was found, whereas higher apoCIII was associated with lower risk of STEMI in the groups divided by quartiles. The highest quartile of apoCIII versus its lowest quartile was associated with an OR of 0.41 (CI 95% 0.24-0.72) for risk of STEMI.

At discharge 100% of the STEMI patients were on statin therapy. The fast majority was on rosuvastatin 10mg daily. Values of continuation samples of conventional lipoproteins were available in STEMI patients after 1 year. Mean TC levels were reduced from 5.63 ± 1.09 mmol/L to 3.96 ± 0.73 mmol/L which is a 27% reduction ($p<0.001$). Mean LDLc was 2.33 ± 0.75 mmol/L compared to 3.48 ± 0.94 at baseline, which is a 27% reduction ($p<0.001$). HDLc raised from 1.27 ± 0.36 mmol/L to 1.40 ± 0.36 which is an increase of 12% ($p<0.001$) Median triglyceride level was 1.31mmol/L (1.00-1.80) at baseline compared to 1.53 mmol/L (1.01-2.22) after 1 year which is a 20% reduction ($p<0.001$). According to medical record review 7 patients (3.2%) were not on statin therapy after 1 year due to pharmacological side effects.

In the patients with STEMI 83 (38%) events were observed after a mean follow-up duration of 8.94 years. For each baseline lipid and apo species the hazard ratio for MACE was calculated after adjustment for age, gender, and statin use. Neither conventional lipid levels nor apo levels were associated with a recurrent event in the STEMI group (table 4).

Discussion

In this case-control study with 10-year follow-up of the STEMI patients the value of extensive lipid and apo profiling to predict STEMI or MACE was studied. Key findings of the study are: (1) apoA1, apoB and the apoB/apoA1 ratio were strongly associated with the risk of developing a STEMI; (2) remnant cholesterol was significantly associated with risk of STEMI; (3) apoCII, apoCIII and apo E were not clearly associated with risk of STEMI; and (4) no significant association of serum lipids or serum apos with MACE in STEMI patients during follow-up who were all treated with statins during follow-up was found.

Despite current standards of care aimed at achieving targets for LDLc and other traditional risk factors, STEMI patients remain at high risk of new cardiovascular events.(8, 13) The results of this study are in line with this, with a 38% event rate during almost 9-year follow-up.

In recent years, it has been demonstrated that apoA1, apoB and apoB/apoA1 ratio can predict CVD better than LDLc.(9-13, 26-28) In this study similar results were found. Adjusted for age, gender and statin therapy, elevated levels of apoB and apoB/apoA1 ratio were associated with an increased risk of developing a STEMI and LDLc was not. Holmes et al. recently confirmed these results in a nested case-control study showing that apoA1, apoB and apo/apoA1 ratio were strongly associated with risk of MI.(15) Similar results were also obtained in a previous meta-analysis of Sniderman et al. leading to the conclusion that apoB is superior to non-HDLc and that non-HDLc is superior to LDLc as a predictor of CVD risk.(16) Taken all this evidence in mind a slow but progressive shift towards the use of serum apos in clinical practice can be observed. Several position or consensus statements and guidelines from medical associations therefore recommend introduction of apoB levels as (secondary) treatment target in clinical practice.(29-32)

ApoB concentration represents the total number of atherogenic particles including VLDL, intermediate-density lipoprotein (IDL), IDL remnants, LDL and lipoprotein (a), as each of these particles carries one molecule of apoB. With the routine measurement of apoB a considerable number of events could be prevented on top of LDLc.(16) The clinical use of apoB levels will be an important step towards precision medicine. It would be ideal if –in the future- events of an individual patient may be predicted and hereby improve risk stratification for future events. For example, recently Hermans et al. showed that premature CAD may occur in patients with an apoCII deficiency with normotriglyceridemia.(33) Despite the fact that these patients had a low *a priori* risk for CAD, they presented with STEMI at young age and had a high relative risk of 10-year reinfarction or revascularization.[30] This contrasts with the phenotype described in textbooks where a total lack of apoCII is assumed to result in intravascular TG accumulation because of inactivation of LPL, whereby delayed intravascular TG lipolysis is a strong and independent predictor of CAD.(34, 35)

So far only serum apo A1 and B were measured in medical laboratories, whereas the apos CI, CII, CIII and E are not routinely measured. To determine the full panel of serum apos in a multiplexed and immunoassay independent way, van den Broek et al.(19) recently developed a quantitative serum apo profiling test using LC-MS/MS. This LC-MS/MS test produces highly accurate test results, which are in concordance with quality requirements for medical tests. Furthermore, the test is not confounded by hypertriglyceridemia.[17] Moreover, the multiplex apo test can be performed in the non-fasting state.(25)

Remnant cholesterol is the cholesterol content of triglyceride-rich lipoproteins, composed of mainly VLDL and IDL.(36, 37) The current study shows that remnant cholesterol level was associated with an increased risk of STEMI. These results are in concordance with the results of several other studies.(36, 38) Varbo et al. implied a causal risk of elevated remnant cholesterol levels for ischemic heart

disease, independent of HDLc levels.(38)

The VLDL-associated apos apoCII, apoE and –to a large extent- apoCIII have recently been identified as potentially important new risk factors.(17, 18) These apos are abundant on TG-rich lipoproteins, strongly modulate their metabolism(39), and might play an important role in the development of atherosclerosis and subsequent CVD.

Recently, Pechlaner et al.(17) showed that apoCII, apoCIII and apoE were strongly associated with incident CVD in the general community. This finding was supported by Van Capelleveen et al.(18) These results could however not be confirmed in the current case-control study. In fact, no clear relation was found between VLDL-associated apos and the risk of STEMI. These conflicting results could have several explanations. First of all, the blood samples of the cases were drawn soon after admission to the hospital, *i.e.* in a non-fasting state. The blood samples of the control group were obtained in a fasting state. Secondly, in both cases and controls the TG levels are 0.5 mmol/L lower than in the EPIC Norfolk population study, so the results in the current study may not be generalizable with the EPIC Norfolk population study. Thirdly, the information about the percentage of controls that have diabetes mellitus (DM) was self-reported (1.7%). In the STEMI group 7.7% had DM.

The relation of apoE with CVD risk is more controversial. Although Pechlaner et al. found a strong association, a meta-analysis with almost 10,000 individuals and 1,400 events recently performed by Sofat et al. found no association of apoE with CVD.(40)

In STEMI patients, no association of baseline serum lipids or serum apos with MACE during long term follow-up was found. Although earlier results from the TNT study (41) suggest that baseline apoB and apoA1 levels are associated with residual risk in a statin-treated secondary prevention population, the current results did not confirm these findings. This can be due to several reasons. First of all, 100% of the patients were put on statin therapy after their STEMI which clearly had an effect on baseline lipid and apo concentrations which could diminish the relation with cardiovascular events. It has been demonstrated that statin therapy reduces 5-year incidence of major coronary events by about 20% per mmol/L reduction in LDL cholesterol.(42) In this cohort a 27% percentage reduction in LDL-c after 1 year was observed. This significant reduction in lipid concentrations together with the limited number of recurrent events could explain why no association was found between apos or lipid concentrations and recurrent events. Unfortunately, all measured apos were only available as baseline samples and not as continuation samples, so the impact of statin therapy on apo levels in this cohort is unknown. For example, van Lennep et al. demonstrated that, in patients with effective statin treatment, on-treatment levels of apoB and apoA1 were significantly predictive for recurrent events in

CAD patients.(43) Secondly, the determinants of residual risk in statin treated patients is multifactorial. For example. smoking, hypertension, diabetes, high BMI and higher inflammation grades all contribute to a higher residual risk.(15, 41, 44, 45) All these (modifiable) risk factors could have played a role in the occurrence of a recurrent event and perhaps did these risk factors obscure the association of apos and lipids with recurrent events. At last, the mean follow-up duration was almost 9 years, instead of 5 years in the TNT study.

The primary goal in STEMI patients is to reduce the residual risk as much as possible. Statin therapy has shown to reduce the residual risk substantially by reducing LDLc levels,(46) but the effect on LDLc levels is confined,(47, 48) so the need for other powerful cholesterol lowering agents or other modifiable risk factors is needed. The IMPROVE-IT study showed that ezetimibe provides an incremental reduction in LDLc of 15-20% which resulted in an increased risk reduction for cardiovascular events. (49) Furthermore, recently the FOURIER studie demonstrated their results with the PCSK9 antagonist evolucumab. They showed an additional lowering of more than 50% of LDLc levels on top of statin therapy with evolucumab compared to placebo in high risk patients. Inhibition of PCSK-9 reduced furthermore the risk of cardiovascular events.(50) In the nearby future the results from the ODDESSEY OUTCOME will be published (51) where 18,000 post ACS patients were administrated with either alirocumab or placebo. Preliminary results showed us that these powerfull cholesterol lowering agents further reduced residual cardiovascular risk by further lowering LDL-c levels. However, as we currently know, LDLc is not discriminating and refined enough to identify high risk patients and we should need to work towards better, more meaningfull and well characterized medical tests suchs as apos to measure pharmacological effects. Current AHA and ESC dyslipidemia guidelines mainly focus on LDLc reduction as primary treatment target. However, in line with several position statements, guidelines and the INTERHEART study, (13, 29-31) valuable effort should be made to substantially modify apoB and apoB/apoA1 ratio. These apos can reliably be measured and a substantial modification could lead to further reduction of the residual cardiovascular risk.

Some potential limitations deserve a comment. First, the controls of the MEGA population were <70 years; so, results from the case-control study only apply to individuals <70 years. Secondly, apoC and E levels were measured in MEGA controls in serum that was thawed twice. However, Bland-Altman plots and scatterplots suggest that this did not lead to measurement error as apoA1 and apoB levels that were measured on both once and twice thawed serum showed equivalent levels with r^2 of 0.89 and 0.93, respectively, but the effect of thawing on apoC and apoE levels is unknown. Furthermore, the models for risk of STEMI and MACE were adjusted for age, gender and statin use. An important factor modifying the association between (apo) lipoproteins and outcome is inflammation. Since C-reactive protein was measured in the acute phase in the STEMI cohort and in the non-acute phase in the MEGA cohort we were not able

to reliably adjust the apo (lipoproteins) for inflammation.

Conclusion

In conclusion, apoA1, apoB, and apoB/apoA1 ratio and remnant cholesterol were strongly associated with risk of STEMI, the apoB/apoA1 ratio being superior to LDLc and non-HDLc. Secondly no clear relation of apoCI, apoCII, apoCIII and apoE with the risk of STEMI as compared with a population based control group was found. Neither serum lipids nor serum apos predicted death, re-infarction or revascularization in statin-treated patients during follow-up after a first STEMI. Valuable effort should be made to further reduce residual cardiovascular risk by intensive life style modification, by testing new powerful cholesterol lowering agents and by using additional more discriminating and more refined treatments targets like apoB and apoB/apoA1 ratio.

Ethical standards

All participants in the MISSION! and MEGA study gave written informed consent in accordance with the Declaration of Helsinki. Both studies were approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands.

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Chapter 5.

Growth differentiation factor-15 levels at admission provide incremental prognostic information on all-cause long-term mortality in ST-segment elevation myocardial infarction patients treated with primary percutaneous coronary intervention

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Abstract

Introduction
To investigate the additive prognostic value of growth differentiation factor (GDF-15) levels in ST-segment elevation myocardial infarction (STEMI) patients treated with primary percutaneously coronary intervention (pPCI) with 10-year mortality on top of clinical characteristics and known cardiac biomarkers.

Methods
Baseline serum GDF-15 levels were measured in 290 STEMI patients treated with pPCI in the MISSION Intervention Trial conducted from February 1, 2004 through October 31, 2006. The incremental prognostic value of GDF-15 and NTproBNP levels were evaluated on top of clinical characteristics using Cox proportional hazards analysis, Chi square models and C-index. Outcome was 10 year all-cause mortality.

Results
Mean age was 59.0 ± 11.5 years and 67 (22.7%) patients were female. A total of 37 patients died during a follow-up of 9.4 (IQR 8.8-10.0) years. Multivariable Cox regression revealed GDF-15 and NTproBNP levels above median to be independently associated with 10 year all-cause mortality (HR GDF-15, 2.453 (95%CI 1.064-5.658), *P*=.04; HR NTproBNP, 2.419 (95%CI 1.043-5.564), *P*=.04) after correction for other clinical variables. Stratified by median GDF-15 (37.78 pmol/L) and NTproBNP (11.74 pmol/L) levels, Kaplan-Meier curves showed significant better survival for patients with GDF-15 and NTproBNP levels below the median versus above the median. The likelihood ratio test showed a significant incremental value of GDF-15 (*P*= .03) as compared with a model with clinically important variables and NTproBNP. The C-statistics for this model improved from 0.82 to 0.84 when adding GDF-15.

Conclusion
GDF-15 levels at admission in STEMI patients are independently associated with 10 year all-cause mortality rates and could improve risk stratification on top of clinical variables and other cardiac biomarkers.

Introduction

Long-term mortality rates in ST-segment elevation myocardial infarction (STEMI) patients treated with primary percutaneous coronary intervention (pPCI) are declining due to more frequent use of reperfusion therapy, modern antithrombotic therapy and secondary prevention measures.[1, 2] However, they are still substantial with a reported 1-year mortality rate of 10%[3, 4] and a 5-year mortality rate of about 23%.[4]

Identification of high-risk patients is essential for optimal monitoring and initiation of appropriate treatment to reduce risk of events. Current risk assessment relies mainly on clinical characteristics such as age, infarct location, Killip class, cardiogenic shock, ejection fraction, diabetes, renal failure and time of ischemia.[5-7] In addition, traditional cardiac biomarkers such as cardiac troponin (cTn) and N-terminal pro-B-type natriuretic peptide (NTproBNP) have been shown to improve risk prediction in STEMI patients on top of clinical characteristics.[8, 9]

Growth differentiation factor-15 (GDF-15) is a systemic stress-responsive member of the transforming growth factor β (TGF-β) superfamily.[10] GDF-15 is a general, relatively novel biomarker which is induced in the myocardium after ischemia and reperfusion[11] and released due to haemodynamic stress.[12] However, GDF-15 is also released in the setting of inflammation or tissue damage and an overexpression has been found in a number of malignancies.[11, 13]

Several studies have shown that GDF-15 levels in STEMI patients provide prognostic information on mortality rates within 1 year additional to established clinical and biochemical biomarkers.[14-18] Apart from this relatively short follow-up duration, these studies were limited since study populations were not always comparable due to differences with respect to timing of GDF-15 measurement, type of thrombolytic therapy and end-points. So, whether GDF-15 levels at admission are also related with long term mortality rate is unknown in STEMI patients treated with pPCI, especially on top of other more recently validated biomarkers such as cTn and NTproBNP.

Therefore, the additive prognostic value of GDF-15 levels at admission in STEMI patients treated with pPCI as to 10-year mortality rate is investigated on top of clinical characteristics and known cardiac biomarkers such as cTn and NTproBNP.

Methods

Study population
In this study, data is used from patients with STEMI, who were included in the prospective MISSION! Intervention trial.[19] The MISSION! Intervention

trail was conducted from February 2004 to October 2006. In this randomized study clinical and angiographic results in patients with STEMI treated with either Bare Metal Stents (BMS) or Sirolimus Eluting Stents (SES) during pPCI were evaluated. In short, patients were eligible if STEMI symptoms started <9h before the procedure and the electrocardiogram (ECG) showed ST-elevation elevation (≥0.2mV in ≥2 leads in V1-V3 or ≥0.1 mV in other leads) or presumed new left bundle branch block (LBBB). Patients were excluded if they were <18 years or >80 years. The study protocol was approved by the institutional ethical committee. This study was conducted according to the declaration of Helsinki and written informed consent was obtained from all patients before enrolment in the study.

Study procedure

During the study, all subsequent patients were treated according to the institutional MISSION! Protocol,[20] based on guidelines of the European Society of Cardiology, American College of Cardiology and the American Heart Association.[21, 22] The pre-hospital protocol included diagnosis by field triage by 12-lead ECG and in-ambulance treatment with a loading dose of clopidogrel, aspirin, heparin, and intravenous glycoprotein IIb/IIIa inhibitors. PPCI was performed according to the clinical guidelines.[21, 22] If tolerated, patients received beta-blockers, ACE-inhibitors and statins within 24 hours. Additionally, patients were prescribed dual antiplatelet therapy, consisting of aspirin 100 mg daily for life and clopidogrel 75 mg daily for 12 months. More than 95% of the patients received a statin, an acetylsalicylic acid, and a thienopyridine and more than 85% of the patients received a β-blocker and an ACE-inhibitor within 24 hours after admission. During admission, patients' demographic characteristics, risk factors and clinical features were collected. Clinical follow-up data was collected during the 30 days, 3, 6 and 12 months outpatient clinic visits. Information on all-cause mortality was obtained from the Dutch Municipality Records registry at 2, 5, and 10 years after admission. Cause of death was retrieved from general practitioners. The primary outcome of this analysis was 10 year all-cause mortality.

GDF-15 and NTproBNP measurement

GDF-15 and NTproBNP levels are expressed in pmol/L which represents the amount of substances. GDF-15 levels are converted to ng/L by dividing the number by 0.02929. NTproBNP levels are converted to ng/L by dividing the number by 0.118.

Blood samples were obtained at presentation before the pPCI procedure was performed. An extra serum sample was Samples were coagulated for at least 60 minutes before centrifugation at 1500 Relative Centrifugal Force (RCF) for 10 minutes at 18^{o} C. Sera were pipetted into 1.1 mL Micronic tubes. Within 2 hours after vena puncture, the serum samples were frozen in a -70/-80^{o} C freezer.

For the in vitro quantitative determination of GDF-15 in human serum a Roche electrochemiluminescence immunoassay "ECLIA" on Cobas e602 series (catalog number 07125933190) is used (Roche Diagnostics, Mannheim, Germany). The test is based on the sandwich principle. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Serum fractions of 250 µL were used for parallel quantification of both serum GDF-15 and NTproBNP. GDF-15 values below the limit of detection were reported as < 11.71 pmol/L. Values exceeding the measuring range were reported as > 585.8 pmol/L.

All GDF-15 measurements were performed at Leiden University Medical Center by investigators who were not aware of patient's characteristics and outcomes.

NTproBNP measurements

A Roche electrochemiluminescence immunoassay "ECLIA" on Cobas e602 series (catalog number 07125933190) is used (Roche Diagnostics, Mannheim, Germany) to determine NTproBNP levels. The test is based on the sandwich principle with a detection limit of 0.59 pmol/L. Values exceeding the measuring range were reported as > 4130 pmol/L. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Statistical analysis

Normally distributed data is presented as mean and standard deviation (SD). Non-normally distributed data is expressed as median with inter-quartile range (IQR). Categorical data is expressed as absolute numbers and percentages. Differences in baseline characteristics between patients below and above the median of GDF-15 were assessed with an independent T-test, the Mann-Whitney U-test or Chi-square test when appropriate. The Pearson correlation coefficient was used to analyse the correlation between GDF-15 and cTnT and NT-proBNP. Event-free survival was analysed with Kaplan-Meijer curves and compared between groups with the log-rank test. To assess the incremental value of GDF-15 levels at admission, we first investigated independent univariate Cox regression analyses to determine the association of potential confounding variables, like body mass index (BMI), hypertension, diabetes, hypercholesterolemia, smoking, history of cardiovascular disease (CVD), prior MI, out of hospital cardiac arrest, cardiogenic shock, culprit vessel, number of vessel disease, type of stent, cTnT, creatine kinase (CK), and creatinine, on 10 year all-cause mortality. We then constructed a base multivariate Cox model which adjusts for age, gender and all variables with a P <0.10 from the univariable analysis. The incremental prognostic value of GDF-15 and NTproBNP levels was then evaluated by independently adding these predictors to the base model and calculated the likelihood-ratio testing for addition of these effects on top of clinical variables. In addition, the overall

C-statistic as proposed by Harrell et al.[23] was calculated. Effects are reported as hazard ratios (HR) with 95% confidence intervals (CI). All statistical tests were performed with SPSS software (Version 24.0, IBM, Armonk, NY). P-values <0.05 assessed by two-sided tests were considered to be statistically significant.

Results

Baseline and clinical characteristics

Baseline serum GDF-15 levels were available in 290 STEMI patients treated with pPCI in the MISSION Intervention Trail. Mean age was 59.0 ± 11.5 years, 67 (22.7%) patients were female, and the median GDF-15 concentration was 37.78 pmol/L (IQR 26.88-55.83 pmol/L). Stratified by median GDF-15 levels, patients with values above the median were older, more often female and had higher NTproBNP, cTnT, creatine kinase (CK) and creatinine levels than the patients below the median (table 1). The correlation coefficient between GDF-15 and NT-proBNP was 0.17 (*P*=0.004) and between GDF-15 and cTnT 0.083 (*P*=0.191).

Table 1. Demographic and clinical characteristics at baseline

Variable		Baseline GDF-15 median (37.8 pmol/L)		*P*
	Total group (n=290)	<median (n=145)	>median (n=145)	
Age, mean (SD), y	59.0 (11.5)	55.7 (11.8)	62.4 (10.9)	<.001
Female gender, n (%)	65 (22.4)	18 (12.4)	47 (32.4)	<.001
Cardiovascular risk factors				
Current smoking	158 (54.5)	82 (56.6)	76 (52.4)	.48
Ex-smoker	33 (11.4)	17 (11.7)	16 (11.0)	.85
NIDDM, n (%)	19 (6.6)	6 (4.1)	13 (9.0)	.10
IDDM, n (%)	11 (3.7)	6 (4.1)	5 (3.4)	.76
Family history of CVD, n (%)	126 (43.4)	70 (48.3)	56 (38.6)	.17
Treated hypercholesterolemia, n (%)	56 (19.3)	25 (17.2)	31 (21.4)	.37
Treated hypertension, n (%)	82 (28.3)	41 (28.3)	41 (28.3)	1.00
Body mass index, mean (SD), kg/m^2	26.6 (4.2)	26.7 (3.8)	26.6 (4.6)	.87
Comorbidities				
Previous myocardial infarction, n (%)	11 (3.8)	6 (4.1)	5 (3.4)	.76
Previous PCI, n (%)	5 (1.7)	3 (2.1)	2 (1.4)	.65
Previous CABG, n (%)	2 (0.7)	2 (1.4)	0 (-)	.16
History of cerebrovascular disease, n (%)	10 (3.4)	3 (2.1)	7 (4.8)	.27

table continues

Variable		Baseline GDF-15 median (37.8 pmol/L)		*P*
	Total group (n=290)	<median (n=145)	>median (n=145)	
Previous medication use				
Beta-blocker, n (%)	36 (12.4)	20 (13.8)	16 (11.0)	.48
ACE-inhibitor/AT2-antagonist, n (%)	34 (11.7)	19 (13.1)	15 (10.3)	.47
Statin, n (%)	31 (10.7)	14 (9.7)	17 (11.7)	.51
Antiplatelet, n (%)	1 (0.3)	1 (0.7)	0 (-)	.37
Ascal, n (%)	28 (9.7)	11 (7.6)	17 (11.7)	.29
Clinical characteristics				
Time of ischemia, median (IQR), min	192 (146-257)	200 (147-260)	191 (146-248)	.58
Number of narrowed coronary arteries				.99
1	158 (54.5)	78 (53.8)	80 (55.2)	
2	115 (39.7)	58 (40.0)	57 (39.3)	
3	15 (5.2)	8 (5.5)	7 (4.8)	
Complete revascularization, n (%)	195 (67.5)	101 (71.1)	94 (65.7)	0.38
Killip class≥2, n (%)	27 (9.3)	11 (7.6)	16 (11.0)	.37
Laboratory results				
Infarct size, median area under the CK curve (IQR), g/m²	8.92 (4.26-15.82)	7.36 (2.93-14.45)	10.54 (5.73-16.99)	.009
Peak cardiac troponin-T, median (IQR), µg/L	5.53 (2.28-10.22)	4.77 (1.64-8.84)	5.91 (3.08-10.72)	.02
NTproBNP, median (IQR), pmol/L	11.74 (4.70-27.53)	9.46 (4.33-22.98)	14.49 (5.40-34.24)	.02
Creatinine, mean (SD), µmol/L	81.6 (18.5)	78.2 (13.8)	85.1 (21.8)	.002

Data are expressed as number (%), median (IQR) or mean ± standard deviation
Abbreviations: ACE, angiotensin converting enzyme; AT2, angiotensin2; CABG, coronary artery bypass surgery; CVD, cardiovascular disease; GDF-15, Growth differentiation factor-15; IDDM, insulin dependent diabetes mellitus; IQR, interquartile range; NIDDM, non-insulin dependent diabetes mellitus; NTproBNP, N-terminal pro b-type natriuretic peptide; PCI, percutaneous coronary intervention; SD, standard deviation. Narrowed coronary artery, defined as on baseline coronary angiogram a ≥ 50% stenosis. Treated hypercholesterolemia, Serum total cholesterol ≥6mmol/L and/or serum TG ≥2.2mmol/L or treatment with lipid lowering drugs. Treated hypertension, Defined as systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensive medication.

Long-term clinical outcome

A total of 37 patients reached the endpoint during a follow-up of 9.4 (IQR 8.8-10.0) years. The cause of death was adjudicated as cardiac origin in 10 patients,

4 patients died of likely cardiac origin, 19 patients died from a non-cardiac cause, and in 4 patients the cause of death is unknown.

Survival analysis

Univariable Cox regression analysis showed that age, diabetes, current smokers, patients with a family history of CVD, cardiogenic shock, more than one vessel disease at the time of STEMI, baseline levels of GDF-15 above the median, baseline NTproBNP level above the median (11.74 pmol/L), and renal dysfunction were associated with unfavourable outcome (table 2). Infarct size expressed by the biomarkers cTnT and area under the curve of CK were not associated with higher mortality rates. Multivariable Cox regression revealed that GDF-15 and NTproBNP levels above median are independently associated with 10 year all-cause mortality (HR GDF-15, 2.453 (95%CI 1.064-5.658), *P*=.04; HR NTproBNP, 2.413 (95%CI 1.043-5.586), *P*=.04) after correction for clinical variables. Furthermore, age (HR 1.095 (95%CI 1.044-1.150), *P*<.001) and cardiogenic shock (HR 13.338 (95%CI 3.374-50.566), *P*<.001) remained significantly associated with all-cause mortality in the multivariable Cox regression analysis.

Table 2. Univariable and multivariable Cox proportional hazard regression analysis to identify independent predictors of all-cause mortality

Parameter		Univariable analysis		Multivariable analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Age, mean, y		1.110 (1.069-1.153)	<.001	1.095 (1.044-1.150)	<.001
Female gender		0.761 (0.334-1.733)	.51	0.413 (0.159-1.072)	.07
Body mass index, mean, kg/m²		0.962 (0.881-1.050)	.39		
Treated hypertension		0.957 (0.463-1.978)	.91		
Diabetes		2.176 (0.956-4.954)	.06	2.374 (0.997-5.654)	.05
Treated hypercholesterolemia		0.875 (0.365-2.098)	.765		
Current smoker		0.576 (0.299-1.109)	.10	1.702 (0.821-3.525)	.15
Family history of CVD		0.453 (0.219-0.936)	.03	0.871 (0.384-1.974)	.74
Prior myocardial infarction		1.322 (0.318-5.500)	.70		
Out of hospital cardiac arrest		0.048 (0.000-568.83)	.53		
Cardiogenic shock		10.76 (3.295-35.15)	<.001	13.062 (3.374-50.566)	<.001
Culprit vessel	RCA	Ref	.65		
	RCX	0.944 (0.359-2.484)	.91		
	LAD	0.727 (0.356-1.483)	.38		
Number of vessel disease (>50%) >1		1.914 (0.993-3.689)	.05	1.383 (0.457-4.183)	.57

table continues

Parameter	Univariable analysis		Multivariable analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Complete revascularization	0.480 (0.252-0.915)	0.026	0.977 (0.329-2.095)	0.97
Drug-eluting stent	1.256 (0.657-2.412)	.49		
Peak cardiac troponin-T level, µg/L	1.023 (0.973-1.076)	.37		
Infarct size, median under the CK curve (IQR), g/m^2	0.979 (0.940-1.019)	.31		
Baseline GDF-15 >median	3.360 (1.585-7.121)	.002	2.453 (1.064-5.658)	.04
Baseline NTproBNP >median	3.332 (1.567-7.042)	.002	2.413 (1.043-5.586)	.04
Creatinine, µmol/L	1.023 (1.007-1.039)	.004	1.005 (0.988-1.022)	.56

Data are expressed as hazard ratios with 95% confidence interval
Abbreviations: CK, creatine kinase; CVD, cardiovascular disease; GDF-15, Growth differentiation factor-15; NTproBNP, N-terminal pro b-type natriuretic peptide; SD, standard deviation. Treated hypercholesterolemia, Serum total cholesterol ≥6mmol/L and/or serum TG ≥2.2mmol/L or treatment with lipid lowering drugs. Treated hypertension, Defined as systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensive medication.

Stratified by median GDF-15 (37.78 pmol/L) and median NTproBNP (11.74 pmol/L) levels, Kaplan-Meier curves showed significantly better survival for patients with GDF-15 and NTproBNP levels below the median than for patients with GDF-15 and NTproBNP levels above the median. In the group with GDF-15 levels below the median, the event free survival was 92.6%, compared to 79.8% in the group with GDF-15 levels above the median (log-rank *P*<.001) (Figure 1a). Similar results were obtained with NTproBNP levels. The event free survival rate was 93.5% with NTproBNP levels below the median, versus 78.8% event free survival in patients with an NTproBNP level above the median (log rank *P* <.001). When these biomarkers are divided in 4 groups (both GDF-15 and NTproBNP < median; GDF-15 > median and NTproBNP < median; GDF-15 < median and NTproBNP > median; and both GDF-15 and NTproBNP > median), the prognostic value improved. In the group with both GDF-15 and NTproBNP levels below their medians the event free survival was 95.7%, whereas in the group with both GDF-15 and NTproBNP levels above their medians (log rank *P*<.001) the event free survival was 70.7% (Figure 2).

Incremental value of GDF-15

Figure 3 shows the incremental value of NTproBNP and GDF-15 on top of other clinically important risk factors for predicting the primary end-point. Model 1 includes all variables that are significant in the univariable Cox regression

analysis. The addition of NTproBNP to the basic model improved the likelihood ratio but this was not statistically significant (*P*=.086). The likelihood ratio test showed a significantly incremental value of GDF-15 (*P*= .027) as compared with a model with clinical important variables and NTproBNP. The C-statistics for this model improved from 0.82 to 0.84 when adding GDF-15 levels to the model with all clinically important risk factors and NTproBNP levels.

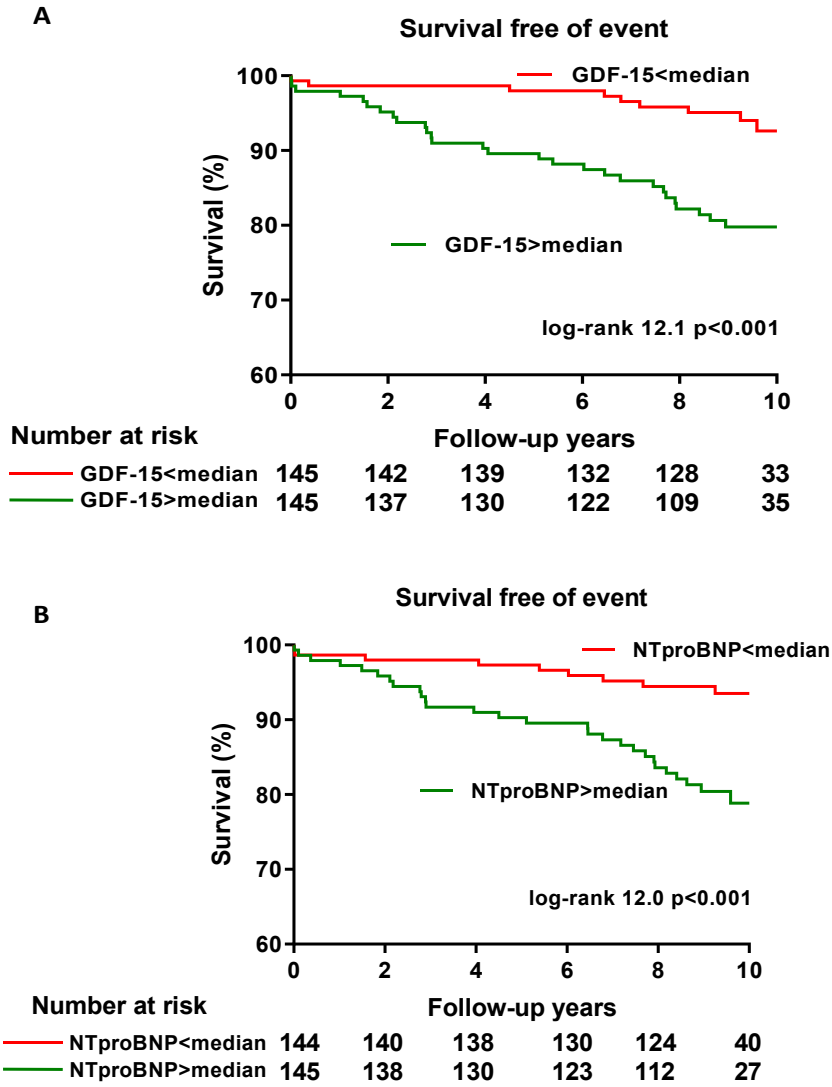

Figure 1. Kaplan-Meier analysis to evaluate the survival free of the primary end-point of all-cause mortality. A = GDF-15. B = NTproBNP. GDF-15 = Growth differentiation factor-15; NTproBNP = N-terminal pro-B-type natriuretic peptide.

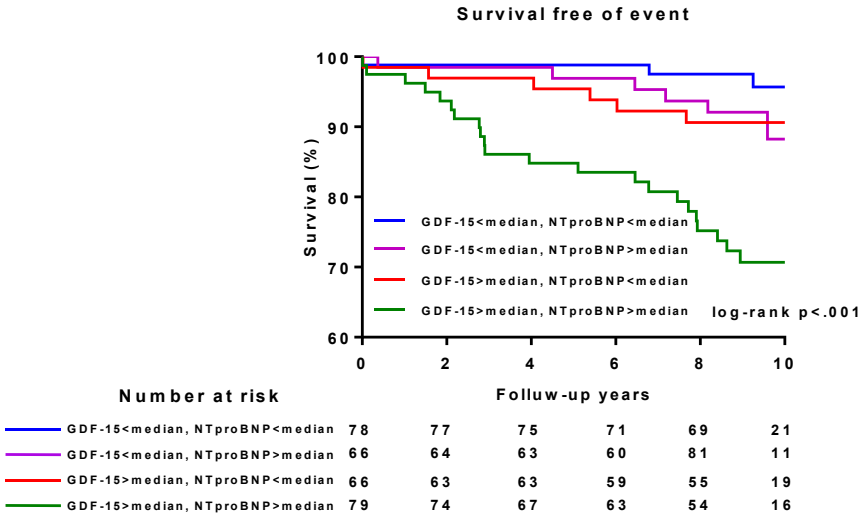

Figure 2. Kaplan-Meier analysis to evaluate the survival free of experiencing the primary end-point of all-cause mortality when combining assessment of GDF-15 and NTproBNP. GDF-15; Growth differentiation factor-15; NTproBNP = N-terminal pro-B-type natriuretic peptide.

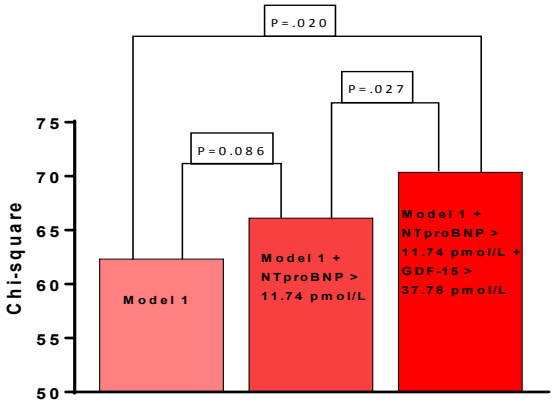

Figure 3. The bar graphs show the incremental value of NTproBNP and GDF-15 on top of other clinically important risk factors for predicting the primary end-point. Harrell C-statistics represents overall adequacy of the risk prediction. Model 1: Clinical variables (age, gender, previous diabetes mellitus, current smoking, family history of CVD, cardiogenic shock, >1 number of vessel disease, complete revascularization, creatinine). Model 2: Model 1 + NTproBNP > median (11.74 pmol/L). Model 3: Model 1 + NTproBNP > median (11.74 pmol/L) + GDF-15 > median (37.78 pmol/L). CVD = cardiovascular disease; GDF-15 = growth differentiation factor-15; NTproBNP = N-terminal pro-B-type natriuretic peptide.

Discussion

This study demonstrates that higher GDF-15 levels at admission are associated with 10 year all-cause mortality in STEMI patients treated with pPCI. This relation was independent of clinical risk factors and biomarkers. Moreover, GFD-15 levels at admission have additional prognostic value beyond identified risk factors and other cardiac biomarkers such as cTn and NTproBNP as analysed by Chi-square test and C-statistics.

When stratified GDF-15 levels by the median, patients with a level below the median show an excellent prognosis with a 10 year all-cause mortality rate of 6.2% compared to 19.3% in the group with a GDF-15 level above the median. NTproBNP levels stratified by the median show the same division of 10 year all-cause mortality rates as GDF-15 levels. However, combining these two biomarkers reveal that they have a complementary relation with 10 year all-cause mortality. So, the combination of these biomarkers seems to identify an interesting group of high risk patients. In the group of patients with both GDF-15 and NTproBNP levels below the median, only 3 patients (3.8%) died within 10 year compared to 22 (27.8%) in the group with both GDF-15 and NTproBNP levels above the median. Furthermore, GDF-15 shows additional prognostic information when adding to clinical information and NTproBNP.

Other studies in a broad spectrum of patients have shown that a high level of GDF-15 is independently associated with mortality and adds extra prognostic value on top of various clinical characteristics and biomarkers.[12, 14-18, 24-26] Several of these studies compared the GDF-15 levels at admission of STEMI patients with outcome.[14-16] Two of these studies used a comparable population with GDF-15 levels at admission of STEMI patient treated with pPCI. Eitel et al. demonstrated that GDF-15 levels at admission are a strong predictor of mortality after 6 years.[16] However two important biomarkers, cTn and NTproBNP, were not available is this cohort. Recently, Velders et al. showed in a large cohort that GDF-15 is independently associated with cardiovascular death after 1 year after adjusting for these biomarkers, the severity of cardiovascular disease and other clinical information.[14] However, the number of studies that compared GDF-15 and NTproBNP levels at admission with all-cause mortality on the long-term in STEMI patients treated with pPCI is virtually absent. To our knowledge the current study is the first that investigated these biomarkers at admission in relation to 10 year all-cause mortality in STEMI patients treated with pPCI.

A relatively low number of patients died in this cohort during the 10 year. In the first year of follow-up after STEMI only 5 patients (1.7%) died, of whom 4 died from a cardiac cause. In the years that followed 32 more patients died of whom 6 died from a cardiac cause and 4 patients died of likely cardiac origin. In total 19 patients died of a non-cardiac cause of which 18 after more than 1 year. By

adding biomarkers that reflect a more general state of disease, one might not only capture patients prone for cardiovascular events but also patients prone for non-cardiac events.

Current risk assessment with for example the TIMI or GRACE risk scores is mainly based upon clinical characteristics.[14] Cardiac biomarkers such as cTn and NTproBNP have shown to improve risk prediction.[8, 9] Using a multimarker strategy that captures a broader spectrum of diseases may have added value since it can reveal novel release mechanisms and therefore potential therapeutic targets. The relation of GDF-15 with cTn and NTproBNP has shown that GDF-15 is involved in cardiac pathologies. GDF-15 is induced in the myocardium after ischemia.[11] Several studies have demonstrated that plasma levels of GDF-15, just as NTproBNP, are associated with mechanical stretch, left ventricular mass, concentric left ventricular hypertrophy, reduced left ventricular ejection fraction, and heart failure.[12, 15, 27] Besides these similarities, the characteristics of GDF-15 release are distinct from that of NTproBNP and cTn. Earlier studies demonstrated that cTn release shows a typical rise and fall pattern, indicating the release from dying cardiomyocytes, whereas GDF-15 values increases within hours after ischemia[11] but remain remarkably stable over time during admission[28] and during 6 months of follow-up.[24] GDF-15 follows several stress pathways that differ from the cardiac-specific biomarkers, like NTproBNP and cTn.[11, 29] Plasma levels of GDF-15 may increase in response to pathological stress associated with vascular inflammation, endothelial activation or tissue damage, and an overexpression has been found in several malignancies.[11, 24, 27, 30, 31] Supported by epidemiological studies, this indicates that GDF-15 seems to be a marker of chronic cardiac and vascular pathologies, and is not *per se* related with acute injury.[27, 32]

The last decade a substantial amount of research has been performed that studied the role of GDF-15 levels for the risk assessment in acute coronary syndrome patients. However, to translate novel biomarkers into clinical practice has shown to be challenging. For example, after the discovery of NTproBNP as a marker for heart failure it took 2 decades to implement it into clinical practice.[33] Recently a multidisciplinary working group defined a strategy and checklist to better identify the clinical unmet needs and how novel biomarkers can satisfy in this need.[34] They provide a practical approach to help assess whether a new biomarker would provide clinical benefit. Purely hypothetical, to set an example, we designed a map for a clinical pathway using this checklist and clinical approach (supplemental figure) on how we could advance GDF-15 into clinical practice.

In this figure we used GDF-15 as an add-on test on top of clinical characteristics and cTn and NTproBNP. We acknowledge that we cannot implement GDF-15 into the clinical practice solely based on the results of our study. Especially since it is currently unclear how GDF-15 levels can be lowered by medical therapy or interventions and whether lowering of these levels result in an improved

outcome. However we do hope that we can encourage other stakeholders to follow this example in larger prospective intervention studies to target this unmet clinical need with the development and implementation of GDF-15 in clinical pathway mapping.

Before this can be considered, further research with regard to the patho-physiological mechanisms and the influence of common and novel medical therapies on plasma levels of GDF-15 should be explored. Two potentially ways to do so might be more aggressive lipid-lowering therapies or by anti-inflammatory therapy. The only study so far who investigated the relation between lipid lowering therapy by statin therapy and GDF-15 levels was conducted by Bonaca et al.[35] They found no interaction of GDF-15 with different kind of statins and moreover, GDF-15 levels did not decline after 4 months. Whether more aggressive medical therapy by for example PCSK-9 inhibitors has beneficial effects on GDF-15 is worth investigating. Another way to influence GDF-15 levels may be by anti-inflammatory therapy. In the recently published CANTOS (Canakinumab Anti-inflammatory Trombosis Outcome Study) trial it was shown that targeting the innate immunity pathway with canakinumab, independent of lipid level lowering, led to a significant lower rate of recurrent cardiovascular events than placebo.[36] It would be of interest to explore whether GDF-15 levels may act as biomarker-guided therapy to evaluate the effect of anti-inflammatory therapy.

Several limitations need to be mentioned. First of all, this study was performed in a single center randomized trial with a limited number of patients; so, the results are limited to the patients eligible for the trial, although the results apply for a broad range of STEMI patients. All patients were treated according to the institutional MISSION! which provided an integrated approach of MI care to optimize treatment. This yielded in a very homogeneous STEMI population. Secondly, limited data is available about the stability of GDF-15 samples after long-term storage at -80°C. However, an earlier study conducted by Daniels at al.[37] measured GDF-15 in 1391 serum samples that were stored for 14-18 years. The fact that GDF-15 levels were prognostic for outcome presents support that there is sufficient stability to preserve a clinical signal.[37] Moreover, the median of the GDF-15 levels in the present cohort was 37.78 pmol/L which is comparable with the median levels of GDF-15 of 38.63 pmol/L in a similar cohort of STEMI patients.[16] Third, a relatively low number of events were noted during follow-up. To make the results more robust, larger cohorts of STEMI patients treated with pPCI should be followed. An explanation for the low number of events could be the exclusion criteria of patients older than 80 years. This could have led to a relatively young cohort with a mean age of 59 years. The last issue that should be addressed is the low number of events in relation to the multivariate Cox model. This paper has investigated the added-value of GDF-15 for the prediction of 10-year all-cause mortality in addition to established risk factors as well as potential confounding variables identified

from univariate analysis on our data. As opposed to fitting a prognostic model, a testing procedure was used, which first estimates a multivariate Cox model to account for the joint effect of these risk factors and potential confounders, after which the added-value was assessed from likelihood ration testing for the addition of GDF-15 and NTproBNP to the base model.

Conclusion

Baseline GDF-15 levels are independently associated with 10 year all-cause mortality rates and improve long term-risk stratification in STEMI patients treated with pPCI on top of clinical variables and other cardiac biomarkers. Before implementation into clinical practice can be considered, the clinical utility needs to be further validated in prospective intervention studies.

Acknowledgements

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Compliance with Ethics Guidelines

The study protocol was approved by the institutional ethical committee. This study was conducted according to the declaration of Helsinki and written informed consent was obtained from all patients before enrolment in the study.

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Chapter 6.

A rapid (differential) effect of rosuvastatin and atorvastatin on high-sensitivity cardiac Troponin-I in subjects with stable cardiovascular disease

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Abstract

Serum troponin within the normal range is an emerging predictor of cardiovascular mortality. We aimed to determine how rapidly high-sensitivity troponin –I (hs-cTnI) levels are lowered by statin therapy in patients with stable cardiovascular disease. In the RADAR substudy, patients were randomized, to atorvastatin 20 mg/day (n = 39) or rosuvastatin 10 mg/day (n = 39) and up-titrated in 6 week intervals to 80 mg of atorvastatin or 40 mg of rosuvastatin. Hs-cTnI concentrations were measured at baseline and at 6 and 18 weeks of follow-up. Statin treatment resulted in a mean change of serum hs-cTnI of -8.2% (p=0.010) after 6 weeks and – 12.3% (p=0.001) after 18 weeks. After 18 weeks, hs-cTnI levels were lowered by 21.8% with atorvastatin and by 4.1% with rosuvastatin (p=0.001 and p=0.133, respectively). During statin therapy serum hs-cTnI levels decreased rapidly within weeks of treatment, suggesting an effect beyond long-term atherosclerosis regression. Mechanisms that mediate this effect require further study.

Introduction

Serum cardiac troponin is a specific marker of myocardial injury and is the cornerstone in the diagnosis and acute management of myocardial infarction.(1) The introduction of high sensitivity assays (hs-cTn), which are up to 100 times more sensitive compared with the first-generation assays, permits the accurate determination of very low levels of circulating cardiac troponin.(2) Minor increases in troponin level might be due to myocardiocyte necrosis or apoptosis which in turn might be caused by subclinical coronary artery disease resulting in transient ischaemia, inflammation, myocardial strain or volume or pressure overload.(3) Interestingly, several studies show that elevated serum hs-cTn levels, even those within the normal range, are an emerging independent predictor of cardiovascular (CV) mortality in patients with and without cardiovascular disease (CVD).(4-8) With the development of these high sensitivity assays, cardiac troponin assessment may potentially be added to other traditional risk factors to predict cardiovascular risk.

Like hs-cTn, elevated brain-type natriuretic peptide (BNP), to levels still normal, is associated with higher risk of heart failure, and interventions in response to these minor increases, improve outcome.(9)

Recently, Ford et al. explored in the West of Scotland Coronary Prevention Study (WOSCOPS) cohort whether hs-cTnI could be modified by statins.(4) They showed that circulating cTnI was lowered by 13% at 1 year in the pravastatin group as compared with placebo ($p<0.001$). Furthermore, the group with the biggest decline of cTnI levels at 1 year was associated with a 5-fold greater reduction of future coronary risk compared with the group with the biggest incline, independent of cholesterol lowering. The authors concluded that serial hs-cTnI measurements have major potential to monitor cardiovascular future coronary risk and may predict future coronary heart disease risk reduction following therapeutic interventions.

However, the earliest onset of this effect and specificity as to the statin used are unknown. The present analysis from a randomized study sought to characterize the effect (magnitude and time of onset) of atorvastatin (ATOR) or rosuvastatin (ROSU) on hs-cTnI levels within the normal range in patients with stable CVD.

Materials and Methods

Study design

The RADAR (Rosuvastatin and Atorvastatin in different Dosages And Reverse cholesterol transport) study was a randomised, open label trial, conducted in 29 centres in The Netherlands.(22) Three pre-selected hospital centres were designated to store biobank samples and to perform extended lab sampling and

storage for their patients to obtain the metabolite profiles. By protocol, a total of 80 patients were included in this present official and prospective sub study of the RADAR study to assess serum hs-cTnI. So it was a prospectively defined subset of patients from those 3 pre-selected hospitals that were included in this sub study. Several RADAR sub studies have been published earlier with the same patient population as was currently used. (23-25)

The RADAR study design has been described in detail elsewhere and is presented in Figure 1.(22) In short, patients with stable CVD entered a 6-week dietary run-in phase. Subsequently, they were, if eligible, randomised to receive either ATOR 20mg or ROSU 10mg. On the following visits, at 6 and 12 weeks the doses were, by protocol, forced up-titrated to 40 mg ATOR or 20mg ROSU (at 6 weeks) and 80 mg ATOR or 40 mg ROSU (at 12 weeks). The study was designed and conducted in accordance with the Declaration of Helsinki and in compliance with the ethical principles of Good Clinical Practice. Appropriate ethics committees or institutional review boards approved the study, and all patients gave written, informed consent.

Study population

Patients aged between 40-80 years were eligible for study participation if they had established CVD, fasting high-density lipoprotein cholesterol (HDL-c) <1.0 mmol/L and fasting triglycerides ≤ 4.5 mmol/L at visits 1 and 2. Exclusion criteria were the use of other lipid-lowering drugs after enrolment, pregnancy or lactation, active arterial disease described as within 2 months of entry into the dietary lead-in phase or likely intervention within 6 months of randomisation, cases of familial hypercholesterolemia, severe concomitant morbidity or indices of impaired renal or liver function.

Measurements and statistical analysis

Hs-cTnI was measured at week 0 (baseline) and after 6 and 18 weeks of treatment. Patients were required to fast for at least 12h before blood collection and abstained from alcohol for the same period prior to blood sampling. Plasma samples were isolated after centrifugation at 4°C, 3000 rpm for 15 min and stored in -80°C freezers until analysis.

Hs-cTnI concentrations were measured using an automated clinically validated assay (i1000SR ARCHITECT, Abbott, UK). The assay has a limit of detection of 1.3 ng/L with a 99th percentile in the general population of 34.2 ng/L for men and 15.6 ng/L for women.(2) Two-level quality controls from the manufacturer ran with coefficients of variation <6%. Pre-specified, by protocol and according to current literature all patients with hs-cTnI levels above the 99th percentile were excluded from further analysis.(4-8)

Comparisons of baseline characteristics between groups were made using chi-square test for categorical variables and independent T-test or Mann-Whitney

U test for continuous variables. First, median changes in hs-cTnI levels between 2 time points were determined using a Wilcoxon-signed rank test. Next, hs-cTnI was log-transformed since it was non-normally distributed. Mean log changes in hs-cTnI and mean low-density-lipoprotein cholesterol (LDL-c) levels between 2 time points were determined using a paired sample T-test. To compare the differences in hs-cTnI levels between treatment groups an independent T-test was performed. A multivariate regression analysis was used to compare the change between baseline and week 18 between the treatment groups which was adjusted for baseline log hs-cTnI concentrations and prior aspirin use. A Pearson correlation coefficient was used for the total group, for the ATOR group as for the ROSU group to assess the association between changes in LDL-c and changes in log hs-cTnI. Analyses were conducted with SPSS 23.0 statistical analysis software (IBM, Armonk, NY, USA).

Results

In all 80 randomized patients sufficient sample material to measure hs-cTnI was available. In 2 patients hs-TnI concentrations were at or above the sex-specific 99th percentile and they were excluded for further study.

Of the 78 remaining study participants mean age was 65 years and 73 (94%) were male. As expected, the baseline characteristics, concomitant medication use and prior cardiovascular disease were similar in the two treatment groups (Table 1). Patients in the ATOR group used significantly more often aspirin than patients in the ROSU group. The lipid profiles at baseline did not differ between the two groups. There was a clear decrease of all the measured lipid levels during follow-up. In short, LDL-c decreased from 3.78 mmol/L to 1.70 mmol/L after 18 weeks in the ROSU group, and from 3.63 mmol/L to 1.77 mmol/L in the ATOR group. The decline of LDL-c did not differ significantly between the two treatment groups.

Effects of increasing doses of statin therapy on hs-cTnI are summarized in table 2. Median baseline hs-cTnI in the combined statin group was 4.50 ng/L (IQR: 3.35-6.67). Divided by treatment group, the median baseline was 3.90 ng/L (IQR: 3.10-5.65) for ATOR and 4.90 ng/L (IQR: 4.20-7.10) for ROSU. The median change from baseline in the combined statin group was -0.7 ng/L (IQR: -2.0 to 0.3), (p-value = 0.005) after 6 weeks and -0.8 ng/L (IQR: -2.1 to 0.4), (p-value <0.001) after 18 weeks. In the ATOR group, median hs-cTnI changes were -0.7 ng/L (IQR: -1.8 to 0.6), (p-value = 0.032) after 6 weeks and -1.1 ng/L (IQR: -1.9 to 0.0), (p-value <0.001) after 18 weeks. In the ROSU group no significant differences were observed. The median changes from baseline were -0.7 ng/L (IQR: -2.0 to 0.2), (p-value = 0.069) after 6 weeks and -0.6 ng/L (IQR: -2.1 to 1.1), (p-value = 0.124) after 18 weeks.

Table 1. Patient demographics and baseline characteristics

	ROSU + ATOR (n=78)	Rosuvastatin (n=39)	Atorvastatin (n=39)	p-value
Patiënt characteristics				
Gender, male (%)	73 (93.6)	37 (95)	36 (92.3)	0.644
Age, years	64.9 (8.9)	65.8 (7.7)	64.1 (9.8)	0.403
Body mass index (kg/m²)	28.5 (3.8)	29.0 (4.0)	28.1 (3.5)	0.279
Systolic blood pressure (mmHg)	139.3 (17.5)	140.1 (17.6)	138.5 (17.6)	0.695
Diastolic blood pressure (mmHg)	81.3 (8.0)	81.7 (7.9)	80.9 (8.1)	0.642
Diabetes mellitus	13 (16.7)	5 (12.8)	8 (20.5)	0.362
Concomitant medication				
Beta blockers	62 (79.5)	28 (71.8)	34 (87.2)	0.092
Antihypertensive medication	49 (59.0)	24 (61.5)	22 (56.4)	0.645
Platelet inhibitors	8 (10.3)	4 (10.3)	4 (10.3)	1.000
Ascal	63 (80.8)	28 (71.8)	35 (89.7)	0.044
Vitamin K antagonist	11 (14.1)	8 (20.5)	3 (7.7)	0.104
Laboratory measurements				
Creatinine, µmol/L	103.8 (16.4)	103.6 (16.5)	104.1 (16.5)	0.886
Total-c, mmol/L	5.78 (1.20)	5.85 (1.25)	5.70 (1.16)	0.569
HDL-c, mmol/L	0.64 (0.10)	0.66 (0.09)	0.62 (0.11)	0.090
LDL-c, mmol/L	3.70 (0.94)	3.78 (0.86)	3.63 (1.01)	0.506
Triglycerides, mmol/L	3.01 (1.54)	2.84 (1.54)	3.17 (1.55)	0.349
Prior cardiovascular disease				
Transient ischaemic attack	5 (6.4)	2 (5.1)	3 (7.7)	0.644
Ischaemic stroke	4 (5.1)	3 (7.7)	1 (2.6)	0.305
Carotid artery disease	0 (-)	0 (-)	0 (-)	-
Angina pectoris	61 (78.2)	31 (79.5)	30 (76.9)	0.784
Myocardial infarction	53(67.9)	29 (74.4)	24 (61.5)	0.225
PTCA	30 (38.5)	15 (38.5)	15 (38.5)	1.000
Peripheral arterial disease	6 (7.7)	2 (5.1)	4 (10.3)	0.395

Categorical variables expressed by number (%)
Numerical variables expressed by mean (SD) or median (IQR)
Comparisons between groups were made using chi-square test for categorical variables and independent T-test or Mann-Whitney U test for continuous variables

Figure 2 illustrates the time course of mean log hs-cTnI during statin therapy. The mean log hscTn-I value at baseline in the combined statin group was 0.70 (SEM: 0.03). After 6 weeks the mean log hs-cTnI was 0.64 (SEM: 0.03) and after 18 week 0.61 (SEM: 0.03). The mean changes of log hs-cTnI were -8.2% (p-value= 0.010) after 6 weeks and -12.3% (p-value < 0.001) after 18 weeks of treatment. The difference between 6 and 18 weeks was -4.5% (p-value = 0.202).

A repeated measure ANOVA determined that mean log hs-cTnI concentrations differed statistically significantly between time points (p-value < 0.001).

Table 2. Troponin concentrations (in ng/L)

	Baseline	Week 6	W6-Baseline	p-value	Week 18	W18-Baseline	p-value
ROSU+ ATOR	4.50 (3.35-6.75)	4.25 (2.80-6.00)	-0.7 (-2.0 to 0.3)	0.005	4.20 (2.68-6.43)	-0.8 (-2.1 to 0.4)	<0.001
ROSU	4.90 (4.20-7.10)	4.90 (3.25-6.63)	-0.7 (-2.0 to 0.2)	0.069	4.90 (3.35-8.00)	-0.6 (-2.1 to 1.1)	0.124
ATOR	3.90 (3.10-5.65)	4.00 (2.45-5.50)	-0.7 (-1.8 to 0.6)	0.032	3.50 (2.05-5.10)	-1.1 (-1.9 to 0.0)	<0.001

Values are presented as median with interquartile range.
The W6-Baseline and W18-Baseline time points are given in absolute values' differences from baseline.
W6 = Week 6, W18 = Week 18

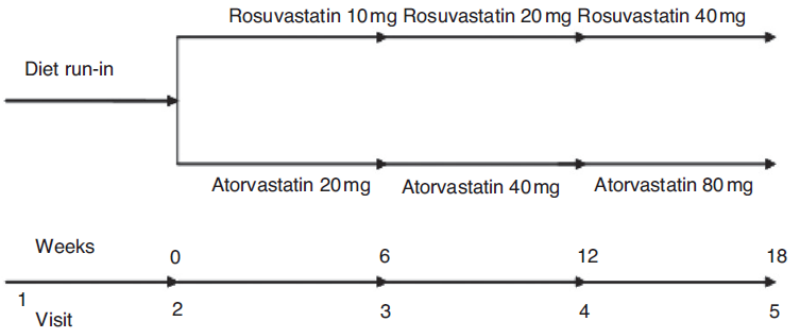

Figure 1. RADAR study design.

Figure 3 presents the time-courses of log hs-cTnI in the separate statin regimens. At baseline the mean log hs-cTnI level was 0.64 (SEM 0.04) in the ATOR group and 0.75 (SEM 0.04) in the ROSU group. Baseline log hs-cTnI levels between the two statin groups tended to differ significant (p-value = 0.053). After 6 weeks mean log hs-cTnI level in the ATOR group was 0.58 (SEM: 0.04) and 0.70 (SEM: 0.05) in the ROSU group (p-value = 0.071), and after 18 weeks 0.50 (SEM: 0.04) in the ATOR group and 0.72 (SEM: 0.05) for the ROSU group. ATOR reduced log hs-cTnI by 9.1% (p-value = 0.074) after 6 weeks and by 21.8% (p<0.001) after 18 weeks. In the ATOR group the difference of log hs-cTnI between 6 and 18 weeks was 14% (p-value = 0.023). The reduction of log hs-cTnI in the ROSU group was 7.2% (p-value = 0.071) after 6 weeks and 4.1% (p-value = 0.133) after 18 weeks. When adjusted for baseline log hs-cTnI levels and prior aspirin use, ATOR reduced hs-cTnI levels between baseline and 18 weeks more distinctly than ROSU (mean

log difference 0.09; p=0.022). Mean changes of hs-cTnI between baseline and 18 weeks of treatment were not correlated using a Pearson correlation with mean changes of LDL-c in the combined group, ATOR or ROSU group (R = 0.011, p-value = 0.928; R = 0.17, p-value = 0.334; and R = -0.072, p-value = 0.698, respectively). (Results are not shown)

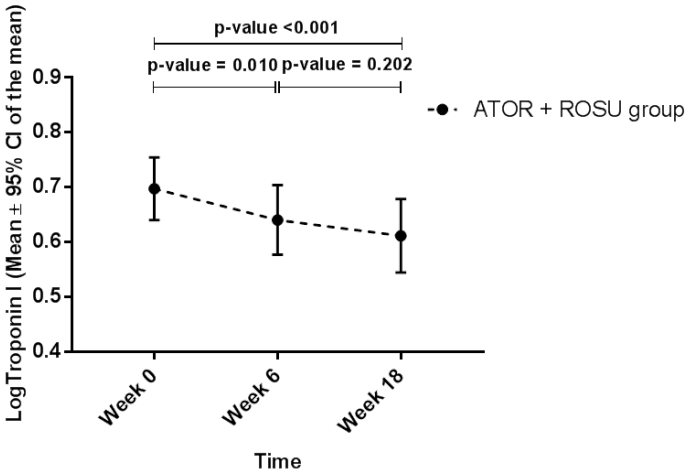

Figure 2. The figures illustrate the effect of statin therapy on the total group. Hs-cTnI was log-transformed since it was non-normally distributed. Hs-cTnI is expressed as mean ± 95% confidence interval of the mean.

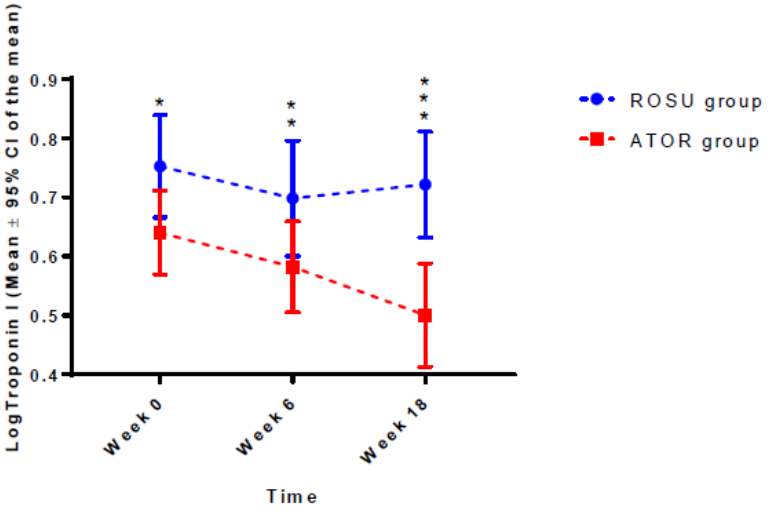

Figure 3. For each time point mean value and 95% confidence interval of the mean are presented. *P values* refer to differences between the two treatment groups for each time point (P=0.053*, P=0.071**, P=0.001***).

Discussion

In this substudy of the RADAR trial, the effect of ATOR and ROSU on serum hs-cTnI was explored. Hs-cTnI levels of patients with stable CVD decreased significantly within weeks of statin treatment. Furthermore, a reduction of hs-cTnI levels occurred with a low dose of statins and in addition, there was evidence that this effect was more pronounced in the ATOR treatment regimen than in the ROSU treatment regimen.

These novel findings suggest a rapid benefit of statin treatment on ongoing subclinical myocardial damage. With the hs-cTn assays, cTn concentrations are emerging as promising biomarkers for the prediction of cardiovascular events and mortality. Several studies demonstrated that increases of cTnI within the normal range are associated with increased cardiovascular risk.(4-7, 10-14) In this relatively uncharted field serial cTn measurements have a major potential to monitor risk and to assess the influence of therapeutic interventions on cardiovascular risk.(4) Our results emphasise this, showing that initiation of lipid lowering treatment provides a rapid, as opposed to long-term, effect on cTn.
Only, limited information is available about the time frame in which statin therapy can reduce serum cTn levels. We show that after 6 weeks of treatment with ATOR 20mg or ROSU 10mg once daily the average decrease of hs-cTnI is 8%. No earlier studies studied the effect of statin therapy in this time frame. Furthermore, it was unknown whether this decline advances with such a low level and whether this decrease associates with a better patient outcome. After 18 weeks serum hs-cTnI levels were on average 12% lower than at baseline in the combined statin group, 22% in the ATOR group and 4% in the ROSU group. Earlier Gravning et al.(15) reported in elderly patients with chronic heart failure that hs-cTnT levels at 3 months of therapy with 10 mg ROSU did not differ from the hs-cTnT levels at 3 months of placebo therapy. This study, however, differs in several aspects from the current study. First of all, an hs-cTnT assay was used instead of an hs-cTnI assay in the present study. Secondly, their study population consisted of elderly patients with heart failure which might have influenced the change of cTn levels and thirdly, in the study of Graving et al.(15) patients received ROSU 10mg daily for 3 months whereas in the present study the doses were up-titrated every 6 week, to ROSU 40mg after 12 weeks.

Ford et al.(4) showed that after 1 year therapy with pravastatin hs-cTnI levels were reduced by 19% (compared to 6% in the placebo group). In twice as many participants in the statin group compared with the placebo group, hs-cTnI levels decreased by >25%. This group was at the lowest risk for future events. White et al.(6) also assessed the effect of 1-year statin therapy on cTnI concentration. These authors found that compared with placebo, pravastatin 40 mg once daily reduced cTnI levels by a median of 3 ng/L. In the placebo group TnI levels did not change in 1 year. However, this treatment effect did not result in important differences in future cardiac events.

In the present study statin therapy led to a decline of hs-cTnI independently of LDL-c reductions. Statins are the most widely prescribed agents in treating CVD and are well-known for their pleiotropic lipid-lowering independent effect. Statins positively interfere with critical components of the atherosclerotic process, and have beneficial anti-inflammatory effects on the vascular wall, improve nitric oxide bioavailability and improve endothelial function.(16) These processes can lead to cardiomyocyte necrosis, apoptosis or reversible injury with increased cardiomyocyte membrane permeability, which in turn can result in cardiac troponin release.(17) Differences between LDL-c lowering effects between statins are well known (18) but variances in pleiotropic effects are less clear. However, there are differences in pharmacokinetics and pharmacodynamics between statins(19) which might explain differences observed between ATOR and ROSU. For example, ATOR is a relatively lipophilic compound while ROSU is relatively hydrophilic.(20) However, whether this physic-chemical difference underlies different hs-cTnI release patterns is unknown as yet.

Some limitations need to be addressed. First of all, our substudy had no placebo controlled group, which makes it difficult to relate statin therapy to the decline of cTnI concentrations observed. However, earlier studies showed that statin therapy compared to placebo reduces hs-cTnI concentrations more effectively after one year.(4) In the present study recruitment in the phase with lifestyle and diet modification might have led to change in hscTnI concentration. However, the first blood withdrawal was after a 6 week diet run-in period and therefore any further changes of hscTnI levels are not likely to depend on dietary or lifestyle changes. Secondly, this study contains patients with known CVD only which makes it difficult to generalize our findings in broader populations. Thirdly, there was no long-term follow up data available to assess the role of our findings on patients' outcome. Fourth, some biological intra-individual variation of hs-cTnI exists, which might have influenced the results. However, the biological intra-individual variation is about 3% for hs-cTnI concentrations.(21) In the present study the average statin-induced decrease of hs-cTnI was 12%, so if any, the biological intra-individual variation can only partly contribute to the decrease of hs-cTnI. Furthermore, only patients who were stable for at least 2 months prior to inclusion were included and none of the patients had a cardiac event during the follow-up which could have affected their serum hs-cTnI concentrations. Lastly, though the difference in the log of the troponin I over time decreased during statin therapy the data on rosuvastatin to atorvastatin only showed no overlap at 18 weeks, while before that time it did. Whether troponin levels are or are not different in ATOR versus ROSU treatment should be handled with caution. Nevertheless, there was an overall decline of troponin levels after statin treatment was initiated.

Conclusion

In patients with stable CVD hs-cTnI decreased significantly during statin therapy, which was independent of decreases of LDL-c concentrations. Statin-induced effect on hs-cTnI was evident as early as after 6 weeks. This effect was more pronounced with atorvastatin than with rosuvastatin. This novel finding suggests a rapid benefit of statin treatment on ongoing subclinical myocardial damage. Whether the short-term effects of statin therapy on hs-cTnI are directly related to improved patient outcome is still unknown, but our findings suggest an urgent need to test this potential.

Study highlights

What is the current knowledge on the topic?
Serum troponin within the normal range is an emerging predictor of cardiovascular mortality.
Recently, a randomized trial showed that circulating troponin I was lowered by 13% at 1 year in the pravastatin group as compared with placebo.

What question did this study address?
The aim of this study was to determine how rapidly high-sensitivity troponin –I (hs-cTnI) levels are lowered by statin therapy in patients with stable cardiovascular disease.

What does this study add to our knowledge?
Hs-cTnI decreased significantly during statin therapy, which was independent of decreases of LDL-c concentrations. Statin-induced effect on hs-cTnI was evident as early as after 6 weeks. This novel finding suggests a rapid benefit of statin treatment on ongoing subclinical myocardial damage.

How might this change clinical pharmacology or translational science?
Whether the short-term effects of statins on hs-cTnI are directly related to improved patient outcome is unknown, but our findings suggest an urgent need to test this potential. Serial troponin measurements could have major potential to assess cardiovascular risk and monitor the impact of therapeutic interventions.

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Chapter 7.

Plasma LDL-cholesterol level at admission is independently associated with infarct size in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention

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Abstract

Introduction
Hypercholesterolemia is a well-known risk factor for developing atherosclerosis and subsequently for the risk of a myocardial infarction (MI). Moreover, it might also be related with the extent of damaged myocardium in the event of a MI. The aim of this study was to evaluate the association of baseline low density lipoprotein-cholesterol (LDL-c) level with infarct size in patients with ST-segment elevation myocardial infarction (STEMI) after primary percutaneously coronary intervention (pPCI).

Methods
Baseline blood samples were obtained from all patients admitted between 2004 and 2014 with STEMI who underwent pPCI. Patients were excluded in case of out of hospital cardiac arrest, treatment delay ≥10 hours or no complete reperfusion after pPCI in the culprit vessel. Peak creatine kinase (CK) level was used for infarct size estimation, defined as the maximal value during admission.

Results
2248 patients were included in this study (mean age 61.8 ± 12.2 years; 25.0% female). Mean LDL-c level was 3.6 ± 1.1 mmol/L and median peak CK level was 1275 U/L (IQR 564-2590 U/L). Baseline LDL-c level (β =0.041; [95%CI 0.019-0.062]; p-value<0.001) was independently associated with peak CK level. Furthermore, left anterior descending artery as culprit vessel, initial TIMI 0-1 flow in the culprit vessel, male gender, and treatment delay were also correlated with high peak CK level (p<0.05). Prior aspirin therapy was associated with lower peak CK level (β=-0.073 [95%CI -0.146 to -0.000], p=0.050).

Conclusion
This study demonstrates that besides the more established predictors of infarct size, elevated LDL-c is associated with augmented infarct size in patients with STEMI treated with pPCI.

Introduction

In patients with ST-segment elevation myocardial infarction (STEMI), primary percutaneously coronary intervention (pPCI) has shown to be an effective strategy to reduce the size of myocardial infarction (MI)[1]. Other well-established determinants of infarct size are long duration of ischemia, left anterior descending (LAD) artery as culprit lesion, and low pre- and post-procedural thrombolysis in myocardial infarction (TIMI) flow[2]. Identifying more determinants that influence infarct size could further aid in reducing infarct size. Hypercholesterolemia is a well-known risk factor for developing atherosclerosis[3, 4] and subsequently for the risk of a MI[5-8]. Moreover, it might also be related to the extent of damaged myocardium in the event of a MI[9-12].

Patient's treatment and prognosis is for an important part determined by the myocardial infarct size.

Myocardial infarct size can be measured with established biomarkers such as creatine kinase (CK) and cardiac Troponin-T (cTnT). Peak CK levels are as accurate as cTnT in estimating myocardial infarct size in STEMI patients who underwent successful pPCI[13], and is an established non-invasive measure of infarct size and severity[14-16]. Furthermore, peak CK levels are strongly associated with clinical outcome[14-16]. Myocardial reperfusion injury (MRI) is the term for further injury to the ischemic myocardium which occurs after restoration of blood flow which reduces the beneficial effect of myocardial reperfusion[17]. It is suggested that approximately 30% of myocardial infarct size is determined by MRI[17] and although the pathophysiological mechanisms of MRI are not fully elucidated[17], this might be preventable. Studies in animal models have demonstrated that hypercholesterolemia may aggravate MRI[9, 10], for example by the no-flow phenomenon[11] or by increased myocardial oxidative stress[10, 12] and inflammation[12].

A relation of hypercholesterolemia with infarct size in clinical studies can have important implications and may lead to more advanced therapies like antioxidant therapy and novel lipid lowering therapy.

So, we hypothesize that high LDL-cholesterol (LDL-c) levels during admission are associated with infarct size in patients with STEMI. Therefore, the aim of this study was to evaluate the association between baseline LDL-c levels with enzymatic infarct size using peak CK in patients with STEMI after pPCI.

Methods

Study population
Consecutive patients admitted with STEMI between February 2004 and January

2014 at the Leiden University Medical Center (LUMC) were included in this retrospective study. All patients were treated with pPCI according to the current guidelines-based institutional MISSION! protocol[18-20]. STEMI was defined as typical electrocardiographic (ECG) changes (ST-segment elevation ≥ 0.2 mV in ≥ 2 contiguous leads in V_1 through V_3, ≥ 0.1 mV in other leads, or presumed new left bundle branch block) and a typical rise and fall of cardiac biomarkers accompanied with chest pain for at least 30 minutes[21]. Pre-defined exclusion criteria were: 1. Patients presenting with an out of hospital cardiac arrest (OHCA); 2Time of ischemia ≥ 10 hours; 3. No complete reperfusion after pPCI in the culprit vessel, defined as TIMI 3 post procedural flow in the culprit vessel; 4. Unknown levels of peak CK during admission. For retrospective analysis of clinically acquired data, the institutional review board waived the need for patient written informed consent. Since the data didn't contain any identifiers that could be traced back to the individual patient and the data are obtained for patient care, the Dutch Central Committee on Human-Related Research permits the use of anonymous data without prior approval of an institutional review board. This study was conducted according to the declaration of Helsinki.

Study procedure

The MISSION! protocol contains a standardized pre-hospital, in-hospital and outpatient clinical framework to optimize treatment. The pre-hospital phase includes diagnosis by a high-quality 12-lead ECG. All patients were treated during the pre-hospital phase with a loading dose of aspirin 300mg orally and a loading dose of either clopidogrel 300mg orally or prasugrel 60mg orally. Furthermore, all patients received abciximab (dose abciximab, 0.25 mg/kg bolus followed by an infusion of 0.125 Ag/kg per minute during 12 hours) in the absence of contraindications. In total, 4.9% of the patients didn't receive abciximab. During the in-hospital phase eligible patients were directly sent to the catheterization lab. Primary PCI was performed according to the clinical guidelines. If tolerated, within 24 hours after admission, patients were prescribed β-blockers, angiotensin-conversing enzyme (ACE) inhibitors, statins, acetylsalicylic acids, and thienopyridines. More than 95% of the patients received a statin, an acetylsalicylic acid, and a thienopyridine and more than 85% of the patients received a β-blocker and an ACE-inhibitor within 24 hours after admission. For the measurement of lipid levels and cardiac biomarkers, plasma samples were obtained from included patients before pPCI was performed. Subsequently, for cardiac biomarker assessment, plasma samples with 6 h interval for at least 48 h were withdrawn. The LDL-c level was calculated from total cholesterol, triglycerides, and HDL-cholesterol concentrations, using the Friedewald formula[22]. CK activity was measured at 37° C with an IFCC-traceable method (Roche Diagnostics) on Modular P analyzers.

Data acquisition/Clinical data

Since the implementation of this protocol in February 2004, clinical and angiographic data and laboratory measurement are systematically collected for

each MISSION! patient in EPD-VISION, using a unique study number.

Study endpoint
Study endpoint was defined as the enzymatic infarct size defined as the maximal level of CK measured during admission. Peak CK level was defined as the maximal CK level measured during admission, provided that maximal CK level is preceded and followed by lower CK levels.

Statistical analysis
Continuous variables with a normal distribution are presented as mean ± standard deviation. The Mann-Whitney U-test was used in case of two groups and the Kruskal-Wallis test was used in case of more than two groups to test differences in non-normally distributed data. These are presented as medians and interquartile range (IQR). Categorical variables are expressed as numbers and percentages. Skewed distributed outcome variables were log-transformed for linear regression analysis. At first, all variables were analyzed in a univariate linear regression model. Secondly, all variables with a p-value <0.10 in univariate analysis were included in a multivariate linear regression model. The fitted beta regression coefficients were compared with their standard errors using the t-test and p-values and 95% confidence intervals were calculated. All statistical tests were 2-tailed, p-values <0.05 were considered statistically significant. Analyses were conducted with SPSS 23.0 statistical analysis software (IBM, Armonk, NY, USA).

Results

Patients' characteristic
In total, 2248 patients were evaluated in the current study (Figure 1). Mean age was 61.8 ± 12.2 years and 562 (25.0%) were female. Cardiovascular risk factors, such as current smoking (45.2%), positive family history of coronary artery disease (39.7%), and hypertension (36.2%), were highly prevalent. Before admission 407 (18.1%) patients were on statin therapy, 17.8% was on a β-blocker, 21.0% was on an ACE-inhibitor or AT2-antagonist, 1.2% was on a thienopyridine and 16.2% was already on an acetylsalicylic acid. In total, 8.5% had a previous myocardial infarction before admission, 6.9% had a PCI before admission and 2.8% were known with chronic kidney disease. All patient characteristics are demonstrated in Table 1. Data about the excluded patients (n=875) are summarized in the supplementary table. Baseline and clinical characteristics of the excluded patients were, essentially, similar compared to the included patients.

Clinical characteristics
Table 2 shows the clinical characteristics of the patients. The LAD artery was identified as the culprit vessel in 42.1% of the cases. 71.2% of the patients had a TIMI flow of 0 or 1 before reperfusion in the culprit vessel. The median time

of ischemia was 167 min (IQR 123-246 min). 56.6 % of the patients had multi-vessel disease. Mean LDL-c at baseline was 3.6 ± 1.1 mmol/L and median peak CK was 1275 U/L (IQR 564-2590 U/L). In the group of patients using a statin before admission the mean LDL-c was 2.74 ± 1.0 mmol/L versus 3.80 ± 1.0 mmol/L in the non-statin users before admission (p<0.001) (results not shown).

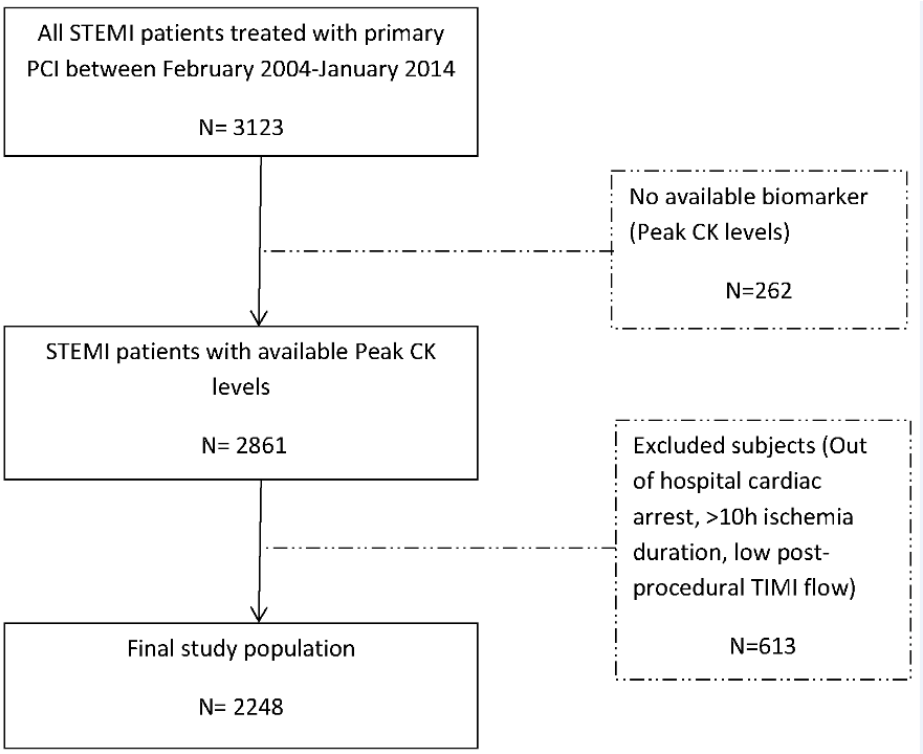

Fig 1. Flowchart of the population. (2-column fitting image). Patients were eligible if they were admitted with STEMI and treated with pPCI. Patients were excluded if peak CK level was not known, if they had an out hospital cardiac arrest (OHCA), if time of ischemia was ≥ 10h, or if post-procedural TIMI flow was <3.

Correlation between variables and enzymatic infarct size

Figure 2 shows the relation between several variables and infarct size. LDL-c values were positively associated with infarct size (Fig. 2A). Infarct size is higher when the culprit vessel is the LAD, than in patients with another vessel as culprit lesion (Fig. 2C). A low TIMI flow (0 or 1) before pPCI is significantly associated with a large infarct size Fig. 2D). A long delay from onset symptoms to balloon time also resulted in a large infarct size (Fig. 2B). Median infarct size in male patients was larger than that in female patients (Fig. 2E) and prior aspirin use was associated with smaller infarct size (Fig. 2F).

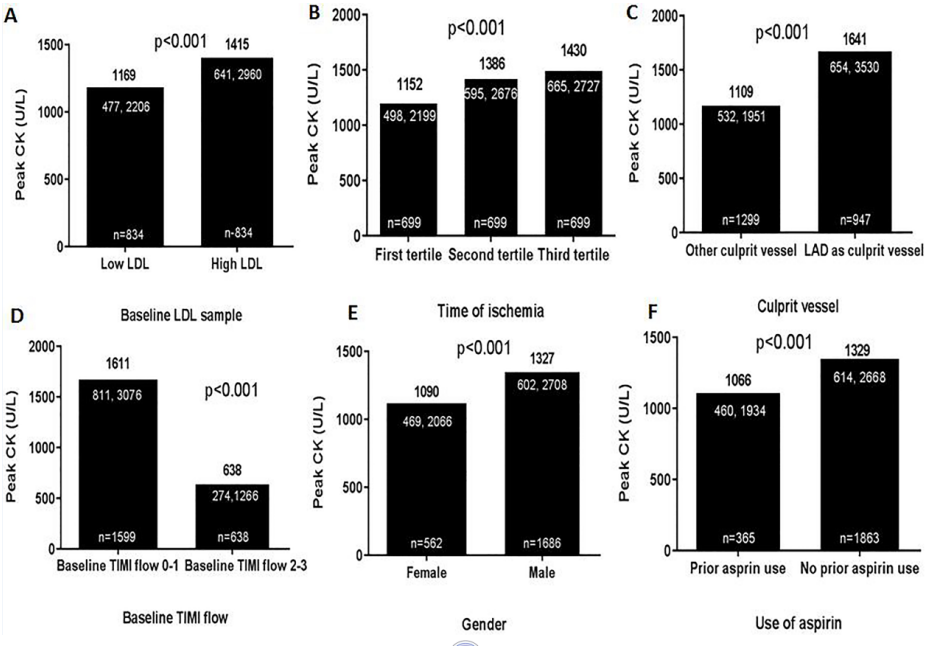

Figure 2. Impact of variable on infarct size. (2-column fitting image). Peak CK expressed as median with interquartile range. (A) Infarct size in patients having LDL-cholesterol below median (3.61 mmol/L) and above the median. (B) Total symptom-onset-to-balloon time divided into 3 tertiles, first tertile <138 min, second tertile 138-215 min, third tertile 216-600 min. (C) Influence of culprit artery on infarct size. (D) Influence of baseline TIMI flow on infarct size. (E) Influence of gender on infarct size. (F) Influence of the use of aspirin prior to myocardial infarction on infarct size. LAD= Left anterior descending artery; Baseline TIMI flow = TIMI flow before primary PCI in the culprit vessel.

Table 3 shows the univariate and multivariate variables that correlate with peak CK level. Multivariate analysis showed that LDL-c levels were independently associated with peak CK level (β=0.041 [95% CI: 0.019-0.062), p<0.001). In addition, male gender (β=0.103 [95% CI: 0.052-0.154], p<0.001), culprit lesion in LAD (β=0.152 [95% CI 0.108-0.195], p<0.001), ischemic time (β=0.206 [95% CI 0.102-0.309], p=0.001), and baseline TIMI flow 0-1 (β=0.431 [95% CI 0.384-0.478], p<0.001) were independently associated with peak CK level. Prior aspirin therapy was significantly associated with lower peak CK level (β=-0.073 [95%CI -0.146 to 0.000], p=0.050). Statin use before admission was in univariate analysis associated with a lower infarct size; however, this effect perished in multivariate analysis.

Table 1. Patients' characteristics

Variable	All patients (n=2248)
Patients characteristics	
Age (years)	61.8 ± 12.2
Female gender, n (%)	562 (25.0)
Cardiovascular risk factors	
Current smoker, n (%)	1022 (45.2)
Ex-smoker, n (%)	274 (12.2)
Non-insulin dependent diabetes mellitus[a], n (%)	177 (7.9)
Insulin dependent diabetes mellitus, n (%)	82 (3.6)
Family history of coronary artery disease[b], n (%)	892 (39.7)
Treated hypercholesterolemia[c], n (%)	447 (19.9)
Treated hypertension[d], n (%)	814 (36.2)
Body mass index (kg/m^2)	26.6 ± 4.0
Comorbidities	
Previous myocardial infarction, n (%)	192 (8.5)
Previous PCI, n (%)	154 (6.9)
Previous CABG, n (%)	42 (1.9)
History of cerebrovascular disease, n (%)	100 (4.4)
Chronic kidney disease[e]	63 (2.8)
Previous medication use	
Betablocker, n (%)	401 (17.8)
ACE-inhibitor/AT2-antagonist, n (%)	472 (21.0)
Statin, n (%)	407 (18.1)
Thienopyridine, n (%)	27 (1.2)
Ascal, n (%)	365 (16.2)

Data are expressed as number (%) or mean ± standard deviation.

[a]*Use of glucose lowering agents or known with glucose>6.9 mmol/L.*

[b]*First degree relative < 60 year with cardiovascular disease.*

[c]*Explicitly stated in patient history or previous pharmacologic treatment.*

[d]*Explicitly stated in patient history or previous pharmacologic treatment.*

[e]*Explicitly stated in patient history.*

Table 2. Clinical characteristics.

Variable	All patients (n=2248)
Clinical characteristics	
Left anterior descending artery as culprit artery, n (%)	947 (42.1)
Number of narrowed coronary arteries[a], n (%)	
1	968 (43.1)
2	798 (35.5)
Door-to-balloon time, (min)	
Median	46
25th, 75th percentile	34, 68
Time of ischemia[b], (min)	
Median	167
25th, 33th , 66th , 75th percentile	123, 136, 210, 246
Killip class, n (%)	
1	2119 (94.3)
2	60 (2.7)
3	11 (0.5)
4	25 (1.1)
Killip class ≥ 2, n (%)	96 (4.3)
Baseline Thrombolysis in Myocardial Infarction flow[c], n (%)	
0	1312 (58.4)
1	287 (12.8)
2	327 (14.6)
3	311 (13.8)
Baseline Thrombolysis in Myocardial Infarction flow < 2, n (%)	1599 (71.1)
Laboratory results	
Peak creatine kinase (U/L)	
Median	1275
25th, 75th percentile	564, 2590
Peak cardiac troponin T (μg/L)	
Median	3.39
25th, 75th percentile	1.30, 7.15
Estimated glomerular filtration rate (ml/min/1.73m^2)	75.0 ± 22.6
Estimated glomerular filtration rate ≤60 (ml/min/1.73m^2), n (%)	149 (6.6)
LDL-cholesterol (mmol/L)	3.6 ± 1.1
HDL-cholesterol (mmol/L)	1.2 ± 0.4
Triglycerides (mmol/L)	1.9 ± 1.3
Total cholesterol (mmol/L)	5.3 ± 1.2

Data are expressed as number (%), mean ± standard deviation, or median (interquartile range).
[a]A narrowed coronary artery was defined as a stenosis of ≥ 50% on baseline coronary angiogram.
[b]Symptom onset to time of reperfusion of the culprit lesion during PCI (in minutes).
[c]Baseline Thrombolysis In Myocardial Infarction (TIMI) flow is the TIMI flow before primary PCI in the culprit vessel.

Table 3. Independent correlates for infarct size measured by (log transformed) peak CK.

Variable	Univariate analysis			Multivariate analysis		
	β	95% CI	P	β	95% CI	P
Age	-0.003	-0.004 to 0.001	0.001	-0.001	-0.003 to 0.001	0.315
Gender(male)	0.091	0.045 to 0.136	<0.001	0.103	0.052 to 0.154	<0.001
Current smoker	0.050	0.011 to 0.090	0.013	0.036	-0.009 to 0.082	0.119
Ex-smoker	-0.048	-0.108 to 0.013	0.121			
Diabetes mellitus	-0.043	-0.105 to 0.018	0.168			
Previous myocardial infarction	-0.039	-0.109 to 0.031	0.278			
Prior statin therapy	-0.087	-0.138 to -0.035	0.001	-0.003	-0.072 to 0.065	0.923
Prior aspirin therapy	-0.098	-0.151 to -0.044	<0.001	-0.073	-0.146 to 0.000	0.050
Prior beta-blocker therapy	-0.073	-0.124 to -0.022	0.005	-0.012	-0.075 to 0.051	0.705
LAD as culprit artery	0.140	0.100 to 0.179	<0.001	0.152	0.108 to 0.195	<0.001
Three vessel disease	-0.001	-0.049 to 0.042	0.981			
Time of ischemia[a]	0.177	0.080 to 0.274	<0.001	0.206	0.102 to 0.309	<0.001
Baseline TIMI flow 0-1	0.394	0.354 to 0.435	<0.001	0.431	0.384 to 0.478	<0.001
LDL-cholesterol (mmol/L)	0.060	0.039 to 0.081	<0.001	0.041	0.019 to 0.062	<0.001

TIMI (Thrombolysis in Myocardial Infarction flow), LAD (Left anterior descending)

[a]Symptom onset to balloon time (in minutes)

β = standardized regression coefficient

Discussion

The purpose of this large cohort study was to assess whether infarct size in patients with STEMI treated with pPCI is determined by pre-existing factors. The primary finding of this study is that higher levels of LDL-c at the time of admission are independently associated with greater infarct size expressed as peak CK level. In addition, anterior infarction, time of ischemia, low pre-procedural TIMI flow, gender, and previous use of aspirin are factors that are related with infarct size in STEMI patients after pPCI.

Further understanding of the variables that affect infarct size in STEMI patients after pPCI can have important implications for the patients' treatment and prognosis. Due to improvements of diagnosis, therapy and care, mortality rates of STEMI patients are reduced at the expense of expanding number of STEMI patients with heart failure. This makes it essential to understand what factors are associated with infarct size, particularly if these factors are potentially modifiable, as this could lead to the earlier detection and development of advanced therapies.

This study demonstrates that higher LDL-c levels are associated with greater infarct size in STEMI patients treated with pPCI. Besides high LDL-c levels, anterior infarction, time of ischemia, low pre-procedural TIMI flow and male gender are shown to be associated with larger infarct size. These determinants are well-established risk factors for a larger infarct size which were earlier identified in a pooled analysis of 4 randomized STEMI trials by Stone et al[2]. Since the mechanisms underlying the effect of higher LDL-c levels on infarct size is still unclear, some explanations may be suggested. Our hypothesis is that higher LDL-c levels itself may aggravate MRI. Several studies with animal models reported that hypercholesterolemia could aggravate ischemia/reperfusion injury[9-12, 23], leading to a greater infarct size. Several mechanisms about the relation between hypercholesterolemia and increased myocardial reperfusion injury have been proposed. For example, by increased oxidative stress[10, 23], reduced extent of the cardioprotective effect of HDL-c[9], activated endoplasmic reticulum stress-mediated apoptosis[12], or by upregulation of inflammatory processes[24]. In human patients, exploration between hypercholesterolemia and infarct size is limited[25-27] and moreover, these studies were not able to consistently confirm the results from animal studies. Marenzi et al. conducted a prospective cohort study which evaluated the effect of statin therapy on myocardial infarct size assessed with cardiac magnetic resonance (CMR) in patients treated with pPCI for STEMI[25]. They observed no significant association between infarct size and LDL-c levels at hospital admission. Some studies suggest that ischemic preconditioning is dependent of serum LDL-c levels present at the time of reperfusion[26, 27]. Ischemic preconditioning is defined by initiating periods of transient myocardial ischemia and reperfusion before the sustained ischemic episode[27]. Ischemic preconditioning is a form of cardioprotection, and appears to inhibit lethal reperfusion injury[26, 27]. Kyriakides et al.[26]

tested the hypothesis that hyperlipidemia inhibits the reduction of myocardial ischemia normally observed after repeated balloon inflations during angioplasty. They showed that hyperlipidemia prevents the reduction of myocardial ischemia on repeated balloon inflations during angioplasty. A similar study, performed by Ungi et al.[27], scrutinized the effect of high serum cholesterol levels on ischemic preconditioning by means of beat-to-beat analysis of ST segments and found that hyperlipidemia accelerates the evolution of myocardial ischemia and delays recovery upon reperfusion.

Aspirin use prior to a MI was associated with smaller infarct size. To our knowledge no earlier studies were able to show this association. Marenzi et al., for example, found no association between prior aspirin use and infarct size determined with CMR[25]. The relatively small population reason may be accountable for the lack of this association. Furthermore, in the present study most of the patients with an earlier MI were on aspirin therapy, which might explain why the infarct size was smaller in the patients on aspirin therapy. Due to earlier damage to the myocardium CK release might be less than in case of first STEMI.

As it is generally believed that a substantial part of the myocardial infarct size is determined by MRI[17], several studies were initiated with the aim to identify cardioprotective agents that may reduce irreversible cell damage upon reperfusion[17, 28]. Due to its pleiotropic effects[29], statin treatment prior to primary PCI in MI patients was proposed as a potential agent that could have an effect on reperfusion induced cell damage; however, this effect is still controversial as they yielded conflicting results. Several hypotheses exist which could explain the potential effect of statin pre-treatment on reperfusion induced cell damage and consequently on clinical outcome. For example, statins are known to suppress active plaque inflammation[30, 31], inhibit thrombosis[32], improve endothelial function[33], inhibit cell adhesion[34] and improve microvascular function[35, 36], all of which contribute to improved clinical outcome after PCI[37].

In the present study, statin pre-treatment was associated with lower peak CK level in univariate analysis, but vanished after multivariate analysis. Several animal studies demonstrated a beneficial effect of statin therapy on infarct size after reperfusion[28, 38], yet, these results could not consistently be reproduced in patients with STEMI[25, 37, 39, 40]. These conflicting results could be explained by several reasons. First, right timing of the statin administration prior to STEMI was diverse, but is of importance since it takes 2-5 hours for statins to reach an optimal blood level during reperfusion[41, 42]. Secondly, the intensity of the statin therapy necessary to attenuate the infarct size differed between the studies. An earlier study conducted in pigs showed that pre-treatment with 160mg of oral rosuvastatin reduced infarct size, whereas 80mg had no significant effect[43]. At last, in STEMI patients' myocardial ischemic injury might be too severe to be prevented or reduced[37].

On the other hand, LDL-c level was significantly lower in patients treated with statin before admission than in patients without statin therapy, which may indicate that not statin use but LDL-c level itself is related with infarct size. Further research, with well conducted randomized trials that address the issues of timing and the intensity of the statin therapy, is needed in STEMI patients to explore the full potential of pre-treatment with statin therapy and other potential cardioprotective agents that can reduce irreversible cell damage upon reperfusion

As expected, a high incidence of traditional risk factors such as smoking, hypertension and dyslipidemia was present in this cohort. Elevated LDL-c is an important risk factor for the development of atherosclerosis and subsequent ischemic heart disease[7], and the association between these risk factors and cardiovascular mortality has been known for decades[44], Therefore, identifying high-risk patients before they develop ischemic heart disease is crucial. The results of this study emphasize that primary prevention and timely management of patients with high cardiovascular risk profiles have not only beneficial effects on the development of ischemic heart disease, but that early identification of patients with high LDL-c levels combined with life style changes and optimal medical treatment might lead to an improved prognosis after having developed ischemic heart disease.

Several limitations of the present study should be mentioned. First, since an observational cohort study was conducted, we could not account for undocumented clinical variables which may possibly have influenced the outcomes. Although this study is retrospectively conducted, all patients are treated according to the institutional MISSION! which provides an integrated approach of MI care to optimize treatment. This yielded in a very homogeneous STEMI population which resulted in a very high number of patients being treated according to the guidelines. Secondly, patients with known coronary artery disease or patients with a previous MI were not excluded in our analysis. Doing so, it was contemplated that it reflects a population-based cohort in the best possible way. Thirdly, information about the number of patient either receiving prasugrel of clopidogrel was not available for this study. Theoretically, prasugrel, a more potent antiplatelet drug could lead to a smaller infarct size. Furthermore, information about statin compliance, intensity of the statin treatment, and treatment duration prior to MI was not available for this study. This could explain the confined association between statin pre-treatment and lower peak CK levels in this study.

At last, peak CK levels during admission were used as an estimate of infarct size. Peak serum CK levels can be used to estimate infarct size, if reperfusion is established rapidly and successfully[14-16] and peak CK is, compared to cardiac magnetic resonance imaging (cMRI), more easily to implement in the daily practice and far less expensive. However, cMRI has emerged as a well-established technique for quantifying myocardial infarct size and has shown to correlate well with clinical outcome and is the gold standard. Therefore, further

studies are warranted to establish the association between cholesterol levels with infarct size on cMRI.

Conclusion

In conclusion, this study demonstrates that besides the more established predictors of infarct size, elevated LDL-c is associated with augmented infarct size in patients with STEMI. Other techniques, such as cMRI, should strengthen the evidence that pre-existing LDL-c levels influence infarct size. Furthermore, in larger patient cohorts it should be established whether elevated pre-infarct LDL-c levels lead to worse clinical outcome and how their prognosis after having developed MI can be improved.

Compliance with Ethics Guidelines
Since the data didn't contain any identifiers that could be traced back to the individual patient and the data are obtained for patient care, the Dutch Central Committee on Human-Related Research permits the use of anonymous data without prior approval of an institutional review board. This study was conducted according to the declaration of Helsinki.

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Chapter 8.

Efficacy of a novel pre-hospital triage protocol for the cardiac emergency department

Under review

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Abstract

Introduction
Overcrowding in emergency departments (ED) is a major public health problem. Pre-hospital triage can help to allocate patients to the appropriate ED and thereby increase the efficacy of acute care in hospitals. The current study aims to evaluate the efficacy of a novel pre-hospital triage protocol for the cardiac ED.

Methods
During 6 months, all consecutive patients admitted to the cardiac ED were included. Eligibility for admission at the cardiac ED was based upon a dedicated pre-hospital triage protocol. Efficacy of the pre-hospital triage protocol was defined as the percentage of patients with a primary cardiac complaint without needing other medical care. Secondly, both HEAR and HEART scores were evaluated for risk stratification in chest pain patients in the pre-hospital setting. Thirdly, a historical control group was added to investigate to what extent the cardiac ED helps to reduce the caseload of the general ED.

Results
Ninety-four percent of the pre-hospital triaged patients (63.3±13.2 years, 56% male) were patients with primary cardiac complaints without needing other medical care. In the subgroup of chest pain patients (n=590), both the HEAR (AUC 0.80) and HEART (AUC 0.85) score adequately identified patients with low and high risk for adverse cardiac events. The cardiac ED reduced the caseload of cardiac patients at the general ED by 34%.

Conclusion
This novel pre-hospital triage protocol is an effective tool to allocate patients to the cardiac ED and may substantially reduce the caseload of the general ED.

Introduction

Overcrowding in emergency departments is a major public health problem.(1) Contributors to overcrowding include a rising number of patients due to the ageing population and a higher severity of illness. Introduction of dedicated cardiac emergency departments may substantially reduce the caseload of the general emergency department. The strength of these cardiac emergency departments is to quickly rule out acute cardiac pathology.(2) However, the majority of cardiac symptoms, such as chest pain, dyspnoea and syncope, may also have a non-cardiac aetiology.(3-5) A thorough analysis of potential non-cardiac pathology at the cardiac emergency department is time consuming since it often requires consultation of other medical specialists and additional diagnostics. This may reduce the quality of care and increase the risk of decisional errors due to potential miscommunication during periods of large patient volumes.(1, 6, 7) Accordingly, it is crucial that patients with primary cardiac symptoms present at the cardiac emergency department. In contrast, patients with a suspicion of a non-cardiac diagnosis should be presented at the general emergency department. Potentially, a pre-hospital triage protocol may help to allocate patients to the appropriate emergency room and thereby increase the quality and efficacy of acute care in hospitals. To achieve this, a dedicated pre-hospital triage protocol for the cardiac emergency department was developed and implemented. The aim of the current study is to evaluate efficacy of this novel pre-hospital triage protocol for the cardiac emergency department.

Methods

Study design and patient population

The current study is a prospective cohort study with a historical control group.

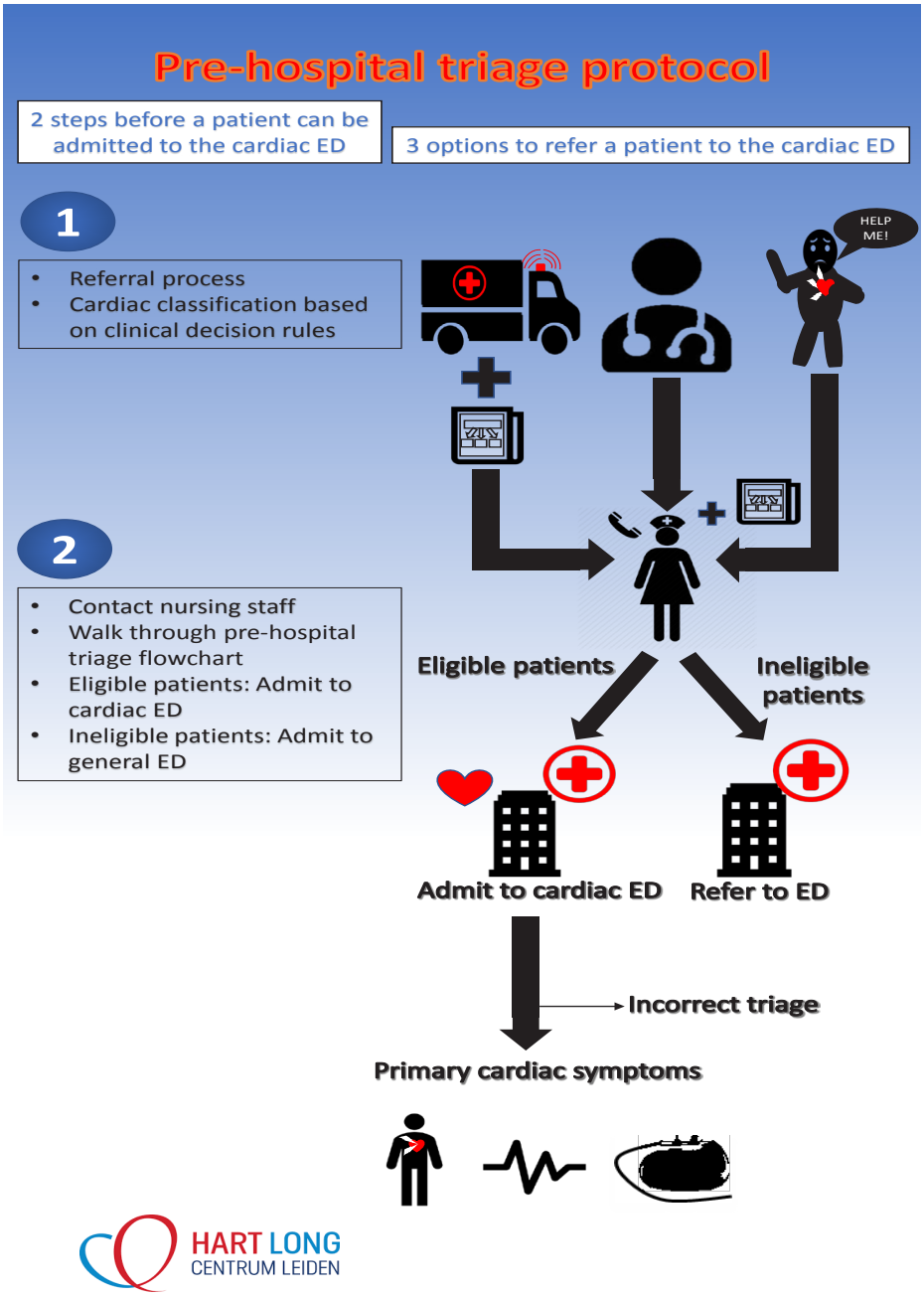

Figure 1. Overview of the pre-hospital admission protocol. Abbreviations: ED, emergency department.

The intervention group comprises all consecutive adult patients admitted to the cardiac emergency department from June 2017 until December 2017. Eligibility for admission at the cardiac emergency department was based upon the novel dedicated pre-hospital triage protocol (Figure 1). The historical control group consists of adult patients visiting the general emergency department with a suspicion of cardiac symptoms in the 2 months before the opening of the cardiac emergency department. This study was conducted according to the declaration of Helsinki and the institutional ethical committee approved the protocol.

Objective and outcome measures

The aim of the current study is to evaluate the efficacy of a novel pre-hospital triage protocol for the cardiac emergency department. The primary outcome is the percentage of patients presenting with a primary cardiac complaint without needing other medical care.

In addition, the following secondary end-points were evaluated:

- The value of the HEAR score as compared to the HEART score in the ability to predict MACE in chest pain patients admitted to the cardiac emergency department. The HEAR score was assessed upon arrival at the cardiac emergency department whereas the HEART score was evaluated after evaluation at the cardiac emergency department. MACE was defined as a composite endpoint of all-cause mortality, myocardial infarction (MI) or coronary revascularization (CABG and/or PCI) within 6 weeks after admission at the cardiac emergency department. (14)
- To what extent the pre-hospital triage protocol could relieve the crowds on the general emergency department.

Pre-hospital triage and patient's inclusion

The Heart Lung Centre of the Leiden University Medical Centre opened a cardiac emergency department in January 2017 and developed a novel dedicated pre-hospital triage protocol in close collaboration with the regional ambulance service "Hollands-Midden". This pre-hospital triage protocol aimed to allocate patients to the appropriate emergency department and consisted of 2 consecutive parts as illustrated in Figure 1.

The first part of the pre-hospital triage protocol concerned the *referral process*, in which patients could either be referred by ambulance or by another health care provider, such as a general practitioner or cardiologist. In addition, self-referral of (known) cardiac patients was possible. Eligibility for referral by ambulance or other health care providers was based on clinical decision rules, pre-defined standard operating procedures and the national protocol ambulance care (LPA)(8).

The second part of the pre-hospital triage protocol consisted of the *pre-hospital triage flowchart*, in which the referring health care provider and the nursing staff of the cardiac emergency department walked through the pre-hospital triage

flowchart step-by-step (Figure 2). In this triage flowchart, patients with a ST-elevation myocardial infarction (STEMI) were directly excluded for admission at the cardiac emergency department and were treated according to the previously described institutional MISSION! infarction protocol.(9) Thereafter, patient's eligibility was assessed by a set of pre-defined exclusion criteria (Figure 2, yellow box). The exclusion criteria were used to identify cardiac patients without major non-cardiac disease. In the absence of these exclusion criteria, patients were eligible for admission at the cardiac emergency department if they presented either with chest pain, palpitations or a cardiac device related problem. In case of self-referral, or when patients were referred by another healthcare provider, the nursing staff used the complete triage flowchart in the same chronological order as the paramedics to consider whether a patient was eligible for admission at the cardiac emergency department. Patients who were classified as eligible based upon clinical decision rules in combination with the novel triage flowchart were subsequently admitted at the cardiac emergency department. Patients who were classified as ineligible were referred to the general emergency department.

Patient admission at cardiac emergency department – Classification and Diagnosis

Patients admitted to the cardiac emergency department were classified into three groups based upon presenting symptoms: chest pain, palpitations or cardiac device related problems. Device related problems were defined as alerts originating from an implanted cardiac device, the suspicion of malfunctioning of a cardiac device as well as appropriate/inappropriate cardiac device therapy. Patients with other primary symptoms who were admitted to the cardiac emergency department were considered as incorrect triage. For each group, the final diagnosis was extracted and evaluated.

Historical control group

The historical control group comprised patients visiting the general emergency department with the suspicion of cardiac symptoms in November 2016 and December 2016 (that is the 2 months before opening of the cardiac emergency department). This historical group was used to evaluate to what extent the cardiac emergency department using the pre-hospital triage protocol could potentially relieve the crowds on the general emergency department. For this purpose, the pre-hospital triage protocol was retrospectively applied to all these patients to evaluate to which ED they would have been allocated.

Risk Stratification using the HEAR and HEART risk scores in chest pain patients

The HEAR and the HEART score were both used for risk stratification of cardiac chest pain patients admitted to the cardiac emergency department.

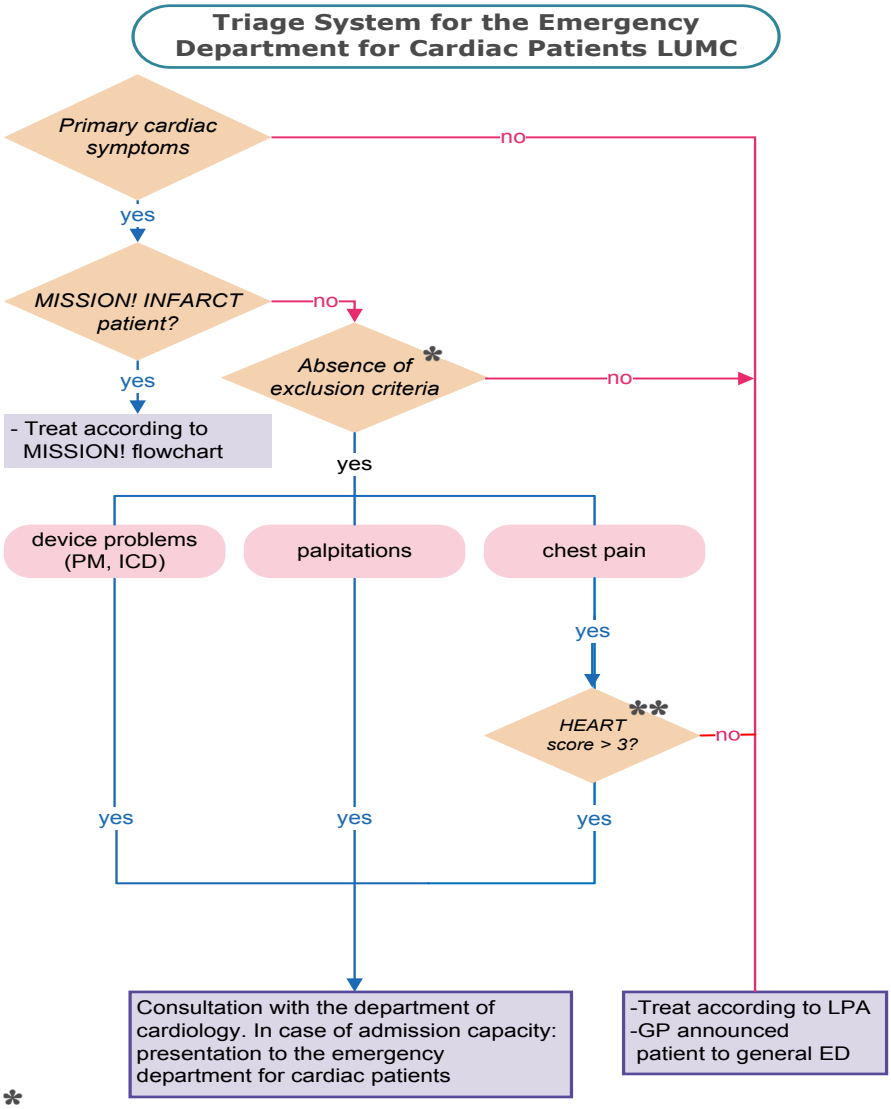

Figure 2. Triage system for the cardiac emergency department for cardiac patients Leiden University Medical Centre (LUMC).

Abbreviations: PM, pacemaker; ICD, internal cardioverter-defibrillator; NPA, national

protocol ambulance care; GP, general practitioner; ED, emergency department; LVAD, left ventricular assisting device; HEART, History, ECG, Age, Risk factors, Troponin.

Upon arrival, the HEAR score was calculated using 4 commonly available parameters: History, Electrocardiogram (ECG), Age and Risk factors. The HEAR score can potentially be used for risk stratification in the pre-hospital setting.(10) Based on the history, ECG, age and cardiovascular risk factors a score between 0 and 8 is calculated, which may predict the risk of a major adverse cardiac event (MACE) within 6 weeks after initial presentation.(10)

After evaluation at the cardiac emergency department, the HEART score was calculated in chest pain patients. The HEART score has shown to be an easy, quick and effective tool to predict outcome in chest pain patients.(11-13) The HEART score was calculated using 5 parameters: History, Electrocardiogram (ECG), Age, Risk factors and Troponin. The score (ranging between 0 and 10) was calculated and can be used to predict the risk of a MACE within 6 weeks after initial presentation. For risk stratification using the HEART score, patients with chest pain were categorised into 3 groups: low risk (score 0-3), intermediate risk (score 4-6) and high risk (score 7-10) (Figure 4).

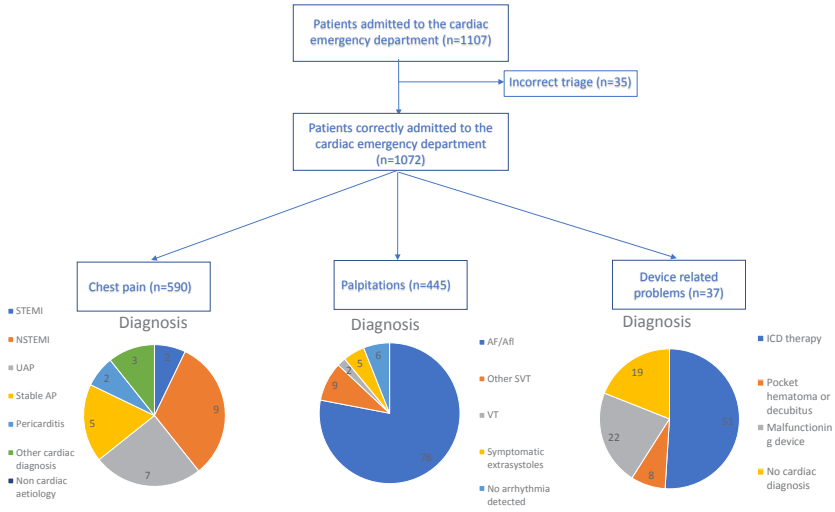

Figure 3. Flowchart with final diagnosis for each admission cause. The percentages represent the proportion of the patients with that diagnosis. Abbreviations: AP, angina pectoris; UAP, unstable angina pectoris; STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; AF, atrial fibrillation; AFl, Atrial flutter; SVT, supraventricular tachycardia; VT, ventricular tachycardia; ICD, internal cardiac defibrillator.

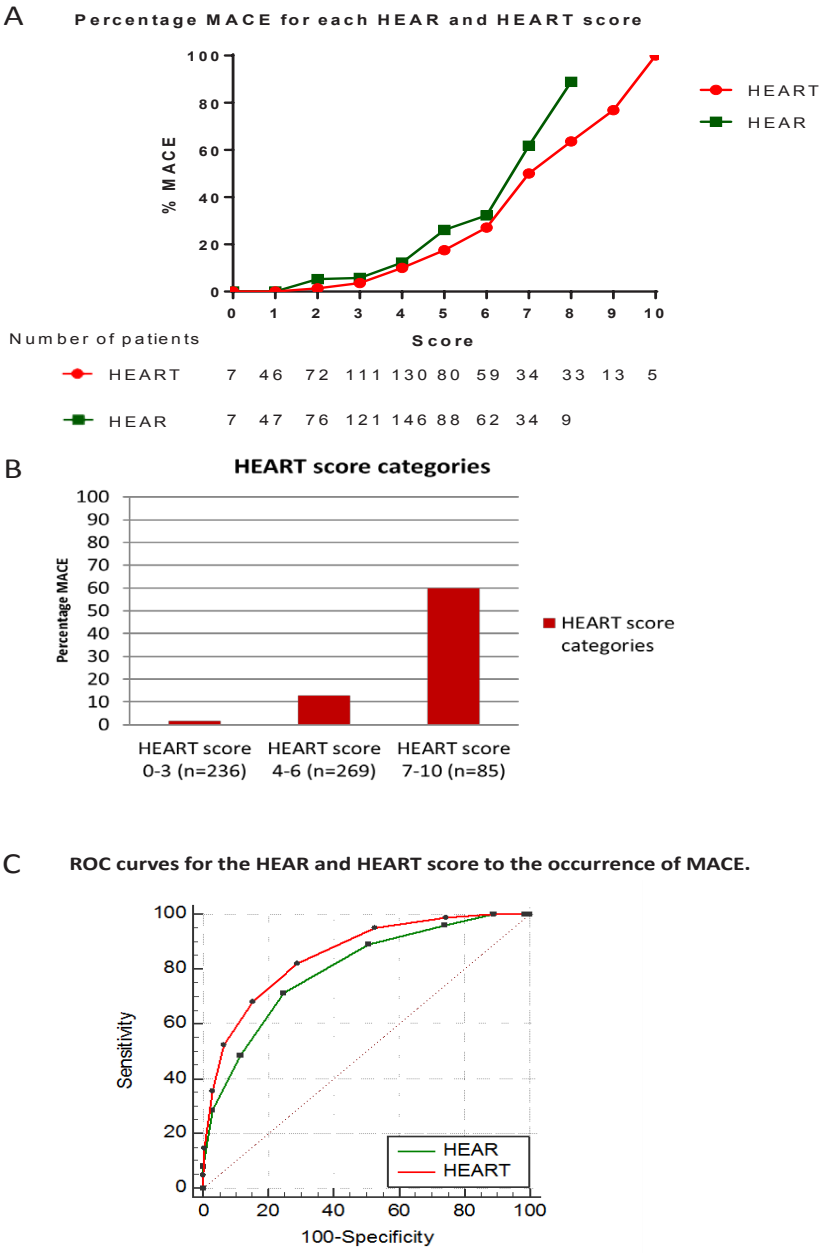

Figure 4ABC. A: Percentage MACE for each HEAR and HEART score in patients admitted with chest pain (n=590). B: Percentage MACE for each HEART score category in patients admitted with chest pain (n=590). HEART scores were dived into 3 categories. Low risk patients with a HEART score from 0-3 (n=237), intermediate risk patients with a HEART score from 4-6 (n=270) and high-risk patients with a HEART score from 7-10 (n=85). C:

ROC curves for the HEAR and HEART score to the occurrence of MACE. Abbreviations: HEART, History, ECG, Age, Risk factors, Troponin; HEAR, History, ECG, Age, Risk factors; MACE, major adverse cardiac event.

Data acquisition
All patient's clinical and follow up data were collected from the institutional electronic patients file system. Information on all-cause mortality was obtained from the Dutch Municipality Records registry.

Statistical analysis
Normally distributed continuous variables were presented as mean ± standard deviation. Non-normally distributed continuous variables were presented as median and 25-75% interquartile range (IQR) and categorical variables were presented as number and percentages.

To evaluate and compare the discriminative power of the HEAR versus the HEART score in their ability to predict MACE, receiver operating characteristics (ROC) curve analysis was performed to determine the area under the curve (AUC) (MedCalc v18.6 (MedCalc software, Belgium). An accuracy of 0.80-0.90 is considered to be good. All statistical analyses were performed with the SPSS software package (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

Results

Pre-hospital triage in the intervention group
During the study period, the pre-hospital triage process allocated 1107 patients to the cardiac emergency department. The majority of patients was referred by ambulance (n=532; 48%) or by another healthcare provider (n=451; 41%). The self-referral rate was relatively low (n=124; 11%). Re-triage upon arrival at the cardiac emergency department revealed that in 35 (3%) patients the pre-hospital triage flowchart was not correctly followed. Accordingly, the pre-hospital triage protocol was correctly followed in 1072 patients (97%). Correct triage was similar for patients transported by ambulance, referred by other healthcare providers and self-referred patients (P=0.126). Analysis of the efficacy end-point was evaluated in the 1072 correctly triaged patients.

Patients' characteristics
Table 1 displays the patient characteristics of the 1072 patients correctly triaged to the cardiac emergency department. Mean age was 63.3±13.2 years and 56% was male. The presenting symptom was chest pain in 590 patients (55%), palpitations in 445 patients (42%) and device-related problems in 37 patients (3%). As shown in Table 1, previously known coronary artery disease and risk

factors for atherosclerosis were highly prevalent in all subgroups.

Primary outcome: efficacy of pre-hospital triage

The pre-hospital triage protocol yielded a high efficacy of 94% for the selection of patients with a primary cardiac complaint without the need for other medical care. In 60 (6%) patients another medical specialist was asked for consultation. In 36 of the 60 patients (60%) the admission cause was chest pain and 23 patients (38%) presented with palpitations. The final diagnosis was atrial fibrillation in 21 patients (33%) and chest pain from non-cardiac aetiology in 23 (38%) patients. Of these patients 5 of them (8%) was admitted to a non-cardiac ward.

Of the total population 608 patients (57%) had a final cardiac diagnosis and 464 patients had a non-cardiac final diagnosis (43%). The most common non-cardiac diagnosis was idiopathic thoracic pain (n=422; 91%) and no arrhythmia detected (n=29; 6%). Figure 3 shows the final diagnosis after evaluation at the cardiac emergency department for each presenting symptom. Of the 590 chest pain patients, 10 patients had a STEMI (2%), 52 patients had a NSTEMI (9%), 40 patients had unstable angina (7%), 29 patients had stable angina (5%) and 13 patients had pericarditis (2%). In total, 18 patients had another cardiac diagnosis (3%) whereas the remaining 428 patients had chest pain without observed cardiac abnormalities (72%). Of the 445 patients evaluated for palpitations, 349 patients had atrial fibrillation or atrial flutter (78%), 39 patients had another type of supraventricular tachycardia (9%), 8 patients a ventricular tachycardia (2%) and 20 patients had symptomatic (supra)ventricular extra systoles (5%). In 29 patients, no arrhythmia could be detected despite the complaints of palpitations (6%). Of the 37 patients evaluated because of a device-related problem, 19 patients experienced an ICD shock (51%), 3 patients had a pocket hematoma or decubitus (8%) and 8 patients had a malfunctioning pacemaker or ICD (22%). In total, 7 patients with potential device related problem had a non-cardiac diagnosis (19%).

After evaluation at the cardiac emergency department most patients were discharged home (N=920; 86%). A total of 34 patients were admitted to the cardiac care unit (3%), 110 to the cardiology ward (10%) and 8 were admitted to a non-cardiac ward (1%).

Added value of HEAR and HEART score for triage of cardiac patients

In the subgroup of 590 patients presenting with chest pain, the HEAR score was calculated upon arrival and the HEART score was calculated after evaluation. Figure 4 depicts the 6-week MACE rate for both the HEAR and HAERT score. The median HEAR score was 4 (IQR 3-5). For both scores, there was a clear relation between a higher score and a higher MACE rate. Similar event rates were observed for the HEAR and the HEART score. As shown in Figure 4B, the 6-week MACE rate for low (score 0-3; n=236), moderate (score 4-6; n=) and high (score 7-10) HEART score patients was 2.1%, 16.0% and 62.4% respectively.

Figure 4C shows the comparison of ROC curves which revealed an AUC of 0.80 for the HEAR score and 0.85 for the HEART score for the occurrence of MACE (P<0.001).

Historical control group

The historical control group consisted of 100 patients (68.0±13.8 years; 65 (65%) male) admitted to the general emergency department (Table 2). The presenting symptom was chest pain in 33 patients (33%), palpitations in 7 patients (7%) and device-related problems in 1 patient (1%). Other common presenting symptoms were collapse (21 patients; 21%), dyspnoea (12 patients; 12%) and out-hospital-cardiac-arrest (9 patients; 9%).

Once the pre-hospital triage protocol was applied to the historical control group, 34 patients (34%) would have been eligible for the cardiac emergency department. Of these 34 patients, 32 patients showed only primary cardiac complaints without the need for other medical care.

In 2 of the 34 patients (6%) another medical specialist was asked for consultation which is in line with the results of the cardiac emergency department. Among the patients that were ineligible for the cardiac emergency department, in 30% of the patients (20 of 66 of the patients) another medical specialist was consulted. These date underline the value of a pre-hospital triage protocol for selection of cardiac patients.

Table 1. Baseline characteristics

	Total group (n=1072)	Chest pain (n=590)	Palpitations (n=445)	Device related (n=37)
Baseline characteristics				
Age (yrs.), mean ± SD	63.3 ±13.2	62.7 ± 13.6	64.1 ± 12.4	63.1 ± 16.0
Male n, (%)	603 (56)	295 (50)	279 (63)	29 (78)
Referred by				
Ambulance n, (%)	520 (48)	413 (70)	97 (22)	10 (27)
Other health care provider n, (%)	435 (41)	147 (25)	268 (60)	20 (54)
Self-referral n, (%)	117 (11)	30 (5)	80 (18)	7 (19)
Clinical characteristics				
Known CHD n, (%)	318 (30)	209 (35)	92 (21)	17 (46)
Diabetes mellitus n, (%)	142 (13)	92 (16)	41 (9)	9 (24)
Hypertension n, (%)	654 (61)	367 (62)	258 (58)	29 (78)
Hypercholesterolemia n, (%)	508 (47)	312 (53)	175 (39)	21 (57)
Current smoking n, (%)	172 (16)	127 (22)	42 (9)	3 (8)
Positive family history n, (%)	318 (30)	217 (37)	98 (22)	3 (8)

Categorical variables expressed by number (%), numerical variables expressed by mean

(SD) CHD, defined as earlier myocardial infarction, percutaneously coronary intervention or coronary artery bypass graft; diabetes mellitus, defined as non-insulin dependent diabetes mellitus, insulin dependent diabetes mellitus or explicitly stated in the medical history; Hypercholesterolemia, defined as treatment with lipid lowering drugs or explicitly stated in the medical history; Hypertension, defined as use of antihypertensive medication or explicitly stated in the medical history.
Abbreviations: SD, standard deviation; CHD, coronary heart disease

Discussion

In this study, the efficacy of a novel pre-hospital triage protocol for the cardiac emergency department was evaluated. Main findings can be summarized as follows. A high efficacy of the pre-hospital triage protocol was achieved as in 94% of the patients the cardiologist was able to answer the acute care demand without consultation of another medical specialist. The HEAR score is a good, easy to apply, risk stratification tool in chest pain patients in the pre-hospital triage setting. Cardiac emergency departments using the current pre-hospital flow-chart may reduce the caseload of cardiac patients at the general emergency department by approximately one third. In addition, a pre-hospital triage protocol enables to select patients with primary cardiac complaints without the need for other medical care.

Rationale of pre-hospital triage

Pre-hospital triage has an established role in emergency medicine. In trauma patients, pre-hospital triage protocols support emergency medical services providers to identify severely injured patients and assure transport of the right patient to the right hospital.(13) Pre-hospital triage in trauma patients has proven to be effective and reduce mortality.(15-17) Similarly, in the setting of STEMI, pre-hospital diagnosis and triage has shown to reduce treatment delay and improve outcome. (18-23) Although cardiac emergency departments and chest pain units are emerging, as far as we know, a pre-hospital triage system for these units has not yet been described.

The added value of pre-hospital triage in trauma patients as well as in STEMI patients inspired us to explore whether pre-hospital triage could be of help to allocate patients with cardiac symptoms in the pre-hospital setting to the appropriate emergency department. Results from this study show that at least one third of the patients admitted to the general emergency department with a suspicion of cardiac complaints could be admitted to the cardiac emergency department. This emphasizes that immediate allocation of patients to “the right place and the right doctor” can make an important contribution to preventing overcrowding of general emergency departments which is associated with high costs and increased mortality.(24)

Added value of the pre-hospital triage protocol
The use of a dedicated pre-hospital triage protocol yielded a high efficacy as in 94% of correctly triaged patients the acute care demand could be answered on the cardiac ED without consulting other medical specialists. Accordingly, the pre-hospital triage protocol can accurately differentiate between cardiac pathology and non-cardiac pathology. Furthermore, the results of the historical comparison substantiate that the cardiac emergency department can substantially reduce the caseload of the general emergency department. Although further study is required, this may eventually lead to shorter admission times in the general emergency department.

Risk stratification in chest pain patients in the pre-hospital setting
Outside the Netherlands, patients with acute chest pain are often referred to specialized chest pain units. These chest pain units are designed with the same rationale as a cardiac emergency department, specifically to provide a rapid approach in the evaluation of cardiac patients. Chest pain units have demonstrated to be feasible, safe and effective alternatives to general emergency departments.(6, 25, 26) However, a high percentage (92%) of patients admitted to these chest pain units have a non-cardiac aetiology of their chest pain.(27) Analysis of non-cardiac pathology can be time consuming and often requires additional diagnostics and consultation of other specialists and could potentially reduce quality of care. Identifying low and high-risk chest pain patients in the pre-hospital triage setting with risk stratification tools (such as a pre-hospital triage protocol) could overcome these issues.

In the current study, the HEAR score was calculated upon arrival to explore the potential as a risk stratification tool in patients with chest pain. The HEAR score showed similar event rates after 6 weeks as compared to the HEART score.(11-13) Furthermore, the AUC of the HEAR score was 0.80, almost similar to the HEART (HEAR score plus troponin measurement) score. When compared to the HEAR score, the HEART score showed a slightly better risk stratification as indicated by a higher AUC, which indicates a good ability to discriminate patients with low and high risk for adverse cardiac events. For this reason, it may enable clinicians to decide whether patients should be either admitted at a cardiac emergency department or may stay at home. Importantly, the HEAR score is applicable in pre-hospital setting as the 4 parameters of the HEAR score can be easily obtained by paramedics without contacting cardiologists or other treating physicians.(10)

These findings are also in line with a study by Bandstein and colleagues(28), who showed that a low single in-hospital troponin level, independent of the timing of the troponin measurement, ruled out myocardial infarction with nearly 100% accuracy. Implementation of troponin measurements in pre-hospital risk scores may be even more preferable (previously referred to as 'modified HEART score') rather than in-hospital troponin measurements. Interestingly, Ishak et al.

evaluated the feasibility of the modified HEART score and showed that patients with a modified HEART score of 0-3 (36%) did not developed a MACE.(29) Using the HEAR or the modified HEART score in the pre-hospital setting has the potential to avoid a substantial number of unnecessary admissions, hereby providing a potential solution against overcrowding of emergency departments and substantially reduce health-care costs.

Clinical implications

The present study illustrates that cardiac emergency departments can significantly contribute in reducing overcrowding general emergency departments. Because of the pre-hospital triage protocol, patients are allocated to the appropriate emergency room and the number of patients with a non-cardiac diagnosis requiring hospital admission to a non-cardiac ward is very low. Future studies are warranted to evaluate whether the pre-hospital triage protocol can also help to decrease admission times and, last but not least, lower health care expenditures. The currently described pre-hospital triage protocol can also be applied to chest pain units, which in concept could lead to improved efficacy and reduced healthcare costs.

Limitations

Several limitations merit consideration when interpreting the results. First, this study was a single centre cohort study. The applicability of the pre-hospital triage protocol to other hospitals inside and outside the Netherlands remains to be explored. Second, based on the current results, the HEAR score seems to be an easy to apply risk stratification tool in the pre-hospital setting. However, prospective validation is required and the intra- and inter-observer variability in the pre-hospital setting remains to be assessed.

Conclusion

In conclusion, this study demonstrates that using a dedicated pre-hospital triage protocol is an effective tool to select patients for admission at the cardiac emergency department which may reduce the caseload of cardiac patients at the general emergency department by approximately one third.

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Chapter 9.

Summary, conclusions and future perspectives

Samenvatting, conclusies en toekomstperspectieven

Summary

The general introduction of this thesis provided an overview of the development of atherosclerosis, treatment and risk stratification in patients with ST-elevation myocardial infarction (STEMI). Despite the impressive improvement that has been achieved in the field of cardiovascular disease it still is of great importance to identify patients at risk for (recurrent) adverse event. The objective of this thesis was to improve risk stratification and identify high risk populations in STEMI patients. Secondly this thesis sought to further optimize the treatment in patients with STEMI or cardiovascular disease.

Chapter 2 was an update on the pathophysiology of atherosclerotic disease and related current and possible future medical interventions with a focus on low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG) and lipoprotein(a) (Lp(a)). Lowering LDL-C by statin therapy remains, to date, the cornerstone for the medical prevention and treatment of atherosclerotic disease. Ezetimibe should be considered in a sub high risk population who do not obtain the desired LDL-c level with intensive statin treatment. Bile acid sequestrants, fibrates and niacin are not recommended. Upcoming PCSK-9 inhibitors are new potent agents for dyslipoproteinaemia. HDL-C modulation through cholesteryl ester transfer protein (CETP) inhibition and apo A-I mimetics did not yet provide evidence for better cardiovascular (CV) outcome. New classes of molecules targeting ANGPTL3 and Lp(a) have shown promising efficacy and good short-term safety profiles in early phase trials and these result warrant further development.

Chapter 3 assessed the long-term prognosis of STEMI patients referred to the general practitioner (GP) after treatment according to the 1-year institutional MISSION! MI protocol. In total, 922 patients were referred to the GP. Median follow-up was 4.55 year. At baseline (at the end of the 1-year MISSON! MI protocol) the mean age was 61 years and 75% was male. After follow-up 93% was still alive and 80% remained event-free. Patient at higher risk for mortality or adverse events were patients with higher age, smokers, history of a malignancy or stroke, not using an ACE-i/AT2-antagonist or aspirin, an impaired left ventricular ejection fraction (LVEF), a mitral regurgitation (MR) grade ≥ 2 and multivessel disease during pPCI. Since there are no recommendations in international guidelines for the appropriate duration of follow-up in the outpatient clinic after a STEMI, this 1-year period might be applied in future guidelines. However, patients with an impaired LVEF and patient with an MR grade ≥ 2 should be considered as higher risk patients and should stay in the outpatient clinic of a cardiologist.

Chapter 4 studied the value of extensive serum apolipoprotein (apo) profiling and the risk of adverse events in patients with STEMI in a case-control design study. It is suggested that the measurement of functional and structural protein

components of lipoproteins, *i.e.* apolipoprotein (apos) has additional value for coronary artery disease (CAD) risk assessment. In total, 220 STEMI patients and 299 control subjects were identified. Overall, the STEMI group had a lipid profile consistent with an increased CV risk. ApoA1 was significantly lower in the STEMI group than in the control group and apoB was significantly higher in the STEMI group than in the control group. High remnant cholesterol, low ApoA1, high ApoB and high apoB/apoA1 ratios were strongly associated with STEMI risk. The VLDL-associated apos gave conflicting results. At discharge 100% of the STEMI patients were on statin therapy. In patients with STEMI 83 events were observed after a mean follow-up of almost 9 years. For each baseline lipid and apo species the hazard ratio for MACE was calculated after adjustment for age, gender and statin use. Neither conventional lipid level nor apo levels were associated with a recurrent event in the STEMI group. This could be explained since 100% of the patient were on statin therapy which significantly reduced the lipid concentrations. This, together with the limited number of recurrent events could explain why no association was found between apos or lipid concentrations and recurrent events.

Chapter 5 was performed to investigate the additive prognostic value of growth-differentiation factor (GDF-15) levels in patients with STEMI with 10-year mortality on top of clinical characteristics and known cardiac biomarkers. In 290 STEMI patients baseline GDF-15 samples were measured. Mean age was 59 years. Stratified by median GDF-15 and median NTproBNP levels, Kaplan-Meier curves showed significantly better survival for patients with GDF-15 and NTproBNP levels below the median than for patients with GDF-15 and NTproBNP levels above the median. Furthermore, an incremental value of GDF-15 was found, as compared with a model with clinical important variables and NTproBNP. So, the combination of these biomarkers seems to identify an interesting group of high risk patients. In the group of patients with both GDF-15 and NTproBNP levels below the median, only 3 patients (3.8%) died within 10 years compared to 22 (27.8%) in the group with both GDF-15 and NTproBNP levels above the median.

Chapter 6 aimed to determine how rapidly high-sensitivity troponin–I (hs-cTnI) levels are lowered by statin therapy in patients with stable cardiovascular disease. A total of 80 patients were included in this present official and prospective sub study of the RADAR (Rosuvastatin and Atorvastatin in different Dosages And Reverse cholesterol transport) study to assess serum hs-cTnI. In the RADAR study, patients with stable CVD entered a 6-week dietary run-in phase. Subsequently, they were randomised to receive either ATOR 20mg or ROSU 10mg. On the following visits, at 6 and 12 weeks the doses were, by protocol, forced up-titrated to 40 mg ATOR or 20mg ROSU (at 6 weeks) and 80 mg ATOR or 40 mg ROSU (at 12 weeks). The study in this thesis, shows that Hs-cTnI decreased significantly during statin therapy, which was independent of decreases of LDL-c concentrations. Interestingly this suggests a rapid benefit of

statin treatment on ongoing subclinical myocardial damage.

Chapter 7 evaluated the association of baseline LDL-c level with infarct size in patients with STEMI treated with a pPCI. In this study, 2248 patients were included with a mean age of 61.8 years. LDL-c values were positively associated with infarct size which was expressed with peak creatine kinase (CK) level. Adjusted for confounders LDL-c levels were independently associated with peak CK level. Due to improvements of diagnosis, therapy and care, mortality rates of STEMI patients are reduced at the expense of expanding number of STEMI patients with heart failure. This makes it essential to understand what factors are associated with infarct size, particularly if these factors are potentially modifiable, as this could lead to the earlier detection and development of advanced therapies.
Chapter 8 investigated the feasibility and efficacy of a novel pre-hospital triage protocol for use in the cardiac emergency department. Eligibility for admission at the cardiac emergency department was based upon the novel dedicated pre-hospital triage protocol. Patients admitted to the cardiac emergency department were classified into three groups based upon presenting symptoms: chest pain, palpitations or cardiac device related problems. Patients presenting with other symptoms at the cardiac emergency department were defined as incorrect triage. Of the 1107 included patients, the incorrect triage rate was 3.2%, with the most common presenting symptoms in the incorrect triaged patients being collapse (n=15; 43%) and dyspnoea (n=8; 23%). After evaluation at the cardiac emergency department the majority of patients were discharged home (n=920; 86%). A total of 34 patients were admitted to the cardiac care unit (3%), 110 to the cardiology ward (10%) and only 8 were admitted to a non-cardiac ward (1%). This demonstrates that using a dedicated pre-hospital triage protocol is a feasible and effective tool to select patients for admission at the cardiac emergency department.

Conclusions and future perspectives

The last decades risk stratification, treatment and prognosis in STEMI patients have dramatically improved. However still a substantial amount of death occurs with a wide variability between patients. The first part of this thesis identified patients at low risk for recurrent events or death after STEMI. It was observed that asymptomatic patients with a LVEF>45% after one year can safely be referred to the GP with mortality rates after STEMI that come close to the rate in the general population. Furthermore, patients were identified who were at higher risk for recurrent events. For example, patients with a MR grade ≥ 2 should be considered as higher risk patients and should stay in the outpatient clinic of a cardiologist**.** Further research is needed to explore and identify all patient eligible for referral to the GP after treatment and the possibilities to refer stable patient with STEMI within one year to the GP should be discovered.

The second part of this thesis focused to identify high-risk subpopulations to improve risk stratification and made a start towards more patient tailored care. It was found that extensive lipid and apo profiling can significantly contribute to predict STEMI or major recurrent events. Mainly, apoA1, apoB and apoB/A1 ratio and remnant cholesterol were strongly associated with risk of STEMI and apoB/A1 ratio being superior to LDLc and non-HDLc. Despite current standards of care aimed at achieving targets for LDLc and other traditional risk factors, STEMI patients remain at high risk of new cardiovascular events. Valuable effort should therefore be made to further reduce residual cardiovascular risk by using additional more discriminating and more refined treatments targets like apoB and apoB/apoA1 ratio. Furthermore, novel biomarkers were identified to improve risk stratification and select high risk sub-populations. GDF-15, a more general marker for disease severity in STEMI patient demonstrated to have an additional prognostic value beyond identified risk factors and other cardiac biomarkers such as cTn and NT-proBNP. Currently it is unclear how GDF-15 levels can be lowered and whether lowering these levels result in an improved outcome. Therefore, further research regarding the pathophysiological mechanisms and the influence of common and novel medical therapies on GDF-15 levels should be explored. Whether more aggressive medical therapy by for example PCSK-9 inhibitors has beneficial effects on GDF-15 is worth investigating. Another way to influence GDF-15 levels may be by anti-inflammatory therapy. It would be of interest to explore whether GDF-15 levels may act as biomarker-guided therapy to evaluate the effect of anti-inflammatory therapy.

Lastly, the third part of this thesis showed that a dedicated pre-hospital triage protocol is a feasible and effective tool to select patients for admission at the cardiac emergency department. Overcrowding is a major public health problem and this thesis shows that the introduction of dedicated cardiac emergency departments can potentially reduce the caseload of the general emergency department. Further studies are needed to evaluate whether pre-hospital triage protocol can also help to reduce the use of medical facilities, decrease admission times and lower health care expenditures.

Samenvatting

De introductie van dit proefschrift laat een overzicht zien van de ontwikkeling van atherosclerose, behandeling en risico stratificatie in patiënten met een ST-elevatie myocard infarct (STEMI). Ondanks de indrukwekkende verbetering die is bereikt in de tak van cardiovasculaire ziekten, is het nog steeds van groot belang om patiënten te identificeren die het risico lopen op een (recidiverend) nadelig event. Het doel van dit proefschrift was om risico stratificatie te verbeteren en om hoog-risico in een populatie met patiënten na een STEMI te identificeren. Ten tweede is er met dit proefschrift getracht de behandeling in patiënten met een STEMI of cardiovasculair lijden te optimaliseren.

Hoofdstuk 2 was een update over de pathofysiologie van atherosclerose en daaraan gerelateerde huidige en mogelijk toekomstige medische interventies met een focus op low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglyceriden en lipoproteine(a) (Lp(a)). Het verlagen van LDL-c door het gebruik van statines blijft op dit moment de hoeksteen voor de medische preventie en behandeling van atherosclerotisch lijden. Ezitimibe moet overwogen worden in een hoog-risico populatie waarbij de streefwaarde van LDL-c niet wordt behaald ondanks intensieve statine therapie. Galzuren, fibraten en niacine worden niet aanbevolen. In opkomst zijn PCSK-9-inhibitoren, een potent medicijn tegen dyslipoproteïnemie. Aanpassing van HDL-c door cholesteryl ester transfer proteïne (CETP) inhibitie en apo A-I mimetica, voorzien vooralsnog van onvoldoende bewijs voor een beter cardiovasculaire (CV) uitkomst. Nieuwe moleculaire klassen die aangrijpen op ANGPTL3 and Lp(a) hebben veelbelovende effectiviteit laten zien met goede korte-termijn bijwerkingen profiel in fase 1 en 2 studies en deze resultaten vragen om verdere ontwikkeling.

Hoofdstuk 3 onderzocht de lange-termijn prognose van patiënten met een STEMI die verwezen zijn na de huisarts nadat ze zijn behandeling volgens het 1-jarig institutionele MISSION! MI protocol. In totaal werden er 922 patiënten verwezen naar de huisarts. De mediane follow-up was 4.55 jaar. De gemiddelde leeftijd was 61 jaar aan het eind van het 1-jarige MISSION! MI protocol en 75% was man. Aan het eind van de follow-up was 93% nog in leven en 80% bleef vrij van nadelige events. Patiënten die een hoger risico liepen om te overlijden waren oudere patiënten, rokers, patiënten met in het verleden een maligniteit of CVA, het niet gebruiken van een ACE-i/AT-2 antagonist of ascal, patiënten met een verminderde linker ventrikel ejectie fractie (LVEF), een mitralisklep insufficiëntie (MI) graad ≥ 2 en meervatslijden gedurende de primaire PCI. Aangezien er vooralsnog geen aanbevelingen bestaan in internationale richtlijnen over wat de geschikte poliklinische follow-up duur is voor patiënten na een STEMI zou deze 1-jarige periode overwogen kunnen worden in toekomstige richtlijnen. Echter patiënten met een verminderde LVEF en patiënten met een MI graad ≥ 2 moeten worden beschouwd als hoog risicopatiënten en moeten vervolgd

worden in de polikliniek bij een cardioloog.

Hoofdstuk 4 onderzocht de waarde van uitgebreide serum apolipropoteine (apo) profilering en het risico op nadelige events in patiënten met een STEMI in een case-control studie ontwerp. Er wordt beschreven dat de meting van functionele en structurele eiwit onderdelen van lipoproteines, *i.e* apolipoprotein (apos) toegevoegde waarde hebben voor de beoordeling van het risico op coronair lijden. In totaal, werden 220 patiënten met een STEMI en 299 controles geïdentificeerd. In het algemeen, had de STEMI groep een lipiden profiel waarmee je een verhoogd risico hebben om CV lijden. ApoA1 was significant lager in de STEMI groep dan in de controle groep en apoB was significant hoger in de STEMI groep dan in de controle groep. Hoog 'remnant' cholesterol, laag ApoA1, hoog ApoB en hoog apoB/apoA1 ratio's waren sterk geassocieerd met een risico op een STEMI. De VLDL-geassocieerde apos gaven wisselende resultaten. Bij ontslag na opname voor een STEMI gebruikte 100% van de STEMI patiënten een statine. In deze groep werden 83 events geobserveerd na een gemiddelde follow-up duur van bijna 9 jaar. Voor elke op baseline gemeten lipide en apo soort werd het risico op een nadelig event berekend, gecorrigeerd voor leeftijd, geslacht en statine gebruik. Zowel conventionele lipide waardes als apo waardes waren niet geassocieerd met een recidiverend event in de STEMI groep. Dit kan verklaard worden omdat 100% van de patiënten een statine gebruikten wat de lipiden waardes significant verlaagde. Dit, samen met het beperkte aantal recidiverende events kan verklaren waarom er geen associatie gevonden werd tussen apos of lipiden waardes en recidiverende events.

Hoofdstuk 5 werd uitgevoerd om te onderzoeken wat de toegevoegde prognostische waarde is van growth-differentation factor 15 (GDF-15) bovenop bekende patiënten karakteristieken en gevestigde cardiale biomarkers bij patiënten met een STEMI. In 290 patiënten met STEMI werden baseline GDF-15 waardes gemeten. De gemiddelde leeftijd was 59 jaar. Gestratificeerd voor de mediane GDF-15 waarde en mediane NTproBNP waardes, lieten Kaplan-Meier curves zien dat er een significante betere overleving is voor patiënten met GDF-15 en NTproBNP waardes onder de mediaan ten opzichte van patiënten met GDF-15 en NTproBNP waardes boven de mediaan. Bovendien, werd er een toegevoegde waarde van GDF-15 gevonden, vergeleken met een model waarbij reeds belangrijke klinische variabelen en NTproBNP zaten. De combinatie van deze biomarkers lijkt daarom een belangrijke hoog-risico groep te identificeren. In de groep waarbij zowel de GDF-15 waarde als de NTproBNP waarde onder de mediaan waren, overleden er slecht 3 patiënten (3.8%) binnen 10 jaar vergeleken met 22 (27.8%) in de groep waarbij de GDF-15 en NTproBNP waardes boven de mediaan waren.

Hoofdstuk 6 had als doel om te bepalen hoe snel hoog sensitiviteits troponine-I (hs-cTnI) werden verlaagd door statine therapie in patiënten met stabiel cardiovasculair lijden. In totaal werden er 80 patiënten geïncludeerd in deze

substudy van de prospectieve RADAR studie (Rosuvastatin and Atorvastatin in different Dosages And Reverse cholesterol transport) waarbij de serum hs-cTnI werd gemeten. In de RADAR studie, begonnen patiënten met stabiel cardiovasculair lijden run-in fase waarbij ze 6 weken lang aan een gezonde levensstijl werden onderworpen. Vervolgens, werden ze gerandomiseerd om danwel ATOR 20mg danwel ROSU 10mg te gebruiken. Gedurende de follow-up bezoeken na 6 en 12 weken werden deze doses volgens protocol verhoogd naar 40mg ATOR of 20mg ROSU (na 6 weken) en naar 80mg ATOR of 40mg ROSU (na 12 weken). Deze studie in het huidige proefschrift laat zien dat hs-cTnI significant verlaagd werd gedurende statine behandeling, onafhankelijk van wat de verlaging van het LDL-c was. Interessant is dat dit suggereert dat er een snel voordeel bestaat van statine behandeling op aanhoudende subklinische myocardiale schade.

Hoofdstuk 7 evalueerde de associatie tussen LDL-c op baseline met infarct grootte in patiënten met een STEMI behandeld met een primaire PCI. In deze studie werden 2248 geanalyseerd met een gemiddelde leeftijd van 61.8 jaar. LDL-c waardes waren geassocieerd met infarct grootte dat was uitgedrukt in piek creatine kinase (CK) waarde. Gecorrigeerd voor confounders waren LDL-c waardes onafhankelijk geassocieerd met piek CK waarde. Door de verbetering van de infarct zorg, met snellere diagnoses, therapie en zorg, zijn de mortaliteitcijfers van STEMI patiënten verbeterd, echter wel ten koste van het aantal patiënten met hartfalen na een STEMI. Dit maakt het belangrijk om beter te begrijpen welke factoren geassocieerd zijn met infarct grootte, met name als deze factoren in potentie aangepast kunnen worden, zodat dit kan leiden tot vroegere detectie en ontwikkeling van nieuwe behandelingen.

Hoofdstuk 8 onderzocht de bruikbaarheid en effectiviteit van een nieuw pre-hospitaal triage protocol voor het gebruik op de Eerste Hart hulp. Patiënten werden beoordeeld of ze geschikt waren om gepresenteerd te worden op de Eerste Hart Hulp door gebruik van dit nieuwe pre-hospitale ziekenhuis protocol. Patiënten opgenomen op de Eerste Hart Hulp werden in drie groepen onderverdeeld gebaseerd op presenterende symptomen; pijn op de borst, palpitaties en problemen gerelateerd aan een cardiaal device. Patiënten die zich presenteerden met andere klachten werden gedefinieerd als incorrecte triage. Van de 1107 geïncludeerde patiënten, de incorrecte triage was 3.2%, met de meest presenterende klachten een collaps (n=15, 43%) en dyspnoe (n=8, 23%). Na evaluatie op de Eerste Hart Hulp kond de meerderheid weer worden ontslagen (n=920, 86%). In totaal werden 34 patiënten opgenomen op de hartbewaking (3%), 110 op de afdeling cardiologie (10%) en slechts 8 patiënten werden opgenomen op een andere niet-cardiale afdeling (1%). Dit demonstreert dat een toegewijd pre-hospitaal triage protocol een goed te gebruiken en een effectief middel is om patiënten te selecteren die geschikt zijn op opgenomen te worden op de Eerste Hart Hulp.

Conclusies en toekomstperspectieven

De laatste decennia zijn risico stratificatie, behandeling en de prognose van patiënten met een STEMI drastisch verbeterd. Echter, er overlijdt nog steeds een substantieel deel met een grote variabiliteit tussen patiënten. Het eerste deel van dit proefschrift identificeerde laag-risico patiënten voor een recidiveerde event na een STEMI. Er werd geconstateerd dat asymptomatische patiënten met een LVEF>45% na 1 jaar veilig kunnen worden verwezen naar de huisarts, waarbij we zagen dat de mortaliteitscijfers in de buurt kwamen van de algehele populatie. Verder werden er hoog-risico patiënten geïdentificeerd. Bijvoorbeeld, patiënten met een MI graad ≥ 2 moeten worden gezien als hoog-risico patiënten en zouden moeten worden vervolgd in de polikliniek van een cardioloog. Aanvullend onderzoek is nodig om verder te verkennen en identificeren welke patiënten geschikt zijn om verwezen te worden naar de huisarts. Daarnaast zou er onderzocht moeten worden of het mogelijk is om stabiele patiënten na een STEMI eerder dan na één jaar te verwijzen naar de huisarts.

Het tweede deel van dit proefschrift richtte zich op hoog-risico populaties om de risico stratificatie te verbeteren en maakte een begin naar meer op individueel gerichte patiënten zorg. Er werd gevonden dat uitgebreid lipiden en apo profilering significant kan bijdragen aan het voorspellen van een STEMI of ernstige recidiverende events. Met name apoA1, apoB en apoB/A1 ratio en 'remnant' cholesterol waren sterk geassocieerd met het krijgen van een STEMI. Bovendien was de apoB/apoA1 ratio superieur ten opzicht van LDL-c en non-HDLc. Ondanks dat de standaard zorg nu gericht is op het bereiken van een bepaalde LDL-c streefwaarde en andere traditionele risico factoren, blijven patiënten met een STEMI een hoog risico houden op een recidiverend event. Het zou de moeite waard zijn om inspanning te verrichten om verder het residuele cardiovasculaire risico te verlagen door gebruik te maken van additionele meer discriminerende en meer verfijnde behandeling streefwaardes zoals apoB en apoB/apoA1 ratio. Verder, werden er nieuwe biomarkers geïdentificeerd om de risico stratificatie te verbeteren en om beter hoog-risico sub populaties te identificeren. GDF-15, wat een meer algemene biomarker is voor ziekte-ernst in patiënten met STEMI, liet zien dat het een toegevoegde prognostische waarde heeft bovenop reeds geïdentificeerde risico factoren en andere cardiale biomarkers zoals cTn en NT-proBNP. Op dit moment is het onduidelijk hoe GDF-15 waardes kunnen worden verlaagd en of deze verlaging dan ook resulteert in een verbeterde uitkomst. Daarom zal er toekomstig onderzoek gedaan moeten worden naar het onderliggende pathofysiologische mechanisme en naar wat de invloed is van reeds gebruikte en nieuwe medische behandelingen op GDF-15 waardes. Het is bijvoorbeeld de moeite waard om te onderzoeken of meer agressieve therapie met bijvoorbeeld PCSK-9 remmers een voordelig effect hebben op GDF-15. Een andere mogelijke manier om GDF-15 waardes te verlagen is met anti-inflammatoire therapie. Het is interessant om te verkennen of GDF-15 waardes mogelijk als biomarker-gerichte therapie kunnen dienen om

het effect van anti-inflammatoire behandeling te evalueren.

Als laatste liet het derde deel van dit proefschrift zien dat een toegewijd pre-hospitaal triage protocol bruikbaar en effectief is om patiënten te selecteren die gepresenteerd kunnen worden op de Eerste Hart Hulp. Overbevolking is een groot maatschappelijk probleem en dit proefschrift laat zien dat de introductie van een toegewijde Eerste Hart Hulp de potentie heeft om de toestroom van patiënten naar de algemene eerste hulp te verminderen. Verdere studies zijn nodig om te evalueren of het pre-hospitale triage protocol ook kan helpen in het verminderen van de diagnostiek, het verkorten van de opname duur en de zorgkosten kan reduceren.

Chapter 10.

List of publications
Dankwoord
Curriculum vitae

List of publications

Bodde MC, van Hattem NE, Abou R, Mertens BJA, van Duijn HJ, Numans ME, Bax JJ, Schalij MJ, Jukema JW. Myocardial infarction patients referred to the primary care physician after 1-year treatment according to a guideline-based protocol have a good prognosis. Neth Heart J. 2019 Nov;27(11):550-558.

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Dankwoord

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Curriculum Vitae

Mathijs Carolus Bodde werd geboren op 23 december 1987 te Leiden. In 2005 haalde hij zijn gymnasiumdiploma aan het Stedelijk Gymnasium te Leiden. In 2005 en 2006 studeerde hij biomedische wetenschappen aan de Katholieke Universiteit te Leuven, alvorens hij aan zijn studie geneeskunde begon aan de Erasmus Universiteit te Rotterdam, alwaar hij in 2014 zijn artsexamen deed. Hiervoor had hij een keuzecoschap algemene geneeskunde gelopen aan het Igogwe Mission Hospital in Tukuyu, Tanzania. In 2014 en 2015 was hij arts-assistent cardiologie niet in opleiding in het toenmalige Medisch Centrum Haaglanden, locatie Westeinde (thans: Haaglanden Medisch Centrum+). In 2015 begon hij aan zijn promotieonderzoek op de afdeling cardiologie van het Leids Universitair Medisch Centrum onder leiding van prof. dr. J.W.J. Jukema en prof dr. M.J. Schalij, waarvan de resultaten in dit proefschrift staan beschreven. Voor een van zijn artikelen won hij in 2017 de Durrer prijs voor het beste klinische artikel in de Netherlands Heart Journal (editor prof. dr. J.J. Piek). In december 2018 startte hij met de opleiding tot cardioloog (opleider: prof. dr. M.J. Schalij). Zijn vooropleiding interne geneeskunde in het Haaglanden Medisch Centrum rondde hij met succes af in augustus 2020 (opleider: dr. A.H. Bootsma). Momenteel is hij werkzaam als arts-assistent in opleiding op de afdeling cardiologie van het Haaglanden Medisch Centrum (opleider: dr. A.P. van Alem).