Innovations to Improve Characterisation and Prognostication of Patients Undergoing Percutaneous Coronary Intervention

Sanneke P.M. de Boer

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Innovaties om de karakterisering en de prognose van patiënten die een percutane coronaire interventie ondergaan te verbeteren

Proefschrift

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Erasmus Universiteit Rotterdam
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Het verschijnen van dit proefschrift werd mede mogelijk gemaakt door de steun van de Nederlandse Hartstichting

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

As a result of improved prevention and treatment, mortality due to coronary artery disease (CAD) has decreased substantially during the last decades.[1, 2] For example, in the Netherlands in 1970 a total of 183 CAD deaths occurred per 100,000 of the population, as compared to 58 per 100,000 in 2012, which corresponds with a relative reduction of 68%. [3] However, CAD remains the leading cause of death in Europe.[4] Further improvements in the existing treatment standards (e.g. percutaneous coronary intervention (PCI)) of CAD only had a marginal effect on outcome. Therefore, it is time to break new grounds. We should improve prognosis in the individual patient, and strive for personalized medicine. There is a growing body of evidence that inflammation plays a central role in all stages of atherosclerotic disease from the preliminary lesion to late-stage plaque rupture.[5-8] The concomitant presence of a vulnerable plaque in the vessel wall and the pro-inflammatory and pro-coagulable milieu as indicated by various biomarkers are necessary in making the patient prone to "vulnerable" plaque rupture and turning stable coronary disease into an unstable phase. However after decades of cardiovascular research the mechanisms linking plaque composition and morphology, vascular inflammation, coagulability and patient outcome are still not fully understood. To understand this we also need to focus on different environmental and genetic factors. Environmental factors can be studied by for example assessing the effect of nutritional and life style choices within different groups of CAD patients. Studying the genetic factors can be done by directly linking genetic variation to clinical traits, i.e. genome-wide association studies, but also by measuring levels of messenger RNA (transcriptome), proteins (proteome), and metabolites (metabolone) as a function of genetics. [9] Besides studying these factors more detailed information on the plaque composition of the vessel wall may provide important information. One technique to do so is intravascular ultrasound-virtual histology (IVUS-VH), which allows visualization of the composition of the vessel wall, and the assessment of four different plaque compositions i.e. fibro-fatty, fibrous tissue, dense calcium and necrotic core.[10, 11] Another upcoming technique that can be used is Near-infrared spectroscopy (NIRS), a novel catheter based imaging modality that determines composition of tissue, and has the potential to identify lipid-core containing coronary plaques (LCP) in patients, using the variation in reflection of the emitted near-infrared light to detect LCP.[12, 13]

The aim of this thesis is to improve characterisation and prognostication of patients undergoing PCI. To do so we first investigated if primary PCI is the best strategy for every patient in every clinical circumstance, and which categories of patients benefit most. We also assessed the effect of life style changes (quitting smoking) on outcome. To assess the best treatment for patients, preferably the result of randomized controlled trials (RCTs) are used. However, external validity of RCT results may be questioned since most clinical trials have strict in- and exclusion criteria, which tend to favour the enrolment of patients at low risk of potential side effects, and therefore may not reflect 'the real world' clinical practice. Therefore we also examined the effect of trial participation on outcome.

In the second part of this thesis we further tried to improve characterisation and prognostication of patients undergoing PCI. We designed the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (ATHEROREMO-IVUS), an exploratory (non-pivotal) clinical study to investigate the associations between genetic profile, circulating biomarkers and coronary atherosclerosis phenotype and vulnerability as determined by IVUS virtual histology and/or NIRS. Finally, the prognostic implications of (the combination) of established and novel biomarkers and plaque phenotypes will be studied.

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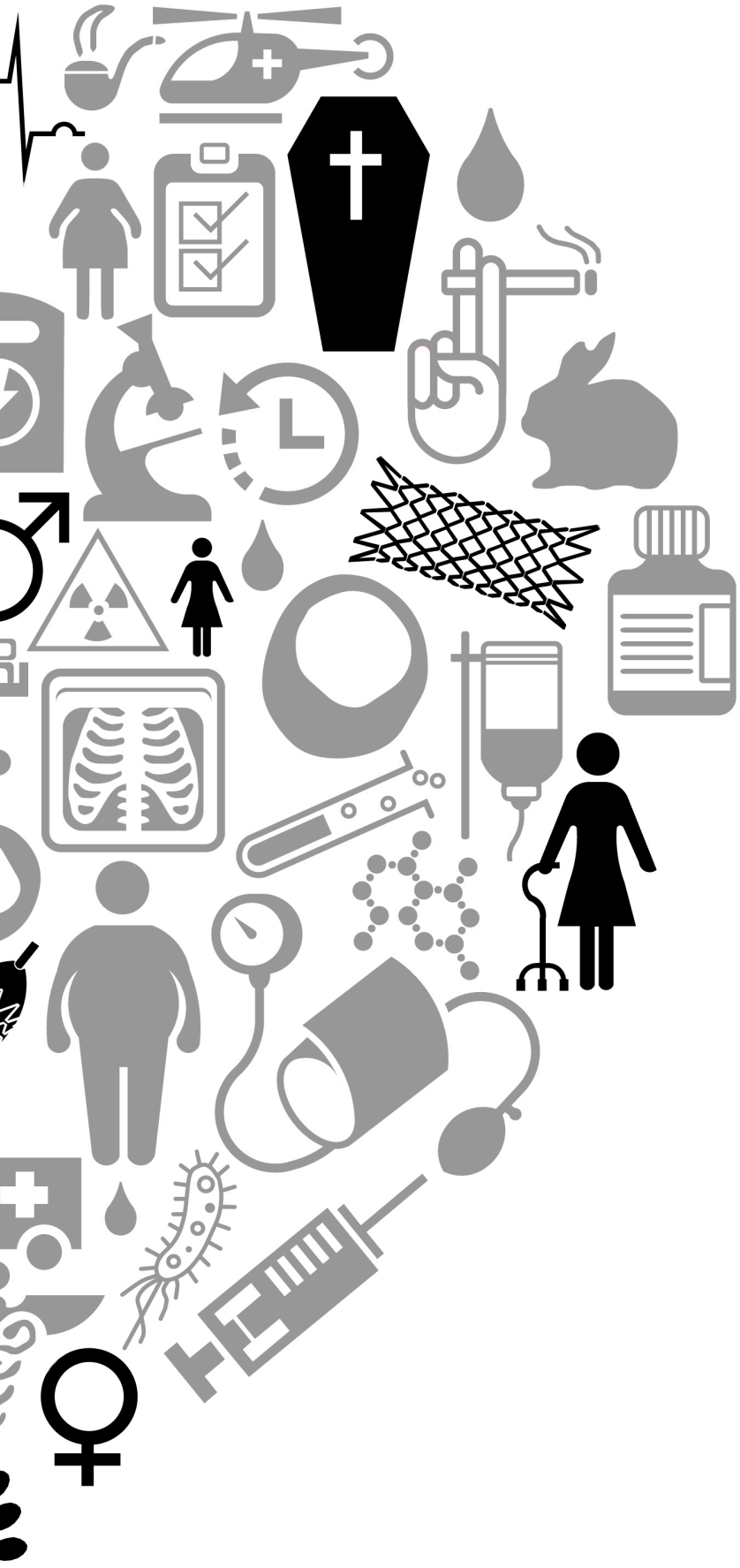
PART I



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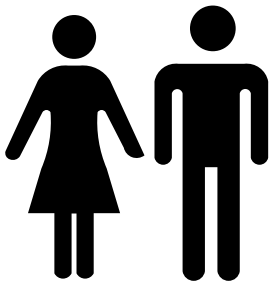
1.1

EXCESS MORTALITY IN WOMEN COMPARED TO MEN AFTER PCI IN STEMI: AN ANALYSIS OF 11931 PATIENTS DURING 2000-2009



EXCESS MORTALITY IN WOMEN COMPARED TO MEN AFTER PCI IN STEMI: AN ANALYSIS OF 11931 PATIENTS DURING 2000-2009

ABSTRACT

Background
Ambiguity exists whether gender affects outcome in patients undergoing percutaneous coronary intervention (PCI).

Methods
To evaluate the relationship between gender and outcome in a large cohort of PCI patients, 11931 consecutive patients who underwent PCI for various indications during 2000 – 2009 were studied, using survival analyses and Cox regression models.

Results
Most patients (N=8588; 72%) were men. Women were older, and more often had a history of hypertension and diabetes mellitus. Men smoked more frequently, had a more extensive cardiovascular history (previous MI, PCI and CABG), a higher prevalence of renal impairment and multi-vessel disease. In STEMI patients, women had higher 31-day mortality rates than men (11.6% vs. 6.5% respectively, $p<0.001$). This difference remained after adjustment for confounders (aHR at 30-days 1.54 and 95% CI 1.22 – 1.96). Likewise, higher mortality was observed at 1-year (15.1% vs. 9.3%) and 4-year follow-up ((21.6% vs. 15.0%, aHR 1.30 and 95% CI 1.10-1.53). There were no differences in mortality between women and men in NSTE-ACS (aHR at 4-years 1.05 and 95% CI 0.85-1.28) or stable angina (HR at 4-years 0.85 and 95% CI 0.68-1.08).

Conclusion
Women undergoing PCI for STEMI had higher mortality than men. The excess mortality in women appeared in the first month after PCI and could only partially be explained by a difference in baseline characteristics. No gender differences in outcome in patients undergoing PCI for NSTE-ACS and stable angina were observed.

INTRODUCTION

As a result of improved primary and secondary prevention and treatment strategies, mortality due to coronary artery disease (CAD) has decreased substantially during the last decades.[1-2] For example, in the Netherlands in 1970 a total of 183 CAD deaths occurred per 100,000 of the population, as compared to 58 per 100,000 in 2012, which corresponds with a relative reduction of 68%. [3] Still, CAD is a major cause of mortality and morbidity in men and women in Western countries.[4-5] Interestingly, the decrease of cardiovascular death has been lower in women than in men.[6] Several studies have found that differences in age, primary and secondary prevention, clinical presentation, risk profile and treatment might account for this sex-based difference in mortality rate. [7-12] Furthermore, despite the well-known benefits of PCI for patients with stable coronary disease (symptom relief) as well as for patients presenting with acute myocardial infarction (improved survival), fewer women than men undergo invasive coronary revascularization.[4] Satisfactory explanations for this phenomenon have not been provided. One of the reasons for the observed discrepancy might be the perception of treating physicians that women benefit less from invasive coronary procedures than men. Indeed, it has been found that women undergoing PCI have higher rates of adverse outcomes than men.[13] Though, when differences in patient-and clinical characteristics are taken into account, ambiguity remains whether gender affects outcome.[8, 14-21]

In our institution, a high-volume tertiary referral center, we developed a database in which we systemically enter all baseline data, procedural data, and follow-up data of all PCI patients. Based on this database we studied the relationship between gender and clinical outcomes during follow-up.

METHODS

Patient population

The Erasmus MC, located in Rotterdam the Netherlands, is a tertiary referral and teaching hospital in the Rotterdam region with approximately 1.9 million inhabitants. The catheterization laboratory of the Thorax center of the Erasmus MC is equipped to provide a 24/7 service for patients with chronic and acute CAD.

Between January 2000 and December 2009 a total of 15,102 PCIs were conducted in 11,931 consecutive patients of 18 years or older, which were the subject of our analysis. The patient and not the PCI procedure was the unit of our analysis. Therefore, the 2,470 patients who underwent multiple PCIs during the research period entered the study database at the moment of their first procedure. The PCIs that were conducted during follow-up were considered as outcome-events. The index PCI was adopted because of stable angina in 4,422 patients, non ST-segment elevation acute coronary syndrome (NSTE-ACS) in 3,280 patients, and ST-segment elevation myocardial infarction (STEMI) in 4,229 patients.

The nomenclature of stable angina - NSTE-ACS - STEMI has developed over time, and definitions have changed. [22-31] In this context, it is important to note that we used the classification that was performed at the time that the patient was treated. In general, stable angina was defined when a patient met two of the following three criteria: 1.substernal chest discomfort of characteristic quality and duration, 2. provoked by exertion or emotional stress, 3. relieved by rest and/or glyceryl trinitrate.[26-27] NSTE-ACS was defined as patients with acute chest pain but without persistent ST-segment elevation.[22-23, 28-29] Patients presenting with ischemic symptoms and persistent (>20 min) ST-segment elevation in at least 2 contiguous precordial leads or at least 2 adjacent limb leads by ECG were classified as STEMI.[25, 30-31]

Patient management

According to the standard policy in our department, all patients underwent stent implantation of (at least) the culprit lesion. The preferred stent varied during the study period. Between January 2000

and April 2002 bare metal stents (BMS) were implanted. Since April 2002 drug-eluting stents were implanted: until March 2003 sirolimus-eluting stents (SES), between March 2003 and March 2007 paclitaxel-eluting stents (PES), and since March 2007 everolimus-eluting stents (EES). Within each period, the preferred stent was almost exclusively used in all patients. However, for specific clinical trials comparing stents, in a small number of patients another type of stent was used.
Patient management was in accordance with the applicable guidelines of the European Society of Cardiology (ESC), which have changed over time. [22-33] Patients received an aspirin and (300 to 600 mg) clopidogrel loading dose before PCI and STEMI patients received both drugs preferably in the ambulance. Clopidogrel (75mg/day) duration was at least one month for patients who received a BMS, at least 3 months for patients who received a SES and at least 6 months for patients with PES or EES. After the procedure, all patients were advised to remain on aspirin (>80mg/day) indefinitely. In the described period the preferred access site was the femoral artery. Periprocedural glycoprotein IIb/IIIa antagonists were used at the discretion of the interventional cardiologist.

Data collection
According to the standard data-management procedures in our department, data are systematically collected on demographics, cardiovascular history, clinical risk factors and treatment characteristics for all patients undergoing PCI. Data-elements are stored in an electronic database, which is filled out immediately after the completion of the PCI by the interventional cardiologist and by the technician who assisted during the procedure. The database, which is maintained by a dedicated IT-officer, is mainly designed for administrative purposes. A systematic evaluation of data-completion and data-integrity is implemented for data that are used for research purposes, including the data that we describe in this manuscript.

Data management and Follow-up
Mortality data for all patients were obtained from municipal civil registries systematically on a yearly basis during the first five years after the initial PCI. Subsequently a health questionnaire was sent to all living patients with specific inquiries on rehospitalisation and major adverse cardiovascular events (MACE). For patients who had adverse events at other centers, medical records or discharge summaries from the other institutions were systematically reviewed. General practitioners, referring cardiologists, and patients were contacted in case additional information was required.

Endpoint definitions
The primary endpoints was all-cause mortality, which was evaluated at 31-days ('early') and at 1 year and 4 years ('late') after the index event. The secondary endpoints included (recurrent) myocardial infarction ((re)MI), repeat revascularization (repeat PCI (rePCI) or coronary artery bypass grafting (CABG)) and the composite endpoint of (re)MI, re-revascularization and all-cause mortality, which were evaluated at 31 day, 1 year and 4 year follow-up. If a patient reported (re)MI at follow-up, the referring hospital was contacted to verify the diagnosis. RePCI was defined as a repeat percutaneous coronary intervention of any lesion located in the epicardial vessels. CABG was defined as a surgical intervention of any lesion located in the epicardial vessels.

Statistical methods
Continuous variables are presented as mean ± standard deviation and categorical variables are expressed as numbers and percentages. Student's t tests and Chi-square test (or Fisher's exact tests) were applied to evaluate differences in baseline variables between women and men, as appropriate.
The incidence of events over time was studied with the use of the Kaplan-Meier method, whereas log-rank tests were applied to evaluate differences between women and men. Patients lost to follow-up were considered at risk until the date of last contact, at which point they were censored.
Cox proportional hazard survival models were applied to evaluate the relationship between gender

	Overall			Stable AP			NSTE-ACS			STEMI		
	Men n=8588	**Women n=3343**	**P-value**	**Men n=3171**	**Women n=1251**	**P-value**	**Men n=2283**	**Women n=997**	**P-value**	**Men n=3134**	**Women n=1095**	**P-value**
Age (years ±SD)	61.3±11.5	66.2±12.1	<0.001	62.0±10.7	66.3±10.7	<0.001	62.6±11.6	67.2±11.8	<0.001	59.6±12.1	65.1±13.6	<0.001
Medical History												
Hypertension n (%)	3726 (43.4)	1851 (55.4)	<0.001	1540 (48.6)	745 (59.6)	<0.001	1065 (46.6)	565 (56.7)	<0.001	1121 (35.8)	541 (49.4)	<0.001
Hypercholesterolemia n (%)	6680 (77.8)	2533 (75.8)	0.019	2598 (82.0)	997 (79.7)	0.08	1813 (79.4)	767 (76.9)	0.11	2269 (72.4)	769 (70.3)	0.18
Diabetes Mellitus n (%)	1364 (15.9)	690 (20.6)	<0.001	559 (18.9)	275 (22.0)	0.020	439 (19.2)	246 (24.7)	<0.001	326 (10.4)	169 (15.4)	<0.001
Family History n (%)	2764 (32.2)	1118 (33.5)	0.18	1113 (35.1)	481 (38.4)	0.037	750 (32.9)	344 (34.5)	0.36	901 (28.7)	293 (26.8)	0.21
Current smokers n (%)	2496 (29.1)	781 (23.4)	<0.001	609 (19.2)	186 (14.9)	0.001	591 (25.9)	198 (19.9)	<0.001	1296 (41.4)	397 (36.3)	0.003
Previous MI n (%)	2418 (28.3)	707 (21.3)	<0.001	1082 (34.5)	284 (23.0)	<0.001	949 (41.9)	325 (32.8)	<0.001	387 (12.4)	98 (8.9)	0.002
Previous PCI n (%)	1048 (12.2)	363 (10.9)	0.041	552 (17.4)	189 (15.1)	0.065	299 (13.1)	128 (12.8)	<0.001	197 (6.3)	46 (4.2)	0.011
Previous CABG n (%)	792 (9.2)	223 (6.7)	<0.001	426 (13.4)	128 (10.2)	0.004	279 (12.2)	79 (7.9)	<0.001	87 (2.8)	16 (1.5)	0.015
Renal impairment n (%)	464 (5.4)	129 (3.9)	<0.001	227 (7.2)	61 (4.9)	0.006	175 (7.7)	52 (5.2)	0.011	62 (2.0)	16 (1.5)	0.27
Procedural characteristics												
Cardiogenic shock n (%)	-	-	-	-	-	-	-	-	-	153 (4.9)	62 (5.7)	0.31
Vessel disease			<0.001			<0.001			<0.001			0.001
0-vessel disease (excluding LM) n (%)	57 (0.7)	24 (0.7)		18 (0.6)	8 (0.6)		17 (0.7)	9 (0.9)		22 (0.7)	7 (0.6)	
1-vessel disease	4051	1835		1384	674 (53.9)		978 (42.8)	498 (49.9)		1689	663 (60.5)	

(excluding LM) n (%)	(47.2)	(54.9)		(43.6)						(53.9)		
2-vessel disease	2629	936 (28.0)		1050	363 (29.0)		748 (32.8)	312 (31.3)		831 (26.5)	261 (23.8)	
(excluding LM) n (%)	(30.6)			(33.1)								
3-vessel disease	1851	548 (16.4)		719 (22.7)	206 (16.5)		540 (23.7)	178 (17.9)		592 (18.9)	164 (15.0)	
(excluding LM) n (%)	(21.6)											
Left main disease	310 (3.6)	112 (3.4)	0.49	112 (3.5)	39 (3.1)	0.49	97 (4.2)	36 (3.6)	0.39	101 (3.2)	37 (3.4)	0.80
Multi-vessel disease n	4585	1524	<0.001	1802	588 (47.0)	<0.001	1323	503 (50.5)	<0.001	1460	433 (39.6)	<0.001
(%)	(53.4)	(45.6)		(56.8)			(58.0)			(46.6)		
Treated vessel												
Left main n (%)	348 (4.1)	151 (4.5)	0.26	161 (5.1)	61 (4.9)	0.78	119 (5.2)	62 (6.2)	0.25	68 (2.2)	28 (2.6)	0.47
RCA n (%)	3165	1328	0.004	1179	515 (41.2)	0.014	820 (35.9)	363 (36.4)	0.79	1166	450 (41.4)	0.023
	(36.9)	(39.7)		(37.2)						(37.2)		
LCX n (%)	2540	(849 (25.4)	<0.001	1214	357 (28.5)	<0.001	817 (35.8)	290 (29.1)	<0.001	509 (16.2)	202 (18.4)	0.09
	(29.6)			(38.3)								
LAD n (%)	4272	1655	0.82	1592	663 (53.0)	0.09	1200	563 (56.5)	0.039	1524	462 (42.4)	<0.001
	(49.7)	(49.5)		(50.2)			(52.6)			(48.6)		
Graft n (%)	1641	695 (20.8)	0.038	895 (28.2)	384 (30.7)	0.10	717 (31.4)	304 (30.5)	0.60	29 (0.9)	7 (0.6)	0.38
	(19.1)											
Glycoprotein IIb/IIIa	1193	341 (10.2)	<0.001	271 (8.5)	87 (7.0)	0.08	308 (13.5)	109 (10.9)	0.043	614 (19.6)	145 (13.2)	<0.001
antagonists n (%)	(13.9)											

Table 1 Baseline characteristics according to gender and indication for PCI

and our primary and secondary endpoints. Baseline and procedural characteristics (i.e. age, medical history, procedural characteristics), as listed in Table 1 were considered as confounders. Subsequently, we performed a landmark analysis for the patients who survived the first 31 days, thereby excluding men and women who were most critical ill. We also performed a sensitivity analysis in the cohort of patients younger than 50 years of age.

Ethics

This is an observational study. For the purpose of this study patients were not subject to acts, neither was any mode of behavior imposed, otherwise than as part of their regular treatment. Therefore according to Dutch law, written informed consent for a patient to be enrolled in this study was not required. This study was conducted according to the privacy policy of the Erasmus MC, and to the Erasmus MC regulations for the appropriate use of data in patient oriented research, which are based on international regulations, including the declaration of Helsinki.

RESULTS

Baseline and procedural characteristics

Between January 1, 2000 and December 31, 2009, a total of 11,931 consecutive patients underwent a PCI in our institution. The majority of these patients were men 8,588 (72%). The indication for PCI was stable angina for 1,251 (37%) women versus 3,171 (37%) men, NSTE-ACS for 997(33%) women and 2,283 (27%) men, and STEMI for 1,095 (33%) women and 3134 (37%) men. There were important differences in baseline and procedural characteristics according to gender and indication (Table 1). Women were older, and had more often hypertension and diabetes mellitus. Men smoked more often, had more often a cardiac history (previous MI, PCI and CABG) and renal impairment. As far as procedural characteristics were concerned, men had more often multi-vessel disease. No difference in the prevalence of left main disease was found. Women more often underwent treatment of the RCA, while men more often underwent treatment of the LCX.

Mortality

Information on survival status at 1-year follow-up was complete for 96.5% of patients. The median follow-up period was 1596 days (IQR 154- 3030 days). During this period a total of 1972 died.

As shown in figure 1A, we found higher mortality in women than in men (p=0.002). When focusing on gender differences within the different diagnostic subgroups (stable angina, NSTE-ACS, STEMI) only in STEMI patients this difference remained.

In patients treated for stable angina the cumulative incidence of all-cause mortality at 31-days follow-up was similar in women compared to men, 0.8% vs. 1.8%, respectively (Kaplan Meier estimates). Similarly, no statistically significant differences were observed in all-cause mortality at 1-year (3.8% vs. 3.5%) and 4-year follow-up (9.1% vs. 10.5%, HR 0.85 and 95% CI 0.68-1.08). Multivariate adjustment for potential confounders (i.e. age, medical history, procedural characteristics) of the relation between gender and the incidence of all-cause mortality did not change this outcome at 1 year, whereas this relation became borderline significant after 4 years (adjusted (a)HR 0.79 and 95% CI 0.63 – 1.00) (Figure 2A).

We found similar short- (3.0% vs. 2.4%) and long-term results (16.2% vs. 14.5%) of all cause mortality in women compared to men treated for NSTE-ACS, (aHR 1.05 and 95% CI 0.85-1.28) (Figure 2).

In STEMI patients undergoing primary (p)PCI a significant gender difference was observed after 30-days (11.6% women vs. 6.5% men, HR 1.82 (1.47-2.27), p<0.001), even after multivariate adjustment for potential confounders (aHR 1.56 and 95% CI 1.25-1.96). Likewise, differences in mortality were also observed at 1-year (15.1% vs. 9.3%) and 4-year follow-up (21.6% vs. 15.0%, aHR 1.28 and 95% CI 1.09-1.51) (Figure 2A).

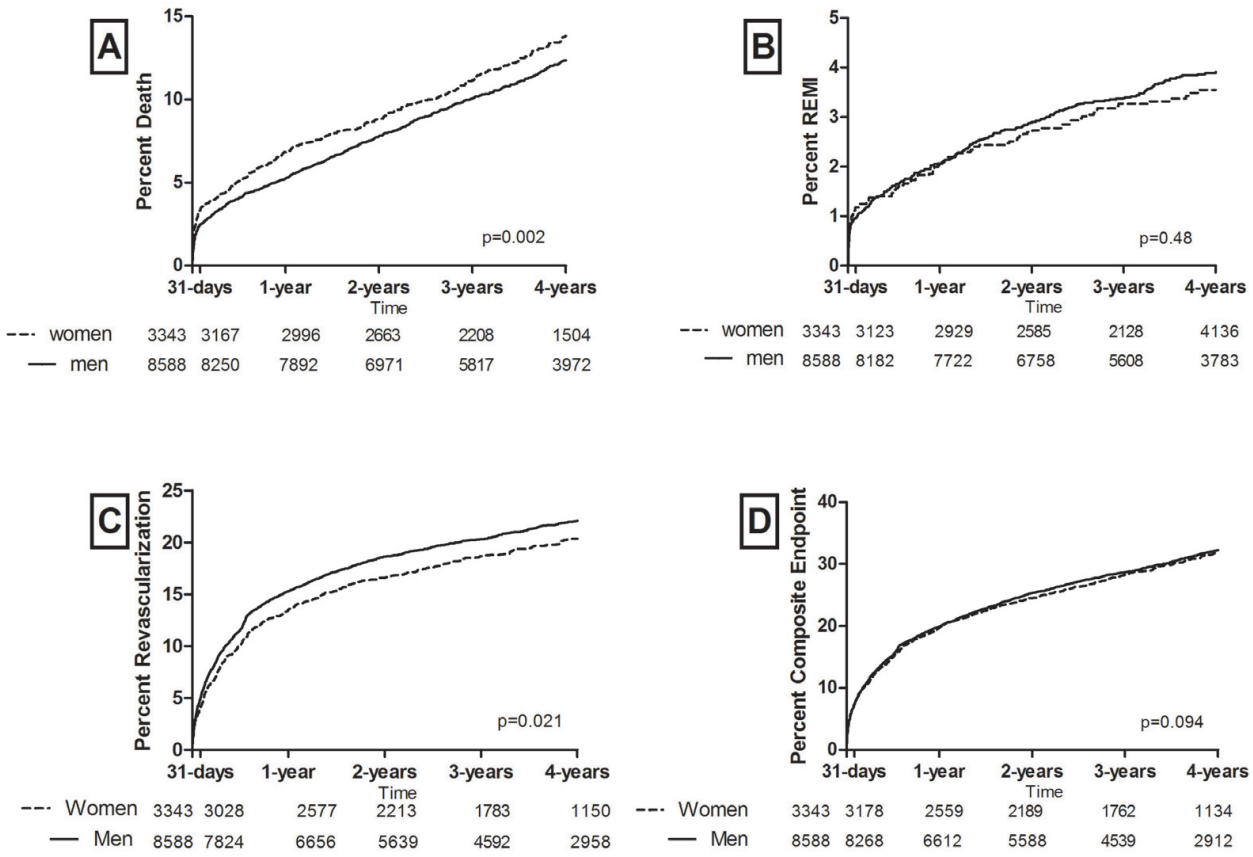


Figure 1 Clinical outomes according to gender
A. all-cause mortality; B. recurrent myocardial infarction; C. repeated revascularization; D. composite endpoint of all-cause mortality, (re)MI and re-revascularization.

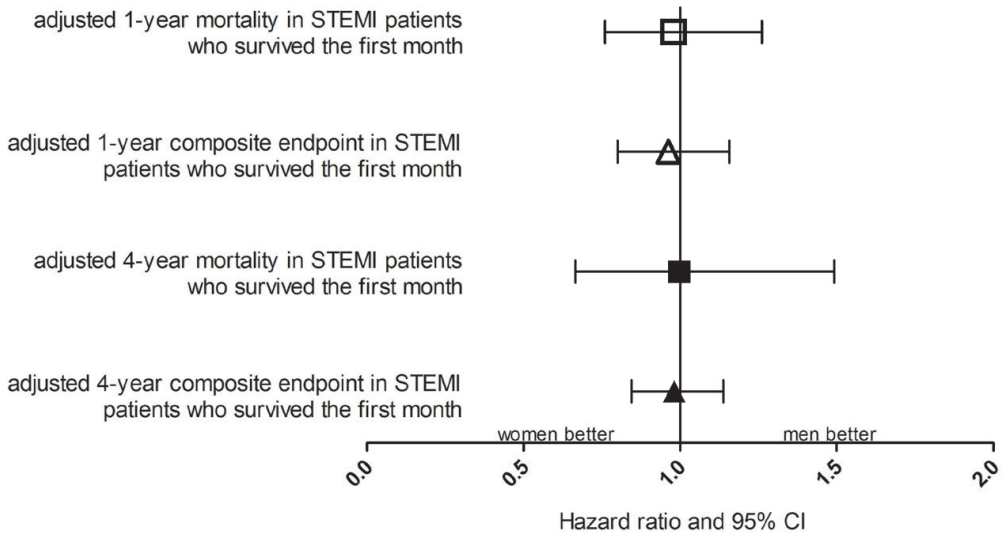


Figure 3 Relation between gender, and the incidence of all-cause mortality and the composite endpoint of all-cause mortality, (re)MI and re-revascularization in 31-days survivors. Adjusted for all confounders as listed in Table 1.

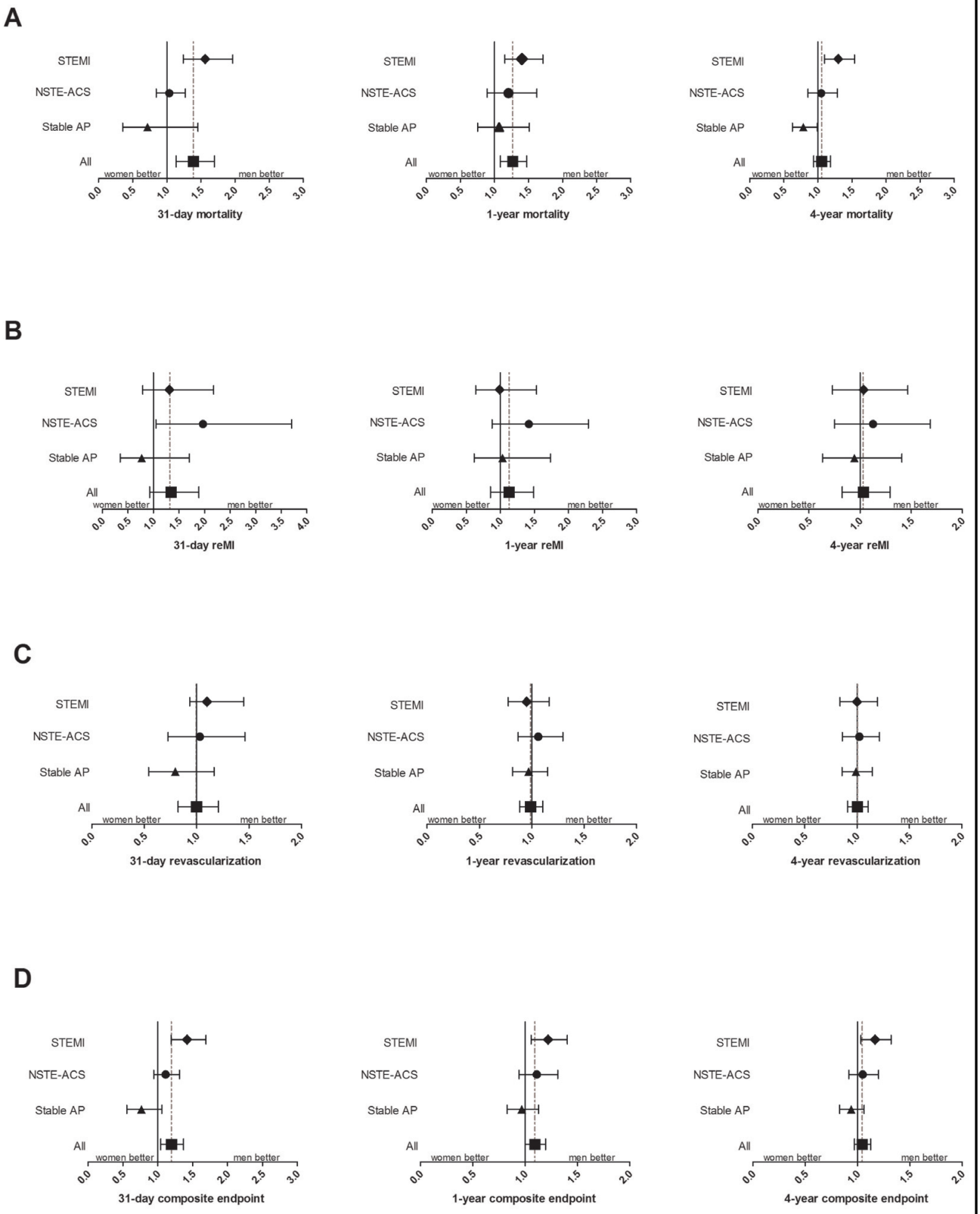


Figure 2 Relation between gender, and outcome according to the various indications for PCI. A. the incidence of all-cause mortality; B. the incidence of recurrent myocardial infarction, C. the incidence of repeated revascularization; D. the incidence of composite endpoint of all-cause mortality, (re)MI and re-revascularization

except that women were older, had less often a history of previous MI and suffered less from multi-vessel disease (table 2)

Differences in mortality rate over time

Figure 4 shows the absolute 31-day mortality risk in women and men according to year of treatment. In the year 2000 the absolute 31-days mortality rate was higher in men than women, thereafter the 31-day mortality rate was consistently higher in women. Moreover there seems to be a trend towards a worse prognosis in women in the last 5 years.

Patients younger than 50 years of age.

Of the female patients 388 (11,6%), and 1465 (17.1%) of the male patients were younger than 50 years of age, p<0.001. The cumulative incidence of all-cause mortality at 31-days follow-up was significantly higher in women as compared to men (5.1% vs. 3.5%, respectively, p<0.001). In STEMI patients this difference was 5.2% vs. 1.6%, p<0.001. No differences in 31-days mortality were found between women and men admitted with stable angina (0.2% vs. 0.2%, p=0.33) and NSTE-ACS (2.2% vs. 0.9% p=0.31).

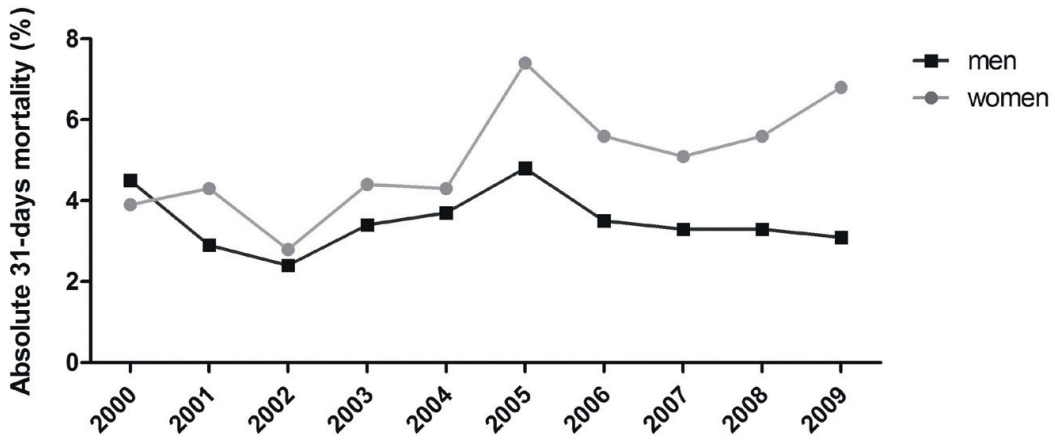


Figure 4 Absolute 30-day mortality rate in men and women according to year of treatment

Non-fatal and composite endpoints

As shown in figure 1B no clinically relevant gender difference in the incidence of (re)mi at short-term and long-term follow-up was found, the cumulative incidence curves were superimposed for this endpoint, p=0.48. With the exception of the occurrence of 31-day (re)MI in NSTE-ACS, also after multivariate adjustment gender did not contribute to the occurrence of (re)MI (Figure 2B).

In contrast, the incidence of re-revascularization was higher in women than men, p=0.021 (Figure 1C). However, no difference in re- revascularization rate was observed between women and men after multivariate adjustment for potential confounders for all indications at all 3 follow-up times (Figure 2C)

As shown in figure 1D, we found no difference in incidence rate for the composite endpoint of all-cause death, (re)MI, re-revascularization between women and men, p=0.94 (Figure 1D). After multivariable adjustment for potential confounders no difference was observed between women and men treated for stable angina pectoris (aHR at 4-years 0.93 and 95% CI 0.82-1.06) (Figure 2D). Similarly, no differences in the composite endpoint at short (7.7% vs. 7.3%) and long-term (34.2% vs. 34.4%) follow-up (aHR at 4-years 1.05 and 95% CI 0.92-1.20) were observed in patients with NSTE-ACS (Figure 2D).

We found a difference in the composite endpoint between women and men treated with PCI for STEMI 36% vs. 33% respectively, p=0.038 (aHR at 4-years 1.18 and 95% CI 1.05-1.39). In the STEMI

	Death within 31 days in STEMI patients			31 –day survivors in STEMI patients		
	Men n=151	Women n=96	P-value	Men n=2925	Women n=966	p-Value
Age (years ±SD)	66.5±12.3	70.7±15.2	0.007	59.1±11.9	64.4±13.2	<0.001
Medical History						
Hypertension n (%)	61 (29.8)	46 (36.2)	0.22	1060 (36.2)	493 (51.0)	<0.001
Hypercholesterolemia n (%)	93 (45.4)	62 (48.8)	0.54	2174 (74.3)	705 (25.7)	0.46
Diabetes Mellitus n (%)	28 (13.7)	23 (18.1)	0.27	298 (10.2)	146 (15.1)	<0.001
Family History n (%)	18 (8.8)	19 (15.0)	0.08	883 (30.2)	273 (28.3)	0.27
Current smokers n (%)	44 (21.5)	26 (20.5)	0.83	1252 (42.8)	370 (38.3)	0.014
Previous MI n (%)	49 (24)	18 (14.2)	0.03	298 (10.2)	146 (15.1)	<0.001
Previous PCI n (%)	5 (2.4)	5 (3.9)	0.43	192 (6.6)	41 (4.2)	0.009
Previous CABG n (%)	12 (5.9)	2 (1.6)	0.06	75 (2.6)	14 (1.5)	0.044
Renal impairment n (%)	11 (5.4)	6 (4.7)	0.80	50 (1.7)	10 (1.0)	0.14
Procedural characteristics						
Cardiogenic shock n (%)	32 (25.2)	56 (27.3)	0.67	97 (3.3)	29 (3.0)	
Vessel disease			0.03			0.001
0-vessel disease (excluding LM) n (%)	9 (4.4)	5 (3.9)		13 (0.4)	2 (0.2)	
1-vessel disease (excluding LM) n (%)	60 (29.3)	56 (44.1)		1629 (55.7)	607 (62.8)	
2-vessel disease (excluding LM) n (%)	50 (24.4)	30 (23.6)		780 (26.6)	231 (23.9)	
3-vessel disease (excluding LM) n (%)	86 (42.0)	36 (28.3)		505 (17.3)	126 (13.0)	
Left main disease	28 (13.7)	14 (11.0)	0.48	73 (2.5)	21 (2.2)	0.57
Multi-vessel disease n (%)	148 (72.2)	72 (56.7)	0.004	1310 (44.8)	360 (37.3)	<0.001
Treated vessel						
Left main n (%)	33 (16.1)	14 (11.0)	0.20	38 (1.3)	14 (1.4)	0.72
RCA n (%)	52 (25.4)	29 (22.8)	0.60	1114 (38.1)	420 (43.5)	0.003
LCX n (%)	50 (24.4)	26 (20.5)	0.41	459 (15.7)	176 (18.2)	0.06
LAD n (%)	98 (47.8)	68 (53.5)	0.31	1425 (48.7)	395 (40.9)	<0.001
Graft n (%)	3 (1.5)	1 (0.8)	0.58	26 (0.9)	6 (0.6)	0.43
Glycoprotein IIb/IIIa antagonists n (%)	26 (12.7)	11 (8.7)	0.29	588 (20.1)	134 (13.9)	<0.001

Table 2 Baseline characteristics according to gender and 31-day survival

patients who survived the first month similar 1-year (aHR at 1-year 0.96 and 95% CI 0.80-1.16) and long term (aHR at 4years 0.98 and 95% CI 0.84-1.14) composite endpoint rates were found for women and men (Figure 2D).

DISCUSSION

In this single center study with long-term follow-up, women undergoing PCI had a substantial different risk profile than men. A higher short and long-term mortality was observed in women compared to men in the STEMI population. This difference in mortality rate did not exist in patients who survived the first month and could only partially be explained by differences in baseline characteristics. No differences in clinical outcome between men and women undergoing PCI for stable angina pectoris and NSTE-ACS were observed.

There were distinct differences in baseline characteristics between women and men. Women were older and, with the exception of smoking, had a higher prevalence of cardiovascular risk factors at baseline. The gender differences in baseline characteristics have been related to the greater age dependency of CAD in women compared to men, women generally start developing CAD predominantly at postmenopausal age, which is 6 to 10 years later than men. [34-35] Still, considering that women had less often an extensive cardiac history and suffered more from single-vessel disease, men had more severe CAD. Whether this observation implies that the observed classical risk factors are less potent in women, is still subject of debate.

Our finding of a higher unadjusted mortality rate in women presenting with STEMI is in accordance with other reports. [8-9, 36-39] Several studies have demonstrated that this higher mortality rate is attributable to the more frequent presence of co-morbidities in these women.[8-9, 39] Contrary to these findings, gender difference in mortality did not disappear in our cohort after adjustment for baseline characteristics. This finding is confirmed by the sub-analyses in patients younger than 50-years of age, in whom we also observed an unfavorable outcome for women as compared to men. An important observation is that this difference in mortality is dominated by the excess mortality of women in the first month after admission. This is in accordance with the finding of Jneid et al. who reported a higher mortality among women presenting with STEMI in the initial 24 hours of hospitalization.[37] Moreover, gender differences are also observed regarding treatment delays, i.e. lower use of early medical treatments and timely reperfusion in women. [37, 40-43] Unfortunately, information needed to calculate the time delay between symptom onset, first (para) medical contact and time to balloon inflation was not available for the majority of patients with STEMI. A factor contributing to a longer treatment delay might be the difference in clinical manifestation of CAD. Women not only present more often with atypical chest pain or complaints of abdominal pain, dyspnea, nausea, and unexplained fatigue instead of chest pain, the higher prevalence of co-morbidity may cause misdiagnosis.[44-45] It is also suggested that this gender difference may be explained by a higher probability of surviving transport to the hospital, despite their poorer risk profile. Subsequently, this group of high risk AMI women are more likely to die in- hospital. [46-47]

With the exception of age, we found no major gender differences in baseline characteristics of the patients who died within the first month, while the observed differences were to the detriment of men. However, when comparing baseline characteristics with the overall STEMI population, the patients who died within the first month had more often diabetes and renal impairment, less likely underwent prior revascularisation, were more likely to have left main disease, presented more often with cardiogenic shock and were less likely treated with Glycoprotein IIb/IIIa platelet receptor antagonist. These characteristics indicated a subgroup of patient with a high-risk profile. Women with STEMI presented more often with single vessel disease, an observation that is in accordance with others. [9]

Moreover, women also are less likely to undergo guideline recommended treatment, undergo less invasive cardiac procedures, and when treated are less likely to be treated aggressively as compared to men.[37, 40-43, 45] In our cohort women were less likely to receive Glycoprotein IIb/IIIa platelet receptor antagonist than men, despite the known benefit [48] and lack of gender difference in protection from major adverse outcomes by GP IIb/IIIa blockade.[49]

No clinical relevant differences in short and long term clinical outcome between men and women undergoing PCI for stable angina pectoris and NSTE-ACS were observed. This is in accordance with

Mehilli et al. who also found identical 1-year outcomes in women and men undergoing PCI for symptomatic CAD. [18]

Limitations

The presented results are based on a single center experience, which may limit the external validity. Nevertheless, the Thorax center Rotterdam can be considered representative for larger tertiary referring and teaching (academic) hospitals in Western populations. For the follow-up on non-fatal endpoints we were dependent on the responses of patients on health questionnaires that were systematically sent to all living patients, with specific inquiries on rehospitalisation and MACE. Consequently, it cannot be excluded that we might have missed some non-fatal endpoints, particularly those that did not result in hospital admissions. We have no indication that underreporting (if any) was related to gender. This phenomenon might have resulted in effect estimates that are biased towards the null. Still, we are confident that similar effects were seen for all ('hard' and 'softer') endpoints.

Conclusion

Women undergoing PCI for STEMI treatment had higher mortality than men. The excess mortality in women appeared in the first month after PCI and could only partially be explained by a difference in baseline characteristics. No gender differences in outcome in patients undergoing PCI for NSTE-ACS and stable angina were observed.

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LIFE YEARS GAINED BY SMOKING CESSATION AFTER PERCUTANEOUS CORONARY INTERVENTION

LIFE YEARS GAINED BY SMOKING CESSATION AFTER PERCUTANEOUS CORONARY INTERVENTION

ABSTRACT

Previous studies have shown that smoking cessation after a cardiac event reduces the risk of subsequent mortality in patients. The aim of this study was to describe the effect of smoking cessation in terms of prolonged life-years gained. The study sample comprised 856 patients who underwent percutaneous coronary intervention (PCI) (balloon angioplasty) during 1980-1985. Patients were followed-up for 30 years and smoking status at 1 year could be retrieved in 806 patients. The 27 patients who died within 1 year were excluded from analysis. The median follow-up was 19.5 years (IQR 6.0-23.0). Cumulative 30-year survival was 29% in the group of patients who quitted smoking and 14% in those who persisted smoking ($p=0.005$). After adjustment for baseline characteristics at the time of PCI, smoking cessation remained an independent predictor of lower mortality (aHR 0.55 and 95% CI 0.44-0.69). The estimated life expectancy was 18.5 years in those who quit smoking and 16.4 years in the persistent smokers ($p<0.0001$). In conclusion, in patients with CHD who underwent PCI in the late 1980s smoking cessation resulted in at least 2.1 life years gained.

INTRODUCTION

Smoking is a well-known risk factor for the development and progression of coronary heart disease (CHD)[1-5] and its relation with increased morbidity and mortality from cardiovascular causes has long been established.[6, 7] Previous studies have demonstrated that smoking cessation after coronary artery bypass grafting (CABG),[8] percutaneous coronary intervention (PCI),[9] and myocardial infarction (MI)[10-12] reduces the rate of subsequent mortality and further cardiovascular events.[13] The aim of our study was to quantify the effect of smoking cessation in terms of prolonged life-years. We followed up the first 856 patients treated with PCI (balloon angioplasty) in the Thorax centre, Rotterdam during 1980-1985 for 30 years. Since this 30-year period almost compromises the whole further life span of these patients, the mean age at admission was 56 years old, we were able to calculate without applying major model assumptions the life expectancy in quitters and persistent smokers.

METHODS

The Erasmus MC, located in Rotterdam the Netherlands, is a tertiary referral and teaching hospital in the Rotterdam region. In the eighties the Erasmus MC was the only hospital with PCI facilities in the Rotterdam region. Historically the first candidates for PCI (balloon angioplasty) were those with proximal, discrete, non-calcified single-vessel obstructions in the presence of recent angina, unacceptable to the patient and uncontrolled by medical therapy.[14] However, due to greater confidence from increased experience and technological advances, more patients with larger numbers of diseased vessels, poorer left ventricular function and more accompanying risk factors were being treated in the following years.[15]

In this report we describe the first 856 consecutive patients of 18 years of age or older who underwent PCI in the Thoraxcenter during September 1980 to December 1985 for various indications. [16] Within 1 year after the PCI 27 patients died and 1 patient was lost to follow up. Among the 828 1-year survivors smoking status could not be retrieved in 22 patients. So, our final study population consisted of 806 1-year survivors with smoking status available. (Figure 1)

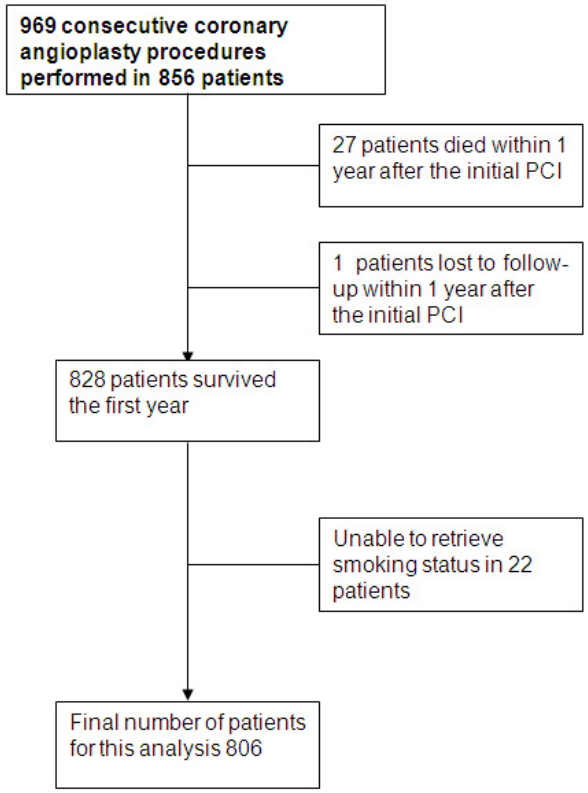

Figure 1 Flowchart patient selection

All data on smoking status are based on self-reporting via phone calls and questionnaires. Patient smoking behaviour was determined before the PCI procedure, and 495 patients (61%) reported to be smokers. One year after the PCI smoking status was obtained again, and 287 patients (36%) smoked 1 year after PCI. Based on this information patients were divided into 3 groups: non-smokers, quitters and persistent smokers. Patients were considered non-smokers if they reported to never have smoked, or quitted smoking before the PCI and did not start smoking within the first year after PCI. Quitters were defined as those patients who smoked prior to PCI and indicated themselves as non-smokers at 1-year follow-up. Patients were assigned to the persistent smoking category when they indicated themselves as smokers before PCI and 1 year after PCI. Two patients who started smoking within the first year after the PCI were considered as persistent smoker for this analysis.

Follow-up data for vital status were obtained from the municipal civil registry in February 2011. We were able to retrieve the vital status for 838 of the 856 patients (97.9%). Of the 18 patients who's vital status could not be retrieved, 7 patients moved abroad. For these 18 patients we used the last know follow-up date, after which point they were considered lost to follow-up.

The endpoint of our analysis was all-cause mortality at maximum follow-up.

Continuous variables are presented as mean ± standard deviation and categorical variables are expressed as numbers and percentages. Student's t tests, one-way ANOVA and Chi-square test (or Fisher's exact tests) were applied, when appropriate, to evaluate differences in baseline variables between non-smokers, quitters and persistent smokers.

We intended to obtain complete information in all patients, but failed to do so for baseline information for a small number of patients. Using missing value analysis (MVA) we evaluated the extent of missing data and searched for patterns of missing data. MVA showed that 1.6% missing values were missing. For the patients with at least one of the variables of interest missing, we decided to impute the missing values by multiple imputation.[17]

The incidence of events over time was studied with the use of the Kaplan-Meier method, whereas log-rank tests were applied to evaluate differences between the different groups (non-smokers, quitters of persistent smokers). Patients lost to follow-up were considered at risk until the date of last contact, at which point they were censored.

Subsequently, Cox proportional hazard models were used to analyse the association between smoking status and mortality during 30-year follow-up. Baseline and procedural characteristics listed in Table 1 were all considered as potential confounders. We report crude and adjusted hazard ratio's (HRs), with their 95% confidence interval (CIs).

Characteristics	Non-smokers (N=309)	Persistent smokers (N=287)	Quitters (N=210)	P-value Persistent smokers vs. quitters	P-value Non- smokers vs. quitters
Age (years±SD)	59.0±8.5	53.8±8.9	54.9±8.9	0.17	<0.001
Male	219 (71%)	251 (88%)	177 (84%)	0.31	<0.001
Indication				0.06	0.041
Stable Angina Pectoris	174 (56%)	149 (52%)	100 (48%)		
Unstable Angina Pectoris	112 (36%)	118 (41%)	81 (39%)		
Acute Myocardial Infarction	23 (7.4%)	20 (7 %)	28 (13%)		
Hypercholesterolemia	87 (29%)	83 (29%)	57 (27%)	0.66	0.78
Hypertension	132 (43%)	101 (36%)	84 (40%)	0.28	0.52
Positive family history for Coronary Heart Disease	131 (43.%)	111 (39%)	79 (38%)	0.84	0.28
Diabetes Mellitus	39 (13%)	31 (11%)	19 (9.1%)	0.53	0.21
Previous Myocardial infarction	113 (38%)	110 (40%)	94 (45%)	0.23	0.08
Previous Coronary Artery Bypass Graft	37 (12%)	18 (6.3%)	11 (5.3%)	0.62	0.010
Multi-vessel disease	122 (40%)	94 (33%)	63 (30%)	0.54	0.026
Clinical success procedure	241 (79%)	236 (83%)	166 (77%)	0.09	0.75

Table 1 Baseline characteristics according to smoking status

Life expectancy after PCI was calculated from the area under the Kaplan-Meier curve.[18] To calculate the exact life expectancy, the Kaplan Meier curves were extended beyond 30 years using the age- and sex-specific mortality data from the Dutch population in 2009, [19] assuming that the 251 patients with PCI who had survived 30 years had similar mortality as their age- and sex-matched peers.
All statistical tests were two-tailed and a p-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS for Windows version 17.0 (SPSS inc., Chicago, Illinois, USA).
RESULTS

The mean age of the patients was 56.2 years, and the majority of patients were men (80%). Key characteristics of the non-smokers, quitters and persistent smokers are presented in Table 1.
The patients that composed our study population were responsible for 14,977 patient years of follow-up; the median follow-up duration was 19.5 years (IQR 6.0-23.0).
The cumulative 30-year survival rate was 29% in the group of patients who quit smoking and 14% in the persistent smokers (p=0.005). (Figure 2). Life expectancy in those who quit smoking was 18.5 years (95% CI 17.3-19.6 years) and 16.4 years (95% CI 15.2-17.4 years) in the persistent smokers (p<0.0001).
Quitters and non-smokers had significant lower mortality than persistent smokers (adjusted hazard ratio (aHR) 0.57 and 95% CI 0.46-0.71), and (aHR) 0.42 and 95% CI 0.34-0.52), respectively. Other significant predictors of long-term all-cause mortality were: multi-vessel disease (aHR 1.45 and 95% CI 1.19-1.75), previous myocardial infarction (aHR 1.27 and 95% CI 1.06-1.53) and hypertension (aHR 1.37 and 95% CI 1.14-1.64).

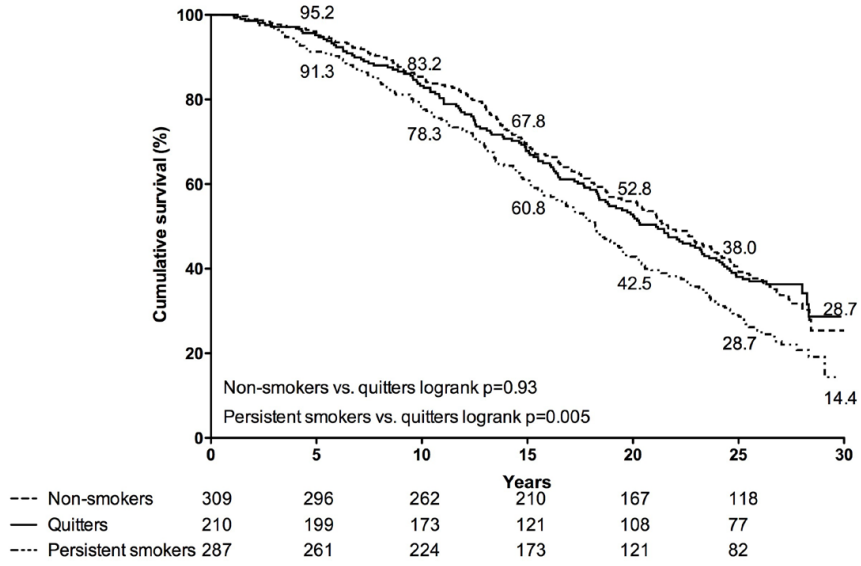

Figure 2 Cumulative survival according to smoking status

DISCUSSION

In this single centre study with long-term follow-up persistent smokers had significantly higher all-cause mortality than quitters. The life expectancy of quitters exceeded the life expectancy of persistent smokers by 2.1 years. Moreover, quitters had a similar all-cause mortality rate as patients who reported to be non-smokers. On average quitters were 4 years younger than non-smokers.
Our results are in accordance with previous studies. A systematic review studying the mortality risk reduction by smoking cessation in coronary heart patients found a substantial reduction in risk of all-

cause mortality and non-fatal myocardial re-infarctions.[13] Van Domburg et al. have shown similar results, smoking cessation after CABG led to a reduction in mortality and a 3 year life gain.[20]

Convincing patients to stop smoking is a difficult task. Pharmacologic therapy including bupropion (Zyban) and varenicline, nicotine patches, inhalers, and behavioral interventions have been developed to improve the chances of smoking cessation.[21-23] These interventions result in a modest increase in smoking abstinence at 12 months when compared with placebo. Unfortunately smoking relapse rates are rather high.[23] Despite the introduction of national health campaigns in the 1980s in the Netherlands, 27% of the general population [24], and 15% of the CHD patients smoke. [25] With our results (the quantification of prolonged life-years), physicians have an additional tool to encourage and convince patients to stop smoking.

The strength of the current study is the long-term follow-up of a cohort of patients undergoing PCI. At the same time, this population was treated with balloon angioplasty only, which is nowadays not the standard of care. Since the 1980s the patient population undergoing PCI has changed as procedures have been improved and refined and novel techniques were introduced, which made it possible to treat increasingly complex lesions and patients with a history of clinically significant cardiac disease, risk factors for coronary artery disease, coexisting conditions, or anatomical risk factors.[26, 27] Furthermore, improvements in supportive medical care (i.e. pharmacological therapy) have been made. Overall, the improved care has led to better prognosis in all patients, including smokers. Our study showed that smoking cessation improved life expectancy. Although it remains to be seen to what extend smoking cessation will benefit current patients, there are no indications that the unfavorable effect of smoking can be made undone by using novel treatment modalities. In addition, it has been shown that stent placement as compared to balloon angioplasty benefits only on target vessel revascularization, not mortality. [28] More research is warranted.

For the classification of smoking status we were dependent on the patients to fill out a questionnaire, patients who continued to smoke may have falsely claimed cessation. Since tests to measure the carbonmonoxide476 concentration were not available in the 1980s we were unable to validate smoking status. However, self-reporting smoking behaviour appears to be accurate as demonstrated in the EuroAspire-I survey, and in a meta-analysis/review. [29, 30] Finally, we did not record smoking status after the one year follow-up; patients may have start or stopped smoking during the follow-up. Nonetheless, since patients had to sustain smoking cessation for one year, the misclassification is limited.

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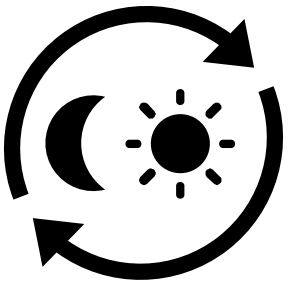
1.3

PRIMARY PCI DURING OFF-HOURS IS NOT RELATED WITH INCREASED MORTALITY



PRIMARY PCI DURING OFF-HOURS IS NOT RELATED WITH INCREASED MORTALITY

ABSTRACT

Purpose

Previous studies have shown contradictory outcomes in ST-segment elevation myocardial infarction (STEMI) patients who underwent primary percutaneous coronary intervention (pPCI) during off-hours versus regular "office" hours. We aimed to evaluate the relationship between pPCI timing (off-hours versus regular hours) and mortality in patients with STEMI undergoing pPCI.

Methods

The study population comprised of 4352 consecutive STEMI patients treated with pPCI in a high volume centre with a 24/7 programme during 2000 - 2009. Descriptive statistics and multivariable survival analyses were applied to evaluate the relation between treatment during off-hours (Monday- Friday, 06.00 PM- 08.00 AM and weekends) versus regular hours and the incidence of all-cause mortality at 30 day and 4 year follow-up.

Results

A total of 2760 patients (63.4%) were treated during off-hours and 1592 patients (36.6%) during regular hours. With the exception of smoking, diabetes mellitus, use of glycoprotein IIb/IIIa antagonists and calcium antagonists, no major differences in baseline characteristics were observed between both groups. Mortality at 30-days follow-up was similar in patients treated during off-hours and those treated during regular hours (7.7% vs. 7.7%; Hazard Ratio adjusted for potential confounders 1.03; 95% CI 0.82 - 1.28). Four-year mortality was similar as well (17.3% vs. 17.3%; adjusted Hazard Ratio 0.95; 95% CI 0.81 - 1.11).

Conclusion

In STEMI patients who present during off-hours in a high volume centre with 24/7 service, pPCI provides similar survival as patients who were treated during regular hours.

INTRODUCTION

Randomised clinical trials have convincingly demonstrated that patients with ST-segment elevation myocardial infarction (STEMI) who undergo primary percutaneous coronary intervention (pPCI) have better event-free survival and clinical outcomes than those treated with fibrinolysis. [1] In order to fully benefit from the instantaneous and long-term effects of pPCI, patients need to be treated as soon as possible after symptom onset. Guidelines recommend that pPCI be performed within 90 minutes after the first medical contact. [2-3] This recommendation is supported by several studies that reported a direct relation between (increased) time delay to pPCI and (worse) clinical outcome. [4-6] Short onset-to-treatment times are best guaranteed in hospitals with an established interventional cardiology program that offers full service 24 hours per day, 7 days per week (24/7 program). [2-3]
Since the occurrence of STEMIs are more or less randomly distributed over time, in a 24/7 program, most patients will be treated during 'off-hours': evening and night shifts and weekends. Previous studies have shown contradictory outcomes in STEMI patients who underwent pPCI during off-hours versus regular "office" hours. However, most of these studies were conducted in centers using both fibrinolysis and pPCI for treatment of patients with STEMI, and did not evaluate long term outcomes. [7-18] In the year 2000 pPCI became the standard treatment for STEMI in our institution and a 24/7 program was established. Baseline, procedural, and follow-up data of all patients undergoing PCI in our institution are systematically collected. Consequently we were able to evaluate the relationship between pPCI timing (off-hours versus regular hours) and short- and long-term outcome in STEMI patients.

METHODS

Patient population

The Erasmus MC is a tertiary referral and teaching hospital in the broader region of Rotterdam (approximately 1.9 million inhabitants), located on the North bank of the Maas river. Between January 2000 and June 2004 the Erasmus MC was the only hospital with pPCI facilities in the region. From July 2004 onwards the Maasstad hospital (also located in Rotterdam, on the south bank of the Maas river) started a 24/7 programme locally and provided regional pPCI service on Mondays and Thursdays and the first weekend of every month to improve service. The Erasmus MC provided pPCI service on the remaining days.
All consecutive patients of 18 years of age or older, who presented within 12 hours of symptom onset with ST-segment elevation myocardial infarction (STEMI) and who subsequently underwent pPCI in our institution between January 2000 and December 2009 were included in the analysis. STEMI is defined as patients presenting with ischaemic symptoms and persistent (>20 min) ST-segment elevation in at least 2 contiguous precordial leads or at least 2 adjacent limb leads by ECG. [3] In total, 4541 pPCIs in 4352 patients were performed. In patients who were admitted more than once for pPCI (n=189), only the initial procedure was used for this analysis.

Patient management

Patient management was in accordance with the applicable guidelines of the European Society of Cardiology (ESC). Patients received an aspirin and (300 to 600 mg) clopidogrel loading dose before pPCI and preferably in the ambulance. Clopidogrel (75mg/day) duration was at least one month for patients treated with bare metal stents (BMS), at least 3 months for patients treated with sirolimus eluting stents (SES) and at least 6 months to patients treated with paclitaxel eluting stents (PES) or everolimus eluting stents (EES). After the procedure, all patients were advised to remain on aspirin (>80mg/day) indefinitely. Periprocedural glycoprotein IIb/IIIa antagonists were left at the discretion of the treating interventional cardiologist.
Since the year 2000, the interventional cardiology department has the policy to us 1 particular stent

as default in a given time interval. The default stent between January 2000 and April 2002 was a BMS, between April 2002 and March 2003 a SES, between March 2003 and March 2007 a PES, and the EES since March 2007. Of note, during the study period a small number of STEMI patients was treated with another stent due to participation in a clinical trial comparing stents.

Data collection

According to the approved standard data-management procedures in our department, data are collected on demographics, cardiovascular history, clinical risk factors and treatment characteristics for all patients undergoing PCI and are stored in an electronic database. Data-elements are filled out immediately after the completion of the PCI by the interventional cardiologist and the technician who assisted during the procedure. The database, which is maintained by a dedicated IT-officer, is mainly designed for administrative purposes. A systematic evaluation of data-completion and data-integrity is implemented for data that are used for research purposes.

Data management and Follow-up

Mortality data related to the entire cohort was obtained from interrogation of municipal civil registries between April and September 2011. A health questionnaire was subsequently sent to all living patients with specific inquiries on rehospitalisation and major adverse cardiovascular events (MACE). For patients who had adverse events at other centres, medical records or discharge summaries from the other institutions were systematically reviewed. General practitioners, referring cardiologists, and patients were contacted in case further information was required.

Endpoint definitions

The primary endpoints were early mortality, which was defined as all-cause mortality within thirty days after the index event, and late mortality, which includes all-cause mortality at 1-year and 4-year follow-up). The secondary endpoints included repeat PCI (rePCI), coronary artery bypass grafting (CABG) or recurrent MI (reMI) and the composite endpoint of reMI, revascularization (rePCI or CABG) and all-cause mortality at 30 day, 1-year and 4-year follow-up. ReMI at follow-up was diagnosed MI was diagnosed by recurrent typical clinical symptoms, the development of ST-segment elevation or left bundle branch block on electrocardiography with a CK-MB rise of three times the upper limit of normal and/or positive troponin levels in the laboratory values. rePCI was defined as a repeat percutaneous intervention of any lesion located in the epicardial vessels. CABG was defined as a surgical intervention of any lesion located in the epicardial vessels.

Statistical methods

Off-hours were defined as weeknights (Monday through Friday from 06.00 PM to 08.00 AM) and weekends (from Friday 06:00 PM to Monday 08:00 AM).

Continuous variables are presented as mean ± standard deviation and categorical variables are expressed as numbers and percentages. Student's t tests, Chi-square tests and Fisher's exact tests were applied to evaluate differences in baseline variables between patients treated during off-hours and regular hours, as appropriate.

We intended to obtain complete information in all patients, but failed to do so for the medication at discharge. Using missing value analysis (MVA) we evaluated the extent of missing data and searched for patterns of missing data. MVA showed that there were 6.2% missing values on average for medication at discharge. For the patients with at least one of the variables of interest missing, we decided to impute the missing values by multiple imputation.[19]

The incidence of events over time was studied with the use of the Kaplan-Meier method, whereas log-rank tests were applied to evaluate differences between the treatment groups (treatment during off-hours versus regular hours). Patients lost to follow-up were considered at risk until the date of last contact at which point they were censored.

Cox proportional hazard (PH) regression models were applied to evaluate the relationship between

	Off-hours n=2760	Regular hours N=1592	P-value
Age (years ±SD)	60.9±12.8	61.5±12.5	0.14
Male (%,n)	73.5, 2029	75.2, 1197	0.23
Medical History			
Hypertension (%,n)	40.1, 1107	38.6, 614	0.32
Hypercholesterolemia	72.4, 1998	71.4, 1137	0.49
Diabetes Mellitus (%, n)	13.0, 358	10.4, 165	0.011
Family History (%, n)	28.6, 790	27.6, 439	0.46
Current smokers (%, n)	41.8, 1154	36.0, 573	<0.001
Previous MI (%, n)	12.1, 334	13.1, 209	0.32
Previous PCI (%, n)	9.2, 147	7.9, 217	0.12
Previous CABG (%, n)	2.4, 66	3.2, 51	0.11
Renal impairment (%, n)	2.1, 57	1.8, 29	0.58
Procedural characteristics			
Cardiogenic shock (%, n)	4.9, 135	4.8, 76	0.86
Vessel disease			0.63
1-vessel disease (%, n)	54.8, 1513	55.2, 878	
2-vessel disease (%, n)	26.9, 742	27.6, 440	
3-vessel disease (%, n)	18.3, 505	17.2, 274	
Multi-vessel disease (%, n)	45.2, 1247	44.8, 714	0.83
Treated vessel			0.35
Left main (%, n)	3.5, 97	4.5, 71	
RCA (%, n)	34.0, 938	35.3, 562	
LCX (%, n)	14.3, 396	13.4, 213	
LAD (%, n)	46.6, 1285	44.9, 715	
Graft (%, n)	0.6, 17	0.9, 15	
Glycoprotein IIb/IIIa antagonists (%, n)	16.5, 456	21.4, 340	<0.001
Discharge medication			
Aspirin (%, n)	90.1, 2599	90.2, 1338	0.91
Calcium antagonist (%, n)	14.5 378	19.8, 294	<0.001

Beta-blockers (%, n)	51.6, 1342	54.0, 801	0.14
RAAS-inhibitors (%, n)	41.7, 1085	39.9, 592	0.25
Statins (%, n)	71,6, 1861	71.2, 1056	0.79

Table 1 Baseline and procedural characteristics according to pPCI timing

treatment during off-hours versus regular hours and the incidence of all-cause death at 30 days, 1 year and 4 years. The baseline clinical and procedural characteristics that are listed in Table 1 were considered as potential confounders for the 1 year and 4 years mortality analysis. As the number of events was limited at 30 days, we were only able to adjust for the following clinically relevant factors: age, sex, multi-vessel disease, shock, previous MI, renal impairment, and diabetes mellitus.
Final results are presented as adjusted hazard ratios (aHR) with 95% confidence interval (CI). All statistical tests were two-tailed and a p-value <0.05 was considered significant. Statistical analyses were performed with SPSS for Windows version 17.0 (SPSS inc., Chicago, Illinois, USA).

RESULTS

Key characteristicsBetween January 1, 2000 and December 31, 2009, a total of 4352 consecutive patients presenting with STEMI underwent pPCI in our institution. A total of 2760 patients (63.4%) were treated during off-hours and 1592 patients (36.6%) during regular hours. Key characteristics of the two cohorts are presented in Table 1. With the exception of diabetes mellitus (13.0% vs. 10.4%, p=0.011), current smoking (41.8% vs. 36.0%, p<0.001), use of glycoprotein IIb/IIIa antagonists (16.5% vs. 21.4%, p<0.001) no statistically significant differences in baseline and procedural characteristics were observed between both groups. Noteworthy, the percentage of patients presenting with cardiogenic shock was similar in both groups (4.9% and 4.8%, respectively, p=0.86). There were also no statistically significant differences in discharge medication, except for the use of calcium antagonists (14.5% vs. 19.8%, p<0.001).

Mortality

Information on survival status at 1-year follow-up was complete for 96.5% of patients. The median follow-up period was 1246 days (IQR 651 - 2228 days).
The cumulative incidence of all-cause mortality at 30-days follow-up was similar in the patients treated during off-hours and those treated during regular hours 7.7% vs. 7.7% respectively (Kaplan Meier estimates). Similarly, no statistically significant differences were observed in all-cause mortality at 1-year (10.9% vs. 12.5%) and 4-year follow-up (17.3% vs. 17.3%). In fact, the cumulative incidence curves were superimposed throughout the entire 4 year follow-up period (figure 1A). Multivariable adjustment for potential confounders of the relation between treatment timing and the incidence of all-cause mortality did not change this message (aHR at 4-years 0.96 and 95% CI 0.81 - 1.12).

Non-fatal and composite endpoints

We did not found any clinically relevant difference in the incidence of non-fatal endpoints between patients treated during off-hours vs. regular hours. Results were similar at short-term and long-term follow-up (table 2, figure 1B-D). At 4-years follow-up, the crude cumulative incidences of reMI were 5.5% and 4.6%, the incidences of CABG were 1.6% and 3.0%, and the incidences of rePCI were 12.9% and 13.4%, respectively. Again, cumulative incidence curves were superimposed for all these endpoints. Treatment timing had no contribution in multivariable Cox PH models that related patient characteristics with non-fatal clinical outcomes. Adjusted HRs of treatment timing were non-significant and close

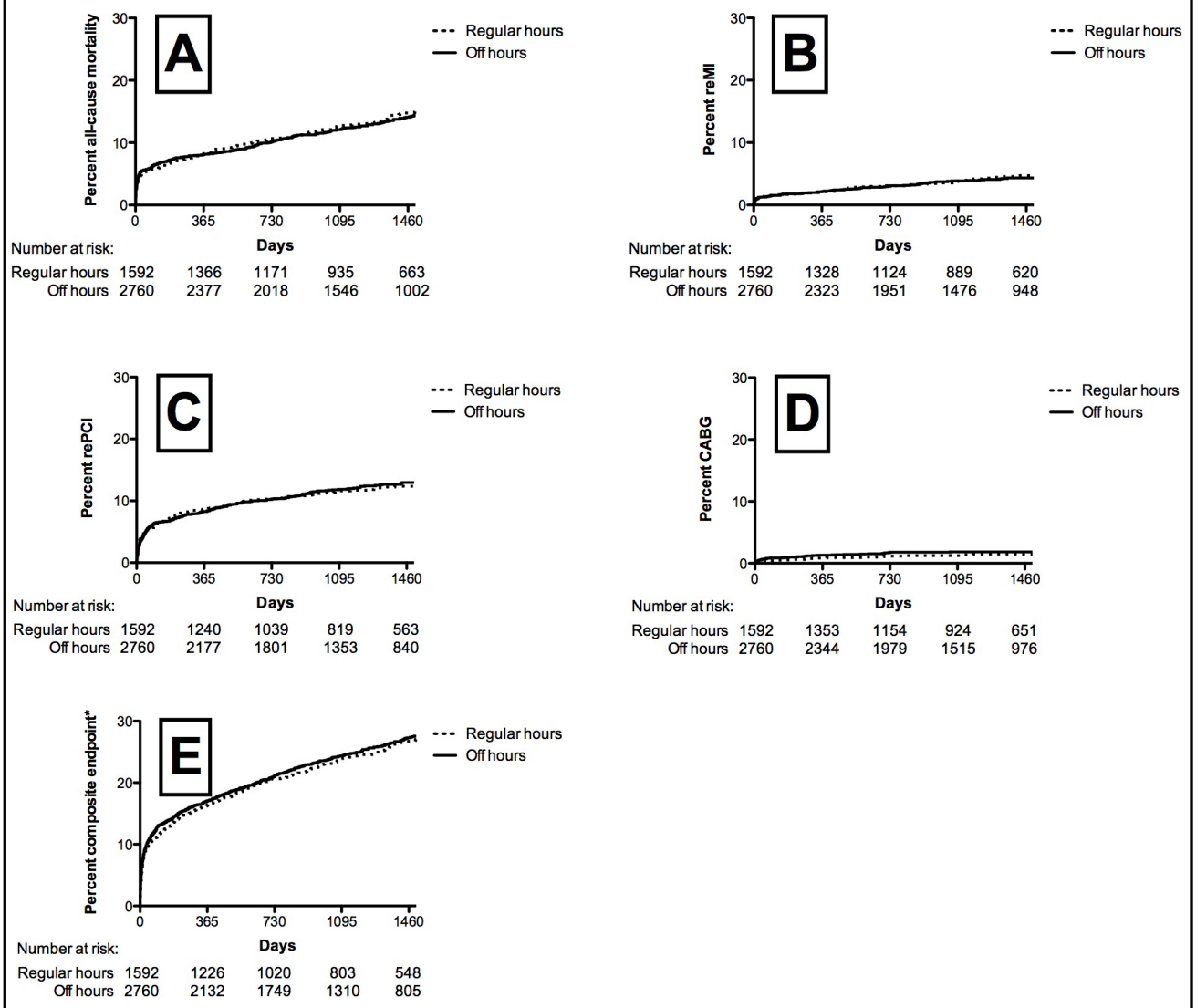


Figure 1 Clinical outomes according to regular hours versus off hours
A: all-cause mortality, B: reMI, C: rePCI, D: CABG, E: composite endpoint of all-cause mortality, reMI, rePCI and CABG.

to 1 for all endpoints at all three follow-up moments that we studied.

The cumulative incidence of the composite endpoint of all-cause death, reMI, PCI or CABG at 4-years follow-up was 29.5% in patients treated during off-hours and 29.5% in those treated during regular hours (table 2, figure 1E). The aHR was 1.05 and the 95% CI ranged from 0.93 to 1.18, indicating that there was no association between treatment timing and the incidence of this composite endpoint.

Findings in subgroups

Figure 2 shows the relation between treatment timing and 30-day all-cause mortality in a number of clinically relevant subgroups. All in all, in the subgroups that we considered, we found no major deviations from the overall result. Except in the strata according to the history of MI, 95% confidence intervals of treatment effect were largely overlapping, and none of the formal heterogeneity tests were statistically significant.

		30-day				1-year				4-year			
		Number of events n	KM estimate %	Crude HR and 95%CI	Adjusted* HR and 95%CI	Number of events n	KM estimate %	Crude HR and 95%CI	Adjusted* HR and 95%CI	Number of events n	KM estimate %	Crude HR and 95%CI	Adjusted* HR and 95%CI
Death	Regular hours	123	7.7	1	1	173	10.9	1	1	251	17.3	1	1
	Off-hours	218	7.7	1.03 (0.82-1.28)	1,05 (0.84-1.31)	291	12.5	0.97 (0.81-1.18)	0.96 (0.79-1.16)	408	17.3	0.92 (0.65-1.18)	0.96 (0.81-1.12)
MI	Regular hours	25	1.6	1	1	43	2.9	1	1	40	5.5	1	1
	Off-hours	40	1.5	0.92 (0.56-1.52)	0.91 (0.55-1.50)	63	2.4	0.84 (0.57-1.24)	0.89 (0.60-1.31)	103	4.6	0.85 (0.63-1.16)	0.88 (0.65-1.19)
CABG	Regular hours	5	0.3	1	1	15	1.0	1	1	22	1.6	1	1
	Off-hours	16	0.6	1,85 (0.68-5.04)	2.06 (0.74-5.72)	35	1.4	1.35 (0.73-2.47)	1.51 (0.82-2.79)	46	3.0	1.22 (0.73-2.02)	1.39 (0.83-2.33)
rePCI	Regular hours	72	4.9	1	1	136	9.2	1	1	177	12.9	1	1
	Off-hours	112	4.2	0.88 (0.65-1.18)	0.87 (0.64-1.17)	217	8.6	0.92 (0.74-1.14)	0.94 (0.76-1.17)	304	13.4	0.99 (0.83-1.20)	1.03 (0.86-1.25)
Composite endpoint*	Regular hours	200	12.6	1	1	310	19.6	1	1	432	29.5	1	1
	Off-hours	339	12.3	0.98 (0.82-1.16)	0.99 (0.83-1.18)	538	19.6	1.00 (0.87-1.15)	1.03 (0.89-1.18)	741	29.5	0.98 (0.87-1.10)	1.046 (0.93-1.18)

Table 2 Clinical outomes according to pPCI timing

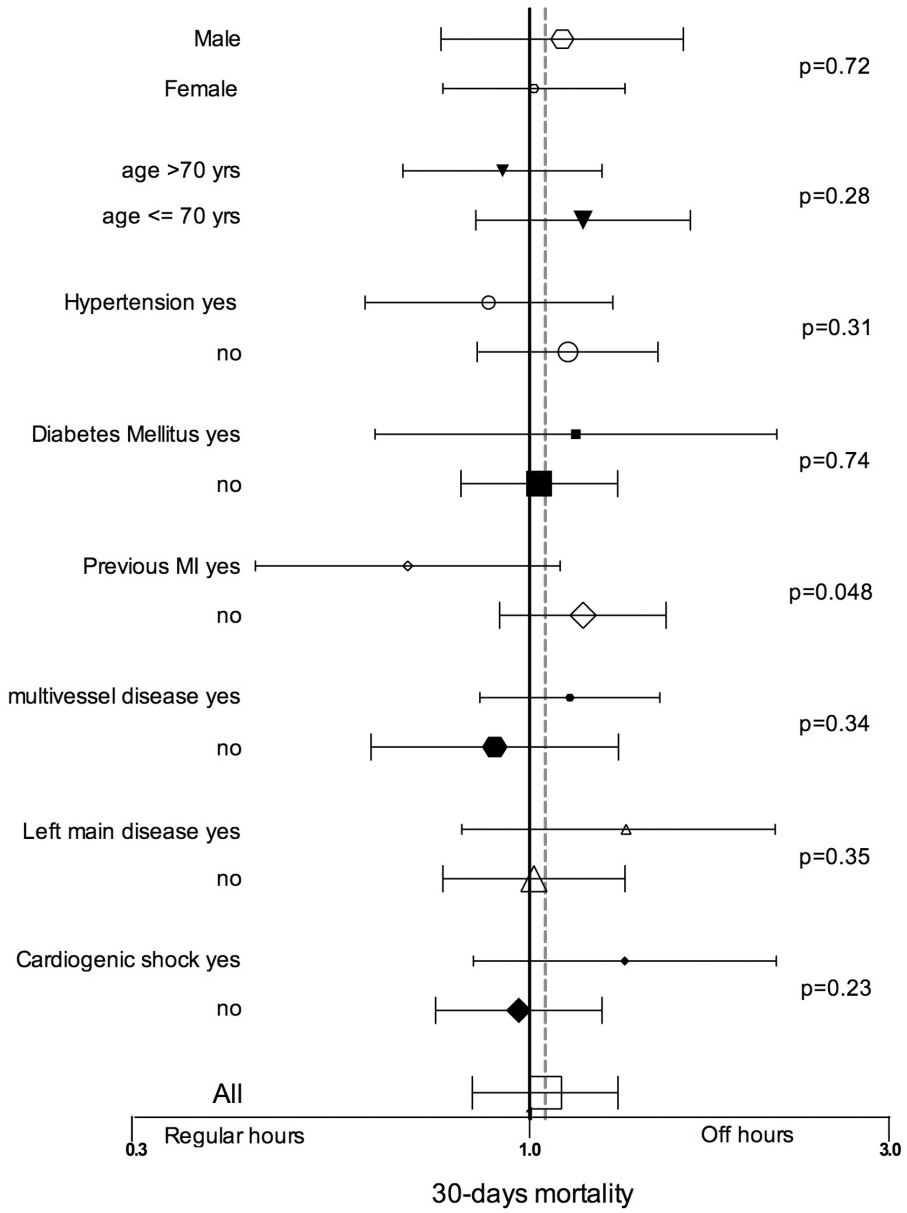


Figure 2 Treatment during off hours in different subgroups

DISCUSSION

In this long-term follow-up study of STEMI patients who were treated during 2000-2009 in a high volume centre with 24/7 service, clinical outcomes were similar in patients undergoing pPCI during off-hours and those undergoing pPCI during regular hours. This similarity in outcome was already observed at 30 days and was maintained until 4 years after the initial procedure. Consistent results were seen in clinically relevant subgroups, including the elderly and patients with multi-vessel disease.

The quality of care delivered to STEMI patients may differ during day and night because of variations in door to balloon time, performance of physicians, catheterization laboratory, and coronary care staff. Compared to regular office hours the hospital staffing is generally reduced during nights and on weekends compared with weekdays. The short-term outcome (after adjustment for any differences in case-mix) may be regarded as a proxy measure of the quality of care during the procedure, whereas the long-term outcome to a greater extent depends on the development of the disease, the use of long-term medication and (probably) the use of coronary revascularisations. Interestingly, we found

few differences in baseline characteristics between patients treated during off-hours and regular hours. Also medical treatment at discharge was similar. Apparently, in the region of Rotterdam, STEMI patients constitute a quite homogeneous population, regardless the timing of presentation. Consequently, the point estimates of the effect of treatment timing didn't change after adjustment for patient characteristics. The estimates of the effect for all the endpoints indicated that treatment during off-hours is as safe and effective as treatment during regular hours.

Previous studies have shown contradictory results in outcome in STEMI patients who underwent pPCI during off-hours versus regular hours. Whereas some studies showed that presentation and treatment during off-hours only had limited impact on in-hospital mortality [7, 9, 12-13, 15-16, 18,] other studies revealed higher in-hospital mortality in pPCI patients during off-hours than regular hours. [8, 10-11, 14] A straightforward comparison of these clinical studies is complicated by differences in patient characteristics and (medical) co-treatment, as well as by the applied definitions. For example, Henriques et al., who reported higher mortality after off-hour treatment, focussed on the circadian patterns of symptom onset, including routine duty hours (0800-1800) versus off-hours irrespective of day of the week.[8] In the study by Kostis et al.[10] the significant difference in 30-day mortality to the disadvantage of the patients treated during off-hours disappeared after adjustment for the use of invasive cardiac procedures, which appeared less often in patients admitted on weekends. Furthermore, in some of the previous studies patients were not only treated with pPCI, but also with fibrinolysis, which may have lead to different results. [9-11, 15, 18] Since 2000 in our centre pPCI is the default strategy for all STEMI patients on a 24/7 basis, precluding potential bias due to fibrinolysis. At the other hand, most patients that are treated in our center are referred for pPCI by hospitals in the larger region of Rotterdam that do not provide a 24/7 pPCI service. Although, apparently, the referral pattern is not dependent on the day of the week and the time of the day (as we found no differences in patient characteristics in relation to treatment timing), the patient selection by referral hospitals might partly explain the consistent outcomes.

Study limitations

Other predictors of outcome after pPCI that have been reported include time delay from symptom-onset to the first balloon inflation [4-6] and physician volume. [20-21] Since the database that we used for our study was not specifically designed to address these issues, several relevant quality parameters have not been recorded prospectively. Still, we retrospectively recorded the time between hospital admission and the start of the pPCI procedure (data were available for 70% of patients), and we found no differences between patients treated during off-hours versus those treated during regular hours (data not shown). The presented results are based on a single centre experience, which limits the external validity. Nevertheless, the Thoraxcenter Rotterdam can be considered representative for larger tertiary referring and teaching (academic) hospitals in Western populations.

For the follow-up on non-fatal endpoints we were dependent on the responses of patients on health questionnaires that were systematically sent to all living patients, with specific inquiries on rehospitalisation and MACE. Thus, we might have missed some non-fatal endpoints, particularly those that did not result in hospital admissions. We have no indication that underreporting (if any) was related to the timing of the initial treatment. This phenomenon might have resulted in effect estimates that are biased towards the null. Still, we are confident that similar effects were seen for all ('hard' and 'softer') endpoints.

Conclusion

STEMI patients who were treated in a high-volume center with a 24/7 program have similar short- and long-term outcomes if they are treated during off-hours or regular hours. Our findings, which are based on systematic monitoring of treatment outcome results, do not give rise to change our practice. Instead, these results may encourage other centers to expand their service.

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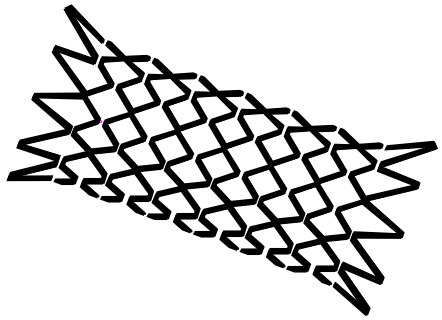
1.4

HIGH-RISK STEMI PATIENTS DERIVE GREATEST ABSOLUTE BENEFIT FROM PRIMARY PERCUTANEOUS CORONARY INTERVENTION: RESULTS FROM THE PRIMARY CORONARY ANGIOPLASTY TRIALIST VERSUS THROMBOLYSIS (PCAT)-2 COLLABORATION



HIGH-RISK STEMI PATIENTS DERIVE GREATEST ABSOLUTE BENEFIT FROM PRIMARY PERCUTANEOUS CORONARY INTERVENTION: RESULTS FROM THE PRIMARY CORONARY ANGIOPLASTY TRIALIST VERSUS THROMBOLYSIS (PCAT)-2 COLLABORATION

ABSTRACT

Background

Meta-analyses of randomised trials show that primary percutaneous coronary intervention (PPCI) results in lower mortality than fibrinolytic therapy in myocardial infarction (MI) patients. We investigated which categories of MI patients would benefit most from the strategy of PPCI, and thus have lowest numbers needed to treat (NNTs) to prevent a death.

Methods

Individual patient data were obtained from 22 (n=6763) randomised trials evaluating efficacy and safety of PPCI vs. fibrinolysis. A risk score was developed and validated to estimate the probability of 30-day death in individuals. Patients were then divided in quartiles according to risk. Subsequent analyses were performed to evaluate if the treatment effect was modified by estimated risk.

Results

Overall, 446 (6.6%) patients died within 30 days after randomisation. The mortality risk score contained clinical characteristics, including the time from symptom onset to randomisation. The c-index was 0.76, and the Hosmer-Lemeshow test was non-significant, reflecting adequate discrimination and calibration. Patients randomised to PPCI had lower mortality than patients randomised to fibrinolysis (5.3% vs. 7.9%; adjusted OR 0.63 95%CI (0.42-0.84); p<0.001). The interaction between risk score and allocated treatment interaction term had no significant contribution (p=0.52) to the model, indicating that the relative mortality reduction by PPCI was similar at all levels of estimated risk. In contrast, the absolute risk reduction was strongly related to estimated risk at baseline: the NNT to prevent a death by PPCI versus fibrinolysis was 516 in the lowest quartile of estimated risk compared to only 17 in the highest quartile.

Conclusion

PPCI is consistently associated with a strong relative reduction in 30 day mortality, irrespective of

patient baseline risk, and should therefore be considered as the first choice reperfusion strategy whenever feasible. If access to PCI is longer than 2 hours, fibrinolysis remains a legitimate option in low risk patients, because of the small absolute risk reduction by PPCI in this particular cohort.

INTRODUCTION

Based on the results of several randomised clinical trials (RCTs) and meta-analyses, it is broadly accepted that the strategy of primary percutaneous coronary intervention (PPCI) compared to fibrinolytic therapy results in better outcomes in patients presenting with acute myocardial infarction (MI).[1,2] In general, PPCI results in fewer deaths, repeat MIs and strokes than fibrinolysis.[3] Recent analyses demonstrated that relative risk reductions are not influenced by the time from onset of symptoms to presentation,[4] nor by the patient's age.[5] Still, these observations do not necessarily imply that PPCI is the best strategy for every patient in every clinical circumstance. Primary PCI results are dependent on operator and site experience and volume,[1] and are reported to be better during on- than off-hours.[6] Although it is true that beneficial results have been observed in clinical trial settings where PPCI was available within 'distances' of 2h from the MI scene,[4] it still might be challenging to perform treatment within that time-window in a wide variety of clinical practice settings. Finally, large relative risk reductions do not necessarily translate into large absolute risk reductions. In fact, the magnitude of absolute risk reductions - and thus the number of patients who need to be treated to prevent an adverse event, the so-called 'number needed to treat' (NNT) - is highly dependent on the patient's baseline risk.
This study was performed to address the issue of which (categories of) MI patients would benefit most from the strategy of PPCI, and thus have lowest NNTs. Based on 6763 individual patients who participated in 22 randomised clinical trials of PPCI versus fibrinolysis, a model of 30-day mortality was developed and validated to estimate risk in these patients. We examined whether the relative and absolute mortality reductions by PPCI were modified by the estimated baseline mortality risk.

METHODS

Details on the applied methodology of the pooled analysis have been published elsewhere.[4] For the purpose of this study, we will briefly describe the trial selection and data collection process, the endpoint definitions, and the applied methods of data analysis.

Trial selection

All clinical trials were considered that enrolled at least 50 MI patients who were randomly assigned to treatment with fibrinolysis or PPCI. Trials published between January 1990 and December 2002 were identified by OVID MEDLINE and ISI Web of Science using a variety of keywords. Each trial identified in this search was evaluated for patient population, study treatment, protocol, and endpoints. The primary investigators of these studies were contacted for verification and access to the individual patient data. In this way, 25 eligible trials were identified, which enrolled a total of 7743 patients. Individual patient data were unavailable in two smaller trials (140 patients), and in the CAPTIM trial (n=840). Consequently, individual patient data were obtained from 22 trials (n=6763), which were pooled for analyses. Data were assessed for completeness, and internal consistency with published reports. Any discrepancies between analysis of the data provided and previously published results were queried and resolved with the primary investigator.

Endpoint definition

The endpoint of the current analysis was all-cause mortality at 30 days after randomisation, which was available for all trials.

Data analysis

Differences in baseline characteristics between patients who reached the primary endpoint and those who remained endpoint free were analysed by unpaired Student's t tests and chi-square tests, as appropriate.

We developed and validated a model to estimate the probability of 30-day death in individual patients. The model was developed on a random selection of 80% of patients (n=5421), and validated on the remaining 20%. First, univariable logistic regression analyses were applied to study the relation between a broad range of potential risk factors (table 1) and the incidence of 30-day death. All variables that had a p-value <0.5 in univariable analysis, based on Wald's chi-square test, entered the multivariable stage. The final multivariable model was then constructed using backward elimination of the least significant variables, until all variables had a p-value ≤0.15. During the iteration process study was included as covariate in all models irrespective of its corresponding p-value. Subsequently, a mortality risk score was determined that included all relevant risk factors, which were weighted according to the natural logarithm of the corresponding odds ratio. For this purpose, the continuous variables age, systolic blood pressure and heart rate were categorized as follows: age <50, 50-60, 60-70, and ≥70 years; systolic blood pressure <115, 115-129, 130-149, and ≥150 mmHg; heart rate <65, 65-74, 75-84, and ≥85 beats per minute. The performance of multivariable models was studied with respect to discrimination (c-index) and calibration (Hosmer-Lemeshow [H-L] goodness-of-fit test).

To examine the influence of baseline risk on treatment effect, a multivariable model was fitted to the entire dataset (n=6763), which included the mortality risk score, allocated treatment (PPCI versus fibrinolysis) and the interaction term between the risk score and allocated treatment. We report relative treatment associations as adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI), and absolute treatment effects by NNTs that are based on the final model.

A total of 1793 (27%) patients had missing data on at least one of the variables that might indicate patient risk. This subset had a higher 30-day mortality than the patients with complete data (8.6% versus 5.9%). Excluding patients with missing data therefore could lead to biased risk estimates.[7] To partly correct for this, all multivariable analyses were performed on a dataset that included imputed predictive variables. The imputation technique used for continuous as well as dichotomous variables was mean substitution. Dichotomous variables were coded as 1 (determinant present) or 0 (determinant absent). Thus, after data imputation, the value of the variable can be interpreted as the probability of the determinant being present. As a comparison we repeated all multivariable analyses on the patients with complete data on all determinants, and consistent results were observed.

All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant. Analyses were performed using the SPSS® System version 15.0.

RESULTS

Patients

This pooled analysis included 6763 patients from 22 trials in which patients were randomised to fibrinolysis (n=3452) or PPCI (n=3451). Overall, 446 (6.6%) patients reached the primary endpoint of all-cause mortality within 30 days of randomisation. There were clinically relevant differences in sex, age, history of MI, diabetes, infarct location, time to randomisation and hemodynamic status at presentation, between patients who died within 30 days and those who survived (table 1). Thirty-day mortality was significantly higher among women, patients with advanced age, those with anterior MI, prior MI, diabetes mellitus, low systolic blood pressure and high pulse rate. The median time between randomisation and the injection (fibrinolysis patients) and between randomisation and the first balloon inflation (PPCI patients) was respectively 19 minutes (interquartile range (IQR) 10 to 30 minutes) and 76 minutes (IQR 62 to 95 minutes)

Characteristic	30 day death %, (n events/n total) *	P-value *	Crude OR (95% CI)¶	Adjusted OR (95% CI) in multivariable analysis¶	P-value in multi-variable analysis¶
Sex		P<0.001			
Female	10.3% (167/1455)		1	1	
Male	5.2% (249/4790)		0.52 (0.42-0.65)	0.77 (0.60-0.98)	0.04
Age, years		P<0.001	1.08 (1.07-1.09)		
<50	1.5% (18/1213)		1	1	
50-60	3.0% (53/1704)		2.0 (1.3-3.6)	2.0 (1.1-3.6)	0.016
60-70	5.9% (115/1823)		4.0 (2.4-6.9)	3.8 (2.2-6.6)	<0.001
≥70	14.2% (260/1577)		10 (6.0-17)	9.5 (5.6-16)	<0.001
Infarct location		P<0.001			
Non-anterior	5,0% (180/3624)		1	1	
Anterior	8.5% (264/3121)		1.8 (1.42-2.2)	1.6 (1.3-2.1)	<0.001
Prior MI		P<0.001			
No	5.9% (325/5480)		1	1	
Yes	9.4% (80/855)		1.6 (1.2-2.1)	1.3 (1.1-2.0)	0.016
Diabetes		P<0.001			
No	5.9% (318/5050)		1	1	
Yes	8.3% (83/864)		1.6 (1.2-2.1)	1.35 (1.0-1.8)	0.05
SBP per 10mmHg			0.87 (0.84-0.91)		
SBP, mmHg		P<0.001			
≥150	4.9% (75/1539)		1	1	
130-149	6.3% (186/2940)		1.5 (1.1-2.0)	1.2 (0.80-1.7)	0.40
115-129	5.3% (53/992)		1.1 (0.76-1.7)	1.7 (1.1-2.6)	0.020
<115	10.2%(132/1292)		2.5 (1.8-3.4)	3.5 (2.5-5.0)	<0.001
Heart rate per 10 beats/min			1.21 (1.15-1.27)		
Heart rate, beats/min		P<0.001			
<65	3.9% (60/1529)		1	1	
65-74	12.8% (57/1103)		1.2 (0.82-1.9)	1.4 (0.89-2.1)	0.16
75-84	6.9% (175/2550)		1.7 (1.2-2.3)	1.4 (0.9-2.2)	0.10
≥85	9.7% (154/1581)		2.5 (1.8-3.5)	2.7 (1.8-3.8)	<0.001

≥2 hours	7.5% (302/3714)		1.5 (1.2-1.9)	1.3 (1.0-1.7)	0.02
Prior CABG		P=0.20			
Yes	8.9% (10/112)		1.46 (0.67-3.19)	-	-
No	6% (316/5277)		1	-	-
Prior PCI		P=0.10			
Yes	3,5% (9/254)		0.57 (0.29-1.19)	-	-
No	6.1% (311/5129)		1	-	-

* Numbers were based on total dataset
¶ Numbers were based on random 80%selection of 80%of the total dataset

Table 1 Relation between baseline characteristics and 30-day all-cause mortality

Characteristic	Ln (OR)	Contribution to the mortality risk score
Woman	0.27	1
Age, years		
<50		0
50 - 60	0.71	2
60 - 70	1.34	5
≥70	2.26	8
Anterior infarct location	0.49	2
Prior MI	0.38	1
Diabetes mellitus	0.28	1
SBP, mmHG		
<115	1.26	4
115 - 129	0.39	1
130 - 149		0
≥150		0
Heart rate, beats/min		
<65		0
65 - 74	0.31	1
75 - 84	0.36	1
≥85	0.97	3
Time to treatment ≥2h	0.29	1

Add up the appropriate points to obtain the **Risk score**

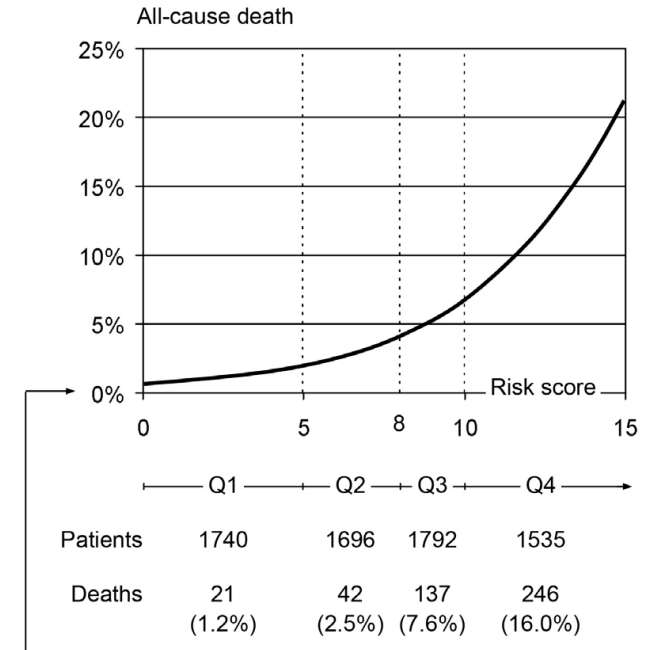


Figure 1 The mortality risk score and the relation between the mortality risk score and 30-day mortality.

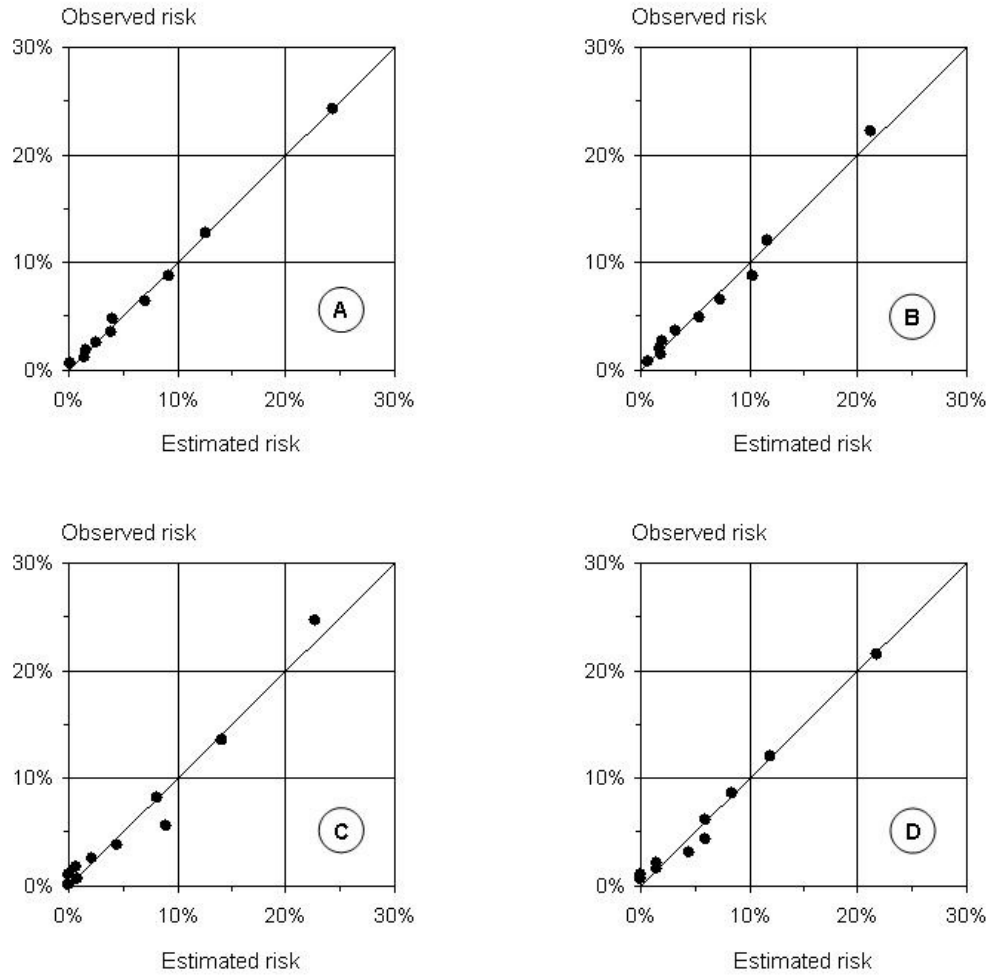


Figure 2 Observed risk versus expected risk for the multivariable model and the simple mortality risk score

Risk factors of 30-day mortality

In the randomly selected development set (n=5421), 30-day mortality was 6.7% (n=363). In univariable analysis, we identified ten potentially relevant determinants of deaths, eight of which remained ($p<0.15$) in the multivariable model (table 1). Age, hemodynamic status, and infarct location were the most dominant risk factors. Patients above the age of 70 years had an almost 10 times higher odds of 30-day death than patients below the age of 50. Patients with a systolic blood pressure lower than 115 mmHg had a 3.5 times higher odds of death than those with a value of 150 mmHg and above. Patients with a heart rate of 85 beats/min or higher had a 3.6 times higher odds of death than patients with a heart rate below 65 beats/min. The mortality risk score that is based on the natural logarithm of the ORs of the variables that compose the final multivariable model is presented in figure 1. This figure also shows the relationship between the mortality risk score (range and quartiles) and 30-day mortality.

Model performance

In the development dataset, the c-index for the multivariable 30-day mortality model was 0.75, reflecting adequate discriminatory power. The H-L test was non-significant ($p=0.90$), which implies that there were only minor differences between the predicted and actually observed mortality risk (i.e. the model showed adequate calibration; figure 2A). The mortality risk score also showed satisfactory discrimina-

tion (c-index 0.76) and calibration (H-L p=0.75; figure 2B).
Thirty-day mortality in the randomly selected 1342 patients who were used to validate the mortality risk score was 6.2% (n=83). There was no difference in the mean score between the patients comprising the development (2.2 ± 1.1) and validation (2.2 ± 1.1) datasets, indicating a successful random (4:1 ratio) assignment of patients to one of these datasets. When applied to the validation set, the full multivariable model showed satisfactory discrimination (c-index 0.78) and calibration (H-L p=0.64; figure 1C) figures, and so did the mortality risk score (c-index 0.78; H-L p=0.82; figure 1D).

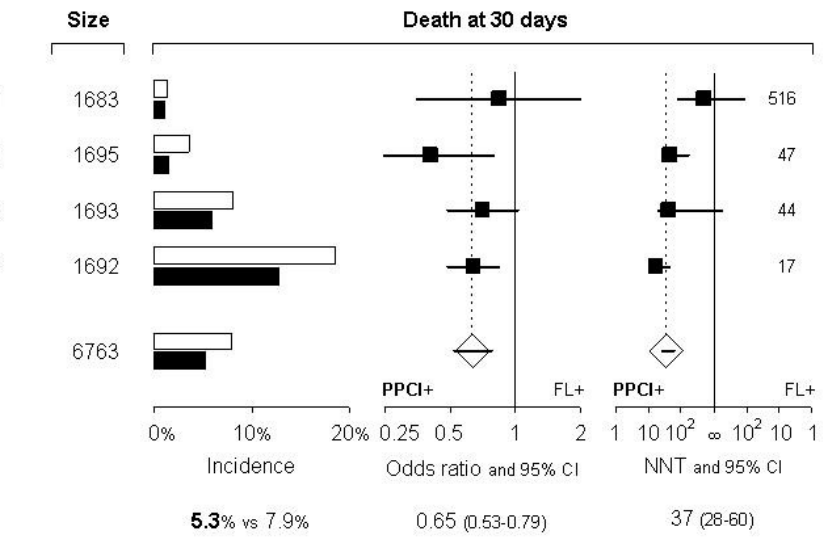


Figure 3 Relation between allocated treatment (PPCI versus fibrinolysis) and NNT, and the incidence of death, according to the quartiles of the distribution of the mortality risk score

Clinical effects of PPCI compared with fibrinolysis
To further evaluate the relation between baseline risk, allocated treatment and 30-day mortality, we determined the risk score for each patient, which was then used as a single variable in the modeling process. Allocated treatment had a significant (p<0.001) contribution to the model. Patients who were randomised to PPCI had lower mortality than those randomised to fibrinolysis (5.3% versus 7.9% events; adjusted OR 0.61 and 95% CI 0.49 to 0.77; p<0.001). The interaction between risk score and allocated treatment had no contribution to the model (p=0.52), indicating that the relative mortality reduction by PPCI (versus fibrinolysis) was not modified by the baseline mortality risk.
This finding is confirmed by an analysis of the treatment effect in subgroups of patients according to the quartiles of the distribution of the mortality risk score (figure 3). In contrast, the absolute mortality reduction by PPCI was strongly and positively associated with the baseline mortality risk. The NNT to prevent one death by PPCI compared with fibrinolysis in patients with a risk score in the lowest risk quartile was 516, whereas the NNT was as low as 17 in patients belonging to the highest risk quartile.

The relation between the risk score and 30-day mortality in patients randomised to PPCI and fibrinolysis (left panel), as well as the absolute mortality reduction by PPCI (right panel) is presented in figure 4 for the risk score based on the multivariable model (Figure 4 A and B) and for the mortality risk score (figure 4 C and D).
As an example, a 71-year old man without co-morbidities, who presents within 2h with a first MI (anterior), with a systolic blood pressure of 115 mmHg and a heart rate of 70 beats/min has a mortality risk score of 10.0 points, which corresponds with an estimated 30-day mortality risk of 13.4% after fibrinolysis and 9.0% after PPCI, and thus an absolute 4.4% mortality reduction in favor of PPCI.

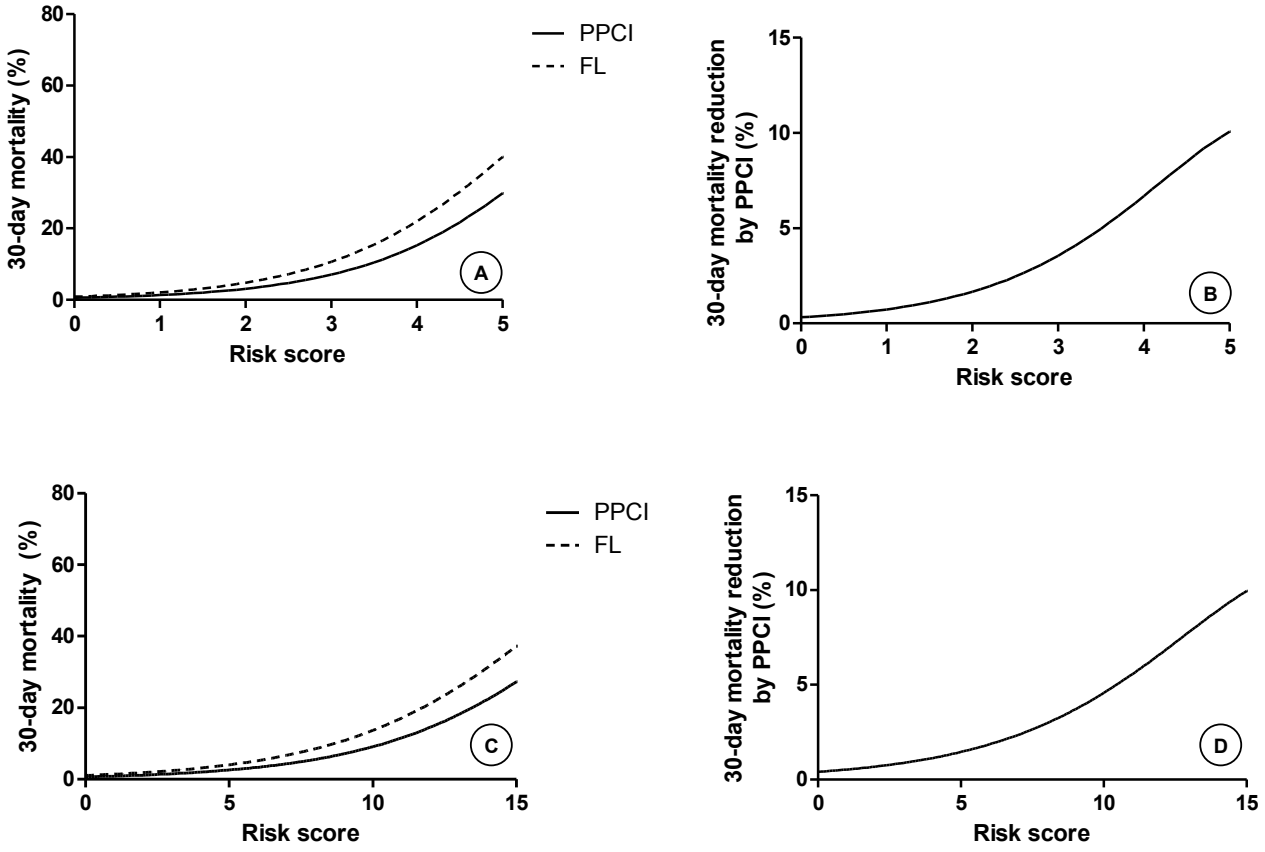


Figure 4 A and C (left panel) Absolute 30-day mortality risk in patients randomised to PPCI versus fibrinolysis (bold) in relation to risk score

Figure 4 B and D (right panel) Absolute 30-day mortality reduction by PPCI in relation to risk score

DISCUSSION

In this analysis of the pooled data of 22 randomised trials, we demonstrated that the relative mortality reduction by PPCI compared with fibrinolysis was not modified by the patient's estimated mortality risk. A consistent 37% relative risk reduction by PPCI was seen across the entire spectrum of estimated risk. In contrast - and as a consequence of the consistent relative risk reduction - absolute mortality reduction was strongly associated with the patient's baseline mortality risk. Patients with high mortality risk scores benefited most from PPCI: their NNTs were lowest.

We based the stratification of patients according to their predicted mortality risk on a simple model that we developed and validated in our dataset. The determinants of adverse outcome that compose the risk model are consistent with the analysis of the PCAT data,[8] as well as with mortality risk models that were developed in other datasets of patients receiving fibrinolysis or PPCI. For example, Lee et al., who studied the GUSTO-1 dataset of 41021 patients who were treated with streptokinase and/or alteplase, described advanced age, low SBP, high heart rate, previous MI, and an anterior location of the current MI as the most relevant determinants of 30-day death. [9] Similar findings were reported by the GRACE and TIMI investigators, who studied the prognosis of acute coronary syndrome and ST-elevation MI patients, respectively. [10-12]

In view of this consistency with the literature, and in view of its adequate discrimination and calibration performance, our mortality risk model might be used for risk stratification purposes. We acknowledge

that the model did not demonstrate excellent discrimination, mainly due to the limited number of baseline characteristics that were collected. The performance of the model might have been better if a broader variation in baseline data were available, including data on baseline ST-deviation, biomarker estimates of infarct size and renal function. [13,14] Still, it is unlikely that an improvement in the model discrimination will influence the main result of this analysis: the relative mortality reduction by PPCI was not modified by the (estimated) patient baseline risk.

One of the challenges for contemporary medicine is to rationally implement available therapies in clinical practice, in the appropriate patients at the appropriate time. Before certain therapy will be initiated, a physician must consider the probability that the patient will improve or deteriorate without such therapy, the chances of improvement if the therapy is initiated, the risks of adverse events, and, last but not least, the therapy-related costs. Our findings provide insight into opportunities to improve evidence-based treatment decisions for MI patients in cost-conscious environments. Since the NNT to prevent one death by PPCI compared with fibrinolysis was strongly related to the baseline mortality risk, it seems logical to first estimate that risk - preferably by using a validated risk score - and then decide on treatment. In our view, fibrinolysis remains a legitimate option for the categories of patients with estimated NNTs that exceed 100, particularly in regions where hospitals with PCI facilities are rare, or where the costs related to PPCI are considered too high to treat the entire MI population, as fibrinolysis compared with conservative treatment has proven to reduce mortality by 25-30% when initiated within 3 hours of symptom onset. If treated later, but within 12h, still a 15% mortality reduction might be realised. [15,16]. According to the AHA/ACC guidelines STEMI patients presenting to a facility without the capability for expert, prompt intervention with PPCI within 90 minutes of first medical contact should undergo fibrinolysis unless contraindicated. Similarly the ESC guidelines indicate fibrinolytic therapy in the absence of contraindications and if primary PCI cannot be performed within the recommended time. Both guidelines valued these recommendations as class 1-A [1,2]. Our analyses do not give rise to change these recommendations.

Patients who participate in clinical trials must satisfy several inclusion criteria and must not meet exclusion criteria, and thus form selected cohorts. In general, patients enrolled in clinical trials tend to be those with the fewest co-morbidities and the lowest risk of potential side effects. We realise that patients who participate in the randomised trials that we considered might therefore not be representative of all patients. Also approximately 80% of all patients in our meta-analysis had a PCI related delay of less than 2 hours, the results of this meta-analysis can therefore not rule on a clinical setting with a delay of more than 2 hours. In the context of our meta-analysis we cannot overcome this issue of external validity. Still, we would like to emphasize that our results are internally valid, since based on random allocation of treatment.

Conclusion

PPCI is consistently associated with a strong relative reduction in 30-day mortality, irrespective of patient baseline risk, and should therefore be considered as the first choice reperfusion strategy whenever feasible. If access to PCI is longer than 2 hours, fibrinolysis remains a legitimate option, especially for patients with a low risk score due to the small absolute risk difference between both treatment modalities in this patient group. Therefore in regions where hospitals with PCI facilities are rare, respectively, where geography does not allow primary PCI in all MI patients, this risk score might be used to select those higher-risk patients who benefit most from PPCI.

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MORTALITY AND MORBIDITY REDUCTION BY PRIMARY PERCUTANEOUS CORONARY INTERVENTION IS INDEPENDENT OF THE PATIENT'S AGE

MORTALITY AND MORBIDITY REDUCTION BY PRIMARY PERCUTANEOUS CORONARY INTERVENTION IS INDEPENDENT OF THE PATIENT'S AGE

ABSTRACT

Objective
The aim of this study was to obtain a valid estimate of the clinical effects of primary percutaneous coronary intervention (PPCI) in relation to age

Background
Treatment with PPCI is most beneficial in high-risk myocardial infarction (MI) patients. Paradoxically, elderly patients, who are at increased risk of adverse outcome, are often withhold PPCI.

Methods
Individual patient data were obtained from 22 randomized trials (6763 patients) evaluating the clinical effects of PPCI vs. fibrinolysis (FL). Differences in 30-day death, repeat myocardial infarction (reMI) and stroke between patients randomized to FL and PPCI were determined in 5 age-strata: ≤50, >50-60, >60-70, >70-80, and >80 years. Treatment effects are reported as odds ratios (ORs) and 95% confidence intervals (CI). Multivariable logistic regression analyses, which included age*treatment interaction, were applied to examine evidence of heterogeneity in age-specific ORs.

Results
Thirty-day death increased with increasing age, and ranged from 1.1% (FL) and 1.8% (PPCI) in patients ≤50 years to 26.4% and 18.3% in patients >80 years. The point estimate of treatment effect (overall adjusted OR 0.65 and 95% CI 0.52-0.79) was compatible with a mortality reduction favoring PPCI in all age-strata (except in patients ≤50 years), and 95% CIs were largely overlapping. There was no evidence of heterogeneity in ORs between age categories. Similar results were observed for reMI and stroke.

Conclusion
In this analysis of randomized trials, the reduction in clinical endpoints by PPCI was not influenced by age. Hence, age per se should not be considered an exclusion criterion for the application of PPCI.

INTRODUCTION

Despite the dominant role of age in the prognosis of coronary artery disease (CAD), elderly patients are often excluded from participation in clinical trials evaluating efficacy and safety of cardiac treatment.[1] The main reason why is that clinical trialists tend to favor the enrolment of patients at low risk of potential side effects, and large proportions of elderly patients meet one or more of the associated exclusion criteria.

Because of the limited availability of clinical trial data, and the increasing variability in treatment response in relation to age, estimates of treatment effect in elderly CAD patients appear to be uncertain for a broad variety of clinical situations. Particularly, uncertainty exists about the balance between beneficial and harmful treatment effects, which hampers the application of evidence-based treatment in clinical practice. For example, the application of reperfusion therapy in patients presenting with ST-elevation myocardial infarction (MI) is strongly related with age: elderly patients less often receive reperfusion therapy than their younger counterparts.[2-4] Most likely the choice for conservative treatment in the elderly is based on negative perceptions of the chances to reduce mortality risk due to limitation of the infarct size (treatment benefit) relative to the chances to cause harm. Particularly, the risk of severe disability, or even death, due to severe hemorrhagic complications is feared in elderly patients receiving fibrinolytic (FL) therapy. Still, the underutilization of reperfusion therapy in older MI patients is also reported with respect to primary percutaneous coronary intervention (PPCI).[5] Apparently, in clinical practice, there is a mismatch between (suspected) patient risk and applied treatment. This treatment-risk paradox has been observed in other settings in clinical cardiology as well.[6,7]

The clinical outcome after PPCI as compared to FL in elderly patients has been evaluated in the randomized trial of De Boer, et al. (patients aged 75+), and in the randomized SENIOR PAMI trial (patients aged 70+).[8,9] Although De Boer et al. demonstrated a favorable clinical outcome after PPCI - a statistically significant reduction was observed in the 30-day incidence of death, reMI and stroke - the sample sizes of both trials were too small to draw definite conclusions. We recently pooled individual patient data from 22 clinical trials of PPCI versus FL in patients with acute MI. This meta-analytic dataset provides a unique and powerful opportunity to study the clinical outcome of PPCI in relation with age.

METHODS

Details on the applied methodology of the pooled analysis have been published previously.[10] In this section, we briefly describe the trial selection and data collection process, endpoint definition, and data analysis for the current study.

Trial selection

All randomized trials that enrolled at least 50 patients presenting with MI assigned to treatment with FL or PPCI were considered. Trials published between January 1990 and December 2002 were identified by OVID MEDLINE and ISI Web science using a broad range of keywords. Each trial identified in this search was evaluated for patient population, study treatment, protocol, and endpoints. The primary investigators of these studies were contacted for verification and access to the individual patient data. In this way, 25 eligible trials were identified, which enrolled a total of 7743 patients. Individual patient data were unavailable in two smaller trials (140 patients),[11,12] whereas the investigators of the larger CAPTIM trial (n=840) elected not to release their data.[13] Consequently, individual patient data from 22 trials (n=6763) were pooled for the primary analysis. Table 1 presents the main design features of the included trials. Data were assessed for completeness, internal consistency with published reports. Any discrepancies between analysis of the data provided and previously published results were queried and resolved with the primary investigator.

Definitions of clinical endpoints

The primary endpoint of this pooled analysis was all-cause mortality at 30 days after randomization. Secondary endpoints included repeat myocardial infarction (reMI) and stroke, as well as the composite endpoints all-cause mortality or reMI, and all-cause mortality, reMI or stroke. Endpoints were counted until 30 days after randomization.

Statistical analysis

Summary statistics for all continuous variables are presented as medians with the corresponding interquartile range (IQR), and discrete variables are presented as counts and percentages. Patients were categorized according to their age into 5 categories: ≤50, >50-60, >60-70, >70-80, and >80 years. Differences in baseline characteristics in relation to age were evaluated by Kruskal-Wallis tests, or Mantel-Haenszel chi-square tests for trend, as appropriate.

All analyses of the relation between randomized treatment and the incidence of clinical endpoints were performed according to the intention-to-treat principle. Age-specific outcome data were pooled using the Cochrane-Mantel-Haenszel method, and odds ratios (OR) and 95% confidence intervals (CI) for clinical endpoints are reported. Breslow-Day tests were applied to examine statistical evidence of heterogeneity among the age-specific ORs. The association between treatment and the clinical endpoints in relation to age were also evaluated by logistic regression (LR). First, crude ORs for study endpoints were determined based on the single fixed effect LR model, stratified by age-category. Subsequently, non-stratified models were fitted with age (as a continuous variable), treatment and the age* treatment interaction term. To adjust for trial effects and for variations in the standards of practice across participating institutions, these models were then extended taking study membership into account. The final multivariable adjustment included study membership, sex, body weight, diabetes mellitus, previous MI, prior revascularization, anterior MI at presentation, heart rate, systolic blood pressure, time to treatment.

Time to treatment was categorized into time from symptom onset to randomization ('presentation delay') and time from randomization to treatment ('treatment delay'). Per definition, the interval between randomization and the actual commencement of treatment is a post-randomization variable, which is influenced by allocated treatment. Hence, analyses in relation to treatment delay that are based on observations in individual patients can result in biased estimates of treatment effect. Moreover, patients are randomized to either FL or PPCI, so on a patient-level time to treatment can only be obtained for one strategy. Analyses based on observations on hospital level may help to overcome this. Thus, the median time between randomization and the start of treatment (i.e., first injection of the fibrinolytic agent or the first balloon inflation) was calculated for each of the 153 participating hospitals. The hospital-specific difference between these median times was then determined, which is hereafter referred to as 'PCI-related delay', and assigned each patient within that hospital. Consequently, PCI-related delay should be interpreted as the additional time that is needed to start the PCI procedure after treatment with a fibrinolytic agent could have been started.

All tests are two-sided with a significance level of 0.05. All statistical analyses were performed using the SAS® System version 8.2 (SAS® Institute Inc., Cary, NC).

RESULTS

Patients

This pooled analysis included 6763 patients from 22 trials in which patients were randomized to FL (n=3383) or PPCI (n=3380). There were important differences in clinical baseline characteristics in relation to age (table 2). Elderly patients had an unfavorable risk profile, as they more often had diabetes mellitus, a history of MI, and a longer presentation delay. For example, 46% of patients in the ≤50-age category were randomized within 2h after the onset of symptoms, in contrast to only 26% in

				Fibrinolysis			Primary PCI	
Trial or author	**Enrollment**	**Inclusion criteria**	**Symptom duration (h)**	**No. of patients**	**30-day mortality (%)**	**Treatment in case of persistent or recurrent ischemia**	**No. of patients**	**30-day moratlity (%)**
Streptokinase trials								
Zijlstra (16)	1990 - 1992	ST↑, ≤75 years	<6	149	7.4	Not specified	152	1.3
Ribeiro (17)	1989 - 1989	ST↑, <75 years	<6	50	6.0	Not specified	50	2.0
Grinfeld (18)	1993 - 1995	ST↑	<12	58	9.3	Not specified	54	13.8
Zijlstra (19)	1993 - 1995	ST↑, low risk	<6	53	2.1	Angiography → possible angioplasty	47	1.9
Akhras (20)	1993 - 1995	ST↑	<12	45	0.0	Not specified	42	8.9
Kedev (21)	1996	ST↑	<12	67	10.4	Not specified	68	2.9
Prague-1 (22)	1997 - 1999	ST↑, LBBB	<6	99	14.1	Not specified	101	6.9
De Boer (9)	1996 - 1999	ST↑, >75 years	<6	41	22.0	Discretion cardiologist	46	6.5
Prague-2 (23)	1999 - 2002	ST↑	<12	421	10.0	Rescue PCI	429	6.8
Duteplase trial								
Gibbons (24)	1989 - 1991	ST↑, <80 years	<12	56	3.6	Discretion cardiologist	47	4.3

(Accelerated) tPA trials								
Grines (25)	1990 - 1992	ST↑	<12	200	6.5	Discretion cardiologist	195	3.1
GUSTO IIb3 (26)	1995 - 1996	ST↑, LBBB	<12	573	7.0	Discretion cardiologist	565	5.7
JIMI (27)	Published in 1997	ST↑, <80 years	<6	62	1.6	Not specified	59	1.7
Ribichini 3 (28)	1993 - 1996	Inferior/anterior ST↓, <80 years	<6	55	5.5	Emergency catheterisation → rescue PTCA	55	1.8
Garcia 3 (29)	1991 - 1996	Anterior MI	<5	94	10.6	Discretion cardiologist	95	3.2
LIMI3 (30)	1995 - 1997	ST↑, <80 years	<6	75	6.7	Not specified	75	5.3
STAT3 (31)	1997 - 1996	ST↑, LBBB	<12	61	3.3	Discretion cardiologist	62	3.2
STOPAMI-13 (32)	1997 - 1999	ST↑	<12	69	7.2	Not specified	71	4.2
AIR PAMI3 (33)	1994 - 1999	<70 years, anterior MI	<12	66	12.1	Emergency catheterisation	71	8.5
C-PORT3 (34)	1996 - 1999	ST↑	<12	226	7.1	Not specified	225	5.3
DANAMI-23 (35)	1997 - 2001	ST↑	<12	782	7.8	Not specified	790	6.6
STOPAMI-23 (36)	1999 - 2001	ST↑, LBBB	<12	81	6.2	Not specified	81	2.5

LBBB, left bunch branch block; Not specified, not specified in the main results paper; ST↑, ST-segment elevation; ST↓, ST-segment depression; h, hours

Table 1 Key design and outcome figures of the trials that are included in this analysis

	Age category, years					P-value
	≤50	>50-60	>60-70	>70-80	>80	
No. of patients	1231	1757	1938	1427	410	
Randomised to Primary PCI, %	50	49	51	49	49	
Men, %	90	84	73	63	48	<0.001
Diabetes Mellitus, %	11	14	17	17	16	<0.001
Prior MI, %	9.7	13	13	16	18	<0.001
Prior PTCA, %	4.3	5.5	4.8	4.5	3.2	0.43
Prior CABG, %	0.86	1.8	2.5	3.1	1.6	0.002
Anterior MI, %	47	45	45	47	52	0.32
Use of Aspirin, %	51	54	54	59	66	<0.001
Use of Betablocker, %	40	35	32	33	38	0.047
Use of Heparin, %	88	90	89	89	82	0.11
Use of GP2B3A, %	23	26	24	27	20	0.62
Systolic bloodpressure, mmHG	133 (120-140)	133 (120-145)	133 (120-145)	133 (123-145)	133 (120-150)	0.022
Pulse, bpm	76 (68-86)	76 (65-80)	76 (65-83)	76 (66-81)	76 (68-88)	<0.001
Presentation delay, min	130 (90-195)	135 (87-195)	143 (91-220)	156 (100-240)	180 (120-300)	0.001
Presentation delay ≤2 h, %	46	45	40	36	26	0.001
PCI related delay, min	55 (37-73)	54 (37-74)	54 (37-74)	56 (38-77)	52 (38-72)	0.030
PCI related delay 0-35 min, %	22	22	22	18	17	0.026

Table 2 Baseline characteristics according to age

	Age category, years					P-value Breslow-Day	Any age
	≤50	>50-60	>60-70	>70-80	>80		
All-cause mortality						0.25	
Fibrionolysis, %	1.1	3.9	6.9	14.8	26.4		7.9
Primary PCI, %	1.8	2.1	5.0	8.7	18.3		5.3
Odds ratio (95% CI) *	1.6 (0.62, 4.2)	0.53 (0.30, 0.94)	0.72 (0.49, 1.0)	0.55 (0.39, 0.76)	0.62 (0.39, 1.0)		0.63 (0.52, 0.77)
Myocardial reinfarction						0.67	
Fibrionolysis, %	5.3	6.4	6.9	8.2	7.0		6.7
Primary PCI, %	2.1	1.6	2.4	3.2	3.9		2.4
Odds ratio (95% CI) *	0.39 (0.20, 0.74)	0.24 (0.13, 0.44)	0.33 (0.21, 0.54)	0.37 (0.23, 0.62)	0.53 (0.21, 1.4)		0.34 (0.26, 0.44)
Stroke						0.40	
Fibrionolysis, %	0.34	0.94	2.4	5.7	7.9		2.6
Primary PCI, %	0.52	0.74	1.6	2.2	5.8		1.5
Odds ratio (95% CI) *	1.5 (0.26, 9.2)	0.78 (0.27, 2.3)	0.64 (0.33, 1.2)	0.36 (0.20, 0.68)	0.72 (0.31, 1.7)		0.57 (0.40, 0.81)
All-cause mortality or reMI						0.37	
Fibrionolysis, %	6.3	9.9	12.5	20.8	30.3		13.5
Primary PCI, %	3.8	3.6	7.2	11.4	19.8		7.3
Odds ratio (95% CI) *	0.58 (0.34, 0.98)	0.34 (0.22, 0.52)	0.55 (0.40, 0.74)	0.49 (0.36, 0.65)	0.57 (0.36, 0.90)		0.49 (0.42, 0.58)
All-cause mortality, reMI, or stroke						0.35	
Fibrionolysis, %	6.6	10.8	14.3	24.2	35.1		15.3
Primary PCI, %	4.1	4.2	8.5	12.5	23.3		8.3
Odds ratio (95% CI) *	0.60 (0.36, 1.0)	0.36 (0.24, 0.53)	0.55 (0.41, 0.74)	0.45 (0.34, 0.59)	0.56 (0.36, 0.86)		0.49 (0.42, 0.57)

* Unadjusted odds ratio

Table 3 Effects of allocated treatment on the incidence of clinical endpoints at 30-day follow-up in relation to age

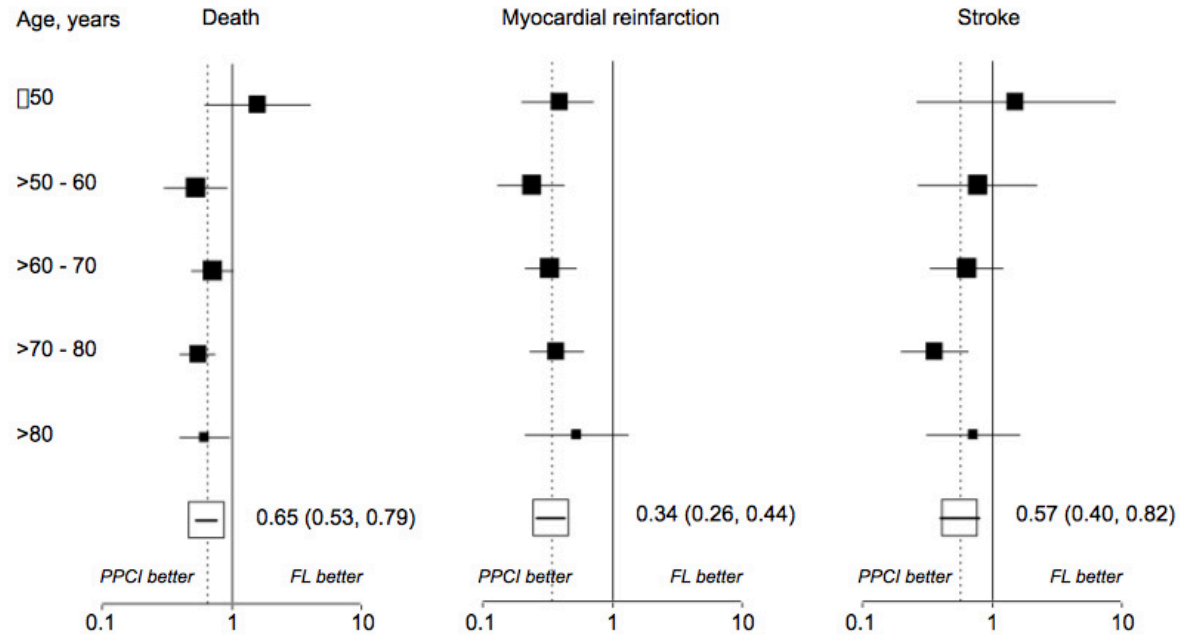

Figure 1 Incidence of death, MI, Stroke/Treatment According to Age

Relation between allocated treatment (PPCI versus FL), and the incidence of death, myocardial infarction, and stroke at 30 days according to age category
Data represent adjusted odds ratios and 95% confidence intervals

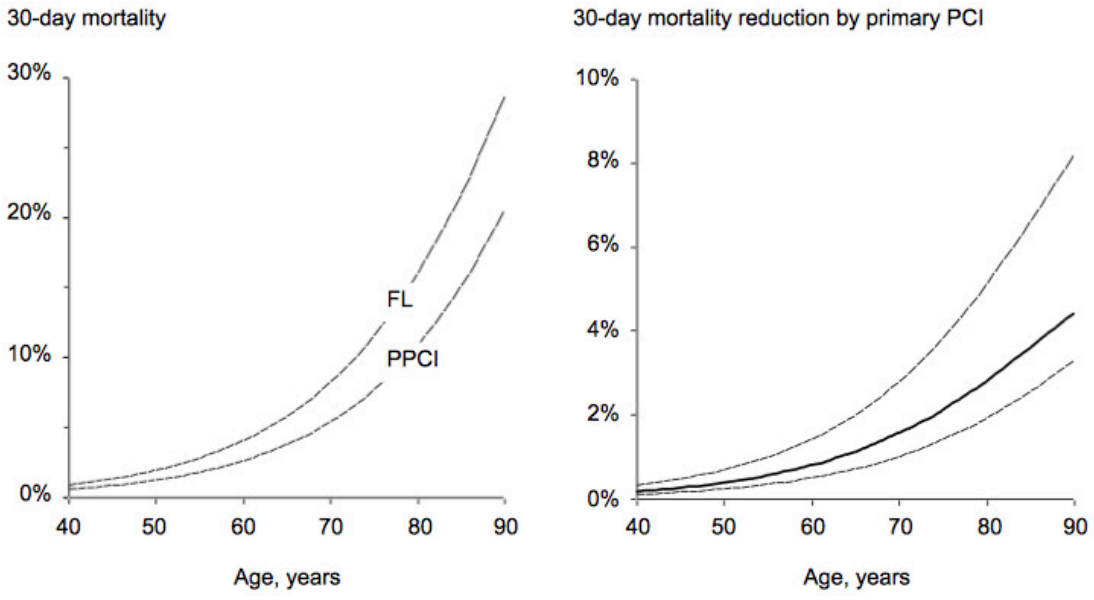

Figure 2 (left panel) Absolute 30-day mortality risk in patients randomised to PPCI versus FL in relation to age
Figure 2 (right panel) Absolute 30-day mortality reduction by PPCI in relation to age

Data are based on results of multivariable regression analysis, with adjustment for the following variables: study membership, sex, body weight, diabetes mellitus, previous MI, prior revascularization, anterior MI at presentation, heart rate, systolic blood pressure, and time to treatment.
Right panel presents point estimates **(middle line)** together with the 95% confidence band.

those older than 80 years. Interestingly, PCI-related delay tended to decrease with age. We observed a statistically significant relation between admission systolic blood pressure, heart rate and age, but the difference in median values of these hemodynamic parameters between younger and elderly patients seemed clinically irrelevant.

Clinical endpoints

Overall, 446 (6.6%) patients died within 30 days of randomization. Mortality was significantly lower in patients randomized to PPCI (n=178; 5.3%) than in patients randomized to FL (n=268; 7.9%). Irrespective of treatment, elderly patients had higher mortality risk than younger patients (table 3). Primary PCI was associated with a lower incidence of death than FL therapy in all age-categories, except in patients ≤50 years (table 3, figure 1). The Breslow-Day test for homogeneity of ORs across age-categories was non-significant (p=0.24), and interaction between age and treatment was not statistically significant (p=0.73). Hence, there was no evidence that the observed mortality reduction by PPCI was heterogeneous with respect to age. After multivariable adjustment, PPCI was associated with a 36% proportional reduction in the odds of death compared to FL therapy, irrespective of age (adjusted OR 0.65 and 95% CI 0.52-0.79).

Repeat myocardial infarction occurred in 225 (6.7%) patients who were randomized to FL and in 80 (2.4%) of those randomized to PPCI. A lower incidence of reMI in patients randomized to PPCI was consistently seen in all age-categories (table 3, figure 1), and there was no evidence of heterogeneity in ORs (p=0.67). PPCI was associated with a 63% proportional reduction in the odds of reMI (adjusted OR 0.37 and 95% CI 0.28-0.48), and a 49% reduction in the composite endpoint of all-cause mortality or reMI (adjusted OR 0.51 and 95% CI 0.43-0.60), irrespective of age.

Similar results were observed with respect to stroke, which occurred in 83 (2.6%) of the patients randomized to FL and in 48 (1.5%) of those randomized to PPCI. Although the incidence of stroke was increased after PPCI in patients younger than 50 years (Figure 1, right panel), the confidence interval of treatment effect was wide, and there was no evidence of heterogeneity in ORs in relation to age (p=0.40). Primary PCI was systematically associated with a lower incidence of the composite endpoint of all-cause mortality, reMI or stroke in all age-categories, and again there was no evidence of heterogeneity in ORs (p=0.35).

Absolute treatment effects

Figure 2 shows 30-day mortality in patients randomized to PPCI and FL (left panel), as well as the absolute mortality reduction by PPCI (right panel) in relation to age as a continuous variable. Apparently, the absolute risk reduction was strongly and positively associated with age. For example, at age 60, PPCI was associated with 1.4 fewer deaths per 100 patients treated compared with FL, whereas the risk reduction amounted to 5.1 per 100 patients at age 80.

Octogenarians

Octogenarians (>80 years old) comprise a clinically relevant cohort and represented 6% of patients. In this cohort, the patients who were randomized to PPCI had a lower incidence of mortality (26.4% versus 18.3% events; p=0.049), reMI (7.0% versus 3.9%; p=0.18) and stroke (7.9% versus 5.8%; p=0.45) than their counterparts who were randomized to FL. Statistical significance was not reached for each of these endpoints, most likely due to small numbers.

DISCUSSION

In this pooled analysis, which was based on randomized trials that involved 6,763 MI patients, age did not modify the mortality reduction by PPCI compared with FL therapy. Since age is a key determinant of mortality in MI patients, the consequence of the consistent relative risk reduction across all age groups is an increasing absolute risk reduction with increasing age, and a decreasing number of patients

that need to undergo PPCI (in stead of FL therapy) to prevent one death - the 'number needed to treat' (NNT). Noteworthy, PPCI was associated with significantly reduced mortality in patients aged 80 years or over. The estimated NNT in these patients was only 20. In view of our results, age per se should not be considered an exclusion criterion for the application of PPCI.

One of the most severe complications of FL therapy is the risk of stroke, particularly intracranial hemorrhage (ICH). This complication occurs in approximately 1% of patients receiving FL therapy, with a case-fatality of around 50%, whereas half of the ICH victims who survive will remain irreversibly disabled.[14] Since age is one of the strongest determinants of ICH risk after pharmacology-based reperfusion therapy,[15] physicians tend to be quite conservative in treating the elderly. Indeed, in our data, among octogenarians who received FL, stroke risk was as high as 8%. We demonstrated that PPCI was associated with a reduced incidence of stroke. Importantly, the risk reduction was particularly strong in elderly patients (although there was no evidence of heterogeneity in relative stroke reduction in relation to age). This observation provides another argument to not systematically exclude elderly patients from treatment with PPCI.

We did not observe significant heterogeneity in treatment effect with respect to the specified outcome events. Indeed, the confidence intervals around the point estimates of treatment effect in the five age-categories were largely overlapping. Still, any heterogeneity cannot be excluded with complete certainty. Despite the considerable amount of included patients, the applied statistical tests of heterogeneity lacked power to reveal small, though clinically relevant differences. The true existence of such differences is unlikely as far as death is concerned, since point estimates were quite consistent across age-groups. However, we acknowledge that age might be a modifier of the influence of PPCI on the incidence of reMI (risk reduction slightly decreases with age) and stroke (risk reduction slightly increases with age). In fact, additional randomized trials are needed to obtain more precise estimates of treatment effects in strata according to age, but, in view of the unambiguous results of the trials that have been conducted, it is unlikely that such new trials will ever be conducted.

We realize that elderly patients who are eligible to participate in a randomized trial might not be representative for real-world patients. As the elderly don't fit the in-and exclusion criteria as the younger patients, they are more likely to be excluded from participation in trials. Indeed, not only suffer elderly often from comorbidities that formally exclude participation in most trials, such as dementia, gastrointestinal bleeds, or malignancy, but also are clinicians often reluctant to include the fragile elderly patient who otherwise doesn't met exclusion criteria. Thus, the elderly who are included in trials form a selected group, more so than younger patients. Consequently, the observed favorable effects in the elderly might probably not fully be extrapolated to the general population. In the context of our pooled analysis we cannot overcome this issue.

The main strength of this study is that it is based on randomized trials. Thus, although one might challenge the external validity, the observed results are at least internally valid (i.e. the results are valid for the type of patients that have entered the included trials). In this respect, the main limitation of our analyses is the fact that the CAPTIM trial data were not included. CAPTIM strongly suggested that the strategy of (prehospital) fibrinolysis, followed by a 'rescue' PCI in case of persistent ischemia, might result in a mortality reduction compared with the PPCI strategy in patients presenting within 2h after onset of symptoms. Although these results were not confirmed in the PCAT-2 main analysis (in fact, a statistically significant mortality reduction was observed in favor of PPCI in patients presenting within 2h),[10] the inclusion of the CAPTIM data would have lead to more precise estimates of treatment effect in patients presenting early. This particularly affected younger patients, since they tend to present earlier than the elderly.

Conclusion

In this pooled analysis of randomized clinical trials, there was no evidence that the reduction in clinical endpoints by PPCI was influenced by age. Hence, age per se should not be considered an exclusion criterion for the application of PPCI.

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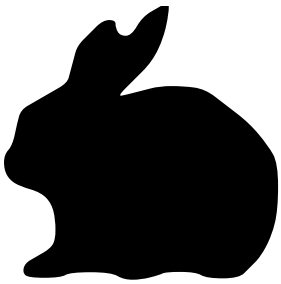
EVALUATING THE 'ALL-COMERS' DESIGN: A COMPARISON OF PARTICIPANTS IN TWO 'ALL-COMERS' PCI TRIALS WITH NON-PARTICIPANTS



EVALUATING THE 'ALL-COMERS' DESIGN: A COMPARISON OF PARTICIPANTS IN TWO 'ALL-COMERS' PCI TRIALS WITH NON-PARTICIPANTS

ABSTRACT

Aims
We aimed to asses the generalizability of two 'all-comers' randomised clinical trials (AC-RCTs) in patients undergoing percutaneous coronary intervention (PCI).

Methods and Results
Recently two large AC-RCT's comparing drug-eluting stents were performed in our institution (LEADERS and RESOLUTE-III). During the inclusion period of these trials 1242 consecutive PCI patients were treated of whom 579 (48%) were actually included. The most important reasons for non-participation were: inability to provide informed consent (33,5%), refused to participate (19%) or patient met one of the other exclusion criteria (26.9%). Trial participants more frequently had stable angina (42.5% versus 34.4%) and less frequently acute myocardial infarction as indication for PCI (31.4% versus 42.4%) than non-participants. Hypertension (52.8% versus 49.1%) and hypercholesterolemia (56.3% versus 49.1%) were seen more frequently in trial participants; heart failure was less common (2.1% versus 4.4%). A significant difference in 30-day mortality was observed between AC-RCT participants and non-participants (0.7% versus 4.5% events; adjusted hazard ratio [aHR] 0.18 and 95% confidence interval [CI] 0.06-0.52). One-year mortality was also lower (3.1% vs. 6.9% events; aHR 0.51 and 95% CI 0.29-0.91, but 1-year mortality in 48 hours survivors was similar (3.1% vs. 4.2% events; aHR 0.74 and 95 CI% 0.41-1.34).

Conclusion
Applying the all-comers design did not result in inclusion of all consecutive patients, as only half of the target population was enrolled. It should be noted, however, that this design included more patients than observed in classical RCTs. AC-RCT participants and non-participants were different in terms of baseline characteristics and outcome.

INTRODUCTION

Randomized controlled trials (RCTs) are the gold standard to evaluate the efficacy and safety of therapeutic options.[1] Randomization results in valid estimates of treatment effect, as it minimizes bias due to differential selection and confounding. [w] Still, external validity of RCT results is often questioned, since clinical trial conditions are highly controlled (strict in- and exclusion criteria are usually applied, which tend to favour the enrolment of patients at low risk of potential side effects) and may not reflect what is sometimes called 'the real world' clinical practice. As a result, sizeable and clinically relevant groups of patients are underrepresented in most RCTs. Elderly patients, women, and patients with cardiac or non-cardiac co morbid conditions are often underrepresented in RCTs that are conducted in the field of cardiology, and thus the generalizability of their results to the broader population can be questioned.[3, 4]

In order to improve the generalizability of RCT results, the 'real world' or 'all-comers' (AC) design is becoming increasingly popular in cardiology. Randomised clinical trials with an 'all-comers' design (AC-RCTs) apply wide inclusion criteria and no (or very few) exclusion criteria, which may result in a more representative sample of the target population. It should be noted, however, that even in an all-comer trial it is not to be expected that each and every consecutive patient will be enrolled. For example, the key principle that patients participate in an RCT on a voluntarily basis only, might still result in non-random patient selection.

Recently, our department participated in two major AC-RCTs (LEADERS and RESOLUTE-III) in patients undergoing percutaneous coronary intervention (PCI). In this paper we studied if and to what extend we succeeded in enrolling a truly representative sample of the PCI patients that form the routine practice in our department, by evaluating baseline characteristics and outcome.

METHODS

Patients and material

The LEADERS trial was designed to compare the clinical safety and efficacy of the BioMatrix Flex (Biolimus A9-Eluting) stent system with the Cypher SELECT (Sirolimus-Eluting) stent system. [5] The RESOLUTE-III trial was designed to compare the clinical results of the Medtronic Endeavor-Resolute (Zotarolimus-Eluting) stent system with the Abbott XIENCE V (Everolimus-Eluting) stent system. [6] Both trials had a prospective, multi-centre, randomized, controlled, non-inferiority design. The broad inclusion criterion in both trials was: patients undergoing routine PCI for any indication. Almost no exclusion criteria were applied, except that patients were not accepted if they already participated in another clinical trial, if they were pregnant, had a known intolerance for aspirin, clopidogrel, ticlopidin, heparin or components of the stents, if surgery was planned within 6 months, had a too large diameter of the study vessel (LEADERS > 3.5 mm, RESOLUTE-III > 4.0 mm) or if no informed consent was provided. Between Nov 27, 2006 and May 18, 2007 LEADERS enrolled a total of 1707 patients in 10 hospitals throughout Europe, and during 22 May, 2008 to 28 October, 2008 RESOLUTE-III enrolled 2292 patients in 19 European hospitals.

According to the standard data-management procedures in our department, data are collected on demographics, cardiovascular history, clinical risk factors and treatment characteristics for all patients undergoing PCI, which are stored in an electronic database. Data-elements are filled out immediately after the completion of the PCI by the interventional cardiologist and the technician who assisted during the procedure. The database, which is maintained by a dedicated IT-officer, is mainly designed for administrative purposes. A systematic evaluation of data-completion and data-integrity is implemented for data that are used for research purposes.

According to this database, during the inclusion period of both AC-RCTs, 1242 consecutive patients underwent PCI in our centre. 579 (47%) participated in the LEADERS or RESOLUTE-III AC-RCT,

whereas, 663 (53%) did not. In the latter patients, the reasons for non-participation were collected by the research staff prior to the intervention.

Follow-up and endpoint definition

We aimed to learn differences in clinical characteristics between patients who participated in an AC-RCT and those who did not, as well as differences in their clinical course during extended follow-up. In that respect, it seems logical to study (differences in) the occurrence of major adverse cardiac events (MACE: re-occurrence of MI (reMI), target lesion revascularisation (TLR), and all-cause mortality) between both patient groups. It should be realised, however, that the drug-eluting stents that were studied in the LEADERS and the RESOLUTE-3 trial, were designed to reduce the incidence of MACE. Consequently, differences (if these appear) in MACE incidence between AC-RCT participants and non-participants are not only to be explained by differences in their baseline characteristics, but, most likely, by the applied (study) stent as well. Therefore, we decided to study all-cause mortality as the single clinical endpoint, as mortality is not influenced by the stent type. Data on vital status was obtained from the municipal civil registries. We report mortality at 30-day and at 1-year follow-up.

Data-analysis

We intended to obtain complete baseline information in all patients, but failed to do so in 17 AC-RCT non-participants, which presented with cardiogenic shock and died within 24h after hospital admission. For these patients with at least one of the variables of interest missing, we decided to impute the missing values. The imputation technique used for continuous as well as dichotomous variables was mean substitution. Dichotomous variables were coded as 1 (determinant present) or 0 (determinant absent). Thus, after data imputation, the value of the variable can be interpreted as the probability of the determinant being present. Continuous data are presented as mean ± standard deviation (SD) and categorical variables are expressed as numbers and percentages. Differences in baseline characteristics between participants and non-participants in an 'all-comer' trial were analyzed by student's t-tests and chi-square tests, as appropriate.

Univariable and multivariable logistic regression (LR) analyses were applied to reveal clinical characteristics that were related to participation in an AC-RCT. AC-RCT participation was considered the dependent variable, and the variables that are listed in table 1 were considered as potential determinants. The final LR model was constructed via backward deletion and all variables with a p-value <0.15 were maintained. Thus, we accepted a type I error of 15% for all variables that composed the final model, in order not to miss clinically relevant explanatory factors. The performance of the final model was studied with respect to discrimination (C-index) and calibration (Hosmer-Lemeshow goodness of fit test for predicted versus observed probabilities).

We applied multivariable logistic regression analysis to develop a propensity score for the likelihood of participating in an AC-RCT. The variables listed in table 1 were included in the model.

The method of Kaplan Meier was used to describe the incidence of all-cause death during follow-up. A logrank test was applied to study differences in survival between AC-RCT participants and non-participants.

Subsequently, Cox proportional hazard (PH) models were used to analyze the relation between AC-RCT participation and mortality at 30 days and 1 year. We adjusted for a range of potential confounders. Since the number of outcome events was limited, we applied a model reduction strategy, which was similar as described above. We forced age and sex in the model, since these are common predictors for mortality. We repeated the analysis and added the propensity score to the model. Subsequently, we repeated the analysis for the patients who survived the first 48h, thereby excluding patient who were unable to give informed consent (IC) due to their critical illness. Finally, Cox PH models were applied to study determinants of mortality in trial non-participants only, combining clinical characteristics and patient-specific reasons for non participation.

We report crude and adjusted odds ratios (ORs) and hazard ratios (HRs) along with their 95% con-

	Non-participant n=663 *	Study participant n=579	P-value
Mean age, yr (±SD)	64.9 ± 12.2	63.7 ± 10.9	0.02
Male sex, n (%)	470 (70.9)	430(70.4)	0.18
Indication			<0.001
Stable Angina, n (%)	228 (34.4)	246 (42.5)	
Unstable Angina, n (%)	154 (23.2)	151(26.1)	
Acute MI, n (%)	281 (42.4)	182 (31.4)	
Cardiogenic shock, n (% of AMI)	17 (6.0)	0 (0)	
Cardiovascular history			
Coronary revascularization, n (%)	176 (26.5)	172 (29.7)	0.22
MI, n (%)	187 (28.2)	171 (29.5)	0.62
Heart failure, n (%)	29 (4.4)	12 (2.1)	0.024
Peripheral artery disease, n (%)	45 (6.8)	36 (6.2)	0.52
Clinical risk factors			
Hypertension, n (%)	325 (49.1)	306 (52.8)	0.022
Hypercholesterolemia, n (%)	327 (49.3)	326 (56.3)	0.001
Diabetes Mellitus, n (%)	125 (18.9)	104 (18.0)	0.68
Renal dysfunction, n (%)	71 (10.7)	54 (9.3)	0.42
Body mass index, kg/m^2 (±SD)	27.2 ± 4.0	27.1 ± 4.1	0.12
Smoking status, n			0.80
-Current, n (%)	192 (29.6)	163 (28.2)	
-quit <=1 year, n (%)	29 (4.5)	25 (4.3)	
-quit > 1 year, n (%)	97 (14.9)	98 (16.9)	
-Never, n (%)	331 (51.0)	293 (50.6)	
Family History, n (%)	232 (35.0)	261 (45.1)	<0.001

* In the non-participants group there are missing values for a few variables, these missing values were imputed for the multivariable analyses.

Table 1 Baseline characteristics

fidence intervals (CIs). For all tests, a two-sided P-value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL).

For the main analysis AC-RCT participants were compared with all non-participants (i.e. the patients who did not participate in any of the AC-RCTs) with respect to baseline characteristics and outcome. As the non-participants form a heterogeneous group, we performed sensitivity analyses including different subgroups of non-participants, to study the robustness of our main findings. We compared the AC-RCT participants with the non-participants, excluding patients who participated in another study.

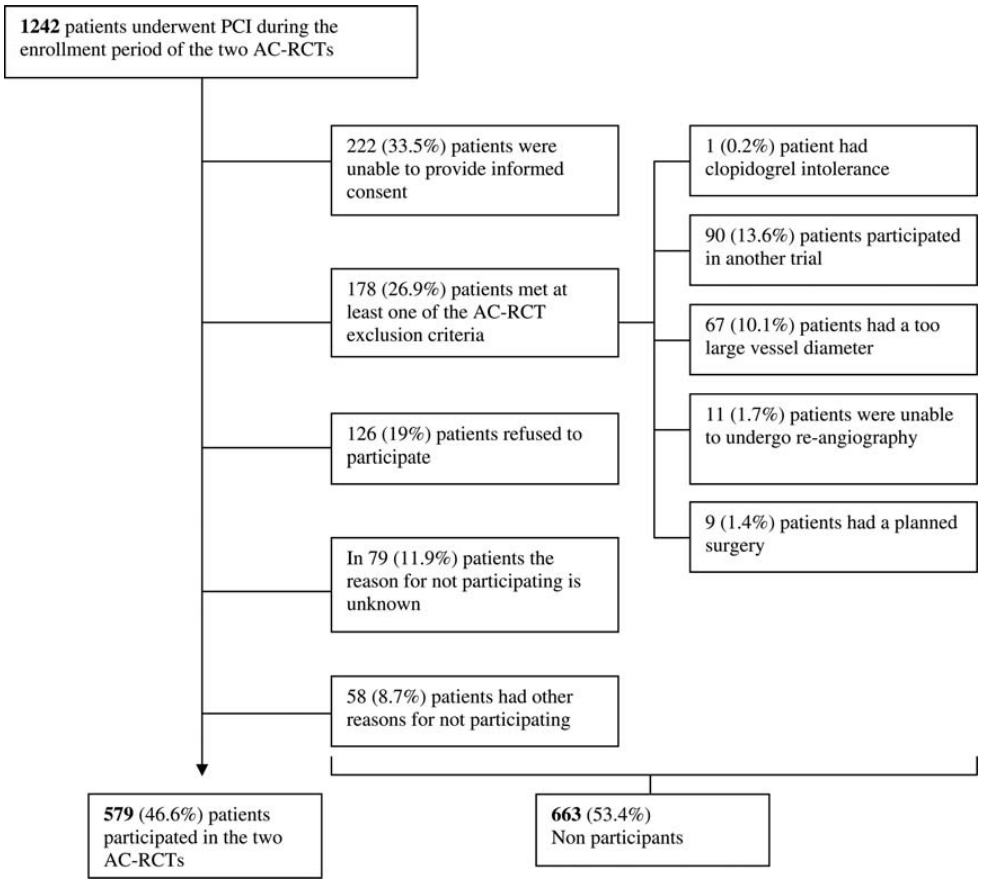


Figure 1 Selection of AC-RCT participants

Furthermore, we compared the AC-RCT participants with the non-participants, excluding the patients who met at least one of the (AC-RCT) exclusion criteria. Since the results of these analyses were highly consistent with the main analysis, we decided to only report the latter.

RESULTS

Baseline characteristics

A total of 579 patients participated in either of the two AC-RCTs during the study period, whereas 663 patients were not enrolled in one of the two AC-RCTs, figure 1.

The most frequent reasons for non participation in an all-comers trial included; inability to provide IC (33, 5%), patient refused to participate (19%) or patient met one of the other exclusion criteria (26, 9%). Of all patients who could not provide IC, 82.9% were admitted with an AMI. The most important reasons to refuse were; their home situation (e.g. a critically ill spouse), severe co-morbidity (cancer, or other severe illness), or study requirements (follow-up visits, repeat angiography, etc.) The reason for non participation was not recorded in 11, 9%.

Baseline characteristics differed considerably between study participants and non-participants with respect to age, the indication for PCI, heart failure, hypertension, hypercholesterolemia, and family history (table 1). Participants in an AC-RCT were younger and had more often stable angina pectoris or unstable angina pectoris as indication for PCI, but suffered less often acute myocardial infarction than the non-participants. Hypertension, hypercholesterolemia and positive family history were seen more often in study participants, whereas heart failure was observed more often in non-participants. None of the study participants were admitted with cardiogenic shock, whereas 17 patients in the non

	Non-participant n=663	Study participant n=579	P-value
Offhours, n (%)	214 (32.3)	133 (23.0)	<0.001
Intervention			<0.001
Balloon only, n (%)	56 (8.4)	21 (3.6)	
Direct stenting, n (%)	276 (41.6)	272 (47.0)	
Stenting after balloon, n (%)	314 (47.4)	282 (48.7)	
Failed procedure, n (%)	17 (2.6)	4 (0.7)	
Treated vessel			0.06
RCA, n (%)	214 (32.3)	178 (30.7)	
LAD, n (%)	279 (42.1)	235 (40.6)	
LCx, n (%)	121 (18.3)	138 (23.8)	
LM, n (%)	23 (3.5)	10 (1.7)	
LIMA, n (%)	0 (0)	1 (0.2)	
Graft, n (%)	26 (3.9)	17 (2.9)	
CTO, n (%)	50 (7.5)	35 (6.0)	0.30
Bifurcation lesion, n (%)	33 (5.0)	54 (9.3)	0.003
Clinical success, n (%)	551 (83.1)	523 (90.3)	<0.001
Complications			
Dissection, n (%)	14 (2.2)	14 (2.5)	0.78
Side branch occlusion, n (%)	2 (0.3)	3 (0.5)	0.57
Perforation, n (%)	4 (0.6)	1 (0.2)	0.22

Table 2 Procedural Characteristics

Characteristic	Crude OR, 95 CI	Adjusted for confounders OR, 95 CI
Age (per year)	0.99 (0.98-1.00)	0.99 (0.98-1.00)
Sex (male)	1.19 (0.92-1.52)	1.17 (0.90-1.52)
Indication for PCI		
Acute MI	0.60 (0.46-0.78)	0.71 (0.51-0.99)
Unstable Angina Pectoris	0.91 (0.68-1.21)	0.94 (0.70-1.26)
Stable Angina Pectoris	1	1
Hypertension (yes)	1.20 (0.96-1.50)	1.20 (0.95-1.52)
Positive Family History for CHD (yes)	1.50 (1.19-1.88)	1.37 (1.08-1.74)
Heart failure (yes)	0.46 (0.23-0.92)	0.42 (0.21-0.84)
Treatment during Off-hours (yes)	0.63 (0.49-0.81)	0.77 (0.55-1.07)

Table 3 Multivariable analysis: predictors for participation in an all-comers trial

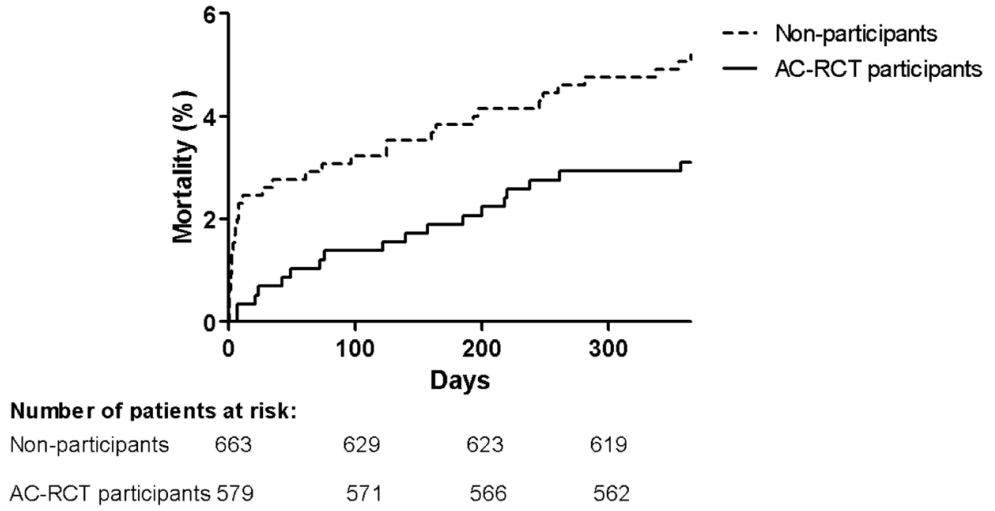


Figure 2 Cumulative incidence for 1-year mortality

participants groups were admitted with cardiogenic shock. As far as procedural characteristics are concerned, trial participants were significantly less often treated during off-hours and were treated more often with balloon angioplasty only, and had significantly more bifurcation lesions and clinical success than non-participants (table 2).

Determinants of AC trial participation

We identified 7 clinical characteristics that were (p-value <0.15) predictors of trial participation; age (adjusted [a]OR 0.99 and 95%CI 0.98-1.00), sex ([a] OR 1.17 (0.90-1.52), indication for PCI (acute MI ([a]OR 0.71 and 95%CI 0.51-0.99) reference is stable angina pectoris), hypertension ([a]OR 1.20 and 95%CI 0,95-1.52) , positive family history for CHD ([a]OR 1.37 and 95%CI 1.08-1.74), heart failure ([a] OR 0.42 and 95%CI 0.21-0.84), and treatment during off-hours (weekends and between 18.00-08.00 during weekdays) ([a]OR 0,77 and 95%CI 0.55-1.07) (table 3). The C-index for the LR model that contained these predictors was 0.60, and the Hosmer-Lemeshow test was non-significant (p=0.94), reflecting poor discrimination and adequate calibration.

Characteristic	Adjusted for confounders HR, 95 CI	Adjusted for confounders and propensity score* HR, 95 CI
Age (per year)	1.05 (1.01-1.08)	1.02 (0.94-1.09)
Sex (male)	2.22 (0.95-5.18)	2.14 (0.92-5.00)
Study participation	0.18 (0.06-0.52)	0.18 (0.06-0.51)
Indication for PCI		
Acute MI	17.12 (3.85-76.07)	18.01 (4.04-80.34)
Unstable Angina Pectoris	5.56 (1.17-26.54)	5.57 (1.17-26.59)
Stable Angina Pectoris	1	1
Heart failure	2.74 (0.76-9.89)	2.51 (0.69-9.13)
Treatment during Off-hours (yes)	0.53 (0.24-1.14)	0.52 (0.24-1.13)

* The propensity score model included all confounders listed in table 1

Table 4 predictors for 30-day mortality

Characteristic	Adjusted for confounders HR, 95 CI
Age (per year)	1.05 (1.02-1.09)
Sex (male)	2.13 (0.86-5.26
Indication for PCI	
Acute MI	8.46 (1.69-42,38)
Unstable Angina Pectoris	4.85 (0.98-24.11)
Stable Angina Pectoris	1
Unable to provide IC	2.89 (1.14-7.33)
Treatment during Off-hours (yes)	0.39 (0.16-0.95)

Table 5 Multivariable model fitted to the non-participants (n=663) indentified four predictors for 30-day mortality

Mortality

As shown in figure 2, study participation was associated with a lower mortality rate than non-participation. Log rank test gave a significant overall difference (p=0.002) in mortality among the two categories (study participants vs. non-participants). In patients surviving the first 48 hours, this difference was non-significant (p=0.31).

After 30-days a significant difference in 30-day mortality was observed between AC-RCT participants and non-participants (n=4 (0.7%) versus n=30 (4.5% (KM estimates)); [a] HR 0.18 and 95% CI 0.06-0.52)). 30-day mortality in AMI patients was 1.6% vs. 7.5% respectively (p=0.006). One-year mortality was also lower in trial participants (n=18 (3.1%) versus n=46 (6, 9% (KM estimates)); p=0.001; aHR 0.51 and 95% CI 0.29-0.91)). In those who survived the first 48 hours (n=1224), no significant differences in 30-day (0.7% vs. 1, 7% (KM estimates), aHR 0.40 and 95 CI% 0.13-1.34) and 1-year mortality were found and (3.1% vs. 4.2% (KM estimates), aHR 0.74 and 95 CI% 0.41-1.34 respectively). None of the study participants died within 48 hours, whereas 18 patients in the non participants group died. Applying the model reduction strategy we indentified predictors of 30-day mortality (table 4): age, sex, study participation, indication for PCI; AMI and unstable angina pectoris, heart failure, and treatment during off hours. After we adjusted for the propensity of AC-RCT participation (by adding the propensity score to the model), the relation between AC-RCT participation and 30-day mortality remained unchanged.

The additionally multivariable model fitted only to the non-participants indentified four predictors for 30-day mortality: age, sex, indication for PCI, unable to give informed consent, and treatment during off hours (table 5).The C-index for this model was 0.80 and the Hosmer-Lemeshow test was non-significant (p=0.56), reflecting both adequate discrimination and calibration. Of the 30 patients who died within 30 days in the non participants group, 12 patients (40%) were admitted with cardiogenic shock.

DISCUSSION

The all-comers design in RCTs is becoming increasingly popular as it is supposed to reflect the real world in a better way than the standard RCTs with strict inclusion and exclusion criteria, resulting in limited generalizability. Although it is not to be expected that all consecutive patients will be included in an AC-RCT, it is important to note that we were only able to enrol 47% of the target population. This raises the question if we enrolled a more representative population than in a classical RCT. Importantly,

differences in baseline characteristics and mortality were observed between participants and non-participants. It should be noted, however, that the observed differences in 30-day and 1-year mortality between participants and non-participants in the two 'all-comers' trials became smaller in those who survived the first 48 hours. These results reveal that the all-comers design does not fully represent daily 'real world' clinical practice. Furthermore, our data indicate that an all-comers design is suitable for studying long term treatment effects, but probably less so for studying acute treatment effects in the broad spectrum of patients undergoing PCI.

The inability of an all-comers design to represent the vast majority of real-life patients is most likely related to the increased likelihood of excluding higher risk patients from these trials, although study criteria would have formally allowed their inclusion without restriction. This phenomenon particularly affects the estimates of early prognosis, whereas the reasons behind are multifactorial. Physicians tend to avoid including patients with a perceived high risk of adverse effects; high-risk patients themselves are less likely to volunteer to participate in a clinical study; and finally regulatory authorities may restrict the inclusion of higher risk subjects (note that the medical ethical committee of our centre prohibited proxy consent).

Although we indentified a number of clinical characteristics for trial participation it is important to note that the low C-index indicated that there are still unidentified predictors for trial participation. Nevertheless it is important to focus on patients who were unable to provide IC. More than 4 out of 5 patients who were unable to provide IC suffered from acute myocardial infarction. This large proportion is most likely due to their poor physical condition, which prevented the interventional cardiologist from asking IC. Not including the most critically ill patients makes the all-comers design less suitable for studying acute treatment effects in PCI patients. As second best option in these critically ill patients IC might be provided by their relatives. Our Medical Ethical Committee, however, did not approve proxy consent.

In addition to the above mentioned study related issues for non-participation, also patient related issues may cause selection bias. [7, 8] Patients with acute myocardial infarction, and acute heart failure participated less often in a study, probably due to their physical condition. In contrast, patients with hypertension, hypercholesterolemia, and positive family history for CHD participated more often, which might be due to the fact that these patients with known risk factors are more aware of their physical condition. Their motivation might be the increased contact (phone/ out patient clinic) with the treating cardiologist which may provide a feeling of greater security, hope for a better intervention, the contribution to science, helping others. [9, 10]

We observed a higher mortality in the AC-RCT non-participants as compared to the participants. Interestingly, this difference appeared in the first 48 hours and did not exceed in the period thereafter. In fact, similar survival was observed in AC-RCT participants and non-participants after they have survived the first 48 hours. Still, we appreciate that a difference in survival might have been missed, due to the relatively low statistical power (note the relatively low number of deaths after 48 hours).

In patients who did not participate in a study an important predictor for mortality was the inability to provide IC of whom the majority was admitted with AMI. Given the high C-index, predictors as age, sex, acute MI, and treatment during off-hours adequately predicted mortality in these patients. All these predictors reflected the most critically ill patients. As discussed above proxy consent could contribute to resolve this issue.

In the context of this paper it is relevant to consider that the nomenclature "all-comers" refers specifically to the trial design, and does not refer (at least not in first instance) to the observed result. In this design inclusion criteria are kept comprehensive, and there are limited exclusion criteria, so that "real world" clinical practice is thought to be reflected. Apparently, as our analysis demonstrates, even in an AC-RCT not all eligible patients will be included, as patients themselves still need to provide informed consent, irrespectively of clinical inclusion and exclusion criteria. Although based on our results the question arises whether the nomenclature "all-comers" is a misnomer, it should be noted that this design included more patients than observed in classical RCTs.[4, 11] Moreover, in a selection of classi-

cal RCTs it was observed that 84-98% of the eligible patients were, due to a variety of reasons, not enrolled.[4]

Limitations

It is important to note that the presented results are a single centre experience only. To further investigate whether multicenter all-comers trials are representative of real-world populations, data on screened patients in all participating sites are needed. Therefore, we recommend that future all-comers studies will maintain a systematic screening log.

Furthermore due to the small sample size and limited number of events the results may have limited external validity. In addition proxy consent was not allowed by our medical ethical committee, resulting in the underrepresentation of the critically ill patients in the AC-RCT.

Conclusion

Applying the all-comers design did not result in inclusion of all consecutive patients, as only half of the target population was enrolled. It should be noted, however, that this design included more patients than observed in classical RCTs. AC-RCT participants and non-participants were different in terms of baseline characteristics and outcome.

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TRIAL-PARTICIPANTS VERSUS EVERY DAY CLINICAL CARE PATIENTS: TRIAL PARTICIPATION AS A DETERMINANT OF ADVERSE OUTCOME IN THE FIELD OF INTERVENTIONAL CARDIOLOGY.

TRIAL-PARTICIPANTS VERSUS EVERY DAY CLINICAL CARE PATIENTS: TRIAL PARTICIPATION AS A DETERMINANT OF ADVERSE OUTCOME IN THE FIELD OF INTERVENTIONAL CARDIOLOGY.

ABSTRACT

Background

This study examines differences in clinical outcome between trial-participants and non-participants after percutaneous coronary intervention (PCI).

Methods and results

This study compromised of 11,931 consecutive patients who underwent PCI in a high volume center, during the period 2000 – 2009. Of these patients, 1787 (15%) participated in an interventional clinical trial with a follow-up period of at least six months. The maximum follow-up duration was 11.8 years, with a median of 3.8 years (IQR: 2.6 – 6.5). Baseline and procedural characteristics differed between trial-participants and non-participants. Trial-participants were more often male, were younger, had more cardiovascular risk factors and were treated more often for stable angina pectoris and single vessel disease. Overall mortality at maximum follow-up was lower for trial-participants compared to non-participants (8.1% versus 17.6%, $p < 0.001$, adjusted HR, 0.62, 95% CI: 0.52–0.74). There was no difference in the incidence of non-fatal MI and CABG. Repeat PCI was seen more often in trial-participants (18.1% versus 30.7%, $p < 0.001$, adjusted HR 1.91, 95%CI 1.73–2.10). Consequently, a higher incidence of the composite of mortality, repeat revascularization, and non-fatal MI was seen in the trail-participants (adjusted HR.1.36 95% CI 1.25 – 1.47), but this association was primarily driven by the occurrence of repeat PCI.

Conclusion

Participants in clinical trials in the field of interventional cardiology with a follow-up of at least six months differed considerably from non-participants in baseline and procedural characteristics. Trial-participants had better survival than non-participants. In contrast, a two-fold higher incidence of repeat PCI was observed in trial-participants.

INTRODUCTION

Randomized controlled trials (RCTs) are the gold standard by which the efficacy and safety of therapeutic strategies are evaluated [1]. Randomization results in valid estimates of treatment effect, as it minimizes bias due to differential selection and confounding [2]. However, patients who participate in these clinical trials are, often due to the strict in- and exclusion criteria, considered being a selective group of patients, questioning the external validity.
Furthermore, generalizability can be limited due to the fact that not all consecutive patients who fulfill the study criteria actually do participate. Due to a number of reasons, including willingness of patients to participate, "burden" of additional procedures (i.e. follow-up angiography), distrust in clinical research among patients and health care providers, it is observed that fewer than half of eligible patients participate in clinical trials [3, 4]. Moreover, study participation is considered as hazardous by some since the therapeutic strategy under investigation may deviate from the applicable standards and guidelines. On the other hand, it is advocated that trial-participants may have a higher likelihood of beneficial outcome due to the most up-to-date treatment provided by qualified physicians embracing novel treatments[5, 6, 7, 8].
A restriction of previous studies comparing trial-participants with non-participants is the heterogeneity of interventions and patient populations. Consequently, it remains unclear whether the observed differences reflect trial participation or differences due to heterogeneity in interventions [9]. In the current study we focus on patients with coronary artery disease (CAD) undergoing a clinically indicated percutaneous coronary intervention (PCI). The objective of this study was to evaluate the extent to which participation in a clinical trial affects clinical outcome of patients with CAD who underwent a PCI.

METHODS

This study was conducted in the Thoraxcenter of the Erasmus MC from January 2000 to December 2009. The Erasmus MC is a tertiary referral and teaching hospital in Rotterdam, The Netherlands that serves a region of over 1 million inhabitants. Approximately 4000 PCIs are performed annually in the Rotterdam region (in three PCI centres), including 1600 patients in the Erasmus MC.
All consecutive patients of 18 years of age or older, who were admitted with stable angina pectoris (sAP), non ST-segment elevation acute coronary syndrome (NSTE-ACS) or ST-segment elevation myocardial infarction (STEMI) and underwent PCI in our institution were included in the analysis. In total, 15,102 PCIs in 11,931 patients were performed. In patients who underwent multiple procedures (n = 2470), only the initial procedure was included in this analysis.
In the context of this study we identified trial participants as those patients who were enrolled in a clinical trial, irrespective of treatment arm, with a follow-up period of at least six months. In total 1787 participants, enrolled in 76 clinical trials (including 35 randomized controlled trials with 1058 patients), were identified. In 59 out of 76 trials (78%), a repeat angiography was mandated, including 1380 patients. Non-participants (*n* = 10,144) were those who fulfilled enrolment criteria but were not included (e.g. patient or physician refusal), participated in a trial without follow-up (e.g. cross-sectional and feasibility studies) evaluating the technical safety and feasibility of a new imaging catheter, using only procedural information) or those who did not fulfil the enrolment criteria.
Patient management was in accordance with the clinical treatment guidelines of the European Society of Cardiology (ESC), which are implemented in our center. The Thoraxcenter has (since the year 2000) the policy to use one particular coronary stent as default in a given time period. The default stent between January 2000 and April 2002 was a bare metal stent (BMS), between April 2002 and March 2003 a sirolimus eluting stent (SES), between March 2003 and March 2007 a paclitaxel eluting stent (PES), and the everolimus eluting stent (EES) since March 2007. Of note, patients could be treated with another stent when participating in a clinical trial.

According to the standard data-management procedures in our department, data are collected prospectively on demographics, cardiovascular history, clinical risk factors and treatment characteristics for all patients undergoing PCI, which are stored in an electronic database. Data-elements are filled out immediately after the completion of the PCI by the interventional cardiologist and the technician who assisted during the procedure. The database, which is maintained by a dedicated IT-officer, is mainly designed for administrative purposes. A systematic evaluation of data-completion and data-integrity is implemented for all data used for research purposes.
Vital status of the entire study cohort was obtained from the municipal civil registries between April and September 2011. Subsequently, a health questionnaire was sent to all living patients with specific inquiries on rehospitalisation and cardiovascular events, including repeat PCI, coronary artery bypass graft (CABG) and non-fatal myocardial infarction (MI). For patients who reported adverse events, medical records or discharge summaries were reviewed systematically. General practitioners, referring cardiologists, and patients were contacted in case further information was required. Patients lost to follow-up were considered at risk until the date of last contact, at which point they were censored.

Endpoint definitions
The primary endpoint was all-cause mortality, evaluated at 31 days, 1 year and 4 years. The secondary endpoints included repeat revascularization, non-fatal MI, and the composite of all-cause mortality, repeat revascularization and non-fatal MI at follow-up (31 days, 1 year and 4 years). Furthermore we also evaluated all-cause mortality at maximum available duration of follow-up (approximately 10 years for the first included patients).
Repeat revascularization was defined as a repeat PCI or CABG of any lesion located in the epicardial vessels. The definition of MI was in accordance with the guidelines of the European Society of Cardiology [10].

Statistical methods
Continuous variables are presented as mean values and corresponding standard deviations (± SD), or median values with corresponding interquartile ranges (IQR). Categorical variables are expressed as numbers and percentages. Student's t tests, Chi-square tests (or Fisher's exact tests), or Mann–Whitney tests were applied to evaluate differences in baseline variables, treatment and outcome between trial-participants and non-participants.
Kaplan–Meier mortality curves were used to describe the incidence of adverse events during follow-up. Log-rank tests were applied to evaluate differences in long-term outcome between trial-participants and non-participants. Subsequently, we repeated the analysis for patients who survived the first month, thereby excluding patients who were unable to participate (i.e. not able to sign the informed consent form and/or not fulfilling study criteria) in a clinical study due to their critical illness. In addition, univariate and multivariate analysis (Cox proportional hazard) were performed to study the association in clinical variables and mortality, repeat revascularization and myocardial infarction between trial-participants and non-participants. In the multivariate analysis we adjusted for a range of potential confounders, including all variables as presented in Table 1. These variables are: age, gender, smoking, hypercholesterolemia, hypertension, diabetes, family history, renal failure, prior PCI, prior CABG, prior myocardial infarction, indication for PCI, off-hours treatment, severity of coronary artery disease, treated coronary vessel (left main, LAD, LCx, RCA, and/or graft), and use of glycoprotein (GP) IIb/IIIa Inhibitors.
For all tests, a p-value of < 0.05 (two-sided) was considered statistically significant. All calculations were performed using the SPSS 20 software package (SPSS Inc. IL. USA).

Ethics
All patients participating in clinical trials provided written informed consent. All trials were approved by the Medical Ethical Committee of the Erasmus MC and were performed in accordance with the

Variable	All	Non-participants	Trial-participants	*p*-value
N (%)	11,931	10,144 (85)	1787 (15)	
Mean age, yr (± SD)	62.2 (± 11.8)	62.4 (± 12.0)	60.8 (± 10.9)	< .001
Male gender, *n* (%)	8588 (72)	7243 (71)	1345 (75)	.001
Smoking status, *n* (%)				.020
Current	3213 (27)	2688 (27)	525 (30)	
Former smoker (≥ 1 yr)	65 (1)	59 (1)	6 (0)	
Medical history, *n* (%)				
Hypercholesterolemia	9213 (77)	7725 (76)	1488 (83)	< .001
Hypertension	5577 (47)	4756 (47)	821 (46)	.46
Diabetes mellitus	2054 (17)	1763 (17)	291 (16)	.26
Family history of CAD	3882 (33)	3197 (32)	685 (38)	< .001
Risk factors[a] per patient, mean (± SD)	1.7 (± 1.0)	1.7 (± 1.0)	1.8 (± 0.9)	.002
Renal failure	593 (5)	518 (5)	75 (4)	.10
Prior PCI	1411 (12)	1209 (12)	202 (11)	.46
Prior CABG	1015 (9)	895 (12)	120 (7)	.003
Prior myocardial infarction	3125 (26)	2699 (27)	426 (24)	.035
Indication for PCI, *n* (%)				< .001
sAP	4422 (37)	3631 (36)	791 (44)	
NSTE-ACS	3280 (28)	2794 (28)	486 (27)	
STEMI	4229 (35)	3719 (37)	510 (29)	
Off-hours[b], *n* (%)	2746 (23)	2404 (24)	342 (19)	< .001
Severity of CAD, *n* (%)				< .001
Single-vessel disease	5886 (49)	4871 (48)	1015 (57)	
Two-vessel disease	3565 (30)	3069 (30)	496 (28)	
Three-vessel disease	2399 (20)	2127 (21)	272 (15)	
Treated vessel, mean (± SD)	1.4 (± 0.7)	1.4 (± 0.7)	1.4 (± 0.6)	.69
RCA, *n* (%)	4492 (38)	3819 (38)	673 (38)	.99
LAD, *n* (%)	6005 (50)	5112 (50)	893 (50)	.74
LCx, *n* (%)	3389 (28)	2887 (29)	502 (28)	.75
LM, *n* (%)	504 (4)	466 (5)	38 (2)	< .001
Graft, *n* (%)	2336 (20)	1947 (19)	389 (22)	.011
GP IIb/IIIa inhibitor, *n* (%)	1534 (13)	1345 (13)	189 (11)	.001

PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; sAP, stable Angina Pectoris; NSTE-ACS, non ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; CAD, coronary artery disease; RCA, right coronary artery; LAD, left anterior descending; LCx, left circumflex artery; LM, left main stem.
‡) Antithrombotic: aspirin, thienopyridines, and/or coumadin.
a) Risk factors included, smoking, diabetes, hypercholesterolemia and hypertension.
b) Off-hours, weeknights (from 06.00 PM to 08.00 AM) and weekends (from Friday 06:00 PM to Monday 08:00 AM).

Table 1 Baseline and procedural characteristics

declaration of Helsinki. Patients not enrolled into clinical trials were not subject to interventions under investigation, neither was any mode of behaviour imposed, otherwise than as part of their regular treatment. Therefore, according to Dutch law, written informed consent was not required for these patients. This study was conducted according to the Privacy Policy of the Erasmus MC, and according to the Erasmus MC regulations for the appropriate use of data in patient oriented research [11].

RESULTS

Baseline and procedural characteristics

Baseline and procedural characteristics of the total study sample (n = 11,931) are displayed in Table 1. Of these patients, 1787 (15%) participated in a clinical trial with a follow-up period of at least six months. Baseline and procedural characteristics differed considerably between trial-participants and non-participants. Trial-participants were more often men (75% versus 71%, p = 0.001) and on average 1.6 years younger (60.8 vs. 62.4, p < 0.001) as compared to non-participants. In addition, trial-participants more often had a history of hypercholesterolemia (p < 0.001) and had a positive family history of CAD (p < 0.001). While most trial-participants were treated for sAP, the leading indication for PCI in non-participants was STEMI, whereas non-participants were also treated more often during off-hours (i.e. during the weekend, and evening-, and night shifts). At the same time, 57% of the trial-participants as compared to 48% non-participants were treated for a single vessel disease. No differences were observed in the treatment of the coronary vessels, with exception of the left main coronary artery (more often in non-participants) and coronary artery bypass grafts (trial-participants more often). The administration of GP IIb/IIIa inhibitors was seen more often in non-participants.

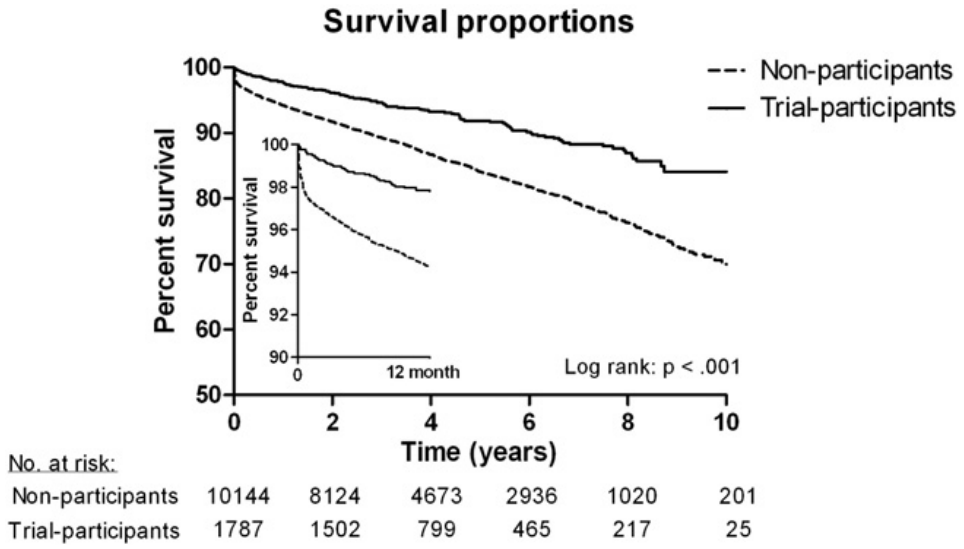


Figure 1 Unadjusted mortality curves for trial-participants and non-participants

Mortality

Information on survival status was complete in 96.4% of all patients (97.3% for trial-participants and 96.3% for non-participants, p = 0.037). The maximum follow-up duration was 11.8 years, with a median of 3.8 years (IQR: 2.6 - 6.5). Based on 1927 fatalities and a total follow-up duration of 52,162 person-years, we observed 36.9 fatal events/1000 person-years in the total cohort. Among trial-participants the number of fatal events/1000 person-years was 18.3 (95% CI 15.5 – 21.4) compared to 40.3 (95% CI 38.4 – 42.2) in non-participants. As also shown in Fig. 1 and Table 2, overall mortality was lower for trial-participants compared to non-participants (8.1% versus 17.6%, (KM-estimate), p

Variable		Non-participants	Trial-participants	Unadjusted HR (95%CI)	Adjusted HR (95%CI)
	All				
n (%)	11,931	10,144 (85)	1787 (15)		
Death	1927 (16.2)	1783 (17.6)	144 (8.1)	0.45 (0.38–0.54)	0.62 (0.52–0.74)
Repeat PCI	2381 (20.0)	1833 (18.1)	548 (30.7)	1.81 (1.65–2.00)	1.91 (1.73–2.10)
(repeat) CABG	337 (2.8)	289 (2.8)	48 (2.7)	0.92 (0.68–1.25)	0.94 (0.69–1.28)
Myocardial infarction	492 (4.1)	413 (4.1)	79 (4.4)	1.06 (0.84–1.35)	1.13 (0.89–1.45)
Any of the above	4283 (36.0)	3603 (35.5)	680 (38.1)	1.17 (1.08–1.27)	1.36 (1.25–1.47)
PCI indication: **sAP**					
n (%)	4422	3631 (82)	791 (18)		
Death	592 (13.4)	528 (14.5)	64 (8.1)	0.51 (0.40–0.67)	0.65 (0.50–0.85)
Repeat PCI	982 (22.2)	730 (20.1)	252 (31.9)	1.68 (1.46–1.94)	1.71 (1.48–1.98)
(repeat) CABG	174 (3.9)	144 (4.0)	30 (3.8)	0.91 (0.61–1.34)	0.97 (0.65–1.44)
Myocardial infarction	156 (3.5)	125 (3.4)	32 (4.0)	1.12 (0.76–1.66)	0.88 (0.57–1.34)
Any of the above	1587 (35.9)	1272 (35.0)	315 (39.8)	1.22 (1.08–1.38)	1.33 (1.17–1.51)
PCI indication: **NSTE-ACS**					
n (%)	3280	2794 (85)	486 (15)		
Death	571 (17.4)	523 (18.7)	48 (9.9)	0.48 (0.35–0.64)	0.65 (0.48–0.88)
Repeat PCI	704 (21.5)	540 (19.3)	164 (33.7)	1.85 (1.55–2.20)	1.90 (1.59–2.27)
(repeat) CABG	85 (2.8)	72 (2.6)	13 (2.7)	0.97 (0.54–1.75)	1.05 (0.57–1.92)
Myocardial infarction	133 (4.1)	111 (4.0)	22 (4.5)	1.07 (0.68–1.69)	0.95 (0.59–1.53)
Any of the above	1241 (37.8)	1039 (37.2)	202 (41.6)	1.18 (1.02–1.37)	1.37 (1.18–1.60)
PCI indication: **STEMI**					
n (%)	4229	3719 (88)	510 (12)		
Death	764 (18.1)	732 (19.7)	32 (6.3)	0.35 (0.25–0.50)	0.46 (0.32–0.66)
Repeat PCI	695 (16.4)	563 (15.1)	132 (25.9)	1.85 (1.53–2.23)	1.90 (1.57–2.31)
(repeat) CABG	78 (1.8)	73 (2.0)	5 (1.0)	0.53 (0.21–1.30)	0.55 (0.22–1.36)
Myocardial infarction	203 (4.8)	178 (4.8)	25 (4.9)	1.10 (0.72–1.68)	1.12 (0.74–1.72)
Any of the above	1455 (34.4)	1292 (34.7)	163 (32.0)	1.05 (0.89–1.24)	1.20 (1.01–1.41)

PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; sAP, stable Angina Pectoris; NSTE-ACS, non ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

Table 2 Outcome

< 0.001, unadjusted Hazard Ratio (uHR) 0.45, 95% CI 0.38 – 0.54). Of note, when the analysis was repeated in those patients who survived the first month, similar results were obtained (uHR 0.56, 95% CI 0.47 – 0.67).

After adjustment for potential confounders, mortality remained lower in trial-participants than in non-participants (adjusted Hazard Ratio (aHR) 0.62, 95% CI: 0.52–0.74) (Table 2). When focusing on the indication for PCI (e.g. sAP, NSTE-ACS, STEMI), similar results were observed. As shown in Fig. 3, trial-participation was associated with better survival at 31 days, 1 year and 4 years of follow-up (5.9% versus 13.6%; aHR 0.58, 95%CI 0.48–0.71 at 4 years).

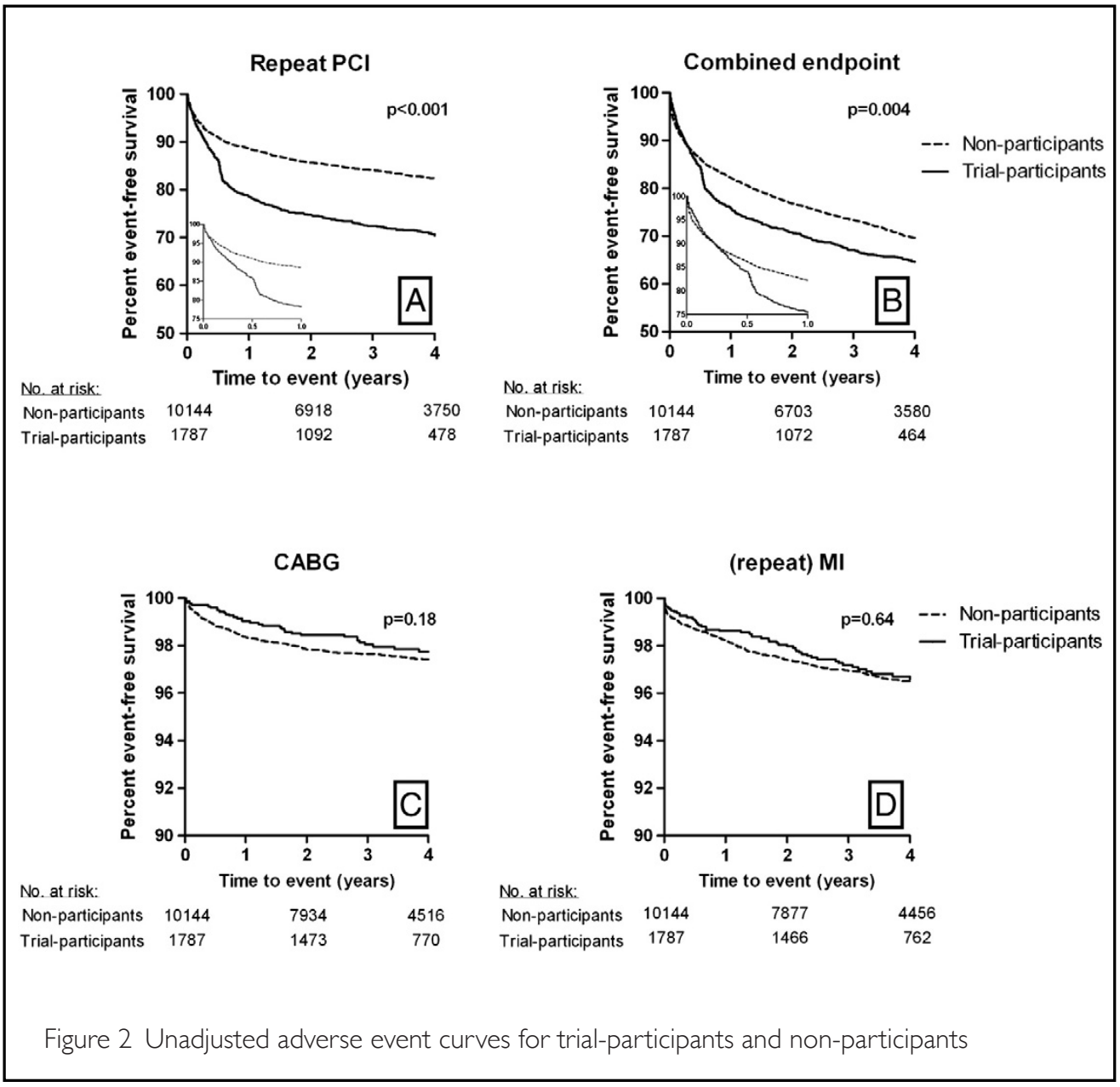


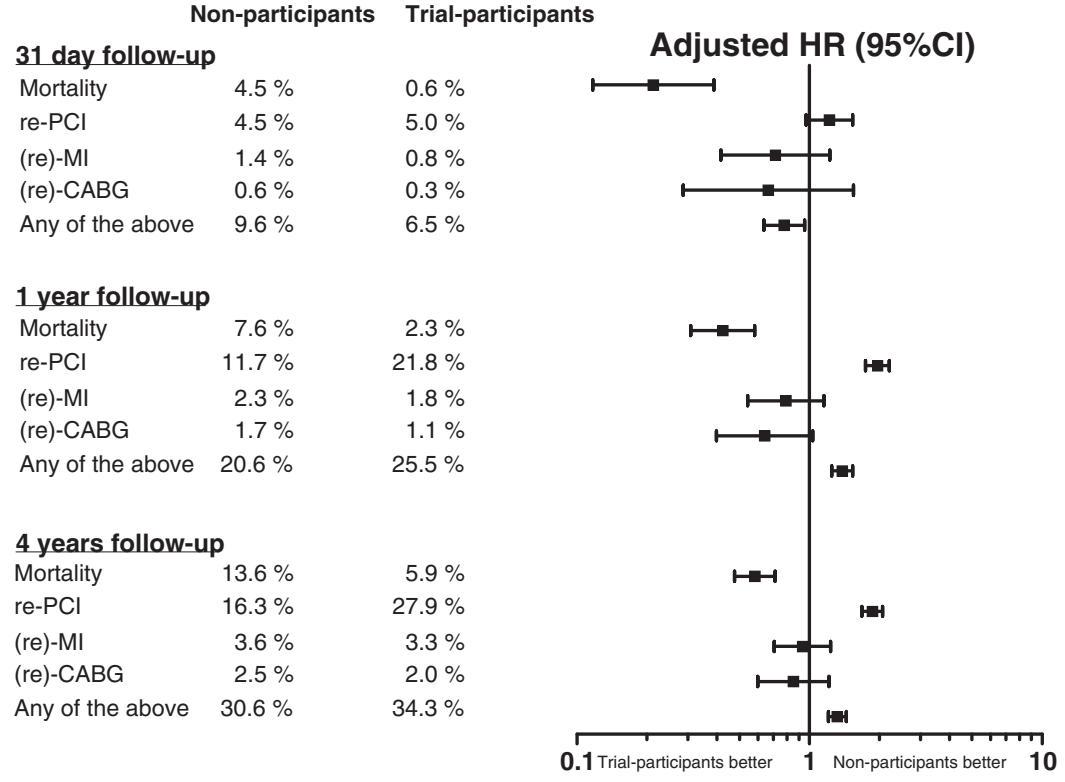
Figure 2 Unadjusted adverse event curves for trial-participants and non-participants

Non-fatal endpoints and the combined endpoint

Repeat PCI was seen more often in trial-participants (18.1% versus 30.7% (KM estimate), p < 0.001); uHR 1.81, 95%CI 1.65–2.00). Similar results were obtained after multivariate adjustment for potential confounders of the relation between trial participation and the incidence of rePCI (aHR 1.91, 95%CI 1.73–2.10), Table 2, and Fig. 2A. Results were comparable at short-term and long-term follow-up (Fig. 3) and in the different indication groups (Table 2). Trial-participants underwent re-PCI in an earlier phase as compared to non-participants (median 114 (IQR: 35 – 195 days) versus 55 days (IQR: 10 – 148 days), $p < .001$ respectively).

We did not find any clinically relevant difference in the incidence of CABG and non-fatal MI between trial participants and non-participants. Results were similar at short-term and long-term follow-up (Fig. 3), and similar in the patients treated for different indications (Table 2). The cumulative incidence curves were superimposed for these endpoints (Fig. 2C and D). Trial participation had no contribution in multivariable Cox PH models that related patient characteristics with CABG and non-fatal MI. Adjusted HRs of trial participation were non-significant and close to 1 for CABG and non-fatal MI at all three investigated follow-up moments (Fig. 3).

The composite endpoint of all-cause mortality, repeat PCI, CABG or MI was observed more often in trial-participants as compared to non-participants (aHR.1.36 95% CI 1.25 – 1.47), Table 2, and Fig. 2B.



Adjusted for age, gender, smoking, hypercholesterolemia, hypertension, diabetes, family history, renal failure, prior PCI, prior CABG, prior myocardial infarction, indication for PCI, off-hours, severity of coronary artery disease, treated coronary vessel (left main, LAD, LCx, RCA, and/or graft), and use of GP IIb/IIIa.
Data for the total follow-up duration are given in table 2.

Figure 3 Adjusted hazard ratios for outcome at different follow-up periods

DISCUSSION

Clinical trials are important to evaluate the efficacy and safety of CAD treatment; however, due to strict in- and exclusion criteria their generalizability is often limited. We studied the effect of trial participation in all consecutive patients undergoing PCI within the last decade at our PCI centre. As expected, we observed important differences in baseline characteristics between trial-participants and non-participants. Mortality was higher in non-participants than in trial-participants, even in those who survived the first month. There was no difference in occurrence of non-fatal MI between both groups. Interestingly, trial-participants had an almost 2-fold higher incidence of repeat PCI. As a result, the composite of mortality, repeat revascularization, and non-fatal MI was (in contrast to mortality) higher in trail-participants. These results show that trial-participation does not fully represent "real world" clinical practice and indicate that the external validity of trial results is limited.

The better survival of trial participants may be related to the increased likelihood (strict inclusion and exclusion criteria) of excluding high risk patients from trials. For example, patients presenting with STEMI (high risk patients) were less likely to be enrolled in clinical trials: 37% of the non-participants had a STEMI versus 29% of the trial participants. Another explanation, as we demonstrated previously in a subset of these patients, is that critically-ill patients are not able to provide informed consent, whereas physicians tend to avoid including a patient with high risk of adverse effects [4]. Secondly, in patients who survived the first month, excluding acute treatment effects, we also observed a higher mortality in the non-participants. This may be due to the fact that trial participants, in the years following the index-procedure, are subjected to strict adherence to the carefully developed protocol and extra visits to

the outpatient clinic. Third, as Within this study we observed a significant difference in subsequent PCI procedures between trial participants and non-participants, whereas CABG and non-fatal MI did not differ between the two groups. Univariate as well as multivariate analysis revealed that almost twice as many trial-participants underwent repeat PCI within 1 year after the initial PCI. Importantly, in the vast majority of trial-participants a follow-up angiography was mandated by the study protocol. This observation is in line with others who reported increased repeat PCI in patients who undergo routine follow-up angiography [14, 15]. In addition, Uchida et al. demonstrated that the majority of those patients were treated for intermediate non-ischemic lesions. Importantly, no survival benefit was observed in patients undergoing angiographic follow-up as compared to clinical follow-up alone (e.g. repeat angiography limited to symptomatic patients only) [16].

Conducting clinical research is an important part of clinical practice in an academic hospital and essential to the further improvement of pharmacological therapies, diagnostics, and interventional devices in the management of patients with CAD. However, trial participation might also be regarded as burdensome for participants and potentially hazardous as the effect of an experimental treatment has not always been proven effective in the population of interest. In contrast, trial participation also includes access to novel and potentially better treatment with an increased focus on standard treatment, comorbid conditions and events as dictated by the protocol and comprehensive case record forms [8]. In addition to this, it is important to note that scientific concerns and ethical dilemmas regarding a clinical trial are judged and approved by an institutional review board [3]. Moreover, the evaluation of the scientific design and assurance of safeguards for the protection of patients, and the patients' autonomy in making a decision to participate are essential parts of the review process.

The present study has several limitations. The presented results are based on a single centre experience, which limits the external validity. Nevertheless, the Thoraxcenter Rotterdam can be considered representative for larger tertiary referring and teaching (academic) hospitals in Western populations. Second, for the follow-up of non-fatal endpoints we were dependent on the patient's response of health questionnaires that were systematically sent to all living patients, with specific inquiries on rehospitalisation and major adverse cardiac events. Thus, we might have missed some non-fatal endpoints, particularly those that did not result in hospital admissions. Possibly, more effort was undertaken in trial participants to collect these data, which may partially explain the higher rate of rePCI in trial participants. Third, it is impossible to study the effects of trial-participation in a randomized controlled trial, which is the gold standard to evaluate treatment efficacy and safety. However, we studied a large population over a long period (10 years) of time in which many trials were performed at our centre. Non-participants represent a heterogeneous group of patients, i.e.: patients who did not meet the study criteria, patients who refused to participate and patients who were unable to provide informed consent. Unfortunately, we did not systematically collect the reasons of non-participation in all studies. Therefore, we were unable to perform a stratified analysis based on the reasons for non-participation.

Conclusion

The current study showed that participants of PCI trials differed considerably in clinical and procedural characteristics from non-participants. Trial-participants had a better survival but a two-fold higher incidence of repeat PCI as compared to non-participants. The higher incidence of the composite of mortality, repeat revascularization, and non-fatal MI in the trial-participants was primarily driven by the occurrence of repeat PCI. These results illustrate that clinical trials do not fully reflect every day clinical practice.

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PART 2

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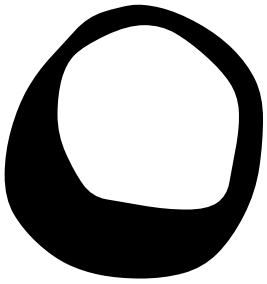
2.1

RELATION OF GENETIC PROFILE AND NOVEL CIRCULATING BIOMARKERS WITH CORONARY PLAQUE PHENOTYPE AS DETERMINED BY INTRAVASCULAR ULTRASOUND: RATIONALE AND DESIGN OF THE ATHEROREMO-IVUS STUDY



RELATION OF GENETIC PROFILE AND NOVEL CIRCULATING BIOMARKERS WITH CORONARY PLAQUE PHENOTYPE AS DETERMINED BY INTRAVASCULAR ULTRASOUND: RATIONALE AND DESIGN OF THE ATHEROREMO-IVUS STUDY

ABSTRACT

Aims

The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study aims to investigate the relations of genetic profile and novel circulating biomarkers with coronary plaque phenotype and vulnerability as determined by intravascular ultrasound (IVUS)

Methods and results: ATHEROREMO-IVUS is a prospective, observational cohort study of 846 patients with stable angina pectoris or acute coronary syndrome (ACS) who are referred for coronary angiography. Prior to the catheterization procedure, blood samples are drawn for biomarker measurements and genetic analyses. During the catheterization procedure, IVUS is performed in a non-culprit coronary artery. The primary endpoint is the presence of vulnerable plaque as determined by IVUS virtual histology. Secondary endpoints include the incidence of major adverse cardiac events during long-term follow-up.

Expected results: Results from ATHEROREMO-IVUS are expected to improve our knowledge on the role of genetic profile and circulating biomarkers in relation to the development of atherosclerosis and vulnerable plaques. Assessment and early validation of the prognostic value of novel biomarkers and intracoronary imaging techniques will be performed. (Clinicaltrials.gov number: NCT01789411)

INTRODUCTION

Coronary artery disease is projected to become the largest single cause of disease-burden worldwide.[1] The traditional view that atherosclerosis is simply a lipid storage disease has been evolved, considering the growing body of evidence that genetic profile, inflammation and blood coagulation play a pivotal role in all stages of atherosclerotic disease, from endothelial dysfunction to late-stage plaque rupture.[2-5] Genetic markers and circulating biomarkers of inflammation, lipids and coagulation may potentially improve risk stratification in patients with atherosclerotic cardiovascular disease, since

they provide information on the biological processes in individuals.[6-7] Furthermore, these markers may also have a role in the development of new therapeutical targets. Genome wide scanning of single nucleotide polymorphisms (SNPs) and plasma lipidomics are two potential methods to identify novel genetic and lipid-related markers of coronary artery disease. In-vivo intracoronary imaging may further improve coronary risk stratification. Intravascular ultrasound (IVUS) backscattering analysis allows for in-vivo differentiation of various plaque phenotypes and may therefore be well suited for detection of plaques that are at high risk to rupture.[8-9]
The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (ATHEROREMO-IVUS) is designed as an exploratory (non-pivotal) clinical study to investigate the associations between genetic profile, circulating biomarkers and coronary atherosclerosis phenotype and vulnerability as determined by IVUS virtual histology Additionally, novel intracoronary imaging techniques, including near-infrared spectroscopy (NIRS), will be explored to identify lipid core plaques in the coronary arterial wall.[10] Finally, the prognostic implications of (the combination) of established and novel biomarkers and plaque phenotypes will be studied.

METHODS

Target population

The ATHEROREMO-IVUS target population consists of patients with stable angina pectoris or acute coronary syndrome (ACS) who are referred for coronary angiography. The in- and exclusion criteria are presented in table 1. Stable angina pectoris was defined as having at least two of the following three criteria: 1. substernal chest discomfort of characteristic quality and duration; 2. provoked by exertion or emotional stress; 3. relieved by rest and/or glyceryl trinitrate.[11] ACS include ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI) or unstable angina pectoris. STEMI was defined by ischaemic symptoms, persistent (>20 min) ST-segment elevation in two contiguous electrocardiogram (ECG) leads and a raise in cardiac enzymes.[12] Patients with acute chest pain and a typical raise and fall in cardiac enzymes but without persistent ST-segment elevation were classified as NSTEMI. Unstable angina was defined by acute or worsened chest pain without persistent ST-segment elevation and without elevated cardiac enzymes.[13]

Study sample

The ATHEROREMO-IVUS study cohort mainly consists of patients who were included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands, which is an academic tertiary referral hospital serving a population of approximately 1.9 million. This cohort was enriched with eligible patients who participated in the Integrated Biomarker and Imaging Study-2 (IBIS-2) trial of darapladib versus placebo (inclusion period 2005-2006).[14]
The ATHEROREMO-IVUS study was approved by the medical ethics committee of the Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all included patients.

Blood sampling

Blood samples were collected to enable genome wide scans, lipid mass spectrometry and the analysis of circulating biomarkers. Blood samples were drawn from the arterial sheath prior to the coronary angiography or percutaneous coronary intervention (PCI) procedure. Blood samples were transported to the local clinical chemistry laboratory for further processing (i.e. centrifugation followed by serum, citrate- and EDTA-plasma aspiration and buffy coat separation from the EDTA tube) and storage at a temperature of -80oC within two hours.

Inclusion criteria:

1. Aged 21 years or older.
2. Presenting with stable angina pectoris (CCS angina class 1, 2, 3 or 4), unstable angina pectoris (Braunwald class 1-3, B-C), documented silent ischemia or acute myocardial infarction (STEMI and NSTEMI).
3. Eligible for coronary revascularization in the native coronary artery/arteries.
4. Willing and able to comply with the specified follow-up evaluation.
5. Willing to sign informed consent.
6. Presence of a flow-limiting stenosis (diameter stenosis ≥50% by QCA or visual estimate) that is held responsible for angina pectoris or acute coronary syndrome
7. The study vessel has not undergone percutaneous coronary intervention in the last 8 months.

Exclusion criteria:

1. Angina caused by a non-cardiac illness (Braunwald class IA, IIA, IIIA).
2. Pregnant women or women of childbearing potential who do not use adequate contraception.
3. Known allergies to aspirin, clopidogrel, ticlopdine, heparin, stainless steel, copper or a sensitivity to contrast media which cannot be adequately pre-medicated.
4. Previous participation in this study or participation in another study with any investigational drug or device within the past 30 days (study participation ends after completion of the final follow-up).
5. Life expectancy of less than one year or factors making clinical and/or angiographic follow-up difficult.
6. Planned or being status post coronary bypass surgery.
7. Planned major non-cardiac surgery.
8. Impaired renal function (creatinine>2 mg/dl or ≥150 μmol/l).
9. History of bleeding diathesis or coagulopathy.
10. History of disabling stroke within the past year.

Exclusion criteria for intravascular ultrasound and near-infrared spectroscopy:

11. Three-vessel coronary artery disease or left main disease with ≥50% stenosis.
12. Minimal lumen diameter <2mm in the segments to be analyzed within the study vessel.
13. Diameter stenosis >70% or total occlusion of the study vessel.
14. In case the study-vessel has been stented previously (>8 months ago), more than 1/3 proximal of the study vessel (at least 40mm in length) should be available for examination (i.e. outside the length of the stent plus 5mm proximal to the stent).
15. Poor left ventricular function as assessed by echocardiography or by angiography.
16. Moderate or severe tortuosity of the study segment (i.e. 2 bends >75° or one bend > 90°).
17. Known tendency for coronary vasospasm.

CCS: Canadian Cardiovascular Society; NSTEMI: non-ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction; QCA: quantitative coronary angiography.

Table 1 Inclusion and exclusion citeria

Genome wide scans

Genome wide scans are preformed to identify a set of genetic variants that correlate with the extent and phenotype of coronary atherosclerosis. The Affymetrix GeneChip Human Mapping 6.0 Array is used for the genome wide scans of 906,.600 SNPs. Quality control was performed, including correction for population structure, removal of related samples or samples with mismatched gender.

Lipid extraction and mass spectrometry

An aliquot of plasma or serum is subjected to lipid extraction. Known amounts of internal standards are added to the samples before extraction and the final lipid extracts are dried under nitrogen. The extracts are reconstituted as described elsewhere.[15] Sphingolipids are analyzed on a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Analytical Technologies) equipped with an ultra-high pressure liquid chromatography (UHPLC) system; CTC PAL autosampler (Leap Technologies) and Rheos Allegro UHPLC (Flux Instruments) using multiple reaction monitoring.[16] Shotgun lipidomics is performed by multiple precursor ion and neutral loss scanning on a QTRAP® 5500 mass spectrometer (Applied Biosystems/MDS Analytical Technologies) equipped with a robotic nanoflow ion source

NanoMate HD (Advion).[17] Mass spectrometry data files are processed using MultiQuant™ 1.1.0.26 or Lipid Profiler™ (Applied Biosystems/MDS Analytical Technologies).[18] Identified lipids are quantified by normalizing against their respective internal standard and tissue wet weight for aorta and volume for plasma. Quality control (QC) samples are utilized to monitor the overall quality of the lipid extraction and mass spectrometry analyses.[19] The QC samples are mainly used to remove technical outliers and lipid species that are detected below the lipid class based lower limit of quantification (LLOQ).

Intravascular imaging

Following the standard coronary angiography, eligibility for intracoronary imaging was assessed. IVUS data was acquired in a non-culprit coronary vessel. The order of preference for selection of the non-culprit vessel was: 1. left anterior descending (LAD) artery; 2. right coronary artery (RCA); 3. left circumflex (LCX) artery. All IVUS data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS catheter (20 MHz). An automatic pullback system was used with a standard pull back speed of 0.5 mm per second.
A number of selected patients in the Erasmus MC also participated in the ATHEROREMO-NIRS substudy (details are described in the online supplement). In these patients, NIRS was performed in the same segment of the non-culprit vessel.

IVUS virtual histology

The IVUS gray-scale and IVUS radiofrequency backscatter analyses, also known as IVUS virtual histology, were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software. The baseline IVUS images were analyzed offline in an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands). The core laboratory personnel were blinded for baseline patient characteristics as well as for biomarker, genetic and clinical outcomes data. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Extent and phenotype of the atherosclerotic plaque were assessed. Plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area. A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames (Figure 1). Using IVUS radiofrequency analyses, the composition of the atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core.[8] In consensus sessions with three investigators who were blinded to the patient characteristics and outcomes, the lesions were further classified into different lesion types (Table 2).[9] A thin-cap fibroatheroma (TCFA) lesion was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen. Remodeling of a lesion was assessed by means of the remodelling index, expressed as the external elastic membrane cross-sectional area at the site of minimal luminal area divided by the reference external elastic membrane cross-sectional area. The reference site was selected <10 mm proximal to the lesion, with no major side branches between the site of the minimal luminal area and the reference.

Follow-up

Clinical follow-up started at inclusion and will last for at least 1 year. Post-discharge survival status will be obtained from municipal civil registries. Post-discharge rehospitalizations will be prospectively assessed during follow-up. Questionnaires focusing on the occurrence of major adverse cardiac events (MACE) will be sent to all living patients. Treating physicians and institutions will be contacted for additional information whenever necessary. If possible and clinically relevant, culprit and non-culprit lesion related events will be distinguished. The occurrence of MACE will be adjudicated by an independent clinical events committee on the basis of original source data and without knowledge of other patient, biomarker, genetic or intracoronary imaging characteristics.

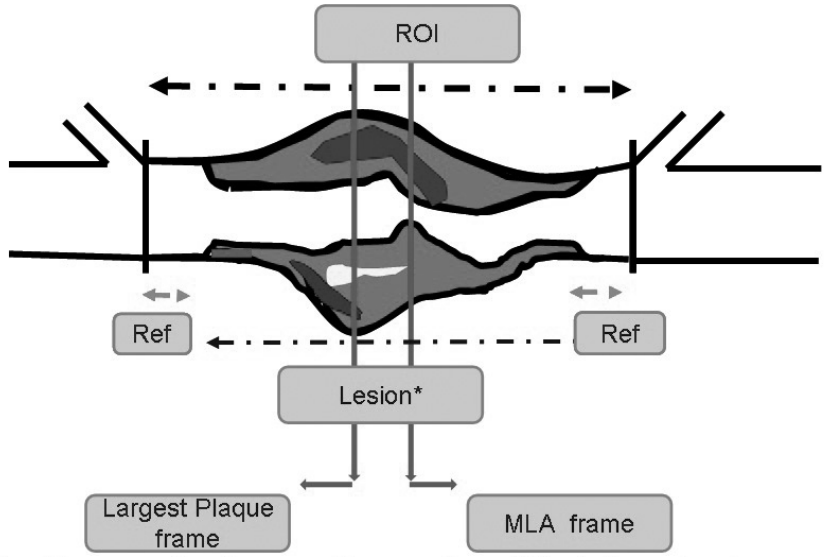


Figure 1 Methodology for detection of lesion and reference segments
MLA: minimal luminal area; REF: reference segments; ROI: region of interest.

Lesion Type	Definition
1. Adaptive intimal thickening	Intimal thickening of <600 μm for <20% of the circumference
2. Pathological intimal thickening	Intimal thickening ≥600 μm for >20% of the circumference with >15% fibrofatty tissue and no confluent necrotic core or dense-calcium
3. Fibrotic plaque	Lesion consisting predominantly of fibrous tissue without confluent necrotic core or dense-calcium
4. Fibrocalcific plaque	Presence of >10% confluent dense-calcium without confluent necrotic core
5. Fibroatheroma	Presence of >10% confluent necrotic core with an overlying layer of fibrous tissue
6. Calcified fibroatheroma	Fibroatheroma containing >10% confluent dense-calcium
7. Thin-cap fibroatheroma	Presence of >10% confluent necrotic core in direct contact with the lumen
8. Calcified thin-cap fibroatheroma	Thin-cap fibroatheroma containing >10% of confluent dense-calcium

Table 2 Classification of lesions on intravascular ultrasound

Study endpoints

The primary objective of ATHEROREMO-IVUS is to correlate genetic markers and circulating (lipid) biomarkers with coronary plaque phenotype as determined by IVUS virtual histology. Therefore, the primary endpoint is defined as the presence of TCFA lesions on the imaged non-culprit coronary segment.

The secondary objective is to assess the prognostic value of established biomarkers, novel genetic and lipid biomarkers and plaque phenotypes as determined by IVUS virtual histology. Therefore, the secondary endpoint is defined as the 1-year incidence of MACE, which includes all-cause mortality, ACS or unplanned coronary revascularization. All-cause mortality is defined as death due to any cause.[20] ACS was defined as the clinical diagnosis of STEMI, NSTEMI or unstable angina pectoris in accordance with the guidelines of the European Society of Cardiology.[21] Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG) due to progressive angina or ACS.

Sample size

ATHEROREMO-IVUS is designed to explore multiple relations. Its sample size is fixed at 800 patients, which is sufficient to reveal relations between 'abnormal' biomarkers (of any kind) and the presence of TCFA lesions with reasonable statistical certainty. We acknowledge that confirmatory studies might be required to more firmly establish relations that we will discover.

Based on prior studies, we expected that 30% of the patients will have a TCFA lesion in at least one coronary artery (i.e. TCFA positive), while 70% of the patients will not have any TCFA lesion (i.e. true TCFA negative) (Figure 2).[9] Since TCFA lesions are more or less randomly distributed across the coronary system, we expected that 10% of the patients will have a TCFA lesion in the imaged coronary artery (i.e. classified as TCFA positive), while 90% of the patient will not have a TCFA lesion in the imaged coronary artery (i.e. classified as TCFA negative). In patients who are classified as TCFA negative, 77.8% is expected to be true TCFA negative.

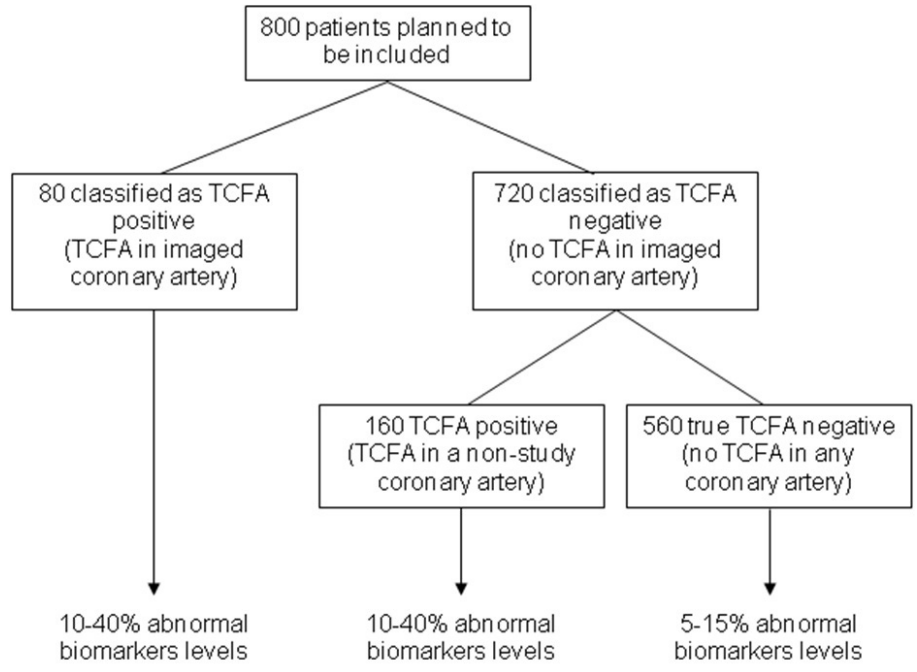


Figure 2 Expected prevalence of thin-cap fibroatheroma lesions
TCFA: thin-cap fibroatheroma.

A biomarker level in the upper quintile of its sample distribution is considered as 'abnormal'. We expect to observe abnormal biomarker levels in 5-15% of the true TCFA negative patients and in 10-40% of the TCFA positive patients. Hence, the proportion of abnormal biomarker levels in the patients who are classified as TCFA negative are expected to range from 6.1-20.6% (Table 3). Table 4 presents the statistical power to detect differences in the frequency of abnormal biomarker levels between patients who are classified as TCFA negative versus those who are classified as TCFA positive (α-error

5%, two-sided test). The power is adequate (≥80%) for the most realistic scenarios.
Power calculations were not performed for the secondary endpoint. Based on the results of other studies and previous registries in our hospital, we expect that MACE will occur in 5-10% of the patients within the first year of follow-up.[22-25]

		Frequency of abnormal biomarker levels in true TCFA negative patients				
		5%	***7.5%***	***10%***	***12.5%***	***15%***
Frequency of abnormal biomarker levels in TCFA positive patients	***10%***	6.1%	6.9%	--	--	--
	15%	7.2%	9.2%	11.1%	13.1%	--
	20%	8.3%	10.3%	12.2%	14.2%	16.1%
	25%	9.4%	11.4%	13.3%	15.3%	17.2%
	30%	10.6%	12.5%	14.4%	16.4%	18.3%
	40%	12.8%	14.7%	16.7%	18.6%	20.6%

Presented data is the expected percentage of patients with abnormal biomarker levels in those who did not have a TCFA in the imaged coronary artery (i.e. *classified* as TCFA negative). The results are displayed for different expected frequencies of abnormal biomarker levels in patients who have a TCFA in the imaged coronary vessel (i.e. TCFA positive) and for different expected frequencies of abnormal biomarker levels in patients who do not have any TCFA in any coronary vessel (i.e. *true* TCFA negative).
TCFA: thin-cap fibroatheroma.

Table 3 Expected percentage of patients with abnormal biomakers levels.

		Frequency of abnormal biomarker levels in true TCFA negative patients				
		5%	***7.5%***	***10%***	***12.5%***	***15%***
Frequency of abnormal biomarker levels in TCFA positive patients	***10%***	18%	12%	--	--	--
	15%	47%	26%	12%	5%	--
	20%	73%	54%	35%	20%	10%
	25%	88%	76%	61%	44%	29%
	30%	96%	90%	81%	68%	54%
	40%	100%	99%	98%	95%	89%

Presented data is the statistical power to detect differences in the frequency of abnormal biomarker levels between patients with a TCFA in the imaged coronary vessel (i.e. TCFA positive) versus patients without a TCFA in the imaged coronary vessel (i.e. *classified* as TCFA negative) (α-error 5%, two-sided test). The results are displayed for different expected frequencies of abnormal biomarker levels in TCFA positive patients and for different expected frequencies of abnormal biomarker levels in patients who do not have a TCFA in any coronary vessel (*true* TCFA negative). The statistical power is adequate for the most realistic scenarios (grey shaded area).
TCFA: thin-cap fibroatheroma.

Table 4 Expected statistical power

Statistical analyses
Conventional linear regression will be applied to relate SNPs, sphingolipids and other biomarkers with IVUS virtual histology measures, corrected for segment length. Mixed linear models will be used for per-lesion analyses. The relation between biomarkers (in a broad sense) and clinical endpoints will be studied by Cox proportional hazard models.
The p-values that appear in the analyses of genetic variants and sphingolipids will be corrected for multiple testing with appropriate methods to adjust for inflation of the type I error (e.g. Bonferroni or simulation). Significant SNPs and lipid fractions need (and will be proposed for) validation in different datasets.

Actual inclusion

A total of 846 patients with complete data for genetic and lipidomics analyses are included in ATHEROREMO-IVUS: 581 patients were enrolled in the Erasmus MC and 265 participated in IBIS-2 (Figure 3).[14] Blood samples are available for 1098 patients. NIRS was performed in 203 patients.

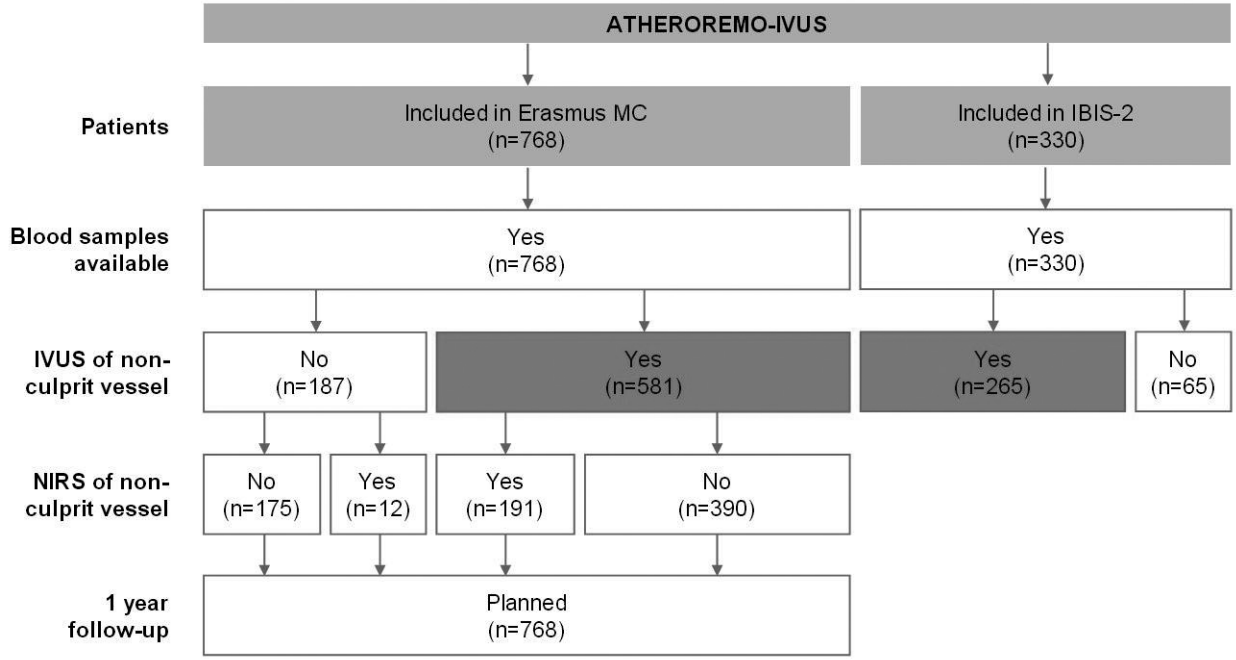


Figure 3 Actual patient inclusion

Finally, blood samples were stored for 1098 patients, IVUS of a non-culprit vessel was performed in 846 patients (red shaded) and NIRS was performed in 203 patients.

IBIS-2: Integrated Biomarker and Imaging Study-2; IVUS: intravascular ultrasound; NIRS: near-infrared spectroscopy.

DISCUSSION

The ATHEROREMO-IVUS study was primarily designed to assess correlations of genetic profile and novel circulating biomarkers with the extent, phenotype and vulnerability of coronary atherosclerotic plaques as determined in-vivo by IVUS. Furthermore we would like to assess the potential prognostic value of novel biomarkers, IVUS and NIRS compositional features of atherosclerotic plaques in major cardiac events at long term follow-up.

Acute coronary syndromes are mostly caused by rupture of TCFA lesions that contain a lipid-rich necrotic core covered by a thin fibrous cap.[2, 26-29] IVUS virtual histology may be suitable for the detection of such vulnerable plaques. The PROSPECT study has shown that TCFA lesions as identified in-vivo by IVUS virtual histology were associated with increased risk for recurrent cardiovascular events in ACS patients. However, the events in PROSPECT were mainly driven by rehospitalizations for unstable or progressive angina, while less is known about the prognostic value of IVUS virtual histology for acute cardiac events as a consequence of spontaneous plaque rupture (i.e. recurrent ACS or death). The prognostic value of IVUS virtual histology in patients with stable angina remains unclear as well. Furthermore, the prognostic value of NIRS for the occurrence of MACE has not yet been investigated. The results of the ATHEROREMO-IVUS study will provide data on these questions.

Coronary artery disease has a strong genetic component. Epidemiological studies suggest that up to

50% of its susceptibility is heritable[30]. Genome wide scans may measure hundreds of thousands of SNPs that can be tested for an association with a coronary atherosclerosis. Although this method is shown to be successful in identifying genetic associations with complex traits,[31] genotyping research programs for atherosclerosis have been of limited importance so far. One of the bottlenecks was the phenotypic complexity of atherosclerotic vascular diseases. The ATHEROREMO-IVUS study is therefore regarded as a unique opportunity to link genotypes with extensive intracoronary imaging data that reach far beyond the limited knowledge of luminal patency (or stenosis) from conventional coronary angiography.

Several biomarkers of inflammation, coagulation, myocardial necrosis and neurohumoral activation (e.g. C-reactive protein, high-sensitive troponin-T and natriuretic peptides) have more or less been established.[27] Our aim is to explore novel lipid biomarkers in first instance, while validation of more established biomarkers will be done in a later stage of the study.

The design of the ATHEROREMO-IVUS study has several strengths. To our best knowledge, this is the first (large-scale) study to combine several novel intracoronary imaging techniques with extensive genetic analyses, biomarker exploration and validation and adverse clinical outcome during follow-up. Secondly, in this study we examine a single non-culprit vessel. Ex vivo as well as in vivo studies using IVUS in patients with myocardial infarction have demonstrated the presence of TCFAs in other than the culprit lesion or even culprit artery.[5] Our approach will allow us to test the hypothesis that the phenotype of a non-culprit artery segment (indicating the patient's atherosclerotic disease burden) can be linked to biomarker, genetic and outcome data. If the imaging characteristics of the non-culprit artery appear to be related to the incidence of MACE, then this can be seen as a confirmation that the non-culprit artery reflects atherosclerotic disease burden of the larger coronary vasculature.

Some limitations of this study have to be acknowledged. Firstly, the genetic profile and biomarkers will be correlated with the phenotype of the imaged non-culprit coronary artery only. Although we expect that the presence of TCFA lesions is randomly distributed through the coronary system, we may miss the patient's dominant phenotypic characteristic if this phenotype is only expressed in a coronary segment that has not been imaged (e.g. culprit lesion). Secondly, the ATHEROREMO-IVUS study was designed to explore and discover new genetic and circulating biomarkers. Newly discovered SNPs and lipid biomarkers remain to be validated in another patient cohort.

The results from the ATHEROREMO-IVUS study will improve our understanding on the role of genetic profile and circulating biomarkers in the development of atherosclerosis and vulnerable plaques. Genome wide scans and lipidomics may identify novel biomarkers in coronary artery disease. Furthermore, the prognostic value of novel circulating biomarkers as well as in-vivo detection of vulnerable plaques by IVUS virtual histology and NIRS will be assessed. These findings may further contribute to improve risk assessment in patients with coronary artery disease, which may be important for the optimal choice of treatment in the individual patient.

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2.2

THE EUROPEAN COLLABORATIVE PROJECT ON INFLAMMATION AND VASCULAR WALL REMODELING IN ATHEROSCLEROSIS - INTRAVASCULAR ULTRASOUND (ATHEROREMO-IVUS)

THE EUROPEAN COLLABORATIVE PROJECT ON INFLAMMATION AND VASCULAR WALL REMODELING IN ATHEROSCLEROSIS - INTRAVASCULAR ULTRASOUND (ATHEROREMO-IVUS)

ABSTRACT

Background

The European Collaborative Project on Inflammation and Vascular Wall Remodelling in Atherosclerosis - Intravascular Ultrasound (ATHEROREMO-IVUS) study has been designed as an exploratory clinical study to investigate the associations between genetic variation, coronary atherosclerosis phenotypes, and plaque vulnerability as determined by IVUS.

Methods

The ATHEROREMO-IVUS study is a prospective, observational study of 846 patients with stable angina pectoris or acute coronary syndrome (ACS) who were referred for coronary angiography to the Thoraxcenter, Rotterdam. Prior to catheterization, blood samples were drawn for genetic analyses. During the catheterization procedure, IVUS was performed in a non-culprit coronary artery. The primary endpoint was the presence of vulnerable plaque as determined by IVUS virtual histology (VH). In addition, we performed a genome wide association study of plaque morphology.

Results

We observed strong signals associated with plaque morphology in several chromosomal regions: twelve SNPs (rs17300022, rs6904106, rs17177818, rs2248165, rs2477539, rs16865681, rs2396058, rs4753663, rs4082252, rs6932, rs12862206, rs6780676) in or near eight different genes (GNA12, NMBR, SFMBT2, CUL3, SESN3, SLC22A25, EFBN2, SEC62) reached significance.

Conclusion

In conclusion, we found twelve SNPS in or in the proximity of eight genes, which were associated with markers of vulnerable plaque.

INTRODUCTION

Despite improved understanding of the pathophysiology of atherosclerosis, the application of novel techniques for early diagnosis, and the availability of more powerful pharmacological therapies, coronary artery disease (CAD) remains the leading cause of death in Europe.[1] The early view that atherosclerosis simply represents increased lipid deposition in the vascular wall has changed, considering the growing body of evidence that inflammation plays a central role in atherosclerotic CAD from early lesion initiation to late-stage plaque rupture.[2-5] Intravascular ultrasound (IVUS) backscattering analysis has the potential of in-vivo differentiation of plaque phenotypes, and may therefore be suited for the detection of inflammatory, vulnerable coronary plaques.[6, 7]

Genetic factors play an important role in the aetiology of CAD,[8 9] and genetic markers may potentially further improve risk stratification. Genetic markers can also be instrumental in the development of new therapeutic targets, as they are stable, and can be objectively measured and evaluated as indicators of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic interventions. Therefore, genome wide association studies in CAD patients are warranted to further unravel the complexity of coronary atherosclerosis.[10] The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (ATHEROREMO-IVUS) was designed as an exploratory clinical study to investigate the associations between the genetic profile and coronary atherosclerosis phenotype and vulnerability as determined by IVUS.[11]

METHODS

Patients

Methods of the ATHEROREMO-IVUS study have been described in detail elsewhere.[11]
Briefly, the target population consisted of patients aged 21 years or older with stable angina pectoris (SAP) or an acute coronary syndrome (ACS) who were referred for coronary angiography (CAG) or percutaneous intervention (PCI). Patients had to have at least one non-culprit coronary artery without obstructive disease (<50% diameter stenosis) of at least 40 mm in length as assessed by on-line angiography to be used as the study vessel. Consequently, patients with significant three-vessel disease were excluded, as were patients with left main disease.
The study cohort mainly consisted of 581 patients who were included between 2008 and 2011 at the Erasmus MC, Rotterdam, the Netherlands. This cohort was enriched with 265 eligible patients who participated in the Integrated Biomarker and Imaging Study-2 (IBIS-2) trial of darapladib versus placebo (inclusion period 2005-2006), with consent of the sponsor.[12] Thus, the total sample comprised 846 patients, slightly more than the anticipated 800.[11]
The ATHEROREMO-IVUS study was approved by the Medical Ethics Committee of the Erasmus MC, performed in accordance to the Declaration of Helsinki (2008, sixth revision) and has been registered at the Dutch Central Committee on Research involving Human Subjects under EMC MEC 2008-210 and at Clinicaltrials.gov under NCT01789411. Written informed consent was obtained from all participants.

Phenotyping by intravascular ultrasound

Intravascular ultrasound imaging of the non-culprit study vessel was conducted for coronary plaque phenotyping. The IVUS gray-scale and IVUS radiofrequency backscatter analyses, also known as IVUS virtual histology (VH), were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software. IVUS images were analyzed offline in an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands). The core laboratory personnel were blinded for baseline patient characteristics as well as for biomarker, genetic and clinical outcomes.

The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Extent and phenotype of the atherosclerotic plaque were assessed. Plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area. A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Using IVUS radiofrequency analyses, the composition of the atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core.6 In consensus sessions with three investigators who were blinded to the patient characteristics and outcomes, the lesions were further classified into different lesion types (Table 2).7 A thin-cap fibroatheroma (TCFA) lesion was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen. Remodelling of a lesion was assessed by means of the remodelling index, expressed as the external elastic membrane cross-sectional area at the site of minimal luminal area divided by the reference external elastic membrane cross-sectional area. The reference site was selected <10 mm proximal to the lesion, with no major side branches between the site of the minimal luminal area and the reference.

Patient baseline characteristics	***AtheroRemo (N=718)***
Age, years	61.4 (53.1, 69.9)
Man, n (%)	558 (77.7)
Diabetes mellitus, n (%)	109 (15.2)
Hypertension, n (%)	404/717 (56.3)
Hypercholesterolemia, n (%)	411/717 (57.3)
Low density lipoprotein, mmol/l	2.56 (2.00, 3.38)/579
High density lipoprotein, mmol/l	1.13 (0.95, 1.37)/592
Total cholesterol, mmol/l	4.5 (3.7, 5.3)/599
Current smoker, n (%)	219 (30.5)
Previous MI, n (%)	147/472 (31.1) ¶
Previous PCI, n (%)	237 (33.0)
Previous CABG, n (%)	18/472 (3.8) ¶
Previous stroke, n (%)	24/472 (5.1) ¶
Peripheral artery disease, n (%)	40 (5.6)
Presentation with ACS, n (%)	334/711 (47.0)
Coronary artery disease	
No significant vessel disease, n (%)	142/615 (23.0) ¶
1-vessel disease, n (%)	315/615 (36.5) ¶
2-vessel disease, n (%)	198/615 (23.0) ¶
3-vessel disease, n (%)	60/615 (7) ¶

¶ Unavailable for the IBIS-2 patients

Table 1 Patient baseline characteristics

Genotyping

Blood samples were drawn from the arterial sheath prior to the CAG or PCI procedure, which were then transported to the clinical chemistry laboratory of the Erasmus MC for further processing and

storage at a temperature of -80°C within two hours. This material was used for the extraction of DNA needed for genome wide scanning, as well as for analyses of serum biomarkers.
Blood samples were transported to the genotyping facility at the Mannheim Medical Faculty, Germany, for batch DNA extraction and genotyping. The Affymetrix GeneChip Human Mapping 6.0 Array was used for genome wide analysis of 906,600 SNPs.

Quality Control
The dataset was filtered to include only autosomal SNPs with non-complementary alleles and genotyping rate >=0.9. PLINK's Multi Dimensional Scaling (MDS) analysis was performed jointly on the study samples and the European samples from the POPRES dataset[13]. First, the POPRES SNPs were pruned to decrease linkage disequilibrium (LD) using PLINK, so that no pair of SNPs with r2 ≥0.8 remained within sliding windows of 50 SNPs, offset by 5. Additional SNPs or samples with genotyping rate ≤0.1 were removed. Next, the study SNP set and the POPRES SNP set were intersected, and MDS was performed on the resulting set. 89 outliers (individuals who were located outside of the main European cluster in the MDS plot) were detected in this step and discarded from further analysis. The results of both the European MDS and a global MDS (using also non-European POPRES samples) appear in Supplementary , Figure 1. The first four axes of variation from the European MDS were used as covariates in all association tests.

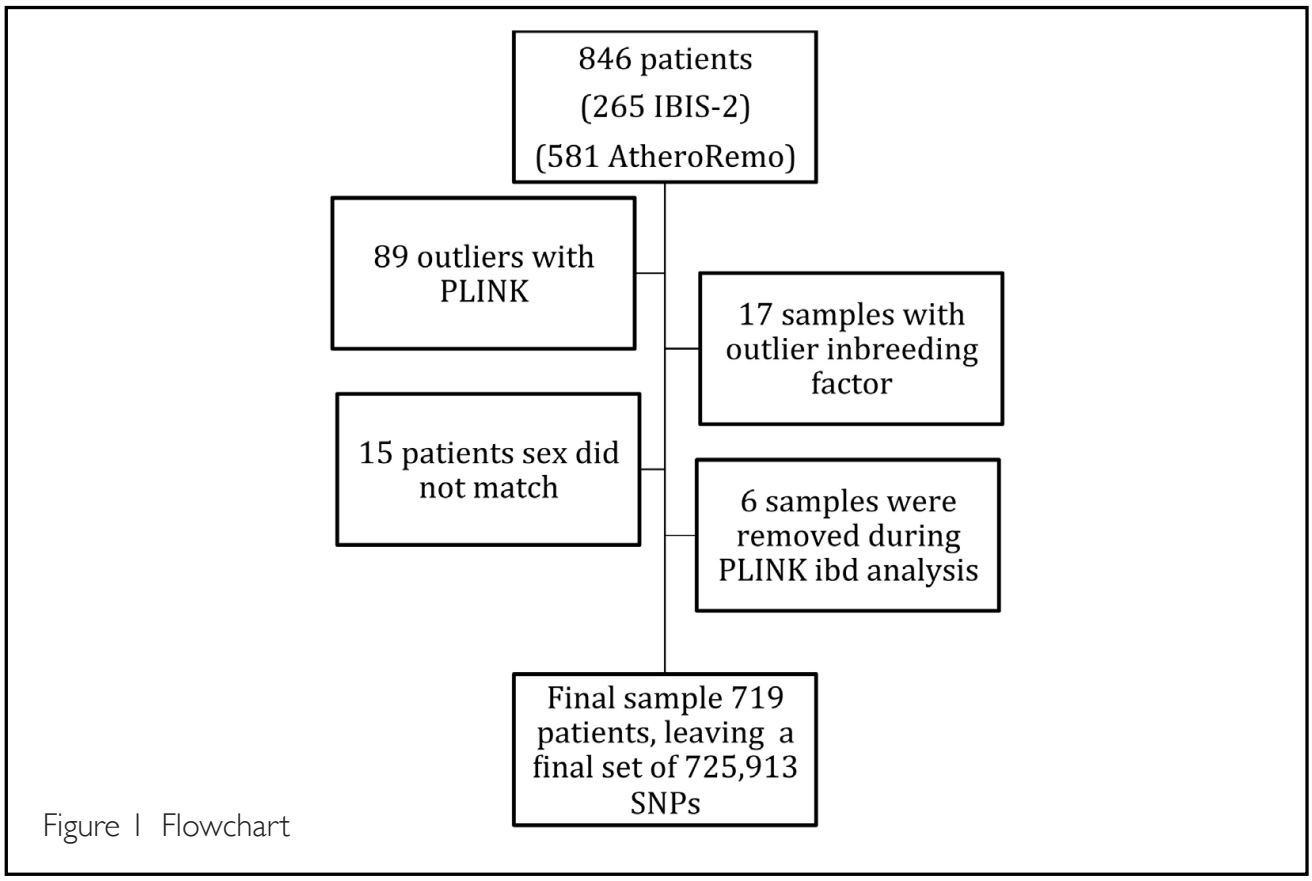

Figure 1 Flowchart

Additional sample filtering and imputation
PLINK's IBD analysis was performed and 6 samples were removed so as to eliminate any pair with estimated proportion of IBD (PLINK's PI_HAT) greater than 0.1875. In addition, following PLINK's heterozygosity analysis 17 samples with outlier inbreeding factor (< -0.15 or > 0.1) were removed. The genotypes used for imputation were filtered to exclude SNPs with genotyping rate ≤0.04 or minor allele frequency ≤0.0125. Imputation was performed with the 1000 genomes European reference panel[14] using BEAGLE version 3.3.2.15 Prior to that, strand consistency between the two datasets was verified using BEAGLE's strand-checking utility.

Association

Samples whose reported sex did not match the sex detected by PLINK [15] were removed to yield a final sample size of 719 individuals, figure 1. SNPs with minor allele frequency ≤10/719 = 0.0139 were discarded.

We used 28 IVUS measurements as phenotypes: 14 quantities describe the vascular segment that was analysed, and 14 quantities described the lesions detected in that segment, up to 4 lesions per individual. For each such phenotype, individuals with missing measurements or outliers (distant from the mean by over 3 standard deviations) were excluded. Phenotypes representing length or tissue percentages were square root-transformed. Phenotypes corresponding to volumes where cubic-root transformed, and divided by the vascular segment length.

Linear and logistic regression models assuming an additive genetic model were used for testing association between SNPs and quantitative and categorical phenotypes, respectively. Each model included, in addition to the SNP and the phenotype, the following covariates: site (Erasmus MC/IBIS-2), sex, age, body mass index (BMI), segment length, and the sample's coordinates along the first 4 MDS axes. Missing BMI values were imputed by the sex-specific median sample BMI.

In addition to the basic SNP vs phenotype association tests, we examined the extended models, which included an interaction effect between SNPs and sex. We used two degrees of freedom test for the combined marginal and interaction effects. An alpha error of 5.0e-07 was considered significant in this genome-wide association study.

Treating special phenotypes

In order to increase the statistical power for the lesion-specific phenotypes, we employed two different strategies. The first approach involved considering as a phenotype the weighted average of multiple lesions in each individual. We used 1, 1.5, 2 and 3 as the weights for lesions 1, 2, 3 and 4, respectively. The second approach was to duplicate individuals with multiple discovered lesions, ending up with one lesion per individual. To account for the strong relatedness introduced by this step, we tested association with this phenotype using EMMAX,[16] which uses a mixed model that corrects for relatedness.

Association with lesion type was tested by dividing the lesions into two categories: The benign category included adaptive intimal thickening, pathological intimal thickening, fibrous and fibrocalcific, and the severe category included fibroatheroma, calcified fibroatheroma, TCFA and calcified thin cap fibroatheroma.

Demographics

Continuous variables are presented as arithmetic mean values and corresponding standard deviations (± SD), or medians together with corresponding interquartile ranges (IQR). Categorical variables are expressed as numbers and percentages. All demographic data were analyzed with SPSS software (SPSS 20.0; IBM Corp., Armonk, NY).

LURIC and CARDIoGRAM

Additionally, we studied within the LURIC and CARDIoGRAM dataset the association between the SNPs we found and different phenotypes, biomarkers, and outcome. LURIC is a prospective, hospital-based study in 3316 patients of German ancestry who underwent coronary angiography. Patients were recruited between July 1997 and January 2000 at a single tertiary care centre in southwestern Germany (Herzzentrum Ludwigshafen). Inclusion criteria were as follows: Caucasian origin, availability of a coronary angiogram and clinical stability with the exception of acute coronary syndromes. Exclusion criteria were: any acute illness other than acute coronary syndromes, any chronic disease where non-cardiac disease predominated and a history of malignancy within the past five years. All participants underwent comprehensive assessment of cardiovascular and metabolic phenotypes. A ten years follow up for mortality and causes of death has been completed. [17] CARDIoGRAM combines data from all published and several unpublished GWAS in individuals with European ancestry, and include

22,000 cases with CAD, MI, or both. [18]

RESULTS

Patient characteristics

Baseline and procedural characteristics of the included patients are presented in Tables 1 and 2. The population consisted mostly of men (78%) and the mean age was 61 years. Most patients were treated for stable angina pectoris (53%), but prior MI (31%) and prior PCI (33%) were common. Hypercholesterolemia, multi-vessel disease and/or hypertension were seen in more than 50% of patients. More than 15% of the patients suffered from diabetes mellitus. The median total cholesterol concentration was 4.5 (IQR 3.7-5.3) mmol/l; low-density lipoprotein (LDL) cholesterol was 2.6 (2.0-3.4) mmol/l, and high-density lipoprotein (HDL) cholesterol was 1.1 (1.0-1.4) mmol/l. The mean length of the studied segment was 45.3 mm, with a mean plaque burden of 40.6%. On average, the plaques consisted mostly of fibrotic tissue (59%), and 19% had a necrotic core. The number of lesions, given as a ratio per patient, was 1.14; 32% of them were TCFA's.

Study segment characteristics	***AtheroRemo (N=718)***
Length, mm	45.3 (35, 56.9) /687
Lumen volume, mm^3	352.5 (243.3, 508.2) /687
Vessel volume, mm^3	604.8 (427.9, 839.3) /687
Plaque volume, mm^3	242.5 (155.3, 347.4) /687
Plaque burden, %	40.6 (32, 47.1) /687
Fibrotic tissue volume, mm^3	61.5 (32.6, 105.3) /705
Fibro-fatty tissue volume, mm^3	11.7 (5.1, 24.7) /705
Necrotic core volume, mm^3	19.4 (7.6, 35.4) /705
Dense calcium volume, mm^3	8.8 (2.7, 18.6) /705
Fibrotic tissue percentage, %	58.7 (51.7, 65.3) /701
Fibro-fatty tissue percentage, %	11.3 (7.4, 17.1) /701
Necrotic core percentage, %	18.7 (12.8, 24.2) /701
Dense calcium percentage, %	8.2 (4.4, 13.5) /701
Number of lesions, n (ratio per patient)	820 (1.14)
TCFA lesion, n (%)	264/820 (32.2%) ¶
Lesion remodeling	0.95 (0.81, 1.02) /819 ¶

¶ Data on lesion level

Table 2 Study segment characters

GWAS analysis of vulnerable plaque

Multiple genomic locations were shown to be potentially associated with vulnerable plaque (Figure 2). We observed strong association signals in several chromosomal regions: twelve SNPs in or near eight different genes reached significance (table 3).

Rs17300022 in the GNA12 gene was significantly associated with fibrotic volume of the vulnerable plaque, p=8.61E-08. The SNP rs6780676 on chromosome 3 was associated with the percentage

of dense calcium within the plaque, p=8.43E-08. A gene in the proximity of rs6780676 is SEC62 at a distance 7kb, SEC 62is part of the protein translocation apparatus in the membrane of the endoplasmic reticulum, and has been linked to prostate and lung cancer[19]. The rs6904106 and rs17177818 were associated with the percentage fibrofatty material within the vulnerable plaque, and are in the proximity (530kb) of the NMBR gene. NMBR gene, a Neuromedin B receptor binds to neuromedin B protein, and is involved in a number of physiological processes including immune defence, thyroid, adrenocortical function and cognition. NMB is also aberrantly expressed by a variety of cancers and is involved in tumour proliferation.[20]

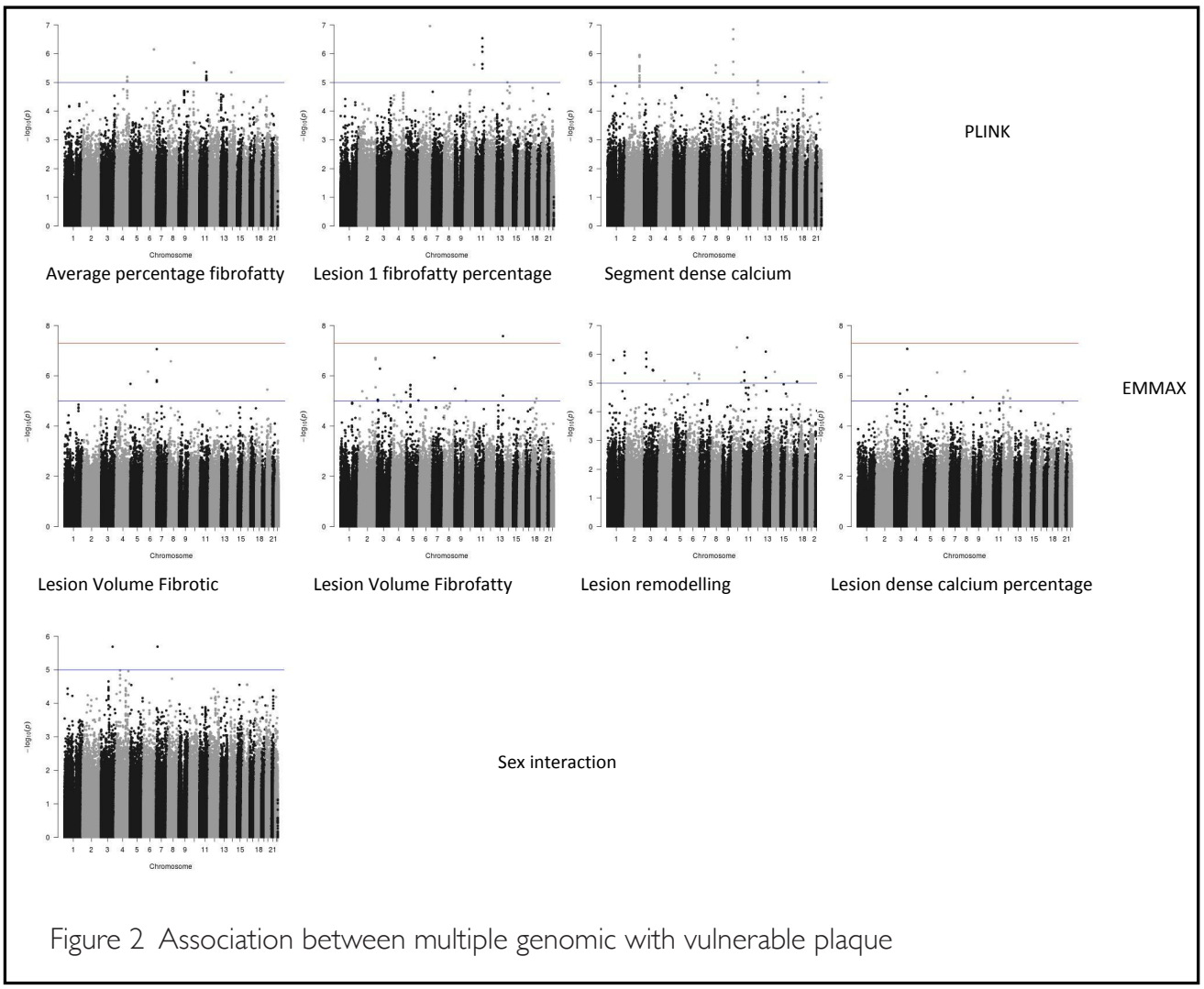

Figure 2 Association between multiple genomic with vulnerable plaque

Associated with dense calcium percentage of the plaque are rs2248165 and rs2477539, which are in the proximity (46kb) of the SFMBT2 (Scm-like with four mbt domains 2) gene, which is a protein-coding gene and has been linked to prostate cancer[21] and placental development[22] Rs16865681 and rs2396058 are linked to volume of fibrofatty material of the plaque, and are close to the CUL3 gene, which encodes a member of the cullin protein family. Rs4753663 and rs4082252 are associated with the percentage of fibrofatty material of the plaque and are in close proximity of the SESN3 gene. This gene encodes for a member of the sestrin family of stress-induced proteins. Rs6932 is associated with remodelling and in closeness of the SLC22A25gene, which encodes for the Solute carrier family [22] member 25 protein, SLC22A25 is also known as organic anion transporter UST6. Rs12862206 is associated with the volume of fibrofatty material of the plaque and is in the proximity of the EFBN2 gene, which encodes for the Ephrin-B2 protein.

SNPS	MAF	Chromosome	Position	Test Performed	Plaque Phenotype	P-Value	Beta	HWE P-value	Closest gene	Distance	Second closest gene	Distance
rs17300022	0.1161	7	2808517	EMMAX	Volume fibrotic (lesion)	8.61E-08	-0.57475	0.02797	GNA12			
rs6904106	0.2872	6	141898808	PLINK (VOL/LEN)	Percentage fibrofatty (lesion)	1.09E-07	0.3016	0.5236	NMBR	530kb		
rs6904106	0.2872	6	141898808	PLINK (VOL/LEN)	Percentage fibrofatty (segment)	7.1E-07	0.2671	0.5236	NMBR	530kb		
rs17177818	0.2872	6	141912038	PLINK (VOL/LEN)	Percentage fibrofatty (lesion)	1.09E-07	0.3016	0.5236	NMBR	530kb		
rs17177818	0.2872	6	141912038	PLINK (VOL/LEN)	Percentage fibrofatty (segment)	7.1E-07	0.2671	0.5236	NMBR	530kb		
rs2248165	0.3428	10	7539534	PLINK (VOL/LEN)	Percentage dence calcium (segment)	1.42E-07	-0.3179	0.7406	SFMBT2	46kb		
rs2248165	0.3428	10	7539534	SEX INTERACTION	Percentage dence calcium (segment)	8.84E-07	-0.1468	0.7406	SFMBT2	46kb		
rs2477539	0.3477	10	7544816	PLINK (VOL/LEN)	Percentage dence calcium (segment)	3.12E-07	-0.3096	0.6811	SFMBT2	46kb		
rs16865681	0.07024	2	225084573	EMMAX	Volume fibrofatty (lesion)	1.94E-07	-0.27607	0.0183	CUL3	41kb		

rs2396058	0.06885	2	225045264	EMMAX	Volume fibrofatty (lesion)	2.25E-07	-0.27576	0.01443	CUL3	41kb		
rs4753663	0.1975	11	94712679	PLINK (VOL/LEN)	Percentage fibrofatty (lesion)	2.89E-07	0.3453	0.1251	SESN3	108kb		
rs4082252	0.1982	11	94710862	PLINK (VOL/LEN)	Percentage fibrofatty (lesion)	5.74E-07	0.3345	0.1599	SESN3	108kb		
rs6932	0.01599	11	62656316	EMMAX	Remodelling (lesion)	2.62E-07	-0.22988	0.1637	SLC22A25	31 kb		
rs12862206	0.09192	13	105515048	EMMAX	Volume fibrofatty (lesion)	2.61E-08	-0.26156	0.1801	EFNB2	425 kb		
rs6780676	0.01624	3	171174616	EMMAX	Percentage dence calcium (lesion)	8.43E-08	-8.59549	1	SEC62	7 kb	SAMD7 GPR169	34kb 64kb

Table 3 Most significant associations bewteen Single-Nucleotide Polymorphisms (SNPs) and plaque phenotypes

LURIC

We studied within the LURIC dataset the association between the SNPs we found and clinical, biomarkers, and outcomes. Several associations with significant p-values were found, however after Bonferroni correction, all were attenuated and p-values became non-significant, Supplementary Table 1.

CARDIOGRAM

Using the Cardiogram[18] dataset we studied the association between the identified SNPs and MI as well as CAD. We found a significant association between rs4082252 (OR 1,05- InSE 0.02, p=0.0009), rs475366 (OR 0.96- InSE 0.02, p=0.012) and MI/ CAD, Supplementary table 2.

eQTL and HaploReg

We searched the Genotype-Tissue Expression (GTEx) project [23] and HaploReg [24] for an association of our SNPs with the expression of nearby genes or the alteration of regulatory sequences. While we did not find any direct association with gene expression, a number of SNPs could alter regulatory motifs (Supplementary Table 3).

DISCUSSION

We performed an exploratory clinical study to investigate the associations between genetic profile and coronary atherosclerosis phenotype and vulnerability as determined by IVUS 11, in a cohort of 719 patients. We found a strong association between indicators of vulnerable plaque and twelve SNPS, in or near 8 different genes.

We further studied these SNPs in the LURIC dataset, and found several significant associations, which however, after Bonferroni correction, lost statistical significance. Therefore we decided to further explore our results in the CARDIoGRAM dataset, in which we found additionally three significant associations between SNPs and CAD/MI; these 3 SNPS encode for 2 genes, namely GNA12 and SESN3.

The GNA12 gene was significantly associated with the vulnerable plaque (dense calcium) and CAD/MI. The GNA12 gene encodes for the Guanine nucleotide-binding protein subunit alpha-12 proteins, which are involved as modulators or transducers in various transmembrane signalling systems, and may play a role in the control of cell migration through the TOR signalling cascade[25].

The SESN3 gene encodes for a member of the sestrin family of stress-induced proteins. The encoded protein reduces the levels of intracellular reactive oxygen species induced by activated Ras downstream of RAC-alpha serine/threonine-protein kinase (Akt) and FoxO transcription factor. The protein is required for normal regulation of blood glucose, insulin resistance, and plays a role in lipid storage in obesity.[26]

Our findings need to be seen in light of several considerations. There may simply be no direct causal link between the SNPs and vulnerable plaque, due to the phenotypic complexity of atherosclerotic vascular disease. Alternatively, there could be indeed a causal relationship, which we had not been able to detect. First, in this study we visualized 40mm of a single non-culprit vessel, and therefore cannot exclude that we might have missed the patient's dominant phenotypic characteristic if this phenotype is only expressed in a coronary segment that has not been imaged (e.g. culprit lesion). However, ex vivo as well as in vivo studies using IVUS in patients with MI have demonstrated the presence of TCFAs in other than the culprit lesion or even culprit artery. 5 Second, is IVUS virtual histology suitable for the detection of such vulnerable plaques? We believe it is, as we demonstrated earlier, based on data from a large subset of this patient population, that the presence of IVUS-VH derived TCFA lesions in a non-culprit coronary artery is strongly and independently predictive for the occurrence of MACE within 1 year, particularly of death and ACS. Also, TCFAs with a large plaque burden carry higher risk than small TCFA lesions, especially on the short-term.[27] Third, the presented results are based on a single centre experience, which may limit its external validity.

However, this is the first (large-scale) study to combine several novel invasive coronary imaging tech-

niques with extensive genetic analyses and we extended our analyses by investigating the associations found in our IVUS study with a CAD/MI phenotype in CARDIoGRAM and with the Friesinger Score, a measure of the extent and severity of coronary atherosclerosis in the LURIC dataset. We identified two genes in particular, which, besides being related to vulnerable plaque, were also related to clinical outcome. Our results are hypothesis generating, and need to be replicated in other, larger populations.

In conclusion, we found twelve SNPs in or in the proximity of eight genes, which were associated with indicators of vulnerable plaque.

The supplementary figures and tables are available online.

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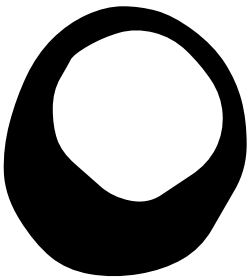
2.3

IN VIVO DETECTION OF HIGH-RISK CORONARY PLAQUES BY RADIOFREQUENCY INTRAVASCULAR ULTRASOUND AND CARDIOVASCULAR OUTCOME: RESULTS OF THE ATHEROREMO-IVUS STUDY



IN VIVO DETECTION OF HIGH-RISK CORONARY PLAQUES BY RADIOFREQUENCY INTRAVASCULAR ULTRASOUND AND CARDIOVASCULAR OUTCOME: RESULTS OF THE ATHEROREMO-IVUS STUDY

ABSTRACT

Aims

Acute coronary syndromes (ACS) are mostly caused by plaque rupture. This study aims to investigate the prognostic value of in vivo detection of high-risk coronary plaques by intravascular ultrasound (IVUS) in patients undergoing coronary angiography.

Methods and results

Between November 2008 and January 2011, IVUS of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography for ACS (n = 318) or stable angina (n = 263). Primary endpoint was major adverse cardiac events (MACEs) defined as mortality, ACS, or unplanned coronary revascularization. Culprit lesion-related events were not counted. Cumulative Kaplan–Meier incidence of 1-year MACE was 7.8%. The presence of IVUS virtual histology-derived thin-cap fibroatheroma (TCFA) lesions (present 10.8% vs. absent 5.6%; adjusted HR: 1.98, 95% CI: 1.09–3.60; P = 0.026) and lesions with a plaque burden of ≥70% (present 16.2% vs. absent 5.5%; adjusted HR: 2.90, 95% CI: 1.60–5.25; P < 0.001) were independently associated with a higher MACE rate. Thin-cap fibroatheroma lesions were also independently associated with the composite of death or ACS only (present 7.5% vs. absent 3.0%; adjusted HR: 2.51, 95% CI: 1.15–5.49; P = 0.021). Thin-cap fibroatheroma lesions with a plaque burden of ≥70% were associated with a higher MACE rate within (P= 0.011) and after (P < 0.001) 6 months of follow-up, while smaller TCFA lesions were only associated with a higher MACE rate after 6 months (P = 0.033).

Conclusion

In patients undergoing coronary angiography, the presence of IVUS virtual histology-derived TCFA lesions in a non-culprit coronary artery is strongly and independently predictive for the occurrence of MACE within 1 year, particularly of death and ACS. Thin-cap fibroatheroma lesions with a large plaque burden carry higher risk than small TCFA lesions, especially on the short term.

INTRODUCTION

Acute coronary syndromes (ACS) are expected to remain the leading cause of mortality and morbidity in the upcoming years.[1] Patients with a history of cardiovascular disease have an increased risk for ACS.[2]Post-mortem studies have shown that ACS is mostly caused by thin-cap fibroatheroma (TCFA) lesions.[3–5] Detection of these coronary lesions that are at high risk to rupture may be highly relevant for further improvement of prognostication and for optimal choice of treatment. However, these high-risk lesions cannot be easily detected by coronary angiography.[6]
Intravascular ultrasound (IVUS) radiofrequency analyses, also known as IVUS virtual histology, allow for differentiation of various plaque phenotypes and may therefore be well suited for detection of plaques that are at high risk to rupture.[7–9] The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study has shown that plaque characteristics as assessed by IVUS were independently predictive for recurrent cardiac events in patients admitted with an ACS.[10] However, the events in PROSPECT were mainly driven by rehospitalizations for unstable or progressive angina, while less is known about the prognostic value of IVUS for acute cardiac events as a consequence of spontaneous plaque rupture (i.e. recurrent ACS or death). Furthermore, the prognostic value of IVUS in patients with stable angina remains unclear. This study aims to investigate the prognostic value of in vivo detection of high-risk plaques by IVUS in patients undergoing coronary angiography for ACS or stable angina.

METHODS

Study population

The design of the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described elsewhere.[11] In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for ACS or stable angina pectoris have been included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands (see Supplementary material online, Figure S1). Although this original ATHEROREMO-IVUS cohort was further enriched with eligible patients who participated in the Integrated Biomarker and Imaging Study-2 (IBIS-2) trial of darapladib vs. placebo, these additional IBIS-2 patients were not included in the present analysis in order to prevent possible treatment interaction from darapladib.[12]
The ATHEROREMO-IVUS study was approved by the medical ethics committee of the Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all included patients. This study is registered with ClinicalTrials.gov, number NCT01789411.

Intravascular ultrasound imaging

Following the standard coronary angiography procedure, IVUS imaging of a non-culprit coronary artery was performed. Selection of the non-culprit vessel was predefined in the study protocol. The order of preference for selection of the non-culprit vessel was: (i) left anterior descending artery; (ii) right coronary artery; (iii) left circumflex artery. All IVUS data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS catheter (20 MHz). An automatic pullback system was used with a standard pull back speed of 0.5 mm/s. The baseline IVUS images were sent to an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for baseline patient characteristics and clinical outcomes data. The IVUS grey-scale and virtual histology analyses were performed using the pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software. The external elastic membrane and luminal borders were contoured for each frame of the virtual histology-derived

data set. Extent and phenotype of the atherosclerotic plaque were assessed. The plaque burden was defined as plaque and media cross-sectional area divided by an external elastic membrane cross-sectional area. A coronary lesion was defined as a segment with a plaque burden of >40% in at least three consecutive frames (see Supplementary material online, Figure S2). Using IVUS virtual histology, the composition of the atherosclerotic lesions was characterized into four different tissue types: fibrous, fibro-fatty, dense calcium, and necrotic core.7 Confluence of the necrotic core and dense calcium and the contact of the necrotic core with the lumen were independently assessed by visual examination, which was performed independently by three investigators (H.M.G., S.P.B., and J.H.H.) who were blinded to the clinical outcomes. Consensus was reached in case of disagreement. The lesions were further classified into: (i) adaptive intimal thickening (intimal thickening of <600 μm for <20% of the circumference); (ii) pathological intimal thickening (intimal thickening ≥600 μm for >20% of the circumference with >15% fibrofatty tissue and no confluent necrotic core or dense calcium); (iii) fibrotic plaque (consisting predominantly of fibrous tissue without confluent necrotic core or dense calcium); (iv) fibrocalcific plaque (presence of >10% confluent dense calcium without confluent necrotic core); (v) fibroatheroma (presence of >10% confluent necrotic core with an overlying layer of fibrous tissue); (vi) calcified fibroatheroma (fibroatheroma containing >10% confluent dense calcium); (vii) non-calcified TCFA (presence of >10% confluent necrotic core in direct contact with the lumen); (viii) calcified TCFA (TCFA containing >10% of confluent dense calcium) (Figure 1).8 All of the above-mentioned criteria should be present in three consecutive frames for a lesion to be considered of a particular category. Thin-cap fibroatheroma lesions with a plaque burden of at least 70% were classified as large TCFA lesions.

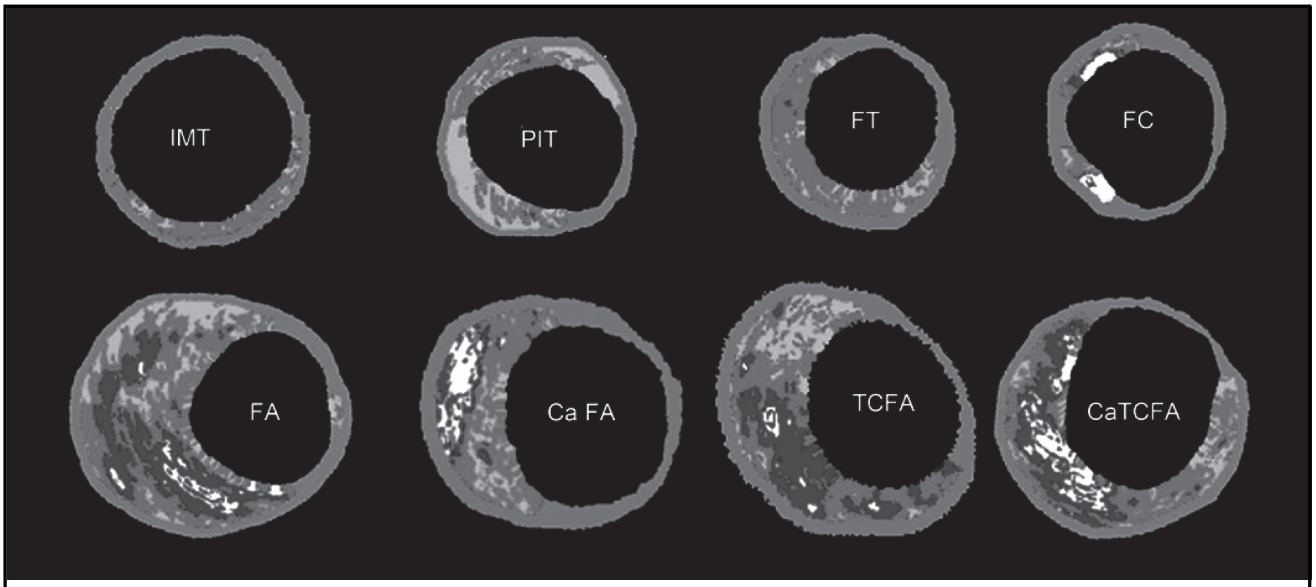


Figure 1 Methodology for detection of lesion and reference segments

Study endpoints

Clinical follow-up started at inclusion and lasted 1 year. Post-discharge survival status was obtained from municipal civil registries. Post-discharge rehospitalizations were prospectively assessed during the follow-up. Questionnaires focusing on the occurrence of MACEs were sent to all living patients. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contacted for additional information whenever necessary. Acute coronary syndrome was defined as the clinical diagnosis of ST-segment elevation myocardial infarction (STEMI), non-STEMI, or unstable angina pectoris in accordance with the guidelines of the European Society of Cardiology.[13] Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG). All events were adjudicated as related to a coronary site that was treated during the index

procedure (culprit lesion-related event) or as related to a coronary site that was not treated during the index procedure (non-culprit lesion-related event). Events that were related to both the culprit lesion and a non-culprit site (e.g. revascularization of multiple vessels with CABG) were classified into both categories. When information was not sufficient to classify an event as either culprit lesion related or non-culprit lesion related, the event was classified as indeterminate.
The primary endpoint was MACE, defined as non-culprit lesion related or indeterminate mortality, ACS, or unplanned coronary revascularization. The secondary endpoint was defined as the composite of non-culprit lesion related or indeterminate mortality or ACS. Definite culprit lesion-related events were not counted in the primary and secondary endpoint. The occurrence of culprit lesion-related events is most probably caused by in-stent restenosis or in-stent thrombosis, while we were only interested in unanticipated, spontaneous MACE. The endpoints were adjudicated by a clinical event committee that had no knowledge of the IVUS data.

Statistical analysis

Under the previously described assumptions (design paper) that high-risk lesions (e.g. TCFA) will be present in 30% of the patients and that MACE will occur in 10% of the total study population, our sample size of 581 patients would provide 85–99% power to detect a hazard ratio (HR) in the range of 2.0–2.5 with a two-sided alpha of 0.05.11
Normally distributed continuous variables are presented as means ± standard deviation. Non-normally distributed continuous variables are presented as median and inter-quartile range. Categorical variables are presented in numbers and percentages. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. Cumulative event rates were estimated according to the Kaplan–Meier method. Cumulative Kaplan–Meier event curves were compared by the
log-rank test. Cox proportional hazards regression analyses were performed to evaluate the associations between IVUS characteristics and study endpoints. In multivariable analyses, the variables age, gender, diabetes mellitus, hypertension, history of PCI, and indication for coronary angiography were considered as potential confounders and were entered into the full model. These covariates (except for indication for coronary angiography) were chosen based on the multivariable model that was used in the PROSPECT study, taking into account the number of events available.[10] The final results are presented as HRs with 95% confidence interval (95% CI). The z-test for heterogeneity was performed to test for heterogeneity in effect estimates between patients admitted with and without ACS. All statistical analyses were performed at the patient level. All data were analysed with the SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). All statistical tests were two-tailed and P-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

The mean age of the study population was 61.6 ± 11.3 years, 75.6% were men and 17.0% had diabetes mellitus (Table 1). Coronary angiography or PCI was performed for various indications: 28.7% of the patients had an acute myocardial infarction (STEMI and non-STEMI), 26.0% of the patients had unstable angina pectoris, and 43.7% of patients had stable angina pectoris. The median length of the imaged coronary segment was 44.3 (33.8–55.4) mm. The median interslice distance was 0.40 mm. A total of 724 lesions were identified in the imaged coronary segment of 508 (87.4%) patients, including 127 (17.5%) lesions with a plaque burden of at least 70% in 124 (21.3%) patients and 206 (28.5%) lesions with a minimal luminal area of 4.0 mm2 or less in 182 (31.3%) patients (Figure 2 and seeSupplementary material online, Table S1). On the basis of radiofrequency IVUS, 271 (37.4%) of the lesions have been classified as TCFA in 242 (41.7%) patients, including 71 (9.8%) TCFA lesions with a

Patient characteristics	***n* = 581 patients**
Age, years	61.6 ± 11.3
Men, *n* (%)	439 (75.6)
Diabetes mellitus, *n* (%)	99 (17.0)
Hypertension, *n* (%)	300 (51.6)
Hypercholesterolaemia, *n* (%)	321 (55.2)
Smoking, *n* (%)	169 (29.1)
Positive family history, *n* (%)	301 (51.8)
Previous MI, *n* (%)	184 (31.7)
Previous PCI, *n* (%)	186 (32.0)
Previous CABG, *n* (%)	18 (3.1)
Previous stroke, *n* (%)	26 (4.5)
History of peripheral artery disease, *n* (%)	36 (6.2)
History of renal insufficiency, *n* (%)	32 (5.5)
History of heart failure, *n* (%)	19 (3.3)
C-reactive protein, mg/L	2.1 (0.9–5.4)
Procedural characteristics	
Indication for angiography	
Acute MI, *n* (%)	167 (28.7)
Unstable angina, *n* (%)	151 (26.0)
Stable angina, *n* (%)	254 (43.7)
Other, *n* (%)	9 (1.5)
Coronary artery disease[a]	
No significant stenosis, *n* (%)	43 (7.4)

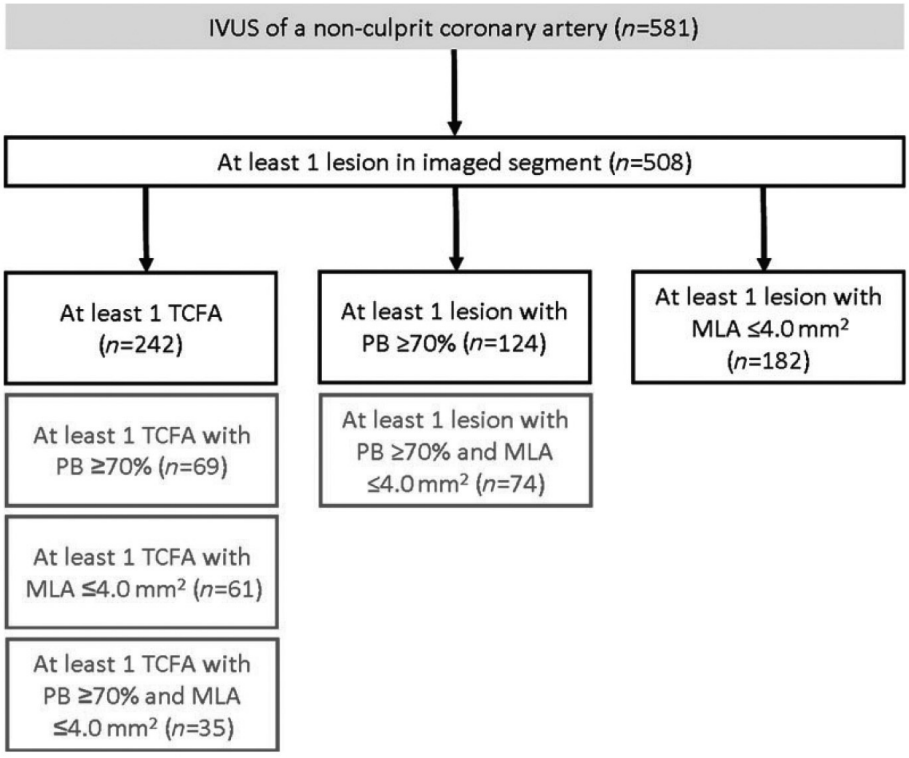
Table 1. Baseline characteristics (see other side)

One-vessel disease, *n* (%)	308 (53.0)
Two-vessel disease, *n* (%)	168 (28.9)
Three-vessel disease, *n* (%)	62 (10.7)
PCI performed, *n* (%)	511 (88.0)
IVUS characteristics	
Imaged coronary artery	
Left anterior descending, *n* (%)	210 (36.1)
Left circumflex, *n* (%)	195 (33.6)
Right coronary artery, *n* (%)	176 (30.3)
Imaged segment length, mm	44.3 (33.8–55.4)

[a]A significant stenosis was defined as a stenosis ≥50% of vessel diameter by visual assessment on the coronary angiogram.

CABG, coronary artery bypass grafting; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Table 1. Baseline characteristics



IVUS, intravascular ultrasound: MLA, minimal lumen area; PB, plaque burden, TCFA, thin cap fibroatheroma

Figure 2. Study participants and intravascular ultrasound-derived coronary lesions

	Definite culprit lesion-related events	**Definite non-culprit lesion-related events**	**Indeterminate events**	**Non-culprit lesion related and indeterminate events combined**	**All events**
Composite of major adverse cardiac events, *n*	11	27	18	45[a]	56
Death from any cause, *n*	1	1	16	17	18
Definite cardiac or unexplained death, *n*	1	1	6	7	8
Acute coronary syndrome, *n*	3	9	2	11	14
Myocardial infarction, *n*	2	3	2	5	7
Unplanned coronary revascularization,*n*	7	17	0	17	24
Composite of death or acute coronary syndrome, *n*	4	10	18	28[b]	32

[a]Primary endpoint.
[b]Secondary endpoint.

Table 2. Patients with major adverse cardiac events

plaque burden of at least 70% in 69 (11.9%) patients, 61 (8.4%) TCFA lesions with a minimal luminal area of 4.0 mm2 or less in 61 (10.5%) patients, and 35 (4.8%) TCFA lesions with a plaque burden of at least 70% and a minimal luminal area of 4.0 mm2 in 35 (6.0%) patients. Antiplatelet medications and statins were prescribed to the majority of patients at the time of discharge (see Supplementary material online, Table S2).

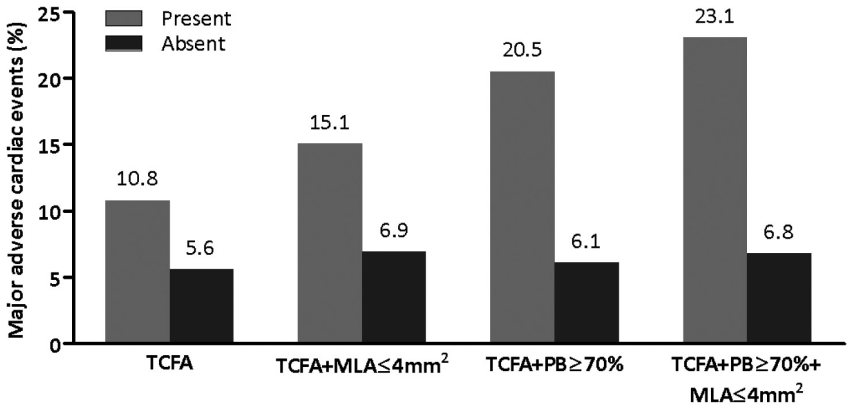
Major adverse cardiac events

Vital status was complete for 580 (99.8%) patients. The response rate of the questionnaires that were sent to all living patients was 91.5%. After 1 year of follow-up, 56 patients had at least 1 event (Table 2). Unplanned coronary revascularization was performed in four patients who did not have PCI during the index procedure. A total of 11 patients had a definite culprit lesion-related event, while 27 patients had a definite non-culprit lesion-related event. Another 18 patients had an event that could not be judged to be either culprit lesion related or non-culprit lesion related and were therefore classified as having an indeterminate event. The cumulative Kaplan–Meier incidence of the 30-day, 6-month, and 1-year MACE (primary endpoint) was 0.7, 4.7, and 7.8%, respectively. The cumulative Kaplan–Meier incidence of the 30-day, 6-month, and 1-year composite of death or ACS (secondary endpoint) was 0.7, 3.1, and 4.8%, respectively.

	Unadjusted model	***P*-value**	**Age and gender adjusted model**	***P*-value**	**Age, gender, and indication for angiography-adjusted model**	***P*-value**	**Full model[a]**	***P*-value**
Major adverse cardiac events (primary endpoint)								
Thin-cap fibroatheroma	HR 1.96 (1.08–3.53)	0.026	HR 1.97 (1.09–3.57)	0.024	HR 2.00 (1.10–3.62)	0.022	HR 1.98 (1.09–3.60)	0.026
Plaque burden ≥70%	HR 3.15 (1.75–5.68)	<0.001	HR 2.83 (1.57–5.13)	0.001	HR 2.83 (1.56–5.12)	0.001	HR 2.90 (1.60–5.25)	<0.001
MLA ≤4.0 mm^2	HR 1.36 (0.74–2.48)	0.32	HR 1.24 (0.68–2.28)	0.48	HR 1.24 (0.68–2.28)	0.48	HR 1.23 (0.67–2.26)	0.50
Composite of death or acute coronary syndrome (secondary endpoint)								
Thin-cap fibroatheroma	HR 2.56 (1.18–5.54)	0.017	HR 2.60 (1.20–5.64)	0.015	HR 2.54 (1.17–5.51)	0.019	HR 2.51 (1.15–5.49)	0.021
Plaque burden ≥70%	HR 2.11 (0.97–4.56)	0.059	HR 1.90 (0.87–4.15)	0.11	HR 1.92 (0.88–4.20)	0.10	HR 2.01 (0.92–4.39)	0.079
MLA ≤4.0 mm^2	HR 1.23 (0.57–2.67)	0.60	HR 1.12 (0.52–2.43)	0.78	HR 1.13 (0.52–2.45)	0.76	HR 1.14 (0.53–2.49)	0.73

[a]Variables entered into the full model were age, gender, diabetes mellitus, hypertension, history of percutaneous coronary intervention, and indication for coronary angiography.
HR, hazard ratio, MLA, minimal luminal area.

Table 3 Associtaions with major adverse cardiac events



Hazard ratio (95% CI)	1.96 (1.08-3.53)	2.26 (1.09-4.69)	3.47 (1.86-6.49)	3.70 (1.72-7.95)
P value	0.024	0.025	<0.001	<0.001
Prevalence (%)	41.7	10.5	11.9	6.0

Figure 3 Cumulative Kaplan-Meier incidence estimates of non-culprit lesion related or indeterminate major adverse cardiac events

Percentages are 1-year cumulative Kaplan-Meier incidence estimates. Hazard ratios are estimated using univariate Cox proportional hazard regression analysis. P values are obtained with log-rank test. MLA; minimal lumen area; PB, plaque burden; TCFA thin-cap fibroatheroma

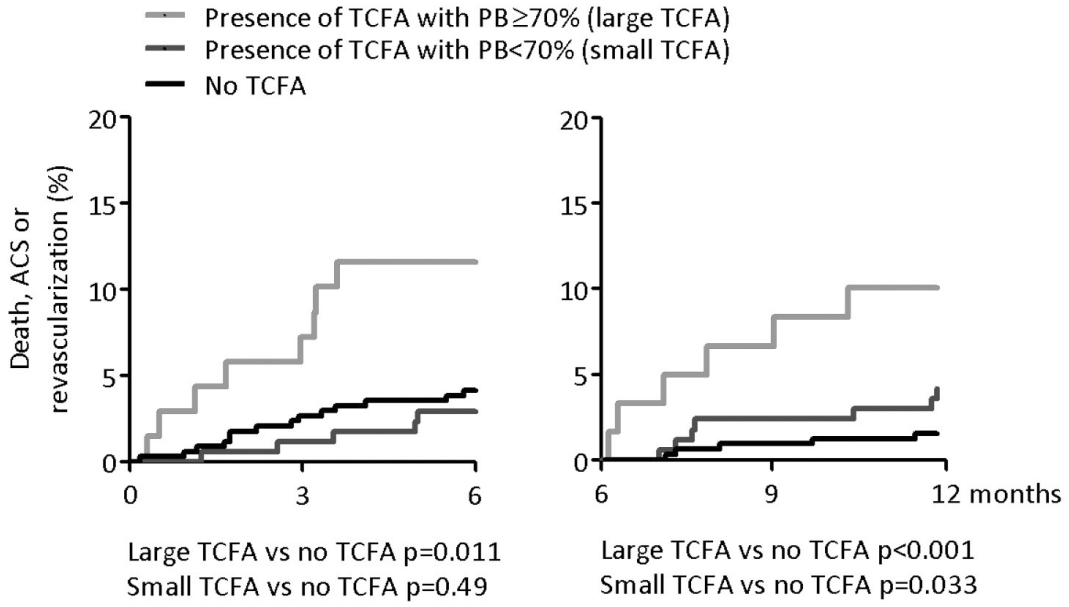


Figure 4 Associations with of short- and long-term major adverse cardiac events

P values are obtained with log-rank test. Overall P value 0-6months is 0.009, overall P value 6-12 months is 0.002.
PB, plaque burden; TCFA, thin-cap fibroatheroma.

Associations with incident major adverse cardiac events

Patients who did not had any lesion in the imaged coronary segment seemed to have a lower occurrence of MACE (absent 4.1% vs. present 8.3%; HR: 0.48, 95% CI: 0.15–1.54; P = 0.22) and a lower occurrence of the composite of death or ACS only (absent 1.4% vs. present 5.4%; HR: 0.25, 95% CI: 0.034–1.83; P = 0.17), although these associations were not statistically significant. The amount of the necrotic core in the imaged coronary segment was associated with MACE (see Supplementary material online, Table S3).

After adjustment for clinical characteristics, the presence of TCFA lesions (present 10.8% vs. absent 5.6%; adjusted HR: 1.98, 95% CI: 1.09–3.60; P = 0.026) and lesions with a plaque burden of at least 70% (present 16.2% vs. absent 5.5%; adjusted HR: 2.90, 95% CI: 1.60–5.25; P < 0.001) were independently associated with a higher occurrence of MACE, while the presence of lesions with a minimal luminal area of 4.0 mm2 or less was not (present 9.4% vs. absent 7.1%; adjusted HR: 1.23, 95% CI: 0.67–2.26; P = 0.50) (Table 3 and see Supplementary material online, Table S4). There was no heterogeneity in the HR estimates between patients admitted with and without ACS (heterogeneity P = 0.31 for TCFA, P = 0.58 for the plaque burden of at least 70% and P = 0.65 for the minimal luminal area of 4.0 mm2 or less). Calcified TCFA lesions seemed to carry higher risk than non-calcified TCFA lesions, although the difference was not statistically significant (P = 0.32) (see Supplementary material online, Figure S3). The presence of TCFA lesions was also significantly associated with the composite of death or ACS only (present 7.5% vs. absent 3.0%; adjusted HR: 2.51, 95% CI: 1.15–5.49; P = 0.021).

Risk for the occurrence of MACE was further increased if the TCFA lesions had a minimal luminal area of 4.0 mm2 or less, had a plaque burden of at least 70%, or a combination of these three characteristics (Figure 3 and see Supplementary material online, Figure S4). Thin-cap fibroatheroma lesions with a plaque burden of at least 70% were associated with a higher MACE rate both in the first 6 months (P = 0.011) and after 6 months (P < 0.001) of follow-up, while smaller TCFA lesions were only associated with a higher MACE rate after 6 months (P = 0.033) (Figure 4).

DISCUSSION

This study investigated the prognostic value of in vivo high-risk plaque detection by IVUS for the occurrence of MACE in patients undergoing coronary angiography. In line with previous studies, we found that the presence of a TCFA lesion as assessed by IVUS in a non-culprit coronary artery was independently predictive for the occurrence of MACE that was not related to the index procedure.[10,14] The event rate was even further increased when patients had a TCFA lesion with a minimal luminal area of 4.0 mm2 or less, a plaque burden of at least 70%, or a combination thereof. Our study is the first to demonstrate that the presence of such vulnerable coronary lesions as assessed in vivo by IVUS is significantly associated with the occurrence of acute cardiac events (composite of death or ACS only) that were not related to the index procedure. Furthermore, we found that patients with a large TCFA lesion (with a plaque burden of at least 70%) were at a higher risk than patients with a small TCFA lesion. The presence of a small TCFA lesion was only predictive for clinical events occurring on the longer term (after 6 months).

Although the PROSPECT and the Virtual histology Intravascular ultrasound in Vulnerable Atherosclerosis (VIVA) studies have previously reported on the prognostic value of vulnerable plaque detection by IVUS, there are some limitations to the conclusion of these studies.[10,14] First, the PROSPECT study only enrolled ACS patients. Therefore, the conclusions of this study cannot be directly extrapolated to patients with stable angina. In contrast, our study presents a patient population that underwent coronary angiography for ACS or stable angina and that may better reflect the 'real world' clinical practice. Secondly, the vast majority of events in the PROSPECT study consisted of rehospitalizations for unstable or progressive angina (69 out of the 74 patients with primary composite endpoint), while the majority of events in the VIVA study consisted of coronary revascularizations (14 out of the 16

patients with primary composite endpoint). Our study demonstrated that vulnerable coronary lesions as assessed in vivo by IVUS are significantly associated with the occurrence of acute cardiac events (composite of death or ACS only) that were not related to the index procedure. Finally, an important difference is that IVUS was performed in three coronary vessels in the PROSPECT and VIVA studies. Our study demonstrated that IVUS in only one non-culprit vessel is sufficient for prognostication. This finding is relevant for the use of IVUS in daily clinical practice, since IVUS acquisition and analysis of three vessels is more time consuming and may increase risk for complications.

Previous studies have demonstrated that coronary atherosclerotic plaque burden as assessed with coronary computed tomography angiography or IVUS is associated with the progression of the lesion and with incident clinical events during the follow-up.[15–17] Similarly, the PROSPECT and the VIVA studies have shown that lesions with a plaque burden of at least 70% were strongly associated with their primary endpoint.[10,14] In the Prediction of Progression of Coronary Artery Disease and Clinical Outcome Using Vascular Profiling of Shear Stress and Wall Morphology (PREDICTION) study, large plaque burden and low local endothelial shear stress were also independently associated with the progression of the lesion and narrowing of the lumen.[18] In accordance with these observations, we found that patients with a coronary lesion that had a plaque burden of at least 70% were at a higher risk for MACE. However, the presence of a lesion with a plaque burden of at least 70% was not significantly predictive for the composite of death or ACS only. These findings suggest that lesions with a high plaque burden are at high risk to cause a flow-limiting stenosis, requiring coronary revascularizations, and rehospitalizations for progressive angina.

Thin-cap fibroatheroma is the most common pathological substrate of ACS and has been found to be associated with incident cardiac events.[19] In the PROSPECT study, non-culprit lesions associated with recurrent events (mainly driven by rehospitalizations) were more likely to be classified as TCFA on the basis of radiofrequency IVUS (adjusted HR: 3.35, 95% CI: 1.77–6.36; $P < 0.001$).[10] In the VIVA study, the presence of a non-calcified TCFA lesion was the only factor that was associated with MACE, which was mainly driven by coronary revascularizations (unadjusted HR: 1.79; 95% CI: 1.20–2.66, $P = 0.004$).[14] Likewise, we found that the presence of TCFA lesions as assessed with IVUS was independently predictive for MACE (adjusted HR: 1.98, 95% CI: 1.09–3.60; $P = 0.026$). Furthermore, the predictive value of TCFA lesions for the occurrence of acute cardiac events (composite of death or ACS only) was even stronger (adjusted HR: 2.51, 95% CI: 1.15–5.49; $P = 0.021$). These findings emphasize the biological importance of TCFA for plaque rupture.

We have also found that patients with a large TCFA lesion (with a plaque burden of at least 70%) were at a higher risk than patients with a small TCFA lesion. Furthermore, large TCFA lesions were associated with a higher MACE rate within and after 6 months of follow-up, while smaller TCFA lesions were only associated with a higher MACE rate after 6 months. Based on these observations, it can be hypothesized that large TCFA lesions are more vulnerable and more prone to rupture, while small TCFA lesions may grow in time and may become more vulnerable in the future. In line with our findings, two previous studies have demonstrated that the majority of the untreated non-culprit TCFA lesions retain their TCFA morphology during the follow-up (6–13 months), and may be accompanied by a decrease in the minimal luminal area and an increase in the necrotic core.[20–21] An other small study of patients with a lower risk profile, however, has demonstrated that the majority of the TCFA lesions was healed after 1 year.[22]

Different MACE definitions have been used in the above-mentioned studies (death, ACS and unplanned revascularization in our study; cardiovascular death, cardiac arrest, myocardial infarction and rehospitalization due to unstable or progressive angina in the PROSPECT study; death, myocardial infarction, and unplanned revascularization in the VIVA study).[10,14] Therefore, MACE rates of these studies cannot be directly compared. Nevertheless, the incidence of MACE seemed to be relatively high in our study population. For example, 18 deaths occurred in 581 patients within 1 year in our study compared with 2 deaths in 170 patients within 625 days in the VIVA, 31 deaths in 697 patients within 3.4 years in the PROSPECT, and 4 deaths in 506 patients within 9 months in the PREDICTION

study.[10,14,18]However, the MACE rate in our study was consistent with that of previous 'all-comer' registries in our hospital, which further emphasizes that our study population may better reflect the 'real world' clinical practice.[23,24]

Some limitations of this study need to be acknowledged. First, this is a prospective observational cohort study. Although we aimed to include a patient population that reflects clinical practice, those patients with any of the exclusion criteria could not be included in this study.11 Secondly, the spatial resolution of IVUS virtual histology (150 μm) is insufficient to exactly replicate histopathological definitions of a thin fibrous cap (<65 μm).25 Therefore, IVUS virtual histology tends to over-estimate the number of TCFA lesions. Nevertheless, the presence of IVUS virtual histology detected TCFA lesions has prognostic information and is therefore clinically relevant. Thirdly, the relatively small number of endpoints did not allow us to evaluate whether adding IVUS imaging to a prognostic model with conventional risk factors would result in improved risk prediction. Finally, repeat intracoronary imaging with IVUS virtual histology was not performed. Therefore, the dynamic nature of coronary artery lesion morphology could not be investigated. Large, future studies (e.g. IBIS-3, www.trialregister.nl identifier NTR2872) may provide useful data in this respect.[26]

In conclusion, IVUS virtual histology appeared to be a useful tool for in vivo detection of high-risk coronary lesions. In patients undergoing coronary angiography, the presence of IVUS virtual histology-derived TCFA lesions in a non-culprit coronary artery is strongly and independently predictive for the occurrence of MACE, particularly of death and ACS. Thin-cap fibroatheroma lesions with a large plaque burden are of a higher risk than small TCFA lesions, especially on the short term.

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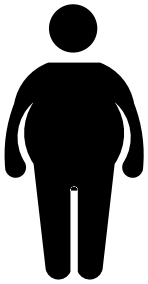
2.4

DETERMINANTS OF HIGH CARDIOVASCULAR RISK IN RELATION TO PLAQUE-COMPOSITION OF A NON-CULPRIT CORONARY SEGMENT VISUALIZED BY NEAR-INFRARED SPECTROSCOPY IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION



DETERMINANTS OF HIGH CARDIOVASCULAR RISK IN RELATION TO PLAQUE-COMPOSITION OF A NON-CULPRIT CORONARY SEGMENT VISUALIZED BY NEAR-INFRARED SPECTROSCOPY IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

ABSTRACT

Aim

The aim of this study was to determine the relation between clinical and blood characteristics of a vascular inflammatory milieu and coronary plaque composition visualized by near-infrared spectroscopy (NIRS) in percutaneous coronary intervention (PCI) patients.

Methods and results

Between April 2009 and January 2011 we performed NIRS in 208 patients who underwent PCI or invasive diagnostic coronary exploration for various indications. Imaging was performed of one non-intervened coronary segment after the initial procedure. Univariate and multivariate linear regression analyses were applied to evaluate the relationship between the acquired NIRS-derived lipid core burden index (LCBI) and clinical and blood (lipids and hs-CRP) characteristics. Patients with a history of hypercholesterolemia (median 48 (interquartile range 21-101) vs. 38 (13-70), p=0.043) and multi-vessel disease (55 (24-104) vs. 32 (12-71), p=0.012) had higher LCBI levels. Men had higher LCBI than women (48 (21-95) vs. 27 (9-59), p=0.003). Hypercholesterolemia and gender remained significant in multivariate regression analysis, whereas also a history of noncardiac vascular disease and beta-blockers were positively associated with LCBI. Altogether 23.2% of the variability in LCBI could be explained by clinical and blood characteristics.

Conclusion

Clinical characteristics reflecting patients with a high cardiovascular risk profile explained 23.2% of the variability in LCBI, whereas blood biomarkers added little. Further research is warranted to evaluate if NIRS has the potential to provide additional prognostic information about patients' cardiovascular risk.

INTRODUCTION

Acute coronary syndromes are a major cause of mortality and morbidity in Western countries.[1, 2] Rupture of vulnerable coronary plaques, often inflamed lipid rich lesions with large necrotic cores with only a thin overlying fibrous layer of intimal tissue, are most likely to cause these acute coronary syndromes. [3-7] Several well-established and emerging cardiovascular risk factors have been related to the occurrence and progression of coronary atherosclerosis [8-10] and the predisposition of patients to plaque rupture or acute coronary thrombosis. [11] An important risk factor for the development of coronary atherosclerosis is the lipid profile[12], which accounts for approximately 50% of the population attributable risk of first acute myocardial infarction (AMI). [13] A meta-analysis of 26 randomised trials showed that lowering low-density lipid (LDL) cholesterol is inversely associated with rates of both major coronary and vascular events.[14] Therefore the lipid profile is widely accepted as a determinant for risk stratification[15] and primary target of therapy. [16] Another established risk factor for coronary atherosclerosis is hs-CRP. Subjects with elevated levels of hs-CRP are at increased risk of coronary heart disease.[17] Furthermore, as demonstrated by the Jupiter trial, rosuvastatin significantly reduced the incidence of major cardiovascular events in seemingly healthy persons without hyperlipidaemia but with elevated hsCRP levels. [18]

Near-infrared spectroscopy (NIRS), a novel catheter based imaging modality that determines composition of tissue, has the potential to identify lipid-core containing coronary plaques (LCP) in patients. The accuracy of NIRS has been validated in coronary autopsy specimens and subsequently in vivo. This intra-coronary system utilizes the variation in reflection of the emitted near-infrared light to detect LCP, which is visualized as a (block) chemogram and qualified by a lipid-core burden index (LCBI) score. [19, 20] Detection of these LCPs in coronary arteries might potentially be used for enhanced risk stratification, prevention and treatment of coronary events. We have reported on the relation between NIRS, Framingham Risk Score and renal function in patients who underwent successful percutaneous coronary intervention (PCI) or invasive diagnostic coronary exploration. [21, 22]

The aim of the current study was to provide a thorough and broad analysis of the patient characteristics that are (potentially) associated with NIRS derived LCBI, in particular blood lipids and hs-CRP. Logically, we do expect higher LCBI levels in patients with a clinical profile corresponding with increased CHD risk.

METHODS

Study population

We studied 208 patients who underwent NIRS, between April 2009 and January 2011, in the Thoraxcenter, Erasmus MC, Rotterdam, the Netherlands, a tertiary referral hospital. Patients were 18 years of age or older, had stable angina pectoris (AP) or an acute coronary syndrome (ACS) and underwent successful PCI or invasive diagnostic coronary exploration of one or more lesions in one or two native coronary arteries. Patients who underwent CABG were not included in this study. After the initial procedure (PCI or invasive diagnostic coronary exploration) patients underwent invasive imaging (NIRS and intravascular ultrasound (IVUS)) of one non-culprit vessel (i.e. the vessel of interest for this study), with a diameter stenosis <50% throughout a target segment of at least 40 mm in length by angiographic visual estimation. These study vessels were accessible to the LipiScan (InfraReDx, Burlington, Massachusetts) catheters and per protocol selected in the following way:

If LAD vessel has been treated, RCA should be selected (if not possible: LCX);
If RCA vessel has been treated, LAD should be selected (if not possible: LCX);
If LCX has been treated, LAD or RCA should be selected.

In case patients underwent an invasive diagnostic procedure, the selection of the study vessel was left to the discretion of the interventional cardiologist.

The medical ethical committee of our hospital approved the study and a written informed consent was obtained of every patient.

Near-infrared spectroscopy (NIRS)

The NIRS system consists of a rapid exchange catheter, a pullback and rotation device, and a console. The catheter is 3.2 F in diameter and 165 cm in usable length. The catheter is intended to be introduced into the vasculature via a 0.014-inch coronary guidewire, which is allowed to exit approximately 25 mm proximal to the distal tip. Two low-profile polymer markers are placed on the stiff proximal shaft to aid the user in locating the exit of the distal tip through the tip of a guiding catheter.

All the study coronary vessels included were accessible to the Lipiscan (InfraReDx, Burlington, Massachusetts) catheters and had a <50% reduction in lumen diameter by angiographic visual estimation throughout a target segment of at least 40 mm in length. Coronary vessels that received a bypass graft with a minimal lumen diameter <2 mm or with a diameter stenosis >50% by angiographic visual estimation in the segments to be analyzed were excluded. There were no complications related to the imaging procedure. A motorized catheter pullback, at 0,5 mm/s and 240rpm, was performed starting distal to a side branch and acquiring 1000 NIRS measurements/12,5 mm of scanned coronary artery. The region of interest (ROI) was defined as the longest coronary segment, between two clearly identifiable landmarks (i.e. side branches).

The measured probability of the LCP from each scanned coronary segment was displayed as a map, the chemogram; yellow regions represent those with highest probability for the presence of the LCP, while red regions represent those with lowest probability. The chemogram displays the pullback position against the circumferential position of the measurement in degrees (Figure 1).

Based on the chemogram the LCBI was calculated, representing the fraction of valid pixels in the chemogram that are yellow, multiplied by a factor of 1000. The LCBI is a summary measure of the amount of LCP along the entire interrogated length of the vessel on a 0-to-1000 scale.

NIRS images were prospectively analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) unaware of the clinical or lab data.

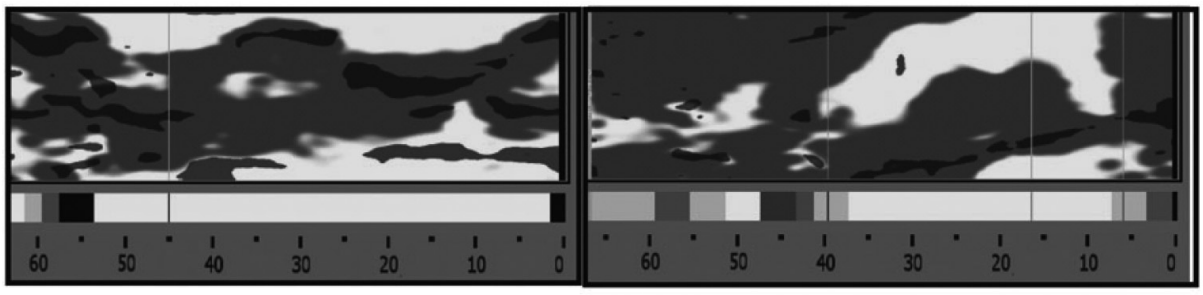


Figure 1 Chemogram of two patients

Intravascular ultra sound (IVUS)

IVUS was acquired with Volcano™ s5/s5i Imaging System using Volcano™ Eagle Eye™ Gold IVUS catheter (20 MHz) at a standard pull back speed of 0.5mm per second with automatic pullback system. During motorized catheter pullback, at 0.5 mm/s, grayscale IVUS and raw radiofrequency data capture

gated to the R-wave were recorded.
All baseline IVUS images were prospectively analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands). The IVUS analyses were performed with VIAS software (Volcano Corporation). Contour detection was performed by experienced IVUS analysts who were blinded to the NIRS results. Quantitative grayscale IVUS measurements included vessel area, lumen area, plaque area (vessel area - lumen area) and plaque burden ([plaque area/vessel area] * 100%).

Biomarker analysis
Routine blood for biomarker assessment was drawn prior to the index procedure and analyzed at our department of medical microbiology & infectious diseases, diagnostic laboratory Erasmus MC, Rotterdam, the Netherlands (accreditation based on ISO/IEC 15189:2003 and CCKL-regulations).

Data analysis
We present patient-level analyses. NIRS and IVUS-VH data are derived from measurements in the non-culprit (study) vessel and are presented as one quantity for each patient.
Continuous variables are presented as mean ± standard deviation or median and interquartile ranges (IQR) as appropriate, categorical variables are expressed as numbers and percentages. The Kolmogorov-Smirnov test was used to analyse whether continuous data were normally distributed. Nonparametric continuous variables were compared using the Mann-Whitney U test, and are presented as median and interquartile range(IQR). If further statistical tests required normal distribution of the data, then log or square root transformations were applied.
We intended to obtain complete information in all patients, but failed to do for the following 7 variables: hs-CRP, HDL, LDL, creatinine, total cholesterol, triglycerides and BMI. Using missing value analysis (MVA) we evaluated the extent of missing data and searched for patterns of missing data. MVA showed that there were 3.3% missing values on average. For the patients with at least one of the variables of interest missing, we decided to impute the missing values by multiple imputation.[23] (We also applied a complete case sensitivity analysis, showing consistent results, which we will not present.)
The baseline creatinine values were used to calculate the creatinine clearance according to the Cockroft and Gault formula: creatinine clearance (millilitres/minute) = (140-age) x weight (kilograms) ÷ 72 x serum creatinine (milligrams/decilitre) (x0.85 for women).[24]
Correlation plots were constructed, and univariate and multivariate linear regression (LR) analyses were applied to reveal clinical characteristics that were related to LCBI. LCBI was considered the dependent variable, and all variables of interest (table1) were considered potential determinants. All baseline variables described in table 1 entered the multivariate stage. All variables that had a p-value <0.5 in univariate analysis, based on Wald's chi-square test, were used to construct the multivariate model. The R-squared statistic is presented, which indicates the percentage of the variance in the dependent variable that can be "explained" by the determinant.
Subsequently, we studied the correlation between NIRS derived LCBI and IVUS-derived plaque burden. We present correlation plots and report Pearson's correlation coefficient. This analysis has caveats, which are mainly due to the fact that we used different cathethers for the IVUS-VH and NIR measurements. The described correlation was based on segment-level data, whereas, for technical and logistic reasons, the analysed segments for IVUS-VH and NIR were not synchronized at the level of frames. Furthermore, the length of the analysed segments was not necessarily the same for both techniques.
Univariate and multivariate linear regression analyses were applied to study the association between NIRS derived LCBI and clinical risk factors (independent variables) and IVUS-derived plaque burden (dependent variable). The data of 12 patients could not be used, because of problems with the IVUS analysis software.
Finally, we studied the relation between blood lipid levels, hs-CRP and LCBI according to the quartiles of their distribution. For all tests, a two-sided P-value of 0.05 or less was considered significant. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL).

Table 1: Baseline and procedural characteristics of the NIRS-patients (n=208)

		Clinical characteristics n (%)	LCBI median (IQR)
Demographic characteristics			
Age mean years ±SD		63.5±10.9	
Male		153 (73.6)	48 (21-98)
Female		55 (26.4)	27 (9-59)
Indication for invasive coronary procedure			
Stable angina		110 (52.9)	37,5 (15-94)
NSTE-ACS		68 (32.7)	48 (16-94)
STEMI		30 (14.4)	36,5 (15-68)
Cardiovascular history			
Cardiac history	Yes	107 (51.4)	46 (15-87)
	No		41 (16-87)
History of PAD, CVA/TIA	Yes	18 (8.7)	63,5 (40-108)
	No		40 (15-82)
Risk factors			
Current smoking	Yes	50 (24.0)	44.5 (14-93)
	No		40.5 (23-70)
Hypertension	Yes	117 (56.3)	43 (16-89)
	No		41 (16-89)
Hypercholesterolemia	Yes	118 (56.7)	47.5 (21-101)
	No		38 (13-70)
Diabetes	Yes	41 (19.7)	55 (12-93)
	No		40 (16-87)
Non-insulin dependent		28 (68.3)	
Insulin dependent		13 (31.7)	
Multi-vessel disease	Yes	84 (59.6)	55 (24-104)
	No		32 (12-71)
Family history	Yes	123 (59.1)	40 (13-95)
	No		43 (21-70)
GFR mean±SD		107±37	
Body-mass index mean±SD		27.7±4.4	
Non-culprit vessel			
RCA		60 (28.8)	46.5 (15-109)
LAD		75 (36.1)	32 (13-67)
LCX		73 (35.1)	48 (24-82)
Medication			
Aspirin		203 (97.5	
Clopidogrel		194 (93.3)	
Beta-blocker		159 (76.4)	
RAAS inhibitor		150 (72.1)	
Statin		187 (89.9)	
Blood lipid levels and hs-CRP (mmol/L)		Median (IQR)	
Total cholesterol		4.20 (3.60-5.20)	
High-density lipoprotein		1.14 (0.92-1.35)	
Low-density lipoprotein		2.46 (1.92-3.20)	
Triglycerides		1.27 (0.92-1.79)	
HS-CRP (mg/l)		3.34 (1.26-5.78)	

Data are presented as percentages or means (±SD). SD = Standard deviation; NSTE-ACS= non-ST-segment elevation acute coronary syndrome (NSTE-ACS) STEMI= ST-segment elevation myocardial infarction; PCI = Percutaneous Coronary Intervention; CABG = Coronary Artery Bypass Graft; PAD = Peripheral Arterial Disease; COPD = Chronic Obstructive Pulmonary Disease; HS-CRP = High-Sensitive CRP; GFR= glomerular filtration rate; LCBI = Lipid Core Burden Index;RCA = Right Coronary Artery; LAD = Left Anterior Descending Coronary Artery; LCX = Left Circumflex Coronary Artery.

Table 1 Baseline and procedural characteristics of the NIRS-patients (in=208)

RESULTS

Study population and baseline characteristics

The baseline and procedural characteristics of the included patients are presented in Table 1. The studied population consisted mostly of men (79.6%) and the mean age was 63.5 years. Most patients were treated for stable AP (52.9%) or non-ST-segment elevation acute coronary syndrome (NSTE-ACS) (32.7%). Prior MI (38.0%) and prior PCI (38.9%) in the cardiac history were common. The prevalence of hypercholesterolemia, positive family history for CHD, multi-vessel disease and/or hypertension was more than 50%. Almost 20% of the patients were diabetic; the non-insulin dependent type was most common. The distribution of the studied coronary vessels (RCA 28.8%, LAD 36.1%, and LCX 35.1%) was about equally divided. The median total cholesterol level was 4.20 (IQR 3.60-5.20) mmol/l, LDL level 2.46 (1.92-3.20) mmol/l, high-density lipoprotein (HDL) of 1.14 (0.92-1.35) mmol/l, triglycerides 1.27 (0.92-1.79) mmol/l, and a high-sensitive CRP 3.34 (1.26-5.78) (mg/dl). The median NIRS-derived LCBI was 42.5 (16.0-85.0).

Univariate	Beta	S.E.	p-value	R-squared
Demographic characteristics				
Age	-0.035	0.025	0.16	0.010
Male	1.75	0.60	0.004	0.039
Indication for invasive coronary procedure				
			0.34	0.011
Stable angina	1			
NSTE-ACS	0.76	0.61		
Acute MI	-0.32	0.80		
Cardiovascular history				
Cardiac history	0.03	0.54	0.96	0.000
history of PAD and/or CVA/TIA	1.51	0.96	0.12	0.007
Risk factors				
Current smoking	-0.17	0.63	0.79	0.000
Hypertension	-0.11	0.55	0.85	0.000
Hypercholesterolemia	1.38	0.54	0.011	0.031
Diabetes	0.02	0.68	0.98	0.000
Multi-vessel disease n, %	1.11	0.58	0.044	0.020
Family history	0.52	0.54	0.34	0.004
GFR	0.006	0.009	0.54	0.006
Body-mass index	-0.02	0.06	0.71	0.001
Medication				
Aspirin	2.14	1.76	0.23	0.007
Clopidogrel	1.00	1.08	0.36	0.004
Beta-blocker	-1.50	0.63	0.019	0.027
RAAS inhibitor	-0.96	0.60	0.11	0.012
Statin	-0.12	0.86	0.89	0.000
Blood lipid levels and hs-CRP				
Total cholesterol (mmol/l)	-0.33	0.29	0.26	0.009
High-density lipoprotein(mmol/l)	0.41	0.32	0.97	0.000
Low-density lipoprotein (mmol/l)	0.20	0.02	0.52	0.003
Triglycerides (mmol/l)	0.41	0.32	0.20	0.011
HS-CRP (mg/l)	0.026	0.02	0.25	0.035

SE = Standard error; MI = Myocardial Infarction; HS-CRP = High-Sensitive CRP; GFR=glomerular filtration rate; LCBI = Lipid Core Burden Index

Table 2a Baseline variables and their unvariable association with LCBI

Multivariate	Beta	S.E.	p-value	R-squared
				0.232
Age	-0.03	0.03	0.23	
Sex	1.62	0.61	0.007	
Stable angina	1			
NSTE-ACS	1.12	0.65	0.08	
Acute MI	-0.36	0.92	0.70	
History of PAD and/or CVA/TIA	2.32	0.96	0.015	
Hypercholesterolemia	1.29	0.55	0.020	
Family history	0.55	0.52	0.29	
Multi-vessel disease	0.94	0.54	0.08	
Aspirin	2.31	1.65	0.16	
Clopidogrel	-1.09	0.63	0.16	
Betablocker	-1.91	0.63	0.003	
RAAS inhibitor	-0.37	0.53	0.49	
Total cholesterol (mmol/l)	0.52	0.30	0.09	
Triglycerides (mmol/l)	0.002	0.35	0.99	
HS-CRP (mg/l)	0.02	0.02	0.23	

SE = Standard error; MI = Myocardial Infarction; HS-CRP = High-Sensitive CRP; LCBI = Lipid Core Burden Index
GFR=glomerular filtration rate

Table 2b Baseline variables and their unvariable association with LCBI

Association between cardiovascular risk factors and LCBI

The univariate and multivariate association between baseline characteristics and LCBI is shown in table 2a and 2b. When patients were stratified on indication for invasive coronary procedure no differences (p=0.49) in LCBI was observed: median (interquartile range) in stable angina 37.5 (15-94), NSTE-ACS 48 (16-94) and STEMI 36.5 (15-68). Patients with history of hypercholesterolemia (median 48 (IQR 21-101) vs. 38 (13-70), p=0.043) and multi-vessel disease (55 (24-104) vs. 32 (12-71), p=0.012) had higher LCBI levels. Men had higher LCBI than women (48 (21-95) vs. 27 (9-59), p=0.003). Except for multi-vessel disease, these variables remained significantly associated with LCBI after adjustment for the selected confounders in multivariate analysis. Also a significant association between history of PAD and/or CVA/TIA, beta-blockers, and LCBI was found. Altogether 23.2% of the variability in LCBI could be explained by the variables that compose the model.

LCBI and and IVUS-derived plaque burden

Figure 2 shows the correlation between LCBI and IVUS-derived plaque burden, as well as the relation between the quartiles of the distributions of LCBI and plaque burden. We observed a significant correlation between the two imaging modalities based on the datapoints in individual patients (Pearson's $r = 0.289$, $p<0.001$), and the quartiles of the distributions of % plaque burden and LCBI, $p<0.001$.

Except for a history of PAD and/or CVA/TIA none of the baseline characteristics were significantly associated with IVUS-derived plaque burden in univariate analysis. This variable remained significantly associated with IVUS-derived plaque burden after adjustment for the selected ($p<0.5$) confounders (age, sex, indication, hypertension, multi vessel disease, aspirin, clopidogrel, beta-blockers, statins and total cholesterol). Altogether 14.0% of the variability in IVUS-derived plaque burden could be explained by the variables that compose the model.

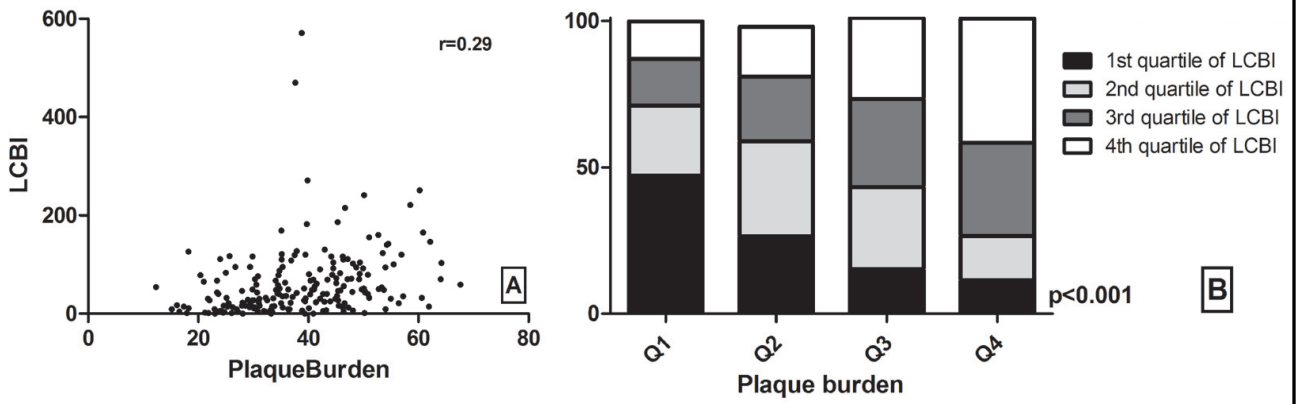


Figure 2A Scatter plot of plaque burden (%) in relationship with LCBI
Figure 2B Quartiles of the distribution of lipid core burden index (LCBI) in relation to the quartiles of plaque burden (%).

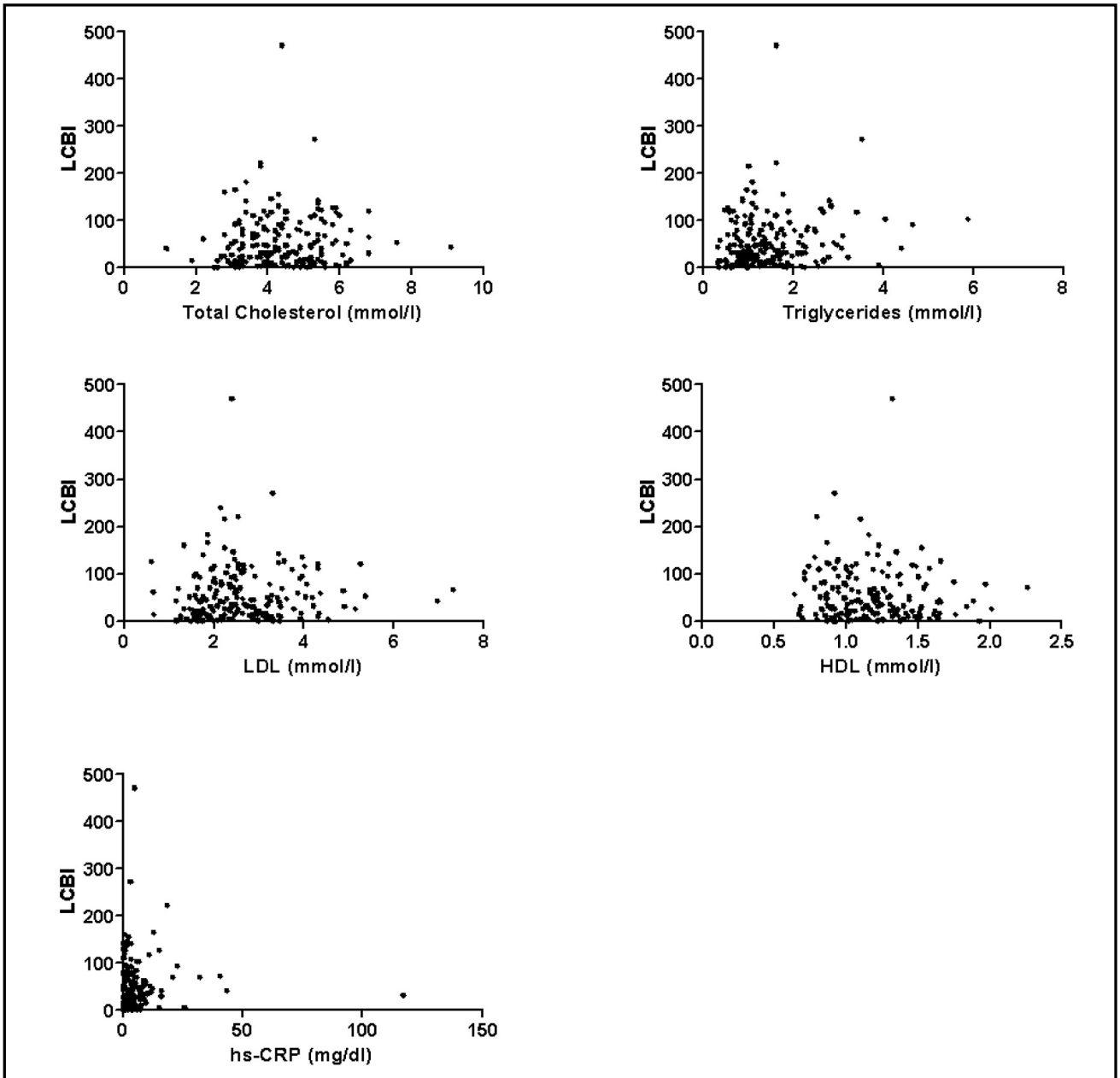


Figure 3 Scatter plots of lipid levels and hs-C-reactive in relationship wit lipid core burden index

Association blood biomarkers and LCBI

Correlation plots (figure 3) and regression analyses showed no (significant) correlation between blood biomarkers and LCBI. The R-squared for blood lipid levels and hs-CRP rated between 0.000 and 0.035 (table 2a), indicating that these variables only explain little of the variability in LCBI between the enrolled patients. Figure 4 shows the lipid core burden index according to quartiles of blood lipid levels and hs-CRP. A weak, but non-significant relation is visible between hs-CRP and LCBI: 40 % of the patients in the lowest quartile (Q1) of hs-CRP had LCBI in Q1, whereas 12 % of the patients in the highest quartile (Q4) of hs-CRP had LCBI a in the first quartile. No relations were visible between other blood biomarkers and LCBI.

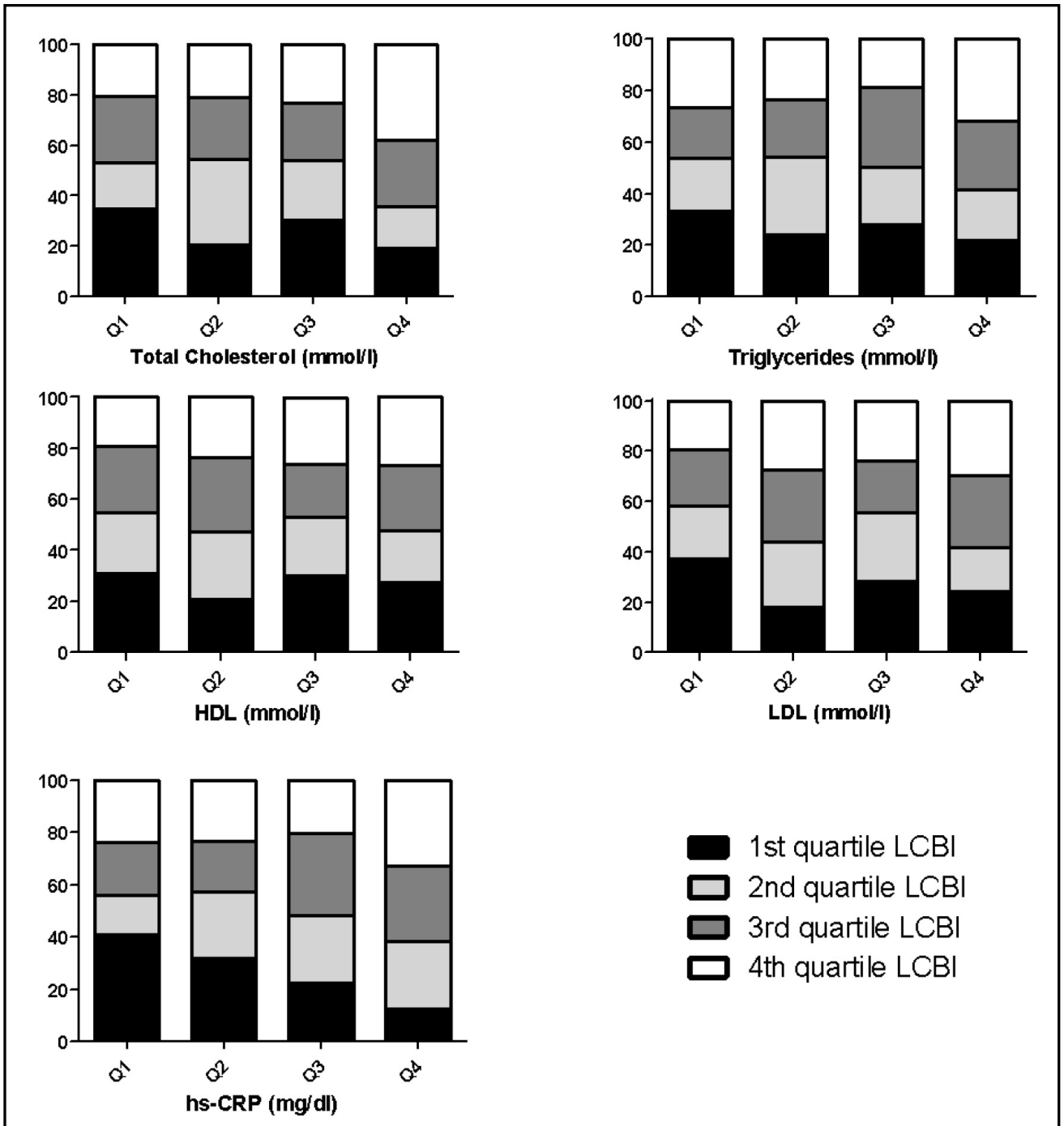


Figure 4 Quartiles of the distribution of lipid core burden index in relation to the quartiles of blood lipid levels and hs-Creactive protein

DISCUSSION

In this single centre cross-sectional study clinical characteristics and blood biomarkers explained 23.2% of the variability in NIRS-derived LCBI score, an indicator for LCP in coronary arteries. As increased levels of lipids and hs-CRP increase the likelihood of LCPs in the coronary vessel, relations between these blood biomarkers and LCBI were expected. However, lipid profile and hs-CRP provided little additional information about the LCBI score in a non-intervened coronary artery segment: a maximum of 3.5 % of the variability in LCBI could be explained by these biomarkers.

The clinical characteristics; hypercholesterolemia, male sex, use of beta-blockers and history of peripheral artery disease PAD and/or cerebral vascular accident/ transient ischemic attack (CVA/TIA) were all significantly associated with LCBI, reflecting patients with a high cardiovascular risk profile. Our finding of an association between history of hypercholesterolemia and LCP in the coronary vessels is in accordance with previous studies that found an association between (treated) hypercholesterolemia and IVUS-derived necrotic core-rich coronary plaques.[25 26] Simsek et al. found no 'dose-dependent' effect of creatinine (as a measure of renal function) on NIRS-derived LCBI score in a previous report of this study.[21]

The relation between gender and LCBI supported other investigations that demonstrated that men have more severe structural and functional atherosclerotic disease in epicardial coronary arteries than women.[27] In fact, the pathophysiology of cardiovascular disease (in particular coronary artery disease) differs significantly between men and women.[28-30] Several study reports suggest that signs and symptoms of CVD experienced by women are related to microvascular ischemia and endothelial dysfunction, more so than in men. Symptomatic women usually have less flow limiting stenoses than men, and are more prone to have plaque erosion with subsequent thrombus formation.[31] Myocardial ischemia in the absence of obstructive coronary disease, but with an increased high risk of adverse outcomes, is an emerging paradigm for women. More research in this field is warranted.

None of the blood biomarkers were independently predictive of LCP in the coronary vessels. This lack of association might have various reasons. First, we might have studied the wrong biomarkers. However both blood lipid levels and hs-CRP are well-established risk factors/ markers for the development/ identification of coronary atherosclerosis. A reason for this lack in association between blood biomarkers and LCBI might be that the vast majority of the patients (89%) were already treated with statins at the time of the procedure. We found relatively low level of cholesterol, LDL and triglycerides in our study population. As shown previously there is a linear relation between LDL cholesterol and annual changes in plaque size, however in patients with low LDL values no plaque progression was found.[32] Apparently, LDL levels influence plaque growth and probably consequently also plaque size. So, since patients treated with statins have lower levels of LDL, they might have as a result relatively small plaques. Furthermore, statins influence coronary plaques via other mechanisms as in patients without hyperlipidaemia the incidence of major cardiovascular events is also reduced by statin use.[18] Consequently, the use of blood biomarkers in statin users as determinant for LCP may be confounded. In accordance, Nicholls et al. found no relation between LDL cholesterol and hsCRP and IVUS derived disease burden. It should be noted that all patients in this study were treated with statins.[33]

Another explanation for the lack of association might be that we visualized 40 mm of a non-culprit vessel, in patients undergoing PCI or invasive diagnostic coronary exploration for various indications, and therefore cannot exclude the possibility that the blood biomarkers are related to the culprit lesion. However ex vivo as well as in vivo studies using IVUS in patients with myocardial infarction have demonstrated the presence of vulnerable plaques in other than the culprit lesion or even culprit artery.[34] Also atherosclerosis in the peripheral vascular system or clinical circumstances can influence blood biomarker levels.

When patients were stratified on indication for invasive coronary procedure (stable angina, NSTE-ACS, or STEMI) no difference in LCBI was observed. This is in contrast with Madder and colleagues who revealed that both target and remote LCPs were more common in patients with ACS than in

those with stable angina.[35]

The expression of atherosclerosis is depended on the interaction of multiple risk factors, including genetic susceptibility, abnormal lipid levels and inflammation. Although we found no association between blood biomarkers and NIRS derived LCBI, there is data to support the hypothesis that blood lipid levels and hs-CRP are independently associated with (the promotion of) plaque vulnerability.[11, 17]

Historical predictors, like blood lipid levels, are frequently used as determinants of cardiovascular risk. If we had demonstrated a strong correlation between these factors and LCP, then NIRS would not provide additional information about a patients' cardiovascular risk. Consequently, as NIRS has the potential of identifying LCP in coronary arteries, indicative of plaque vulnerability, this novel imaging technique might be a promising tool to provide additional information about patients' cardiovascular risk, independent of blood lipid levels. It should be noted; however that NIRS has not yet proved to be a predictor of cardiovascular events at long-term follow-up. Nevertheless, since NIRS is able to indentify extensive LCPs that are associated with a high risk of peri-procedural MI,[36] it is a promising tool that needs further exploration.

This study has some limitations. First, as only one non-culprit vessel was imaged, we lack information on the culprit artery, which may flaw our efforts to assess a relationship between LCBI and blood biomarkers. For the same reason, we were unable to assess the correlation of clinical presentation with LCBI findings as reasons for intervention are closely related to the culprit lesions. Second, this study might lack power to show strong correlations. Third, this is a cross-sectional study; therefore no information is available on the (reversed) progression of LCPs and its relation with lipid levels in this population over time. Future research might focus on the effect of lipid level changes on LCP. Finally, although we had unique information on NIRS-derived and IVUS-derived parameters in the same segment, the observed results should be interpreted with caution, because the analysed segments were not synchronized at the level of frames.

The single centre design of this study guarantees limited variability in the NIRS and blood collection procedures (consistent environment and care protocols) and the use of an independent core laboratory to analyze the NIRS images, assures an independent and accurate analysis according to uniform standards.

Concluding, clinical characteristics reflecting patients with a high cardiovascular risk profile explained 23.2% of the variability in LCBI, whereas blood biomarkers added little. Further research is needed whether NIRS has the potential to provide additional information about patients' cardiovascular risk.

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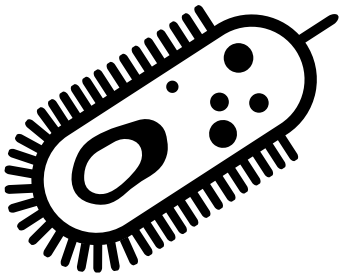
2.5

ANTIBODIES TO PERIODONTAL PATHOGENS ARE ASSOCIATED WITH CORONARY PLAQUE REMODELING BUT NOT WITH VULNERABILITY OR BURDEN ATHEROSCLEROSIS



ANTIBODIES TO PERIODONTAL PATHOGENS ARE ASSOCIATED WITH CORONARY PLAQUE REMODELING BUT NOT WITH VULNERABILITY OR BURDEN

ABSTRACT

Objective

Previous studies have suggested positive associations between periodontal infection and cardiovascular disease. We aimed to investigate the associations of circulating antibodies against periodontal pathogens with 1-year cardiovascular outcome, as well as the extent of coronary atherosclerosis, plaque vulnerability and lesion remodeling on intravascular ultrasound (IVUS) imaging.

Methods

Between 2008 and 2011, radiofrequency IVUS imaging of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography. Immunoglobulin G (IgG) and A (IgA) against P.gingivalis, A.actinomycetemcomitans, T.forsythia and P.intermedia were measured in plasma.

Results

None of the antibody levels were associated with coronary plaque burden, radiofreqeuncy-IVUS-derived thin-cap fibroatheroma lesion morphology or 1-year incidence of major adverse cardiac events (MACE), which included all-cause mortality, acute coronary syndrome and unplanned coronary revascularization. IgA against A.actinomycetemcomitans, T.forsythia and P.intermedia were inversely associated with extent of positive lesion remodeling (OR for highest versus lowest tertile 0.55, 95%CI 0.35-0.88, p=0.012; 0.53, 95%CI 0.32-0.87, p=0.012; and 0.64, 95%CI 0.40-1.02, p=0.061, respectively). In diabetic patients specifically, IgG against P.gingivalis tended to be associated with coronary plaque burden (p=0.080), while IgA against P.gingivalis tended to be associated with incident MACE (p=0.060).

Conclusion

Plasma IgG and IgA against major periodontal pathogens were not associated with the extent of coronary atherosclerosis (with the exception of a trend in diabetics) nor with coronary plaque vulnerability. IgA against periodontal pathogens were inversely associated with extent of coronary remodeling. Altogether, these results do not add evidence for a substantial role of systemic exposure to periodontal pathogens in coronary artery disease.

INTRODUCTION

Periodontitis is a bacterially induced chronic inflammatory disease of tissues supporting the teeth and is highly prevalent (20 to 50%) in the adult population.[1] In the past decades, several epidemiological studies have suggested positive associations between clinically established periodontal and cardiovascular disease,[2-4] and several small experimental studies have proposed potential mechanisms underlying these associations.[2, 5] The mechanism that has been most advocated is bacteraemia followed by vascular contamination by periodontal pathogens.[2, 6] On the other hand, there have also been many studies that failed to demonstrate such associations between periodontal infection and atherosclerosis, particularly after adjusting for confounding variables.[2] Furthermore, evidence that periodontal interventions or systemic antibiotic treatment result in improved cardiovascular outcomes is currently lacking.[2, 7] Therefore, the proposed independent association between periodontal disease and atherosclerosis may still be considered as controversial.

This controversy is further underscored by the nature of the measures that have been used for periodontal infection (i.e. exposure) and atherosclerotic disease (i.e. outcome) in previous studies. The measures for periodontal infection were mostly subjective or based on clinical findings, usually in studies with limited sample size.[2] However, circulating immunoglobulin G (IgG) and immunoglobulin A (IgA) levels against periodontal pathogens may be more accurate measures of periodontal infection and its severity.[8, 9] Furthermore, they may be used in large epidemiological studies. Major periodontal pathogens include Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia and Prevotella intermedia.2 The outcome measures that have been used in previous studies mostly consisted of clinical diagnosis of coronary heart disease (such as history of myocardial infarction), which is a clinical manifestation of the underlying atherosclerosis, but may not be an accurately measure of the extent of atherosclerosis. Conversely, intravascular ultrasound (IVUS) imaging of the coronary arteries allows for accurate measurement of coronary plaque burden, as well as measurement of remodeling of coronary lesions.[10-12] Additionally, IVUS virtual histology (IVUS-VH) (i.e. analysis of IVUS radiofrequency backscatter), allows for tissue characterization and for identification of virtual histology-derived thin-cap fibroatheroma (VH-TCFA) lesions, which have previously been shown to be predictive for future coronary events.[10-15]

This study aims to investigate whether there are positive associations between plasma IgG and IgA-class immunoglobulin levels against four major periodontal pathogens (i.e. P.gingivalis, A.actinomycetemcomitans, T.forsythia and P.intermedia) and the extent of coronary atherosclerosis, coronary plaque vulnerability and coronary remodeling as measured by IVUS, as well as 1-year cardiovascular outcome.

METHODS

Study population

The design of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described in detail elsewhere.[12, 16] In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) (n=318) or stable coronary artery disease (n=263) have been included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands. The ATHEROREMO-IVUS study was approved by the medical ethics committee of the Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all included patients. This study is registered in ClinicalTrials.gov, number NCT01789411.

Antibodies against periodontal pathogens

Blood samples were drawn from the arterial sheath prior to the coronary angiography procedure. The blood samples were further processed in the clinical laboratory of the Erasmus MC, and stored at a temperature of -80oC within 2 hours after blood collection. Subsequently, EDTA-plasma samples (n=575) were transported under controlled conditions (at a temperature of -80oC) to the Laboratory for Vascular Translational Science, INSERM UMRS 1148 , Paris, France, where levels of IgG and IgA against A.actinomycetemcomitans, P.intermedia, P.gingivalis and T.forsythia were measured. In 6 patients, EDTA samples were not available for measurement of antibodies against the periodontal pathogens.

Determination of antibodies against the periodontal bacteria were performed by enzyme-linked immuno sorbent assay (ELISA). One strain of P.intermedia, one strain of T.forsythia, a mixture of six A.actinomycetemcomitans strains and two P.gingivalis strains were used as antigens. The strains were: P.intermedia CIP 104480, T.forsythia CIP 105220, A.actinomycetemcomitans ATCC 29523, ATCC 43718, ATCC 33384, IDH 781, IDH 1705 and C59 (representing serotypes a, b, c, d, e and one nonserotypeable strain respectively) and P.gingivalis CIP 103683, OMGS 434 (representing serotypes a and c). The bacteria were cultivated in medium 20 (composition available on line at www.crbip.pasteur.fr) for the CIP strains and according to the multiserotype ELISA protocol published by Pussinen et al.9 Briefly, the suspensions of fixed bacteria (0.5% formalin-PBS) were centrifuged (5500g for 15 minutes at 4oC) and washed 3 times in PBS. The pellets were resuspended in 0.05 M bicarbonate buffer to obtain an optical density of 0.15 at 580 nm. For serotype mixture, equal volumes of the six A. actinomycetemcomitans or two P.gingivalis strains were mixed and used to coat the 96-well plates. The bacteria were dispended in the plates and were incubated at 37oC for 4 hours and overnight at 4oC. The excess bacteria were removed by washing in 0.05% Tween 20 in PBS and the unspecific binding was blocked by 5% bovine serum albumin in PBS at room temperature for 30 minutes. Two dilutions of each serum were used in each assay. The dilutions used were 1:1500 and 1: 3000 for A.actinomycetemcomitans and P.intermedia, 1:100 and 1:200 for P.gingivalis, 1:400 and 1:800 for T.forsythia. After washing, 100 µl of peroxidase-labelled goat anti-human IgG or IgA (dilution 1:30,000 in 1% BSA-PBS) were added to each well and incubated for 1 hour at room temperature. After washing, o-phenylenediamine dihydrochloride (SigmaFast™ OPD) was used as substrate for peroxidase and the reaction was stopped with 0.5N H_2SO_4 before reading at 492 nm. On each plate, two dilutions of a high and a low control serum in duplicate were added to monitor for the inter-assay variations. The results are expressed in mean optical absorbances.

Coronary intravascular ultrasound imaging

Following the standard coronary angiography procedure, IVUS imaging of the most proximal part of a non-culprit coronary artery was performed. Selection of the non-culprit vessel was predefined in the study protocol. The order of preference for selection of the non-culprit vessel was: 1. left anterior descending (LAD) artery; 2. right coronary artery (RCA); 3. left circumflex (LCX) artery. All IVUS data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS catheter (20 MHz). An automatic pullback system was used with a standard pull back speed of 0.5 mm per second. The baseline IVUS images were sent to an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for baseline patient characteristics, data on anti-periodontal pathogens antibodies and clinical outcomes. The IVUS virtual histology analyses were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software.

The extent, phenotype and remodeling of the coronary atherosclerosis were assessed. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Plaque burden was defined as plaque and media cross-sectional area divided by external elastic membrane cross-sectional area (Figure 1A). A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Using IVUS-VH, the composition of the atherosclerotic lesions was characterized into 4 different tissue types: fibrous, fibro-fatty, dense

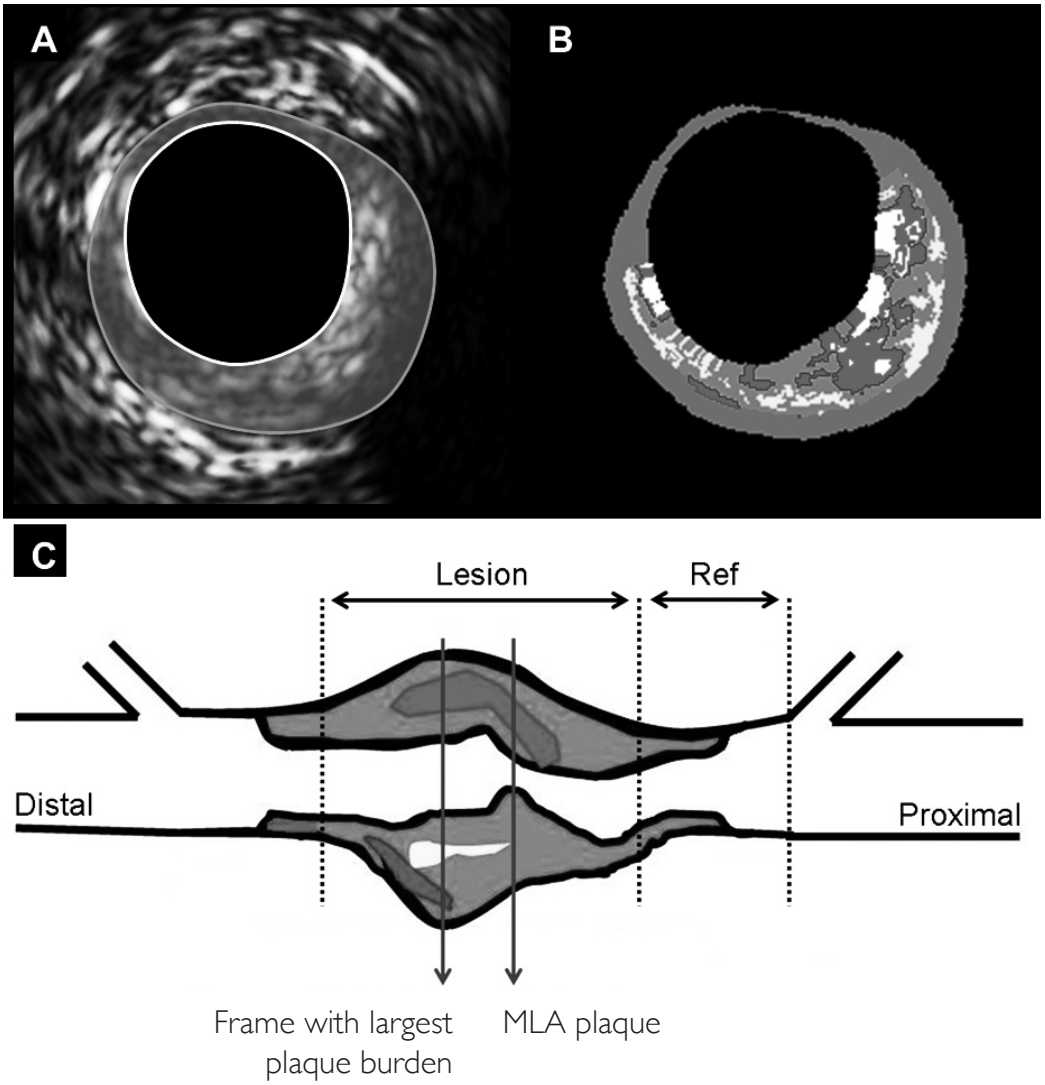


Figure 1. Methodology for intravascular ultrasound measurements
A: Plaque burden is defined as plaque and media cross-sectional area (green) divided by external elastic membrane cross-sectional area (contoured in blue). B: Thin-cap fibroatheroma lesion, defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. C: A lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Remodeling index was calculated by dividing the external elastic membrane cross-sectional area at the site of minimal luminal area (MLA) by the reference external elastic membrane cross-sectional area. Positive remodeling was defined as a remodeling index of >1.05.

calcium and necrotic core.[14] IVUS-VH derived thin-cap fibroatheroma (VH-TCFA) lesion was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen in at least three consecutive frames (Figure 1B).10-13, 15 Remodeling of the plaque lesion was assessed by means of the remodeling index, expressed as the external elastic membrane cross-sectional area at the site of minimal luminal area divided by the reference external elastic membrane cross-sectional area. The reference site was selected <10 mm proximal to the lesion. Positive remodeling (arterial expansion) was defined as a remodeling index of >1.05 (Figure 1C). Negative remodeling (arterial shrinkage) was defined as a remodeling index of <0.95.

Clinical endpoints

Clinical follow-up started at inclusion and lasted 1 year. Post-discharge survival status was obtained from municipal civil registries. Post-discharge rehospitalizations were prospectively assessed during follow-up. Questionnaires focusing on the occurrence of major adverse cardiac events (MACE) were sent to all living patients. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contacted for additional information whenever necessary.

The primary clinical endpoint was MACE, defined as all-cause mortality, ACS or unplanned coronary revascularization. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction (STEMI), non-STEMI or unstable angina pectoris in accordance with the guidelines of the European Society of Cardiology.[17] Unplanned coronary revascularization was defined as unplanned repeat PCI (either culprit or non-culprit coronary artery) or coronary artery bypass grafting (CABG). The endpoints were adjudicated by a clinical event committee that had no knowledge of the anti-periodontal pathogen antibodies and IVUS data.

Statistical analysis

The distributions of the continuous variables, including IgG and IgA levels against periodontal pathogens and the IVUS parameters, were tested for normality. Normally distributed continuous variables are presented as mean ± standard deviation (SD). Non-normally distributed continuous variables are presented as median and interquartile range (IQR). IgG and IgA levels were not normally distributed and were therefore ln-transformed when analyzed as continuous variable. IgG and IgA levels were also analyzed when categorized into tertiles. Categorical variables are presented as numbers and percentages.

We examined associations of concentration of IgG and IgA against individual periodontal pathogens, as well as of the sum of all IgG and all IgA levels, with (1) plaque burden in the imaged coronary segment (a measure of the extent of coronary atherosclerosis), (2) VH-TCFA morphology of the coronary lesions (a measure of coronary plaque vulnerability), (3) positive lesion remodeling and (4) incident MACE during the follow-up period. Linear regression analyses were performed to evaluate the associations with plaque burden. The results are presented as β with 95% confidence interval (95% CI) per standard deviation increase in ln-transformed IgG or IgA level. Generalized estimating equation analyses were performed to evaluate the associations with VH-TCFA and positive lesion remodeling. This method accounts for the presence of multiple lesions within one individual patient. The results are presented as odds ratio (OR) with 95% CI. Cumulative event rates of MACE were estimated according to the Kaplan-Meier method. Cox proportional hazards regression analyses were performed to evaluate the associations with MACE. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. The results are presented as hazard ratios (HR) with 95% confidence interval (95% CI).

In multivariable analyses, the variables age, gender, diabetes mellitus and smoking status (current smoking vs. never/past smoking) were considered as potential confounders and were entered into the full model. These variables were chosen because they were considered the most important confounders based on etiologic consideration and previous literature. Additionally, stratified analyses by smoking status and diabetes were performed to assess effect modification. All data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Mean age of the patients was 61.5 ± 11.3 years and 76% were men (Table 1). Coronary angiography was performed for various indications: 46% of patients had stable coronary artery disease, 26% of the

	n = 575 patients
Clinical characteristics	
Age, years	61.5 ± 11.3
Men, n (%)	435 (75.7)
Diabetes Mellitus, n (%)	99 (17.2)
Hypertension. n (%)	299 (52.0)
Hypercholesterolemia, n (%)	319 (55.5)
Smoking, n (%)	165 (28.7)
Positive family history, n (%)	298 (51.8)
Previous MI, n (%)	183 (31.8)
Previous PCI, n (%)	186 (32.3)
Previous CABG, n (%)	18 (3.1)
Previous stroke, n (%)	25 (4.3)
History of peripheral artery disease, n (%)	36 (6.3)
History of renal insufficiency, n (%)	32 (5.6)
History of heart failure, n(%)	19 (3.3)
Plasma immunoglobulin levels	
IgG against P. gingivalis, OD	0.61 [0.52-0.72]
IgG against A. actinomycetemcomitans, OD	0.13 [0.08-0.19]
IgG against T. forsythia, OD	1.00 [0.77-1.22]
IgG against P. intermedia, OD	0.31 [0.18-0.55]
IgA against P. gingivalis, OD	0.28 [0.17-0.47]
IgA against A. actinomycetemcomitans, OD	0.16 [0.11-0.23]
IgA against T. forsythia, OD	0.36 [0.19-0.60]
IgA against P. intermedia, OD	0.10 [0.06-0.15]
Procedural characteristics	
Indication for angiography	
Acute MI, n (%)	163 (28.3)
Unstable angina, n (%)	150 (26.1)
Stable angina, n (%)	262 (45.6)
Coronary artery disease*	
No significant stenosis, n (%)	43 (7.5)
1-vessel disease, n (%)	306 (53.2)
2-vessel disease, n (%)	165 (28.7)
3-vessel disease, n (%)	61 (10.6)
PCI performed, n (%)	505 (87.8)
IVUS characteristics	
Imaged coronary artery	
Left anterior descending, n (%)	206 (35.8)
Left circumflex, n (%)	193 (33.6)
Right coronary artery, n (%)	176 (30.6)
Median imaged segment length, mm	44.3 [33.9-55.4]
Mean plaque burden, %	38.2 ± 11.5

* A significant stenosis was defined as a stenosis ≥50% of vessel diameter by visual assessment on t coronary angiogram.

Table 1. Baseline patient characteristics

patients had unstable angina pectoris and 28% of the patients had an acute myocardial infarction. ACS patients had slightly lower IgG-class antibody levels against A. actinomycetemcomitans compared to patients with stable coronary artery disease (Supplemental table 1). Antibody levels were similar in

patients with and without multivessel disease (Supplemental table 1). The median length of the imaged coronary segment was 44.3 [33.9-55.4] mm. Mean plaque burden in the imaged coronary segment was 38.2 ± 11.5 percent. In the imaged coronary segments, a total of 723 coronary lesions have identified in 505 (87%) patients, including 271 VH-TCFA lesions in 240 (42%) patients.

Antibodies to periodontal pathogens and extent and vulnerability of coronary plaque

For reasons of conciseness, multivariable estimates are displayed in the tables. All univariable estimates are displayed in the online supplement. In the total study population, plasma levels of IgG and IgA against P. gingivalis, A. actinomycetemcomitans, T. forsythia and P. intermedia were not associated with plaque burden in the imaged coronary segment (Table 2 and supplemental table 2). Plasma levels of IgG and IgA against P. gingivalis, A. actinomycetemcomitans, T. forsythia and P. intermedia were not associated with VH-TCFA lesion morphology either (Table 3 and supplemental table 3).

		Adjusted β* (95% CI)	**P**
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	-0.70 (-2.99 ; 1.60)	0.55
	tertile 3 vs. 1	1.21 (-1.07 ; -3.49)	0.30
	Linear association		0.62
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.04 (-1.27 ; 3.36)	0.38
	tertile 3 vs. 1	-1.06 (-3.28 ; 1.16)	0.35
	Linear association		0.70
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.01 (-2.22 ; 2.24)	0.99
	tertile 3 vs. 1	-0.04 (-2.35 ; 2.27)	0.97
	Linear association		0.89
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	-2.33 (-4.61 ; -0.04)	0.046
	tertile 3 vs. 1	-0.22 (-2.49 ; 2.05)	0.85
	Linear association		0.69
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	-0.53 (-2.71 ; 1.66)	0.64
	tertile 3 vs. 1	-1.31 (-3.61 ; 0.99)	0.26
	Linear association		0.58
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.28 (-1.01 ; 3.58)	0.27
	tertile 3 vs. 1	-0.22 (-2.47 ; 2.02)	0.85
	Linear association		0.29
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	-1.24 (-3.53 ; 1.06)	0.29
	tertile 3 vs. 1	-1.68 (-3.87 ; 0.51)	0.13
	Linear association		0.27
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	-1.90 (-4.17 ; 0.38)	0.10
	tertile 3 vs. 1	-0.79 (-2.98 ; 1.40)	0.48
	Linear association		0.56

* β indicates the increase in plaque burden per standard deviation increase in ln-transformed immunoglobulin level. Adjusted for age, gender, smoking and diabetes.

Table 2. Association between periodontal pathogens and coronary plaque burden

		Adjusted OR* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.99 (0.67-1.45)	0.94
	tertile 3 vs. 1	1.01 (0.68-1.49)	0.98
	Linear association		0.91
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.20 (0.82-1.76)	0.36
	tertile 3 vs. 1	0.85 (0.57-1.27)	0.43
	Linear association		0.52
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.01 (0.69-1.49)	0.95
	tertile 3 vs. 1	1.05 (0.71-1.56)	0.81
	Linear association		0.96
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	0.88 (0.60-1.30)	0.52
	tertile 3 vs. 1	0.95 (0.65-1.39)	0.80
	Linear association		0.45
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.81 (0.56-1.18)	0.28
	tertile 3 vs. 1	0.84 (0.57-1.25)	0.40
	Linear association		0.97
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.12 (0.77-1.64)	0.55
	tertile 3 vs. 1	0.78 (0.53-1.16)	0.22
	Linear association		0.32
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.91 (0.63-1.32)	0.62
	tertile 3 vs. 1	0.94 (0.64-1.40)	0.78
	Linear association		0.80
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.10 (0.75-1.61)	0.64
	tertile 3 vs. 1	1.16 (0.79-1.71)	0.44
	Linear association		0.92

* Adjusted for age, gender, smoking and diabetes.

Table 3. Association between periodontal pathogens and thin-cap fibroatheroma morphology

Antibodies to periodontal pathogens and lesion remodeling

IgA-class antibody levels against A. actinomycetemcomitans were inversely associated with the extent of positive lesion remodeling (p for linear association 0.013) and directly with the extent of negative lesion remodeling (p for linear association 0.034). Similar associations with positive lesion remodeling were also seen with the highest tertiles of T. forsythia (p=0.012) and P. intermedia (p=0.012) IgA levels (Table 4, Figure 2, Supplemental table 4 and supplemental table 5).

Antibodies to periodontal pathogens and cardiovascular outcome

Vital status at 1-year follow-up could be acquired for 573 (99.7%) patients. Response rate of the questionnaires that were sent to all living patients was 92.7%. After 1 year of follow-up, 56 patients had experienced a MACE. The cumulative Kaplan-Meier incidences of the 30-day, 6-month and 1-year MACE were 0.9%, 5.6%, and 9.8%, respectively. High plasma concentrations of IgG and IgA against P. gingivalis, A. actinomycetemcomitans, T. forsythia and P. intermedia were not associated with the occurrence of MACE during 1 year of follow-up (Table 5 and supplemental table 6).

		Adjusted OR* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	1.20 (0.75-1.92)	0.46
	tertile 3 vs. 1	1.07 (0.66-1.75)	0.79
	Linear association		0.36
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	0.71 (0.45-1.14)	0.71
	tertile 3 vs. 1	0.74 (0.47-1.19)	0.22
	Linear association		0.18
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.17 (0.74-1.83)	0.50
	tertile 3 vs. 1	0.75 (0.46-1.25)	0.27
	Linear association		0.63
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	0.81 (0.51-1.28)	0.37
	tertile 3 vs. 1	0.73 (0.46-1.15)	0.18
	Linear association		0.14
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.72 (0.45-1.13)	0.15
	tertile 3 vs. 1	0.71 (0.44-1.14)	0.15
	Linear association		0.42
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	0.63 (0.39-1.00)	0.048
	tertile 3 vs. 1	0.55 (0.35-0.88)	0.012
	Linear association		0.013
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	1.08 (0.69-1.67)	0.74
	tertile 3 vs. 1	0.53 (0.32-0.87)	0.012
	Linear association		0.19
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.05 (0.66-1.66)	0.85
	tertile 3 vs. 1	0.64 (0.40-1.02)	0.061
	Linear association		0.20

* Adjusted for age, gender, smoking and diabetes.

Table 4. Association between periodontal pathogens and positive lesion remodeling

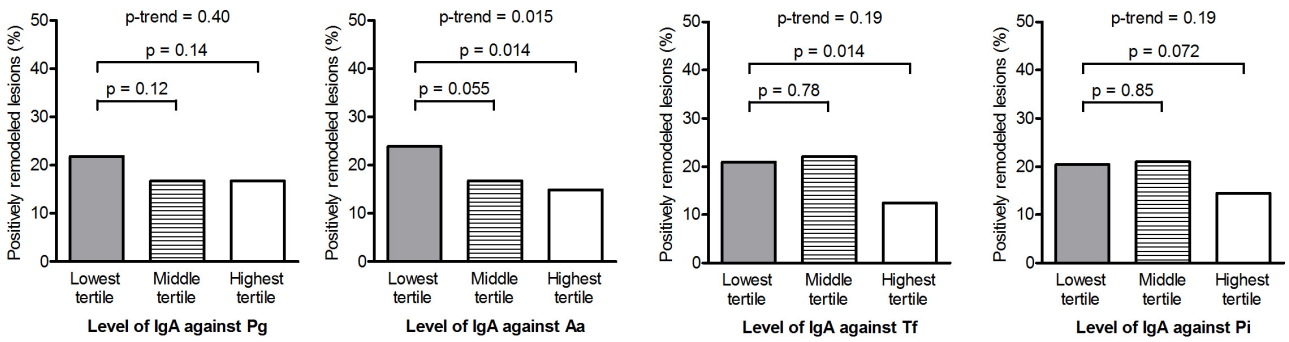


Figure 2 Association between level of immunoglobulin A against periodontal pathogens and positively remodeled lesions

Aa = Aggregatibacter actinomycetemcomitans; IgA = immunoglobulin A; Pi = Prevotella intermedia; Pg = Porphyromonas gingivalis; Tf = Tannerella forsythia.

		Adjusted HR* (95% CI)	P
IgG against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.85 (0.43-1.68)	0.63
	tertile 3 vs. 1	1.03 (0.55-1.92)	0.94
	Linear association		0.95
IgG against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.31 (0.69-2.49)	0.41
	tertile 3 vs. 1	0.91 (0.46-1.79)	0.91
	Linear association		1.00
IgG against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.51 (0.26-1.00)	0.048
	tertile 3 vs. 1	0.68 (0.36-1.27)	0.23
	Linear association		0.96
IgG against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.67 (0.90-3.08)	0.10
	tertile 3 vs. 1	0.63 (0.29-1.36)	0.24
	Linear association		0.91
IgA against	tertile 1	reference	
P.gingivalis	tertile 2 vs. 1	0.92 (0.48-1.77)	0.81
	tertile 3 vs. 1	0.87 (0.46-1.67)	0.68
	Linear association		0.67
IgA against	tertile 1	reference	
A.actinomycetemcomitans	tertile 2 vs. 1	1.78 (0.92-3.46)	0.087
	tertile 3 vs. 1	1.19 (0.58-2.41)	0.64
	Linear association		0.65
IgA against	tertile 1	reference	
T.forsythia	tertile 2 vs. 1	0.81 (0.41-1.60)	0.55
	tertile 3 vs. 1	1.13 (0.59-2.14)	0.72
	Linear association		0.91
IgA against	tertile 1	reference	
P.intermedia	tertile 2 vs. 1	1.20 (0.62-2.34)	0.59
	tertile 3 vs. 1	1.18 (0.61-2.28)	0.63
	Linear association		0.97

* Adjusted for age, gender, smoking and diabetes.

Table 5. Association between periodontal pathogens and 1-year major adverse cardiac events

Sum of IgG and IgA levels

The sum of IgG levels and the sum of IgA levels were neither associated with coronary plaque burden (p=0.86 and p=0.30 respectively), nor with VH-TCFA lesion morphology (p=0.60 and p=0.97 respectively), nor with MACE (p=0.15 and p=0.67 respectively). The sum of IgA levels was inversely associated with the extent of positive lesion remodeling (highest versus lowest tertile OR 0.50, 95% CI 0.31-0.82, p=0.006), while the sum of IgG levels was not (p=0.68).

Analyses stratified by diabetes and smoking status

In patients with diabetes, the highest IgG tertile against P. gingivalis was associated with coronary plaque burden (p=0.020) while the corresponding IgA antibody levels tended to associate with MACE (p=0.16) (Figure 3). Although statistical significance mostly disappeared because of lower power in the subgroups, the estimates of the remaining associations that were found in the total study population remained materially the same after stratification on diabetes. The same applied to the estimates found in smokers and non-smokers.

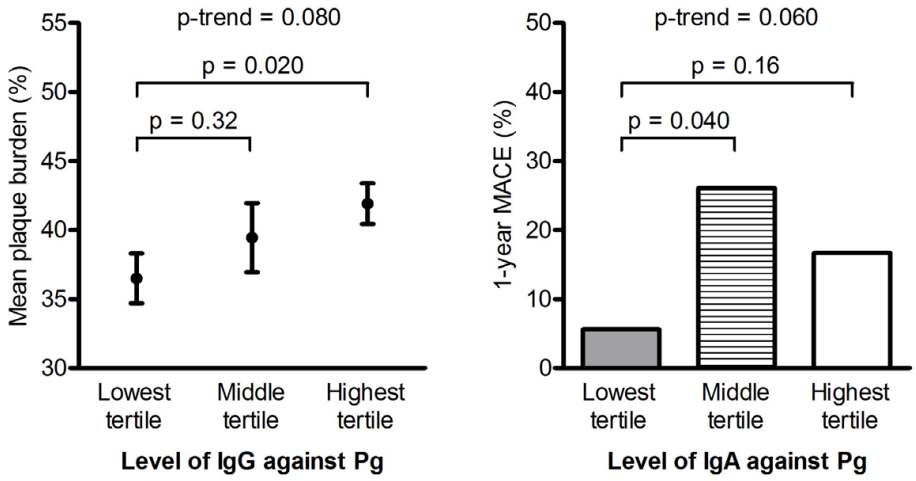


Figure 3 Associations of Porphyromonas gingivalis with extent of coronary atherosclerosis and with cardiovascular outcome in patients with diabetes

IgA = immunoglobulin A; IgG = immunoglobulin G; MACE = major adverse cardiac event; Pg = Porphyromonas gingivalis

DISCUSSION

This study investigated the associations between antibodies to major periodontal pathogens and coronary atherosclerosis on IVUS-VH. We found that IgG and IgA against P.gingivalis, A.actinomycetemcomitans, T.forsythia and P.intermedia were not associated with coronary plaque burden or VH-TCFA lesion morphology in the overall study population. High levels of IgA against A.actinomycetemcomitans, T.forsythia and P.intermedia, however, were associated with lower extent of positive lesion remodeling. The serologic markers of periodontal infection were not predictive for 1-year MACE. In stratified analyses, antibodies against P.gingivalis tended to be associated with the extent of coronary atherosclerosis and with incident MACE during 1 year follow-up in patients with diabetes.

The major strengths of this study include the objective measurements of the exposure (i.e. periodontal infection as assessed by antibodies), the precise measurement of the extent of coronary atherosclerosis, as well as the assessment of the composition of coronary atherosclerosis (atherosclerotic plaque morphology and lesion remodeling). The validated multiserotype ELISAs that have been used for the measurement of P.gingivalis and A. actinomycetemcomitans antibodies have a good sensitivity (71%) and specificity (90%) for clinically and radiographically diagnosed periodontitis, and allowed us to measure the infection by these periodontal pathogens.[18] IVUS enables for accurate measurements of various coronary plaque characteristics, including plaque burden, plaque morphology and lesion remodeling.[12, 16] To our best knowledge, this is the first study on periodontal disease that used IVUS to measure the extent of coronary atherosclerosis, and the associations between periodontal infection and plaque morphology and lesion remodeling have so far not been investigated at all.

Many observational studies have investigated the association between periodontal disease and cardiovascular disease; the latter mostly defined as extent of atherosclerosis or occurrence of clinical cardiovascular events.[2] However, in these studies, periodontal disease has been broadly defined by a variety of measures, including self-reported assessment of tooth loss or periodontal status, and clinically or radiographically assessed gingival inflammation or pathological periodontal pockets. Few studies have assessed the periodontal infection by measuring IgG and IgA antibody levels against several bacteria.[19-26] A study by Colhoun et al. found an association between IgG against P.gingivalis and

A.actinomycetemcomitans and coronary artery calcification as measured by computed tomography in diabetic subjects, although this association did not appear to be independent of baseline characteristics.[20] In line with this finding, our study showed an association between IgG against P.gingivalis and the extent of coronary atherosclerosis on IVUS in diabetic patients with established coronary artery disease. Cross-sectional and case-control studies by Pussinen et al., Beck et al, and Spahr et al. have reported positive associations between antibody levels against various periodontal pathogens and the presence of coronary heart disease within large population-based cohorts.[19, 23-26] Furthermore, two prospective studies have investigated the association between serum antibody titers and risk of incident MACE during follow-up. Lund Haheim et al. measured IgG to P.gingivalis, A.actinomycetemcomitans, T.forsythia, and Treponema denticola, and found that no single bacterium but rather combinations were related to increasing risk for incident myocardial infarction.[21] Pussinen et al, measured IgG and IgA to P.gingivalis and A.actinomycetemcomitans, and found that IgA against P. gingivalis was associated with increased risk for occurrence of myocardial infarction during 10 years of follow-up.[24] The latter is in line with our finding that IgA against P.gingivalis is associated with 1-year MACE in diabetic patients with coronary artery disease. The lack of further associations with extent of coronary atherosclerosis and with MACE in our full cohort may be explained by the fact that ours was a cohort of patients with known coronary artery disease, and associations may be different compared to population-based cohorts or case-control studies, which contain healthy control subjects.

In the current study, we did not find an association between antibody levels against periodontal pathogens and VH-TCFA lesion morphology, which suggests that periodontal infection and its inflammatory reaction is not associated with coronary plaque vulnerability. However, we did find that IgA against A.actinomycetemcomitans, T.forsythia and P.intermedia (but not P.gingivalis) were inversely associated with extent of positive lesion remodeling. Modest positive lesion remodeling may be considered as a physiological, and thus favourable, response to progression of atherosclerotic plaque, also known as the Glagov adaptive phenomenon.[27] In this light, infection with A.actinomycetemcomitans, T.forsythia and P.intermedia may be considered as being associated with a lower adaptive capacity to atherosclerotic burden.

Some limitations of this study need to be acknowledged. Firstly, clinical periodontal examination was not performed in our study. Although measurement of antibodies provides good sensitivity and specificity for clinically and radiographically diagnosed periodontitis, data on actual presence of periodontitis or history of periodontal destruction is lacking. Furthermore, IgG and IgA levels reflect both past and recent exposure to the periodontal pathogens.[28] In the current study, we could not investigate the effects of the active phase of periodontitis, which can only be determined by clinical measurements of changes in attachment level, on coronary atherosclerosis.[8] The ideal analysis includes both the clinical diagnosis of periodontitis and the antibodies against periodontal pathogens.

Secondly, a single non-culprit coronary vessel was imaged in this study. This approach was chosen based on the hypothesis that the phenotype of a non-culprit artery segment represents the patient's systemic atherosclerotic disease burden.[16] Although this hypothesis may be debated, it is highly supported by our previous finding that IVUS in only one non-culprit vessel has strong prognostic value.[12] Finally, the spatial resolution of IVUS-VH (150μm) is insufficient to exactly replicate histopathologic definitions of a thin fibrous cap (<65μm).[29] Therefore, IVUS-VH tends to over-estimate the number of histopathological TCFA lesions. Nevertheless, the presence of VH-TCFA lesions has been shown to have prognostic information.[10-12]

In conclusion, in the current study, plasma levels of IgG and IgA against four major periodontal pathogens were neither associated with the extent of coronary atherosclerosis, nor with coronary plaque vulnerability, as measured with IVUS-VH in a population of patients with established coronary artery disease. IgA against A.actinomycetemcomitans, T.forsythia and P.Intermedia were inversely associated with extent of positive coronary remodeling. IgG and IgA against the four major periodontal pathogens that we tested were not predictive for cardiovascular outcome at 1 year. Only in the subgroup of patients with diabetes, antibodies against P.gingivalis tended to be associated with the extent of coronary

atherosclerosis and with incident MACE during 1-year follow-up. Altogether, the results of the current study do not add evidence for the hypothesis that systemic exposure to periodontal pathogens, as assessed by antibodies against periodontal pathogens, plays a substantial role in coronary artery disease. Further studies in diabetic patients are needed to clarify the potential role of periodontal pathogens in coronary atherogenesis and associated cardiovascular events.

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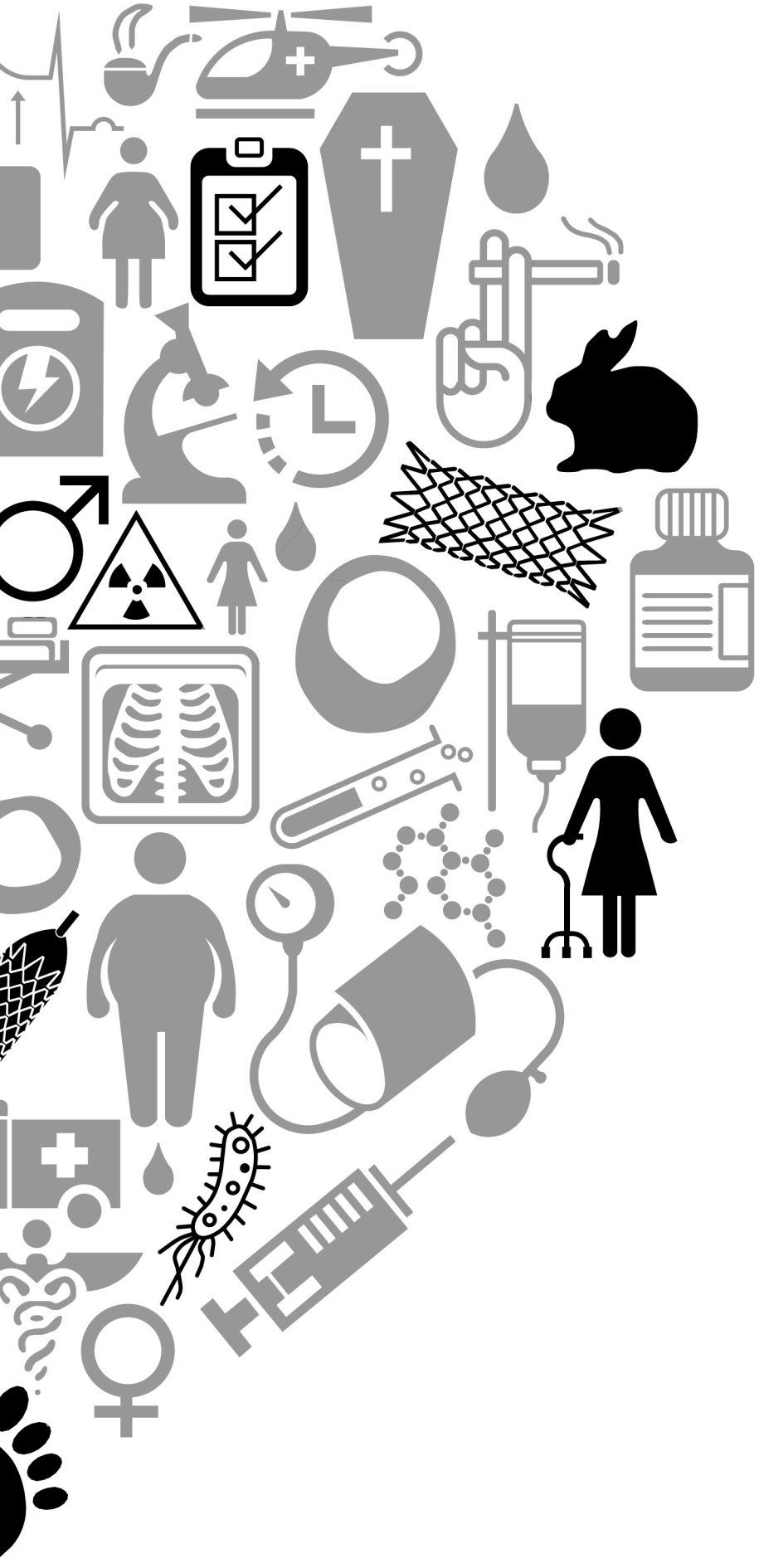

GENERAL DISCUSSION AND SUMMARY
ALGEMENE DISCUSSIE EN SAMENVATTING

GENERAL DISCUSSION AND SUMMARY

The main objective of this thesis was to improve characterisation and prognostication of patients undergoing percutaneous coronary intervention (PCI). We will discuss our main findings and their impact on clinical practice and give some directions for further research.

PART I

Since the introduction of reperfusion therapy a significant improvement in outcome has been observed in patients presenting with acute ST-elevation myocardial infarction (STEMI). Meta-analyses of randomised clinical trials showed that so-called primary PCI (PPCI) results in lower mortality than fibrinolytic therapy in these patients. However, various reports indicate that outcome after PPCI depends on clinical parameters such as infarct size, presence of cardiogenic shock, and treatment delay, as well as on patient-related risk factors. Age, gender, heart rate and blood pressure are relevant factors in this respect. Furthermore, several studies indicate the importance of logistic aspects, such as operator and site experience, and the ability to provide state-of-the-art treatment during off hours.

Ambiguity remains whether gender affects outcome the Erasmus MC.[1-9] Therefore, we analysed almost 12,000 consecutive patients who underwent PCI in and found that women undergoing PCI for STEMI had higher mortality than men. Importantly, the excess mortality in women appeared in the first month after PCI and could only partially be explained by differences in baseline characteristics. No gender differences were observed in outcome in patients undergoing PCI for non-ST-elevation acute coronary syndrome (NSTE-ACS) or stable angina pectoris. Our findings are in agreement with a recent meta-analysis of over 700,000 patients, which showed that women tend to have higher mortality post-PCI at both short and intermediate term follow-up.[10] These observations demonstrated that research is warranted to study the presentation and treatment of coronary artery disease (CAD) in relation to gender. Ideally, this will eventually result in better-fitted diagnostic and treatment strategies for both genders, and consequently the disappearance of gender disparities in patient outcomes.

Smoking is a well-known risk factor for the development and progression of CAD[11-15], and its relation with increased morbidity and mortality from cardiovascular causes has long been established.[16], [17] However, the effect of smoking cessation in terms of prolonged life-years is unknown. In order to quantify the effect of smoking cessation, we performed a follow-up of 1980-1985 cohort of PCI (balloon angioplasty only) patients treated in the Erasmus MC. Based on this 30-year follow-up period, we estimated that (at least) 2.1 life-years were gained by patients who stopped smoking as compared to continuing smokers. Despite the introduction of national health campaigns in the 1980s, 27% of the general population in the Netherlands still smokes. [18] In patients with established CAD, this figure is as high as 15%, [19] sadly making smoking cessation still a current topic. We believe, our results should encourage physicians to keep convincing their patients to stop smoking.

Several studies report that treatment during off-hours is related with poorer outcomes after PPCI than treatment during on-hours, although ambiguity exists. [20-26] [27-30] In a cohort of more then 4,000 consecutive STEMI patients treated with PPCI in the Erasmus MC we found no outcome differences between those treated during off- and on-hours. Absence of outcome differences was consistently found in clinically relevant subgroups, including the elderly and patients with multi-vessel disease. These findings, which are based on a systematic monitoring of treatment outcomes, do not give rise to change our practice. Instead, these results may encourage other centres to expand their service.

Another important issue that has been addressed in this thesis refers to the identification of categories of MI patients who may benefit most from a PPCI, and thus have lowest numbers needed to treat (NNTs) to prevent a death. Based on an analysis of 22 randomized trials, we concluded that PPCI is consistently associated with a strong relative reduction in 30 day mortality, irrespective of patient baseline risk, and should therefore be considered as the first choice reperfusion strategy whenever

feasible. In contrast, the absolute risk reduction was strongly related to estimated risk at baseline: the NNT to prevent one death by PPCI versus fibrinolysis was 516 in the lowest quartile (i.e. low risk based on baseline characteristics) of estimated risk compared to only 17 in the highest quartile (i.e. high risk based on baseline characteristics). If access to PCI is longer than 2 hours, however fibrinolysis remains a legitimate option in low risk patients, because of the small absolute risk reduction by PPCI in this particular cohort.

Age is an important determinant of adverse outcome, however, elderly patient are often withhold PPCI. Clinicians tend to make decisions influenced by the presence of comorbid conditions and/or frailty of elderly patients rather than on the guidelines. Unfortunately, there is little clinical evidence, as most elderly patients are excluded from trial-participation due to the favour of clinical trialists to enrol patients at low risk of potential side effects [31]. Therefore another analysis based on the individual data of the above mentioned 22 randomized control trials (RCT's) was performed, which revealed that the reduction in clinical endpoints by PPCI was not influenced by age, and therefore strengthening the fact that age per se should not be considered an exclusion criterion for the application of PPCI.

To improve the generalizability of RCT results, the 'real world' or 'all-comers' (AC) design is becoming increasingly popular in cardiology. This design can potentially overcome the exclusion of patients with a perceived high risk of potential side effects. We studied the participants of two large AC trials that were enrolled in the Erasmus MC, and compared their characteristics with non-enrolled patients. It appeared that still half of the eligible patients who belonged to the target population were not included in the AC trial AC trial participants were significantly younger than non-participants and had better survival The observed differences in 30-day and 1-year mortality between participants and non-participants became smaller in those who survived the first 48 hours. These results reveal that the AC design does not fully represent daily 'real world' clinical practice. We speculate that the perception of clinical trialists still plays an important role in the selection of trial participants. It is to be expected that this is hard to overcome in future studies, although it is remains worth attempting.

PART 2.

CAD is a complex disease, and after decades of cardiovascular research the mechanisms linking plaque composition and morphology, vascular inflammation, coagulability and patient outcome are still not fully understood. The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (AtheroRemo-IVUS) was designed as an exploratory (non-pivotal) clinical study to investigate the associations between genetic profile, circulating biomarkers, and coronary atherosclerosis phenotype and vulnerability as determined by IVUS virtual histology or NIRS. Furthermore, the prognostic implications of (the combination) of established and novel biomarkers and plaque phenotypes were assessed. The AtheroRemo had an AC design, which included patients with an ACS and stable angina pectoris.

CAD has a strong genetic component with epidemiological studies suggesting 50% of susceptibility is heritable[32]. Genome wide association studies, in which hundreds of thousands of single-nucleotide polymorphisms (SNPs) are tested for association with a disease, have proved successful in identifying genetic associations with complex traits. [33] Atherosclerosis genotyping programs have so far been of limited importance. In AtheroRemo a total of twelve SNPs in or in the proximity of eight genes were associated with indicators of vulnerable plaque. Two of these genes, GNA12 and SESN3 were also related to clinical outcome. We consider these findings hypothesis generating, and further validation of these SNPs in other (larger) cohorts of CAD patients is warranted.

Ex vivo as well as in vivo studies using IVUS in patients with myocardial infarction have demonstrated the presence of thin caped fibroatheroma's (TCFAs) in other than the culprit lesion or even culprit artery[34] In AtheroRemo, we demonstrated that the presence of IVUS-derived TCFA lesions in a non-culprit coronary artery was predictive of the occurrence of major adverse cardiac events within 1 year,

including death and ACS. TCFA lesions with a large plaque burden carry higher risk than small TCFA lesions. Thus, IVUS imaging of only part of the coronary system seems sufficient for prognostication.
Near-infrared spectroscopy (NIRS) is a catheter based imaging modality with high potential to identify lipid-core containing coronary plaques (LCP) in CAD patients. This intra-coronary imaging technique utilizes the variation in reflection of the emitted near-infrared light to detect LCP, which is visualized as a (block) chemogram and qualified by a lipid-core burden index (LCBI) score. [35, 36] In AtheroRemo, LCBI was associated with clinical features that represent high cardiovascular risk. Furhtermore, NIRS had prognostic value, since patients with an LCBI equal or above the median had a 4-fold risk of adverse cardiovascular events during 1-year follow-up. [37] These early results are promising, but further research is warranted to evaluate the longer-term outcomes. Currently, the Lipid Rich Plaque (LRP) study is being conducted, aiming to further explore the relation between NIRS findings and adverse cardiac events in a large series of European and American patients.
Finally, in AtheroRemo we sought to study the relation between a broad range of novel circulating biomarkers and coronary atherosclerosis phenotype and vulnerability. We studied circulating cytokines38, acute phase proteins,[39] chemokines[40], antibodies to periodontal pathogens, and plasma concentrations of molecular lipid species. Although statistically significant relations with plaque composition and clinical outcomes were found for various markers, including TNF-α[38], RANTES[40,] and several molecular lipids species[41], we consider these finding hypothesis generating. We continue expanding our line of research to replicate our observations, and to study the relevance of the biomarker and imaging findings for clinical outcomes during longer-term follow-up.

ALGEMENE DISCUSSIE EN SAMENVATTING

De belangrijkste doelstelling van dit proefschrift was om middels innovaties de karakterisering en de prognose te verbeteren van patiënten die een percutane coronaire interventie ondergaan. In dit proefschrift bespreken we onze bevindingen en (de mogelijke) impact op de klinische praktijk en geven enkele voorstellen voor richtingen waarin verder onderzoek kan plaatsvinden.

DEEL I

Sinds de introductie van reperfusietherapie is een aanzienlijke verbetering in mortaliteit en morbiditeit waargenomen bij patiënten die zich presenteren met een acuut ST-elevatie myocardinfarct (STEMI). Meta-analyses van gerandomiseerde klinische studies hebben aangetoond dat primaire PCI (PPCI) resulteert in lagere mortaliteit dan fibrinolytische therapie bij deze patiënten. Echter, uit diverse onderzoeken blijkt dat uitkomst na PPCI ook afhankelijk is van klinische parameters zoals infarct grootte, aanwezigheid van cardiogene shock en vertraging in behandeling, alsmede patiënt-gerelateerde risicofactoren zoals leeftijd, geslacht, hartslag en bloeddruk. Bovendien, hebben een aantal studies het belang aangetoond van de logistieke aspecten, zoals de ervaring van de interventie-cardioloog, het ziekenhuis en de mogelijkheid om state-of-the-art behandeling buiten kantooruren te bieden.
Momenteel bestaat nog onduidelijkheid of het geslacht van de patiënt die een PCI ondergaat van invloed is op uitkomst.[1-9] Om deze vraag te beantwoorden hebben we dit bij ongeveer 12.000 opeenvolgende patiënten die een PCI ondergingen voor verscheidende indicaties geanalyseerd en concludeerden dat vrouwen die een PCI ondergingen voor STEMI een hoger sterftecijfer hadden dan mannen. Wel is het belangrijk te melden dat de oversterfte bij vrouwen alleen optrad tijdens de eerste maand na PCI en slechts ten dele kon worden verklaard door verschillen in patiëntkenmerken. Bij patiënten die een PCI ondergingen voor niet-ST-elevatie acuut coronair syndroom (NSTE-ACS) of stabiele angina pectoris werden geen geslacht verschillen in uitkomst waargenomen. Deze bevindingen zijn in overeenstemming met een recente meta-analyse van meer dan 700.000 patiënten, waaruit blijkt dat vrouwen een hogere sterfte post-PCI hebben op zowel korte als middellange termijn follow-up.[10] Deze observaties laten zien dat meer onderzoek noodzakelijk is naar de presentatie en behandeling van coronaire hartziekte (CHZ) bij vrouwen. Idealiter zal dit uiteindelijk resulteren in betere diagnostische en therapeutische strategieën en verdwijnen daarmee de verschillen in uitkomst tussen mannen en vrouwen.
Een bekende risicofactor voor de ontwikkeling en progressie van CHZ is roken[11-15], waarvan het verband met een verhoogde morbiditeit en mortaliteit door cardiovasculaire oorzaken reeds lang bekend is.[16, 17] Ook het positieve effect van stoppen met roken op de lange termijn is bekend, echter is dit effect nog niet gekwantificeerd. Om dit effect van stoppen met roken te kunnen kwantificeren hebben we gekeken naar de lange termijn follow-up van patiënten uit een PCI cohort (alleen ballondilatatie) uit de jaren 1980-1985, behandeld in het Erasmus MC. Op basis van een follow-up periode van 30 jaar, schatten we dat patiënten die stopten met roken (ten minste) 2,1 jaar langer leefden in vergelijking met 'doorrokers'. In Nederland rookt 27% van de bevolking nog steeds, ondanks de invoer van diverse nationale campagnes om het stoppen met roken te bevorderen in de jaren tachtig.[18] Bij patiënten met vastgestelde CHZ, is dit cijfer nog steeds 15%,[19] wat stoppen met roken helaas nog steeds een actueel onderwerp maakt. Wij hopen dat met onze resultaten artsen een middel in handen hebben om patiënten te kunnen overtuigen te stoppen met roken.
Verschillende studies melden dat behandeling buiten kantooruren is gerelateerd aan slechtere resultaten na PPCI dan behandeling tijdens kantooruren, hoewel onduidelijkheid bestaat.[20-30] We hebben in een cohort van meer dan 4000 opeenvolgende STEMI patiënten behandeld met PPCI in het Erasmus MC gekeken of er verschil in uitkomst was na PPCI tijdens kantooruren versus buiten kantooruren en vonden geen verschil. Gelijke uitkomsten werden eveneens gevonden in klinisch relevante subgroepen,

waaronder ouderen en patiënten met meerdere aangedane kransslagvaten. Deze bevindingen, die zijn gebaseerd op een systematische monitoring van de resultaten van de behandeling, gaven geen aanleiding om onze praktijk te veranderen. In plaats daarvan, kunnen deze resultaten mogelijk andere centra stimuleren om hun dienstverlening uit te breiden.
Een andere belangrijke kwestie die in dit proefschrift aanbod komt, is de identificatie van de categorieën van patiënten met MI die het meest van een PPCI kunnen profiteren. We hebben 22 gerandomiseerde studies geanalyseerd en vonden dat PPCI consequent geassocieerd was met een sterke relatieve reductie van mortaliteit op 30 dagen, ongeacht het risicoprofiel van patiënt. Reden om reperfusiestrategie als behandeling van eerste keuze te beschouwen. Daarentegen was de absolute risicoreductie sterk gerelateerd aan het geschatte risico op baseline: het aantal patiënten dat behandeld moest worden om één sterfgeval te voorkomen door PPCI versus fibrinolyse was 516 in het laagste kwartiel (d.w.z. een laag risico op basis van de uitgangskenmerken) in vergelijking met slechts 17 in de hoogste kwartiel (d.w.z. met een hoog risico op basis van de uitgangskenmerken). Indien een PCI centrum op meer dan 2 uur afstand is, blijft vanwege de lage absolute risicoreductie van PPCI in dit cohort, fibrinolyse een legitieme optie bij laag risico patiënten,
De oudere patiënt wordt vaak PPCI onthouden, dit komt omdat artsen de neiging hebben om beslissingen te maken gebaseerd op basis van co-morbiditeit en/of broosheid van de oudere patiënt in plaats van op de richtlijnen. Helaas is er weinig wetenschappelijke bewijs over de behandeling van de oudere patiënt, omdat de meeste oudere patiënten vanwege de eerder genoemde co-morbiditeit / broosheid zijn uitgesloten van deelname aan wetenschappelijk onderzoek. Wetenschappers hebben een voorkeur voor de categorie patiënten die het laagste risico lopen op mogelijke bijwerkingen/ slechte uitkomst[31]. Daarom hebben we de individuele gegevens van de bovengenoemde 22 gerandomiseerde studies opnieuw geanalyseerd, hieruit bleek dat de reductie in klinische eindpunten door behandeling met PPCI onafhankelijk is van de leeftijd. Dit laat zien dat leeftijd op zich geen reden is om af te zien van PPCI in de oudere patiënt.
Het 'dagelijkse klinische praktijk' of 'all-comers' (AC) design om de generaliseerbaarheid van gerandomiseerde studies te vergroten, wordt steeds populairder binnen de cardiologie. Dit design heeft als doel zowel patiënten met een met een hoog als laag risico op mogelijke bijwerkingen in wetenschappelijk onderzoek te includeren. Om te evalueren in hoeverre het AC design hierin slaagt hebben we de patiëntkenmerken van deelnemers aan twee grote AC studies in het Erasmus MC vergeleken met niet- deelnemers. Ondanks de ruime inclusiecriteria, bleek dat nog steeds maar de helft van alle mogelijke deelnemers, deelnam aan het wetenschappelijk onderzoek. AC deelnemers waren significant jonger dan niet-deelnemers en hadden een betere overleving. De waargenomen verschillen in de 30-dagen en 1-jaars mortaliteit tussen de deelnemers en niet-deelnemers werden kleiner in zij die de eerste 48 uur overleefden. Deze resultaten tonen aan dat de AC-ontwerp niet geheel representatief is voor de dagelijkse klinische praktijk. We denken dat de perceptie van de klinische onderzoekers nog steeds een belangrijke rol speelt bij de selectie van de deelnemers aan het wetenschappelijk onderzoek. Dit probleem is moeilijk op te lossen, hoewel het, het proberen waard blijft.

DEEL 2.

CHZ is een complexe ziekte, en na decennia van cardiovasculair onderzoek zijn de mechanismen die plaque samenstelling, plaque morfologie, vasculaire ontsteking, coagulatie en uitkomst met elkaar verbinden nog steeds niet volledig begrepen. Het Europese samenwerkingsproject over Inflammation and Vascular Wall
Remodeling in Atherosclerosis - Intravascular Ultrasound (AtheroRemo-IVUS) is ontworpen als een verkennende klinische studie naar de associatie tussen genetische profiel, circulerende biomarkers, en plaquemorfologie als bepaald door IVUS virtuele histologie en/of NIRS. Bovendien zullen de prognostische implicaties (de combinatie) van bekende en nieuwe biomarkers en plaque fenotypes worden

bekeken. De AtheroRemo had een AC ontwerp, zowel patiënten met een ACS als stabiele angina pectoris werden geïncludeerd.
Epidemiologische studies suggereren dat 50% van de gevoeligheid voor CHZ genetisch bepaald is [32]. Genome wide assosciation studies, waarin honderdduizenden single-nucleotide polymorfismen (SNP's) worden getest op associatie met een ziekte, zijn succesvol in het identificeren van genetische associaties met complexe aandoeningen. [33] Programma's met atherosclerose genotypering hebben tot op heden geen belangrijke doorbraak laten zien. In de AtheroRemo studie werden in totaal twaalf SNPs in of in de nabijheid van acht genen geassocieerd met indicatoren van een kwetsbare plaque. Eerder onderzoek heeft laten zien dat twee van deze genen, GNA12 en SESN3 ook geassocieerd zijn met klinische uitkomst. We beschouwen onze bevindingen als hypothese genererend en zijn van mening dat verdere validatie van deze SNPs in andere (grotere) cohorten van CHZ patiënten noodzakelijk is.
Zowel 'ex vivo' alsook 'in vivo' studies waarbij met behulp van IVUS de kenmerken van atherosclerotische plaques bij patiënten met een hartinfarct geëvalueerd werden hebben de aanwezigheid van thin capped fibroatheroma's (TCFAs; deze plaques hebben de hoogste kans op ruptuur) aangetoond in coronairen die niet de primaire oorzaak van het infarct waren.[34] In AtheroRemo hebben we laten zien dat de aanwezigheid TCFA laesies (met behulp van IVUS) in deze zogenaamde non-culprit kransslagaders voorspellend waren voor het optreden van cardiale incidenten binnen 1 jaar, met inbegrip van de dood en (recidief) ACS. TCFA laesies met een hoge plaque burden (de hoeveelheid plaque) brengen een groter risico met zich mee dan met een lagere plaque burden. Hieruit concludeerden we dat ook wanneer slechts een deel van de kransslagvaten in beeld wordt gebracht, IVUS voldoende prognostische waarde lijkt te hebben.
Near-infrarood spectroscopie (NIRS) is een beeldvormende modaliteit op een katheter met een hoge potentieel om lipide-rijke coronaire plaques (LCP) bij CHZ-patiënten te identificeren. Deze intracoronaire beeldvormende techniek maakt gebruik van de variatie in het reflecteren van uitgezonden near-infrarood licht om LCP te detecteren. Dit gereflecteerde near-infrarood licht wordt gevisualiseerd als een (blok) chemogram en gekwalificeerd door een lipid core burden index (LCBI) score. [35, 36] In AtheroRemo, werd een associatie aangetoond tussen LCBI en relevante (hoog cardiovasculair risico) klinische kenmerken. Ook bleek NIRS een prognostische waarde te hebben, aangezien patiënten met LCBI gelijk aan of boven de mediaan een 4 keer zo groot risico hadden op cardiovasculaire incidenten geobserveerd tijdens een 1 jaar durende follow-up. [37] Deze eerste resultaten zijn veelbelovend, maar verder onderzoek is geboden. Momenteel wordt het Lipid Rich Plaque (LRP) onderzoek uitgevoerd, met als doel de relatie tussen NIRS bevindingen en cardiale incidenten bij een groot aantal Europese en Amerikaanse patiënten nader te bestuderen.
Concluderend, in AtheroRemo zochten we naar de relatie tussen een breed scala van nieuwe circulerende biomarkers en de kwetsbaarheid/fenotype van plaques in de coronairen. We bekeken cytokines[38], acute fase eiwitten,[39] chemokines[40], antilichamen tegen parodontale pathogenen en plasma concentraties van moleculaire lipide soorten.[41] Hoewel we statistisch significante relaties vonden tussen diverse markers, plaque samenstelling en uitkomst, beschouwen we deze resultaat vooral hypothese genererend. We blijven ons lijn van onderzoek uitbreiden met als doel onze waarnemingen te repliceren en het effect van onze bevindingen (biomarker en beeldvorming) op uitkomst te bestuderen gedurende de langere-termijn follow-up.

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PhD PORTFOLIO
SUMMARY OF PHD TRAINING AND TEACHING ACTIVITIES

1. PHD TRAINING

Research skills	Year	Workload ECTS
NIHES Master's degree Clinical Epidemiology	2010	70
BROK course	2010	1,5
Courses		
Cardiovascular imaging and diagnostics	2010	1,5
Clinical cardiovascular epidemiology	2010	1,5
Research seminars		
Cardiac and vascular remodeling and repair	2009	0,4
NT-pro BNP measurements in clinical practice	2009	0,4
New Developments in Percutaneous Revascularization	2009	0,4
Farmacogenetica	2009	0,4
International conferences		
Cardiology & Vascular Medicine, Rotterdam	2009	1,5
ACC congress, Atlanta, USA oral and poster presentation	2010	1,5
EuroPCR Parijs, Frankrijk	2010	1,5
ESC congress, Stockholm, Zweden 2poster presentations	2010	1,5
ESC congress, Parijs, Frankrijk	2011	1,5
AHA congress, Orlando, USA oral Presentation	2011	1,5
Vulnerable plaque meeting, Cascais, Portugal	2011	1,5

2. TEACHING

KLEP journal club	2011-2012	0,6
Staflunch	2008-2011	0,9
Supervising 2nd year medical students performing a systematic review	2009-2011	6,0

ABOUT THE AUTHOR

Curriculum vitae

Sanneke Petronella Maria de Boer was born on April 8th, 1983 in Delft, The Netherlands. After graduating from secondary school in 2001 (VWO, Stanislas college Westplantsoen, Delft), she started her medicine study at the University of Leiden. In 2007 she graduated cum laude from medical school. After she started a research project within the field of interventional cardiology at the AMC in Amsterdam, she switched to the Erasmus MC Thoraxcenter in Rotterdam in April 2008. There she started the research project named Innovations to improve characterisation and prognostication of patients undergoing percutaneous coronary intervention, under the supervision of Prof. dr. P.W.J.C. Serruys and Prof. dr. E. Boersma. She completed the Master of Science in Epidemiology programme as part of The Netherlands Institute for Health Sciences (NIHES) curriculum of the Erasmus University in 2010. April 2012 she started working in the internal medicine department at the Albert Schweitzer Ziekenhuis in Dordrecht for two years (supervision: Dr. E.H.J. van Bommel), as part of her cardiology training (supervision: Dr. F.J ten Cate). Afterwards she worked for one year at the cardiology department at the same hospital (supervision: Dr. M.J.M. Kofflard and Dr. E.J. van den Bos). Thereafter she continued her cardiology training at the Erasmus MC Thoraxcenter (supervision: Prof. dr. J. Deckers).

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