Circulating chemokines in relation to coronary plaque characteristics on radiofrequency intravascular ultrasound and cardiovascular outcome

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ABSTRACT

Objective: To investigate relations of several circulating chemokines with extent and phenotype of coronary atherosclerosis and with 1-year clinical outcome.

Methods: Intravascular ultrasound virtual histology imaging of a coronary artery was performed in 581 patients. MCP-1, MIP-1α, MIP-1β and RANTES were measured in plasma.

Results: Higher MCP-1, MIP-1α and lower RANTES were associated with coronary plaque burden. Higher MCP-1, MIP-1α and lower RANTES were associated with the presence of IVUS-VH-derived thin-cap fibroatheroma lesions. RANTES was associated with major adverse cardiac events.

Conclusions: RANTES is a promising biomarker that is inversely associated with coronary plaque burden and vulnerability, as well as with death and ACS.

INTRODUCTION

Inflammation has been recognized as an important contributing factor in all phases of atherosclerosis.[1-3] In particular, inflammation is believed to play a crucial role in the development and rupture of vulnerable plaques, resulting in major cardiovascular problems such as myocardial infarction and stroke.[1-3] Circulating inflammatory biomarkers may potentially improve prognostication of patients with atherosclerotic cardiovascular disease.[4]

Chemokines are involved in the recruitment of various leukocytes, such as monocytes, macrophages and T lymphocytes, into the atherosclerotic plaque.[5,6] Monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), MIP-1β and regulated upon activation normal T cell expressed and secreted (RANTES) are typical C-C motif chemokines that have been studied extensively.[5,6] Several studies have shown that these chemokines have an important role throughout the entire atherosclerotic process from atherogenesis to plaque destabilization.[5,6] However, their clinical utility as biomarker remains unclear.[5,6] Furthermore, prospective data on associations of these biomarkers with in-vivo measurements of extensiveness, phenotype and vulnerability of coronary atherosclerosis is currently lacking. This study aims to evaluate the usefulness of MCP-1, MIP-1α, MIP-1β and RANTES by investigating their relations with intravascular ultrasound virtual histology (IVUS-VH)-derived measures of coronary plaque burden, quantity of necrotic core, and presence of thin-cap fibroatheroma lesions (TCFA), and by investigating their prognostic value for major adverse cardiac events.

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.[7,8] In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina pectoris (SAP) 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.

Data collection

Baseline characteristics of all patients were collected prospectively by trained research physicians. These physicians reviewed the medical charts of the patients at the time of inclusion in the study, and extracted variables regarding demographics, medical history, cardiovascular risk factors and procedural characteristics. Medical history and cardiovascular risk factors are a routine part of clinical patient assessment at the department of Cardiology. Thus, presence of diabetes mellitus, hypertension, hypercholesterolemia, history of renal insufficiency and history of heart failure were defined as a clinical diagnosis of these conditions as reported by the treating physician in the medical chart. Smoking was defined as current smoking, reported by the patient. Procedural characteristics were prospectively extracted from the catheterization report.

Biomarkers

Blood samples were drawn from the arterial sheath prior to the diagnostic coronary angiography or PCI procedure. The blood samples were transported to the clinical laboratory of Erasmus MC for further processing and storage at temperature of −80°C within 2 hours after blood collection. MCP-1, MIP-1α, MIP-1β and RANTES were measured in the stored EDTA-plasma samples (n=570) using a validated multiplex assay (Custom Human Map, Myriad RBM, Austin, Texas, USA).

Intravascular ultrasound

Following the standard coronary angiography or PCI procedure, IVUS data were acquired in a non-culprit coronary vessel. 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 IVUS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) that had no knowledge of clinical data. The IVUS gray-scale and IVUS radiofrequency analyses, also known as IVUS virtual histology, were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software. 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 plaque and media cross-sectional area divided by external elastic membrane cross-sectional area and is presented as a percentage. The composition of the atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core.[9] A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive

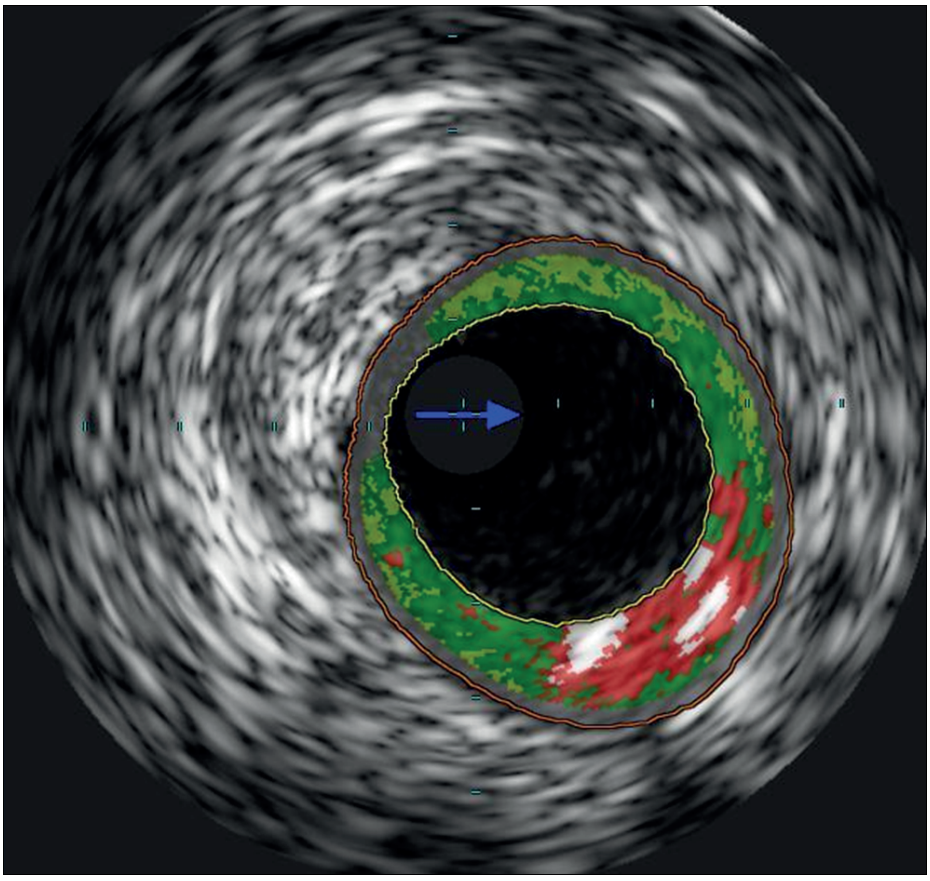

Figure 1. Thin-cap fibroatheroma lesion on intravascular ultrasound virtual histology
Thin-cap fibroatheroma lesion on intravascular ultrasound virtual histology is defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. White indicates dense calcium, light green indicates fibrofatty tissue, dark green indicates fibrous tissue.

frames. A thin-cap fibroatheroma (TCFA) lesion on IVUS-VH was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen (Figure 1).[10,11] TCFA lesions with a plaque burden of at least 70% were classified as large TCFA lesions.

Study endpoints

In this study, follow-up started at inclusion and lasted up to 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. Treating physicians and institutions were contacted for additional information whenever necessary. 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.[12-14] 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 the 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 all-cause mortality, ACS or unplanned coronary revascularization. The secondary endpoint was defined as the composite of non-culprit lesion related or indeterminate all-cause mortality or ACS. Definite culprit lesion related events were excluded from the primary and secondary endpoints, because the pathophysiology of culprit lesions related events (e.g. in-stent restenosis or in-stent thrombosis) differs from our primary research focus on spontaneous plaque rupture leading to unanticipated, spontaneous MACE. The endpoints were adjudicated by a clinical event committee that had no knowledge of biomarkers and IVUS data.

Statistical analysis

The distributions of the continuous variables, including biomarker levels and the IVUS parameters, were tested for normality by visual examination of the histogram. Normally distributed continuous variables are presented as mean ± standard deviation (SD), while non-normally distributed continuous variables are presented as median and interquartile range (IQR). MCP-1, MIP-1α, MIP-1β and RANTES concentrations were not normally distributed and were therefore ln-tranformed for further analysis. Categorical variables are presented in percentages. We examined associations of biomarker concentrations with plaque burden and necrotic core fraction in the imaged coronary segment. Specifically, we calculated means of plaque burden and necrotic core fraction according to tertiles of biomarker concentration. To test for trends, we used linear regression analyses with continuous ln-transformed biomarker concentrations as the independent variable. The final results are presented as β (per SD increase in ln-transformed biomarker concentration) with 95% confidence interval (95% CI). Furthermore, we have examined the relation between biomarker concentrations and the presence of IVUS-VH derived TCFA lesions using logistic regression analyses with continuous ln-tranformed biomarker concentration as the independent variable. The final results are presented as odds ratio (OR) per SD increase in ln-transformed biomarker concentration with 95% CI.

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 log-rank test. Cox proportional hazards regression analyses were performed to evaluate the relationship between biomarker concentration and clinical endpoints. Biomarkers that were significantly associated with occurrence of MACE in univariable analysis were further evaluated in multivariable analyses. The variables age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, statin use, history of MI and indication for coronary angiography were considered as potential confounders and were entered into the full model. These covariates were a priori chosen, taking into account the number of events available. Subsequently, C-reactive protein (CRP) was also entered into the model to evaluate whether the associations between biomarkers and MACE were independent of CRP concentration. The final results are presented as hazard ratio (HR) per SD increase in ln-transformed biomarker concentration with 95% CI.

All statistical analyses were primarily performed in the overall study population. Heterogeneity in effect estimates between patients with ACS and patients with stable angina were examined using the Z-test for heterogeneity. If there was no heterogeneity, conclusions were based on the effect estimates belonging to the total study population. If there was significant heterogeneity between patients admitted with and without ACS, conclusions were based on effect estimates of the separate groups.

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 ± 11.4 years, 75.4% were men and 17.4% had diabetes mellitus (Table 1). Coronary angiography or PCI was performed for various indications: 159 (27.9%) patients had an acute myocardial infarction, 150 (26.3%) patients had unstable angina pectoris and 261 (45.8%) patients had stable angina pectoris. Some patients had biomarker concentrations beneath the lowest detection limit of the assay, which especially pertains to MIP-1α (measurable in 84% of patients). The median length of the imaged coronary segment was 44.1 [33.7-55.4] mm. On basis of radiofrequency IVUS, a total of 239 (41.9%) patients had at least 1 IVUS-VH-derived TCFA, including 69 (12.1%) patients with at least 1 IVUS-VH-derived TCFA with a plaque burden ≥70%.

Table 1. Baseline characteristics

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Patient characteristics			
Age, years	61.5 ± 11.4	59.7 ± 11.9	63.6 ± 10.3
Men, n (%)	430 (75.4)	227 (73.5)	203 (77.8)
Diabetes mellitus, n (%)	99 (17.4)	40 (12.9)	59 (22.6)
Hypertension. n (%)	295 (51.8)	134 (43.4)	161 (61.7)
Hypercholesterolemia, n (%)	317 (55.6)	137 (44.3)	180 (69.0)
Smoking, n (%)	164 (28.8)	115 (37.2)	49 (18.8)
Positive family history, n (%)	293 (51.4)	140 (45.3)	153 (58.6)
Previous MI, n (%)	184 (32.3)	80 (25.9)	104 (39.8)
Previous PCI, n (%)	185 (32.5)	57 (18.4)	128 (49.0)
Previous CABG, n (%)	18 (3.2)	7 (2.3)	11 (4.2)
Previous stroke, n (%)	23 (4.0)	10 (3.2)	13 (5.0)
Peripheral artery disease, n (%)	36 (6.3)	12 (3.9)	24 (9.2)
History of renal insufficiency, n (%)	32 (5.6)	13 (4.2)	19 (7.3)
History of heart failure, n (%)	19 (3.3)	6 (1.9)	13 (5.0)
C-reactive protein, mg/L	2.1 [0.8-5.3]	2.8 [1.1-7.0]	1.5 [0.6-3.1]
Statin use, n (%)	359 (63.0)	146 (47.2)	213 (81.6)
Procedural characteristics			
Indication for catheterization			
Acute coronary syndrome, n (%)	309 (54.2)	309 (100)	0 (0)
Myocardial infarction, n (%)	159 (27.9)	159 (51.5)	0 (0)
Unstable angina pectoris, n (%)	150 (26.3)	150 (48.5	0 (0)
Stable angina pectoris, n (%)	261 (45.8)	0 (0)	261 (100)
Coronary artery disease			
No significant stenosis, n (%)	42 (7.4)	18 (5.8)	24 (9.2)
1-vessel disease, n (%)	301 (52.8)	168 (54.4)	133 (51.0)
2-vessel disease, n (%)	166 (29.1)	88 (28.5)	78 (29.9)
3-vessel disease, n (%)	61 (10.7)	35 (11.3)	26 (10.0)
PCI performed, n (%)	501 (87.9)	287 (92.9)	214 (82.0%)
Serum biomarker concentrations			
MCP-1, pg/ml *	91 [70-122]	92 [70-133]	88 [71-111]
MIP-1α, pg/ml †	16.0 [12.0-21.9]	15.0 [12.0-21.9]	17.0 [12.0-21.9]
MIP-1β, pg/ml *	123 [92-165]	130 [95-179]	114 [89-146]
RANTES, ng/ml ‡	11.0 [6.4-19.0]	14.0 [7.6-23.0]	9.1 [5.0-14.3]
IVUS segment characteristics			
Imaged coronary artery			
Left anterior descending, n (%)	204 (35.8)	117 (37.9)	87 (33.3)

Table 1 *(continued)*

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Left circumflex, n (%)	190 (33.3)	107 (34.6)	83 (31.8)
Right coronary artery, n (%)	176 (30.9)	85 (27.5)	91 (34.9)
Segment length, mm	44.1 [33.7-55.4]	43.9 [32.9- 54.1]	44.8 [34.2-57.2]
At least 1 TCFA	239 (41.9)	140 (45.3)	99 (37.9)
At least 1 TCFA with PB≥70%	69 (12.1)	32 (10.4)	37 (14.2)

* Measurable in >99% of patients; below limit of detection in <1% of patients.

† Measurable in 84% of patients; below limit of detection in 16% of patients.

‡ Measurable in all patients.

ACS indicates acute coronary syndrome; CABG, coronary artery bypass grafting; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; PB, plaque burden; PCI, percutaneous coronary intervention; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris; TCFA, thin-cap fibroatheroma.

Associations with coronary atherosclerosis

In patients who were admitted with stable angina pectoris, higher plasma MCP-1 concentrations were associated with higher coronary plaque burden (per SD increase of ln-transformed MCP-1: β=2.56, 95% CI 0.91-4.21, p=0.002) and a higher fraction of plaque consisting of necrotic core (per SD increase of ln-transformed MCP-1: β=1.14, 95% CI 0.02-2.25, p=0.045) (Table 2). Higher MCP-1 concentrations also seemed to be associated with the presence of IVUS-VH derived TCFA lesions (OR per SD increase in ln-transformed MCP-1 1.90, 95% CI 1.00-3.61, p=0.052) in patients who were admitted with stable angina pectoris (Table 3).

Higher MIP-1α concentrations were associated with higher plaque burden (per SD increase of ln-transformed MIP-1α: β=1.66, 95% CI 0.72-2.61, p=0.001), higher necrotic core fraction (per SD increase of ln-transformed MIP-1α: β=0.89, 95% CI 0.23-1.55, p=0.008) and with the presence of IVUS-VH derived TCFA lesions with plaque burden ≥70% (OR per SD increase in ln-transformed MIP-1α 1.75, 95% CI 1.09-2.81, p=0.021) in the total study population.

In patients who were admitted with ACS, lower RANTES concentrations were associated with higher plaque burden (per SD increase of ln-transformed RANTES: β=−1.57, 95% CI −2.94;−0.20, p=0.025) (Figure 2). Furthermore, lower RANTES concentrations also seemed to be associated with the presence of IVUS-VH derived TCFA lesions with plaque burden ≥70% in the overall patient population (OR per SD increase in ln-transformed RANTES 0.76, 95% CI 0.57-1.02, p=0.067).

Table 2. Associations with plaque burden and necrotic core fraction in imaged coronary segment

	Total study population (n=570)				ACS patients (n=309)				SAP patients (n=261)				Hetero-geneity
	*Tertile 1**	*Tertile 2**	*Tertile 3**	*P*	*Tertile 1**	*Tertile 2**	*Tertile 3**	*P*	*Tertile 1**	*Tertile 2**	*Tertile 3**	*P*	*P*
Mean values of plaque burden (%)													
MCP-1	38.0 ± 11.0	37.8 ± 11.3	38.9 ± 12.4	0.46	38.4 ± 11.9	35.7 ± 11.0	36.9 ± 12.5	0.49	37.7 ± 9.9	40.2 ± 10.7	41.0 ± 12.3	0.002	0.004
MIP-1α	36.9 ± 10.8	37.8 ± 9.8	39.0 ± 11.9	0.001	35.1 ± 10.6	36.5 ± 10.1	39.3 ± 12.2	0.001	38.8 ± 10.9	39.8 ± 9.0	38.6 ± 11.7	0.38	0.10
MIP-1β	36.7 ± 11.2	39.0 ± 11.5	39.0 ± 11.8	0.31	36.5 ± 12.2	38.6 ± 11.4	36.0 ± 11.8	0.84	37.3 ± 10.1	39.5 ± 11.9	42.1 ± 10.8	0.015	0.071
RANTES	39.5 ± 10.9	37.7 ± 12.2	37.5 ± 11.4	0.089	38.8 ± 11.4	37.3 ± 12.0	34.9 ± 11.8	0.025	39.4 ± 10.3	38.3 ± 12.0	41.2 ± 10.8	0.32	0.022
Mean values of necrotic core fraction (%													
MCP-1	21.3 ± 8.1	21.3 ± 7.3	21.6 ± 8.8	0.84	22.6 ± 8.4	21.1 ± 8.2	21.5 ± 9.2	0.32	19.6 ± 7.3	21.6 ± 6.5	21.9 ± 8.1	0.045	0.027
MIP-1α	21.1 ± 7.6	21.6 ± 7.2	21.5 ± 8.7	0.008	21.7 ± 7.9	21.0 ± 7.4	23.0 ± 9.3	0.009	20.1 ± 7.2	22.4 ± 6.7	19.9 ± 7.7	0.33	0.27
MIP-1β	21.4 ± 8.0	21.4 ± 7.5	21.4 ± 8.7	0.76	21.9 ± 8.1	21.3 ± 8.0	22.0 ± 9.6	0.84	20.8 ± 7.8	21.5 ± 6.1	20.9 ± 8.1	0.91	0.83
RANTES	21.8 ± 7.3	21.1 ± 9.1	21.4 ± 7.8	0.53	22.8 ± 8.1	21.6 ± 9.1	20.8 ± 8.5	0.17	21.0 ± 6.4	20.4 ± 8.3	21.8 ± 7.4	0.81	0.24

P-values were obtained with linear regression analyses with continuous ln-transformed biomarker concentration as independent variable.

* Tertiles of biomarker levels.

ACS indicates acute coronary syndrome; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris.

Table 3. Associations with presence of intravascular ultrasound virtual histology-derived thin-cap fibroatheroma lesions

	Total study population (n=570)		ACS patients (n=309)		SAP patients (n=261)		Hetero-geneity
	OR (95% CI)	*P*	*OR (95% CI)*	*P*	*OR (95% CI)*	*P*	*P*
Presence of at least 1 thin-cap fibroatheroma							
MCP-1	1.03 (0.74-1.45)	0.85	0.77 (0.51-1.17)	0.22	1.90 (1.00-3.61)	0.052	0.022
MIP-1α	0.87 (0.63-1.21)	0.42	0.94 (0.61-1.42)	0.75	0.83 (0.49-1.39)	0.47	0.72
MIP-1β	1.16 (0.85-1.60)	0.36	1.18 (0.79-1.76)	0.42	0.97 (0.55-1.70)	0.91	0.69
RANTES	0.97 (0.80-1.18)	0.75	0.87 (0.66-1.15)	0.33	0.98 (0.72-1.33)	0.90	0.57
Presence of at least 1 thin-cap fibroatheroma with plaque burden ≥70%							
MCP-1	1.23 (0.75-2.04)	0.41	0.94 (0.48-1.83)	0.86	2.16 (0.95-4.93)	0.067	0.12
MIP-1α	1.75 (1.09-2.81)	0.021	2.15 (1.13-4.09)	0.020	1.29 (0.63-2.66)	0.49	0.30
MIP-1β	0.89 (0.54-1.47)	0.66	0.91 (0.47-1.78)	0.79	1.01 (0.46-2.20)	0.98	0.85
RANTES	0.76 (0.57-1.02)	0.067	0.73 (0.47-1.15)	0.17	0.84 (0.55-1.28)	0.41	0.67

Odds ratios are per standard deviation increase in ln-transformed biomarker concentration.
ACS indicates acute coronary syndrome; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris.

Major adverse cardiac events

Vital status was acquired for 569 (99.8%) patients. Response rate of the questionnaires that were sent to all living patients was 92.3%. After 1 year of follow-up, 56 patients had at least 1 event (Supplemental table 1). 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 incidences of the 30-day, 6-month and 1-year composite of non-culprit lesion related or indeterminate death, ACS or unplanned coronary revascularization were 0.7%, 4.7%, and 7.9%, respectively. The cumulative Kaplan-Meier incidences of the 30-day, 6-month and 1-year composite of non-culprit lesion related or indeterminate death or ACS were 0.7%, 3.2%, and 4.9%, respectively.

Associations with non-culprit lesion related and indeterminate events

In univariable analysis, RANTES (HR per SD increase of ln-transformed RANTES 0.67, 95% CI 0.50-0.89, p=0.005) was associated with occurrence of the primary endpoint of non-culprit lesion related and indeterminate MACE during follow-up (Table 4, Figure 2). There was no heterogeneity in the hazard ratio estimate between ACS patients and patients with stable angina (heterogeneity p=0.39). RANTES (HR per SD increase of ln-transformed RANTES 0.64, 95% CI 0.45-0.91, p=0.013) was also significantly associated

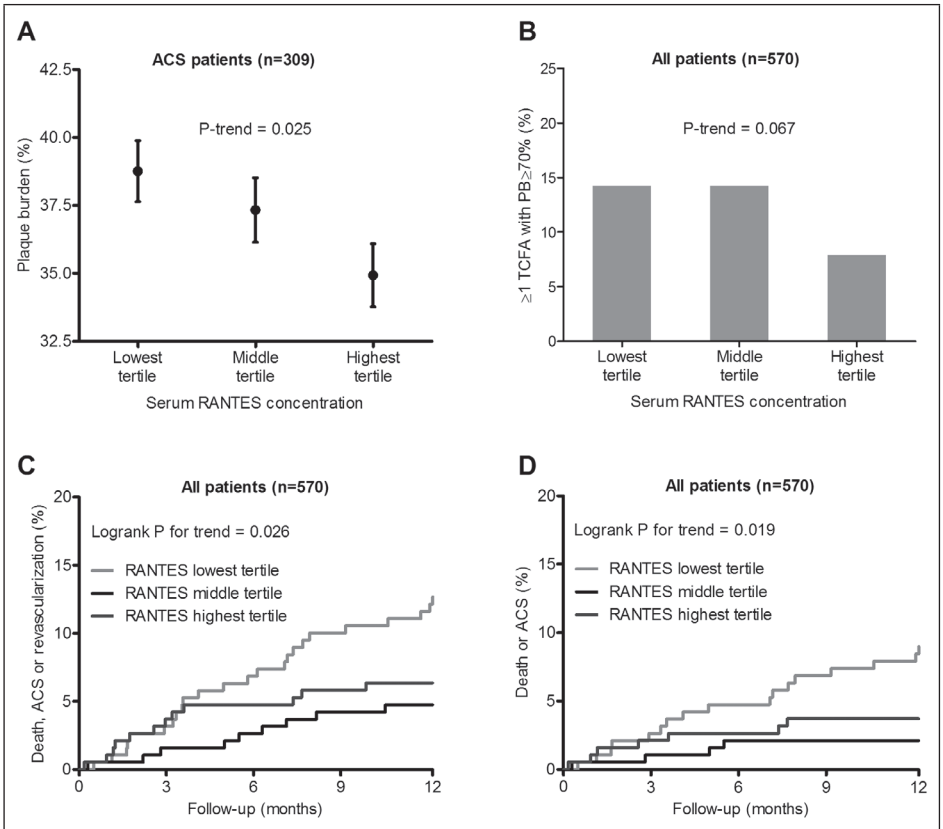

Figure 2. Associations of circulating RANTES concentrations with coronary atherosclerosis and clinical outcome

A. Association with intravascular ultrasound-derived measures of coronary plaque burden in patients admitted with acute coronary syndrome.

B. Association with presence of thin-cap fibroatheroma lesions with plaque burden ≥70% as assessed by intravascular ultrasound virtual histolgy.

C. Association with occurrence of non-culprit lesion related and indeterminate death, acute coronary syndrome or coronary revascularization. The lowest RANTES tertile was associated with the highest event rate (lowest tertile vs. middle tertile p=0.006; lowest tertile vs. highest tertile p=0.042; middle tertile vs. highest tertile p=0.50; logrank p for trend=0.026).

D. Association with occurrence of non-culprit lesion related and indeterminate death or acute coronary syndrome. The lowest RANTES tertile was associated with the highest event rate (lowest tertile vs. middle tertile p=0.004; lowest tertile vs. highest tertile p=0.039; middle tertile vs. highest tertile p=0.86; logrank p for trend=0.019).

ACS indicates acute coronary syndrome; PB, plaque burden; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; TCFA, thin-cap fibroatheroma.

with the composite of non-culprit lesion related and indeterminate death or ACS only. After adjustment for conventional cardiovascular risk factors in multivariable analysis, RANTES remained independently predictive for non-culprit lesion related and inde-

Table 4. Associations with non-culprit lesion related and indeterminate major adverse cardiac events

	Total study population (n=570)		ACS patients (n=309)		SAP patients (n=261)		Hetero-geneity
	HR (95% CI)	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*	*P*
Major adverse cardiac events (primary endpoint)							
MCP-1	0.87 (0.64-1.18)	0.37	0.81 (0.55-1.20)	0.29	1.00 (0.61-1.65)	1.00	0.51
MIP-1α	1.13 (0.85-1.49)	0.40	1.16 (0.82-1.66)	0.40	1.06 (0.69-1.64)	0.80	0.74
MIP-1β	1.00 (0.74-1.34)	0.99	1.15 (0.82-1.62)	0.42	0.82 (0.50-1.34)	0.42	0.26
RANTES	0.67 (0.50-0.89)	0.005	0.77 (0.50-1.18)	0.23	0.59 (0.40-0.88)	0.009	0.39
Composite of death or acute coronary syndrome (secondary endpoint)							
MCP-1	0.73 (0.48-1.09)	0.12	0.74 (0.47-1.16)	0.19	0.69 (0.31-1.53)	0.36	0.88
MIP-1α	1.11 (0.77-1.58)	0.58	1.12 (0.73-1.70)	0.61	1.11 (0.59-2.09)	0.74	0.99
MIP-1β	1.11 (0.78-1.57)	0.57	1.34 (0.98-1.84)	0.071	0.48 (0.24-0.98)	0.043	0.010
RANTES	0.64 (0.45-0.91)	0.013	0.58 (0.36-0.94)	0.028	0.62 (0.35-1.10)	0.10	0.86

Hazard ratios are per standard deviation increase in ln-transformed biomarker concentration.
MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; SAP, stable angina pectoris.

terminate MACE (HR per SD increase of ln-transformed RANTES 0.69, 95% CI 0.52-0.93, p=0.016) and for non-culprit lesion related and indeterminate death or ACS only (HR per SD increase of ln-transformed RANTES 0.60, 95% CI 0.41-0.88, p=0.010) (Table 5). RANTES also remained independently associated with MACE (HR per SD increase of ln-transformed RANTES 0.69, 95% CI 0.51-0.93, p=0.014) and the composite of death or ACS

Table 5. Multivariable analysis on non-culprit lesion related and indeterminate major adverse cardiac events

	Adjusted for age and gender		Adjusted for age, gender and indication for angiography		Adjusted for conventional risk factors and indication for angiography*		Adjusted for conventional risk factors, indication for angiography and CRP*	
	HR (95% CI)	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
Major adverse cardiac events (primary endpoint)								
RANTES	0.72 (0.54-0.96)	0.024	0.71 (0.53-0.95)	0.023	0.69 (0.52-0.93)	0.016	0.69 (0.51-0.93)	0.014
Composite of death or acute coronary syndrome (secondary endpoint)								
RANTES	0.69 (0.48-0.99)	0.046	0.64 (0.44-0.93)	0.021	0.60 (0.41-0.88)	0.010	0.59 (0.40-0.88)	0.010

Hazard ratios are per standard deviation increase in ln-transformed biomarker concentration.
* Conventional risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, statin use and history of myocardial infarction.
CRP indicates C-reactive protein; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted.

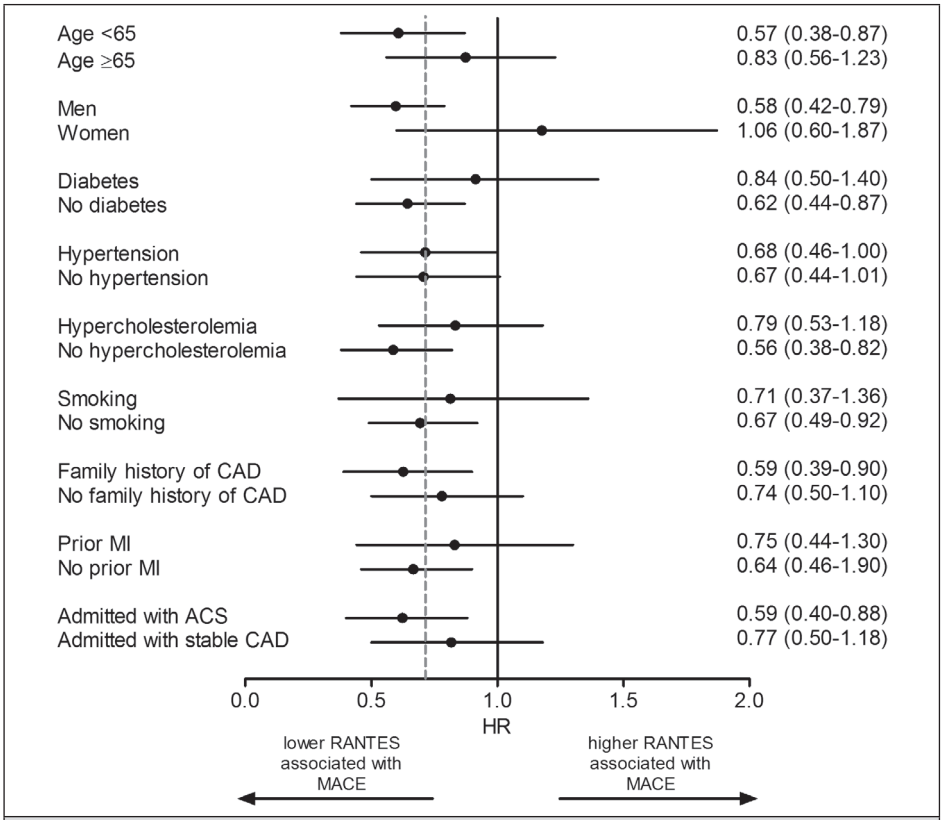

Figure 3. Association between RANTES level and major adverse cardiac events stratified by patient subgroups

Hazard ratios (95% confidence intervals) are per standard deviation increase in ln-transformed RANTES concentration. Dotted line indicates the hazard ratio estimate in the total study population.

ACS indicates acute coronary syndrome; CAD, coronary artery disease; HR, hazard ratio; MI, myocardial infarction; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted.

only (HR per SD increase of ln-transformed RANTES 0.59, 95% CI 0.40-0.88, p=0.010) after additional adjustment for baseline CRP levels. Subgroup analysis showed that the inverse association between RANTES level and MACE was present in all patient subgroups (Figure 3). There was no significant heterogeneity in the hazard ratio estimate between the evaluated patient subgroups.

DISCUSSION

This study investigated the relations of circulating chemokine concentrations with extensiveness of coronary atherosclerosis, amount of necrotic core, the presence of

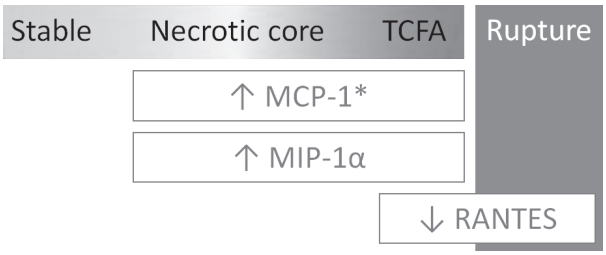

Figure 4. Circulating chemokine concentrations as biomarkers for phenotype of coronary atherosclerosis and risk of plaque rupture
Hypothesized model based on findings in this study. Plasma MCP-1, MIP-1α and RANTES concentrations were all associated with coronary plaque burden and different plaque phenotypes. RANTES was associated with major adverse cardiac events that were most probably caused by plaque rupture.
* Only for patients with stable angina pectoris.
↑ indicates higher biomarker concentrations.
↓ indicates lower biomarker concentrations.
MCP-1 indicates monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; RANTES, Regulated upon Activation Normal T cell Expressed and Secreted; TCFA, thin-cap fibroatheroma.

IVUS-VH derived TCFA lesions and occurrence of future major adverse cardiac events in patients who underwent coronary angiography for ACS or stable angina pectoris. To our best knowledge, this is the first study that correlates circulating chemokines with in-vivo measurements of coronary atherosclerosis using IVUS-VH. Higher plasma MCP-1, MIP-1α, and lower RANTES concentrations were all associated with higher coronary plaque burden and more advanced plaque phenotypes as determined by IVUS-VH (Figure 4). However, only RANTES was found to be independently predictive for the occurrence of MACE, particularly of death and ACS.

Chemokines are small cytokines that have the ability to induce directed chemotaxis of nearby leukocytes. MCP-1, MIP-1α, MIP-1β and RANTES belong to the C-C motif chemokine ligand (CCL) family and are also known as CCL2, CCL3, CCL4 and CCL5, respectively.[5,6] Pathologic studies have shown that these chemokines are highly expressed in atherosclerotic plaques.[15-17] Animal studies have shown that these chemokines are actively involved in atherogenesis and plaque destabilization.[5,6] Furthermore, several epidemiological studies have indicated that serum or plasma levels of MCP-1, MIP-1α, MIP-1β and RANTES may predict future cardiac events.[5] However, their clinical utility as biomarker for cardiovascular risk stratification remains unclear.[5,6] We sought to further elucidate the correlations of circulating chemokine concentrations with in-vivo measurements of extensiveness, phenotype and vulnerability of coronary atherosclerosis by using IVUS-VH.

Grey-scale IVUS allows for in-vivo measurements of coronary plaque burden. Additionally, radiofrequency IVUS allows for differentiation of the composition of the athero-

sclerotic plaque and is therefore also known as IVUS-VH.[10] Necrotic core is often found in the more advanced and rupture-prone plaques.[18] The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study has demonstrated that TCFA lesions as determined by IVUS-VH are associated with MACE.[19] The strong and independent associations (adjusted hazard ratios ranging from 1.79 to 3.35) of IVUS-VH-derived TCFA with MACE emphasize its biological importance.[18-20] However, there are several reasons why IVUS is currently not suitable for use as diagnostic and prognostic tool in the overall population of patients with coronary artery disease.[19] Its invasiveness is probably the most important limitation in this respect. Therefore, circulating biomarkers may have an important role in cardiovascular risk assessment.

In our study, lower plasma RANTES concentrations were independently associated with adverse outcomes during 1 year of follow-up. The association was independent of CRP. Its association with acute cardiac events (death or ACS; HR 0.59) seemed to be even stronger than with all major adverse cardiac events (death, ACS or unplanned coronary revascularization; HR 0.69). This may indicate that RANTES is especially predictive for plaque rupture rather than plaque growth. Our finding that low serum RANTES concentrations, rather than high, are associated with adverse coronary events may seem counterintuitive, since animal studies have shown that RANTES and its receptor are actively involved in atherogenesis and that RANTES was found to be highly expressed within atheromous lesions.[6,21] However, the inverse associations of RANTES may be explained by increased deposition of RANTES on the vascular endothelium, resulting in lower free circulating serum concentrations.[22,23] The inverse associations of RANTES are also consistent with observations from previous studies. A large case-control study reported that serum RANTES levels were lower in coronary heart disease patients compared with age- and gender-matched controls.[22] Another study reported that low plasma RANTES levels were independently associated with cardiac mortality in 389 male patients who underwent coronary angiography.[23] Such an association was not found in a population-based case-cohort study that included 363 individuals with incident coronary events and 1908 non-cases.[24]

We found that higher plasma MCP-1 concentrations were associated with higher coronary plaque burden in patients who were admitted with stable angina pectoris. These findings are in line with a previous study that measured MCP-1 concentrations in blood from the coronary sinus and found that these levels were associated with the extent of coronary atherosclerosis as assessed on the coronary angiogram.[25] Although we observed that high MCP-1 concentrations were associated with a more advanced plaque phenotype (i.e. higher necrotic core fraction) and with the presence of IVUS-VH derived TCFA lesions, MCP-1 was not predictive for future events. Previous epidemiological studies have shown that the ability of MCP-1 to predict subclinical coronary artery disease is somewhat disappointing, but that MCP-1 may have some value in predicting

cardiovascular events in patients with overt coronary artery disease.[5] For example, a previous study found that MCP-1 was independently associated with the composite of death or myocardial infarction in a large cohort of 4244 patients with ACS.[26] This study also demonstrated that high MCP-1 values at 4 months after the initial ACS were still predictive for long-term mortality afterwards. A major difference with our study is that both culprit lesion related and non-culprit lesion related events were included in their study endpoints, while definite culprit lesion related events were excluded from our study endpoints. Furthermore, we may have lacked statistical power to detect the previously reported association.

MIP-1α has been studied less extensively. We found that MIP-1α was associated with coronary plaque burden, necrotic core fraction and with the presence of large TCFA lesions on IVUS-VH. However, we did not observe a correlation between MIP-1α concentration and occurrence of MACE. Another study, however, found that MIP-1α was predictive for recurrent ACS in a relatively small cohort of 54 patients with unstable angina pectoris.[27] Further research is required to elucidate the role of MIP-1α in patients with coronary artery disease.

CONCLUSIONS

Higher circulating MCP-1, MIP-1α, and lower RANTES concentrations were associated with a higher extent, a more advanced phenotype and a higher vulnerability of coronary atherosclerosis. Such associations were not present for MIP-1β. In addition, RANTES was independently associated with occurrence of MACE, particularly of death and ACS. Its prognostic value was similar in patients with and without ACS. Its inverse associations are consistent with observations from previous studies and may be explained by increased deposition of RANTES on the endothelium, resulting in lower free circulating concentrations. The findings in this study demonstrate that RANTES may be a useful biomarker for assessment of cardiovascular risk. Further research on the incremental prognostic value of RANTES over established clinical covariates in large, prospective studies is warranted.

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SUPPLEMENTAL TABLES

Supplemental table 1. Patients with major adverse cardiac events

	Culprit lesion related events	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	56
Death from any cause, n	1	1	16	17	18
Definite cardiac or unexplained sudden death, n	1	1	6	7	8
Acute coronary syndrome, n	3	9	2	11	14
Myocardial infarction, n	2	3	2	5	7
Elective coronary revascularization, n	7	17	0	17	24
Composite of death or acute coronary syndrome, n	4	10	18	28	32