Circulating cytokines in relation to the extent and composition of coronary atherosclerosis

Battes LC*, Cheng JM*, Oemrawsingh RM, Boersma E, Garcia-Garcia HM, De Boer SP, Buljubasic N, Mieghem NA, Regar E, Geuns RJ, Serruys PW, Akkerhuis KM, Kardys I.

** These authors contributed equally.*

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ABSTRACT

Objective: We investigated whether concentrations of TNF-α, TNF-β, TNF-receptor 2, interferon-γ, IL-6, IL-8, IL-10 and IL-18 are associated with cardiovascular outcome, as well as extent and composition of coronary atherosclerosis determined by grayscale and virtual histology (VH)-intravascular ultrasound (IVUS).

Methods: Between 2008-2011, IVUS(-VH) imaging of a non-culprit coronary artery was performed in 581 patients (stable angina pectoris (SAP), n=261; acute coronary syndrome (ACS), n=309) undergoing coronary angiography from the ATHEROREMO-IVUS study. Coronary plaque burden and VH-derived thin-cap fibroatheroma (TCFA) lesions were assessed. Major adverse cardiac events (MACE: all-cause mortality, ACS, unplanned coronary revascularization) were registered during 1-year follow-up. We applied linear and logistic regression.

Results: TNF-α levels were positively associated with plaque burden (beta (β) [95%CI]: 4.45 [0.99-7.91], for highest vs lowest TNF-α tertile) and presence of VH-TCFA lesions (odds ratio (OR) [95%CI] 2.30 (1.17-4.52), highest vs lowest TNF-α tertile) in SAP patients. Overall, an inverse association was found between IL-10 concentration and plaque burden (β [95%CI]: −1.52 [−2.49 – −0.55], per Ln(pg/mL) IL-10) as well as IL-10 and VH-TCFA lesions with plaque burden ≥70% (OR: 0.31 [0.12-0.80],highest vs lowest IL-10 tertile). These effects did not reach statistical significance in the separate SAP and ACS groups. Fifty-six (9.8%) patients had MACE. No statistically significant associations were present between biomarkers and MACE.

Conclusion: Higher circulating TNF-α was associated with higher plaque burden and VH-TCFA lesions in SAP patients. Lower circulating IL-10 was associated with higher plaque burden and large VH-TCFA lesions. These in-vivo findings suggest a role for these cytokines in extent and vulnerability of atherosclerosis.

INTRODUCTION

Inflammation is known to play a major role in atherosclerosis[1-3].The development of atherosclerosis includes, among others, expression of adhesion molecules by inflamed endothelium, migration of leukocytes into the intima, uptake of modified lipoprotein particles, and formation of lipid-laden macrophages[4]. During the evolution of atherosclerotic lesions, T-lymphocytes join the macrophages in the intima[4]. This T-cell infiltrate produces proinflammatory cytokines (including tumor necrosis factors (TNFs), interferons (IFNs), and interleukins (ILs)), but may also stimulate a T helper cell type 2 (Th2) response which can promote anti-inflammatory actions (and cytokines such as IL-10 and transforming growth factor β) [2, 5]. This dual role of cytokines is believed to control the subsequent development and destabilization of arherosclerotic plaques in coronary (among other) arteries[6], potentially leading to plaque rupture or erosion and ultimately resulting in adverse clinical events such as myocardial infarction or sudden cardiac death [7].

While previous research has provided ample insights into the signalling cascades of cytokines and their roles in the pathogenesis of atherosclerosis, studies on the associations of cytokines with in-vivo determined extent and particularly composition of coronary atherosclerosis are currently scarce. Cytokines are located both inside the affected vessel walls and in the circulation [8]. We hypothesize that circulating cytokines are associated with in-vivo measures of plaque burden and features of plaque vulnerability, and consequently may be useful for clinical risk stratification with regard to cardiovascular outcome.

The aim of this study is to examine the associations of the cytokines TNF-α, TNF-β, interferon γ (IFNγ), IL-6, IL-8, IL-10 and IL-18 and of circulating TNF receptor 2 (TNF R2) with the extent and composition of coronary atherosclerosis as determined in-vivo by intravascular ultrasound (IVUS) and IVUS-virtual histology (IVUS-VH), in a non-culprit vessel in patients undergoing coronary angiography. Furthermore, the prognostic value of the cytokines for major adverse cardiac events (MACE) in these patients is studied.

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[9]. In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS; n=309) or stable angina pectoris (SAP; n=261) have been included from November

2008 to January 2011 in the Erasmus MC, Rotterdam, the Netherlands. Intravascular ultrasound (IVUS) of a non-culprit coronary artery was performed subsequent to angiography. The ATHEROREMO-IVUS study has been approved by the human research ethics committee of Erasmus MC, Rotterdam, the Netherlands. Written informed consent was obtained from all included patients and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

Biomarkers

Blood samples were drawn from the arterial sheath prior to the diagnostic coronary angiography or PCI procedure, and were available in 570 patients for the current study. The blood samples were transported to the clinical laboratory of Erasmus MC for further processing and storage at a temperature of −80°C within two hours after blood collection.

C-reactive protein (CRP) was measured in serum samples using a immunoturbidimetric high sensitivity assay (Roche Diagnostics Ltd., Rotkreuz, Switzerland) on the Cobas 8000 modular analyzer platform (Roche Diagnostics Ltd., Rotkreuz, Switzerland). These analyses were performed in the clinical laboratory of Erasmus MC.

Frozen EDTA-plasma samples were transported under controlled conditions (at a temperature of −80°C) to Myriad RBM, Austin, Texas, USA, where the concentrations of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 were determined using a validated multiplex assay (Custom Human Map, Myriad RBM, Austin, Texas, USA). While TNF-α, TNF R2, IL-6, and IL-8 were determined in the full cohort of 570 patients, TNF-β, INFγ, IL-10 and IL-18, were determined in a random subset of 473 patients. This difference in numbers resulted from batch-wise handling of the samples in combination with an update of the composition of the multiplex assay by the manufacturer in-between two batches. None of the biomarker laboratories had knowledge of clinical or intracoronary imaging data.

Intravascular ultrasound

Following the standard coronary angiography or PCI procedure, IVUS data were acquired in a non-culprit, non-treated, coronary vessel, without significant luminal narrowing. 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 or biomarker data. The IVUS gray-scale and IVUS radiofrequency analyses, also known as IVUS virtual histology (IVUS-VH), 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 the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area and is presented as a percentage. A coronary lesion was defined as a segment with a plaque burden of 40% in at least three consecutive frames[9]. Using IVUS-VH, the composition of the atherosclerotic plaque was characterized into 4 different types: fibrous, fibro-fatty, dense calcium and necrotic core [10]. A VH-IVUS-derived thin-cap fibroatheroma (TCFA) lesion was defined as a lesion with presence of > 10% confluent necrotic core in direct contact with the lumen[11].

Clinical study endpoints

In this study, follow-up lasted up to 1 year post angiography. Post-discharge survival status was obtained from municipal civil registries. Post-discharge rehospitalizations were prospectively assessed. 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. 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). The primary endpoint was MACE, defined as all-cause mortality, ACS or unplanned coronary revascularization. The endpoints were adjudicated by a clinical event committee that had no knowledge of biomarkers and IVUS data.

Statistical analysis

Categorical variables are presented in percentages. The distributions of continuous variables, including biomarker levels and IVUS parameters, were examined for normality by visual inspection of the histogram and calculation of the skewness coefficient. 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). For reasons of uniformity, all biomarkers are presented as median (IQR).

In further analyses, biomarker concentrations were examined both as continuous and as categorical variables (the latter by dividing the variables into tertiles). Biomarkers with a non-normal distribution were ln-transformed. Biomarkers in which the concentrations were too low to detect in more than 20% of the patients, were not examined as continuous variables. They were examined as tertiles, or else as dichotomous variables (measurable vs not measurable).

To take into account possible effect modification by indication for coronary angiography, we performed all analyses separately in patients with SAP and patients with

ACS. We also present the results for the full cohort, in order to evaluate the effect of higher statistical power in those cases where associations were present in both groups of patients.

First, we examined associations of biomarker concentrations with the extent of atherosclerosis according to IVUS. We applied linear regression analyses with biomarker concentrations as the independent variable (ln-transformed or categorized when appropriate) and segmental plaque burden in the imaged coronary segment as the dependent variable. The results are presented as βs (per unit increase in ln-transformed biomarker concentration or per category of biomarker concentration) with 95% confidence intervals (95% CI). Subsequently, we examined the associations between biomarker concentrations and composition of atherosclerosis, specifically the presence of VH-TCFA lesions as well as VH-TCFA lesions with plaque burden ≥ 70%. We used logistic regression analyses with biomarker concentrations as the independent variable (ln-transformed or categorized when appropriate). The results are presented as odds ratios (ORs) per unit increase in ln-transformed biomarker concentration or per category of biomarker concentration, with 95% CIs.

Moreover, we examined associations of biomarker concentrations with MACE during 1 year follow-up. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. We used Cox proportional hazard regression analyses with biomarker concentration as the independent variable (ln-transformed or categorized when appropriate). The results are presented as hazard ratios (HRs) per unit increase in ln-transformed biomarker concentration or per category of biomarker concentration, with 95% CIs.

First, all above-described analyses were performed univariably. Subsequently, we adjusted for age, gender, indication for coronary angiography, diabetes, hypertension and CRP.

All data were analyzed with SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics are summarized in Table 1. Mean age was 61.5 ± 11.4 years and 75.4% were men. Coronary angiography or PCI was performed for several indications: 159 (27.9%) patients had an acute myocardial infarction, 150 (26.3%) patients had unstable angina pectoris and 261 (45.8%) had SAP. The median length of the imaged coronary segment was 44.1 [33.7-55.4] mm. Based on IVUS-VH, a total of 239 (41.9%)

Table 1. Baseline characteristics.

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Patient characteristics			
Age, years (mean±SD)	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 (51.5)	140 (45.5)	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)
Procedural characteristics			
Indication for coronary angiography			
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)
IVUS characteristics			
Segment length (mm), median (IQR)	44.1 (33.7-55.4)	43.9 (32.9-54.1)	44.8 (34.2-57.2)
Plaque burden (%), median (IQR)	39.2 (30.0-46.4)	37.2 (28.0-45.5)	40.2 (31.8-47.8)
Presence of VH-TCFA, n(%)	239 (41.9)	140 (45.3)	99 (37.9)
Presence of VH-TCFA with PB ≥ 70%, n(%)	69 (12.1)	32 (10.4)	37 (14.2)
Serum biomarker concentrations			
C-reactive protein (mg/L), median (IQR)	2.1 [0.8-5.3]	2.8 [1.1-7.0]	1.5 [0.6-3.1]
Tumor Necrosis Factor α (pg/mL) median (IQR)[+]	2.0 [1.4-2.9]	1.8 [1.4-2.6]	2.0 [1.4-3.3]
Tumor Necrosis Factor β (pg/mL) median (IQR)[†§]	35.0 [18.0-116.0]	20.5 [16.5-44.3]	36.5 [27.0-152.8]
Tumor necrosis factor receptor 2 (ng/mL) median (IQR)[#]	4.5 [3.6-5.7]	4.4 [3.5-5.8]	4.5 [3.7-5.6]

Table 1. *(continued)*

	Total (n=570)	ACS patients (n=309)	SAP patients (n=261)
Interferon γ (pg/mL) median (IQR)*§	5.1 [3.9-7.3]	4.8 [3.8-6.6]	5.7 [4.2-8.2]
Interleukin-6 (pg/mL) median (IQR)⁻	3.5 [2.2-5.8]	3.7 [2.5-6.8]	2.5 [2.1-4.1]
Interleukin-8 (pg/mL) median (IQR)#§	8.9 [6.8-12.0]	9.9 [7.1-12.6]	8.3 [6.5-10.3]
Interleukin-10 (pg/mL) median (IQR)#§	5.2 [3.6-9.4]	6.9 [4.1-15.0]	4.4 [3.0-6.0]
Interleukin-18 (pg/mL) median (IQR)*	171.0 [132.3-215.0]	173.0 [133.0-216.3]	169.5 [130.5-211.3]

*Measurable in all patients
#Measurable in >99% of patients, too low to detect in <1%
+Measurable in 76% of patients, too low to detect in 24%
⁻ Measurable in 38% of patients, too low to detect in 62%
†Measurable in 8% of patients, too low to detect in 92%
§ TNFβ, IFNγ, IL-10 and IL-18: total n= 473, ACS n=309, SAP n= 261

patients had at least 1 TCFA lesion, including 69 (12.1%) patients with at least 1 TCFA lesion with a plaque burden ≥ 70%. Concentrations of INFγ, TNF R2, IL-8, IL-10 and IL-18 were not normally distributed; these biomarkers were therefore ln-tranformed for further analyses. TNF-α, TNF-β and IL-6 were too low to detect in a large part of the patients, and thus were not examined as continuous variables in the statistical models. TNF-α was too low to detect in 24%, and hence was categorized into tertiles for further analyses. TNF-β and IL-6 were too low to detect in 92% and 62% of the patients, respectively, and these markers were dichotomized into measurable versus not measurable for further analyses. IL-10 concentrations could be measured in 99%. TNF R2, IL-8, IL-18 and IFNγ were measurable in all patients.

Biomarkers and extent of atherosclerosis

The results of the analyses for plaque burden of the entire measured segment are shown in Figure 1 and supplemental tables 1a,b and c. Higher TNF-α was associated with higher coronary plaque burden in patients with SAP (β [95%CI]: 4.45 [0.99-7.91], for the highest vs the lowest tertile of TNF-α). Such an effect could not be demonstrated in patients with ACS.

Furthermore, lower IL-10 concentrations were associated with higher coronary plaque burden in the full cohort (β [95%CI]: −3.88 [−6.00 – −1.76], for the highest vs the lowest tertile of IL-10). This effect was driven by both the SAP patients and the ACS patients. Although effect estimates for the highest tertile of IL-10 were similar in both groups (SAP: −2.95 [−6.23-0.33], ACS: −3.42 [−6.57 – −0.27], in the SAP patients the estimates, as well as the linear trend, did not reach statistical significance.

After multivariable adjustment, associations remained essentially the same for both TNF-α and IL-10.

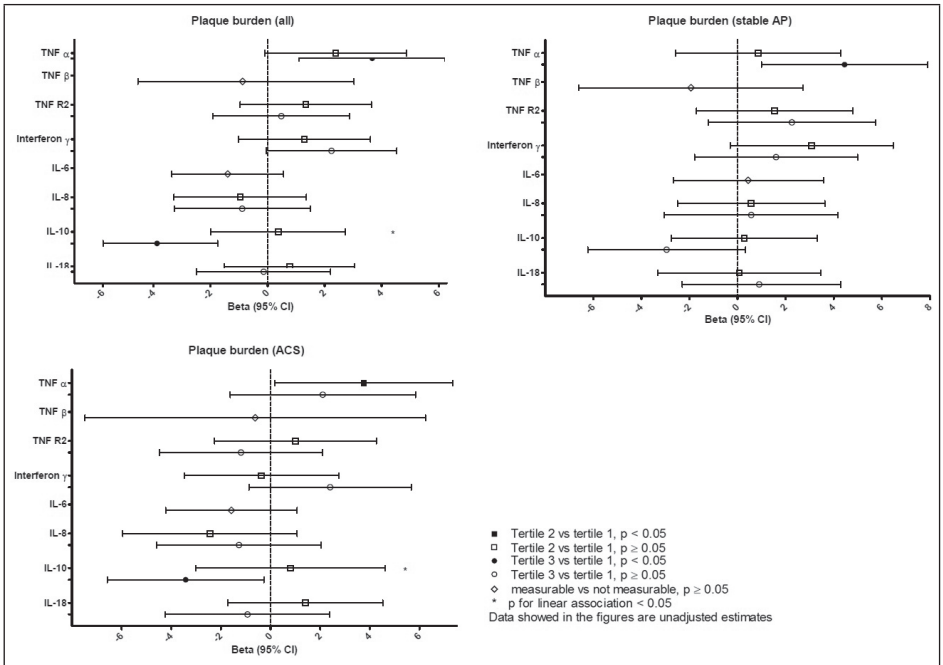

Figure 1. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in all patients, patients with stable AP and patients with ACS.

Biomarkers and composition of atherosclerosis

The results of the analyses for VH-TCFA lesions are displayed in Figure 2 and supplemental tables 2a, b and c. High TNF-α was positively associated with presence of VH-TCFA lesions in patients with SAP (OR[95%CI]: 2.30 [1.17-4.52] for the highest vs the lowest tertile of TNF-α). Such an effect was absent in patients with ACS. Furthermore, higher IL-8 seemed to confer lower risk of VH-TCFA in ACS patients; however, this effect was mainly driven by tertile 2. No associations were present between any of the other biomarkers and VH-TCFA.

Higher TNF-α was positively associated with presence of VH-TCFA lesions with a plaque burden ≥ 70% in the full cohort (OR[95%CI]: 2.85 [1.28-6.31] for the highest vs the lowest tertile of TNF-α) (table 4). This effect was driven by both patients with SAP and patients with ACS. Although the effect estimate reached statistical significance in the full cohort, this was not the case in the SAP and ACS groups. Nevertheless, the effect estimates for the highest tertile of TNF-α were similar in magnitude in both groups (SAP: 3.44 [0.89-13.29], ACS: 2.39 [0.89-6.45]. Higher IL-10 displayed an inverse association with presence of VH-TCFA lesions with a plaque burden ≥ 70% in the full cohort (OR[95%CI]: 0.31 [0.12-0.80] for the highest vs the lowest tertile of IL-10, p for trend=0.037). Again, effect estimates did not reach statistical significance in these separate groups.

After multivariable adjustment, associations remained essentially the same.

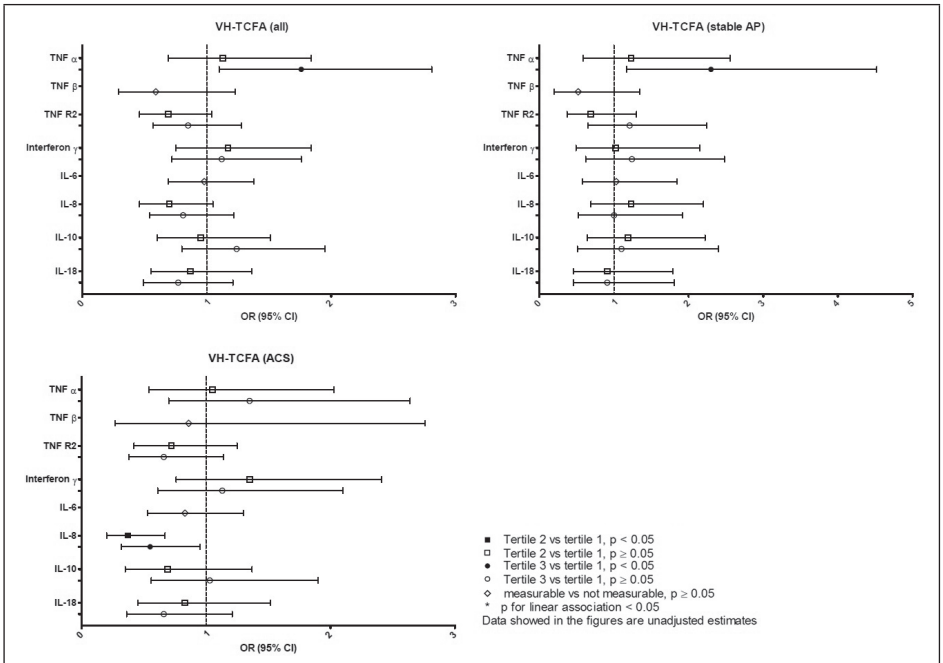

Figure 2. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in all patients, patients with stable AP and patients with ACS.

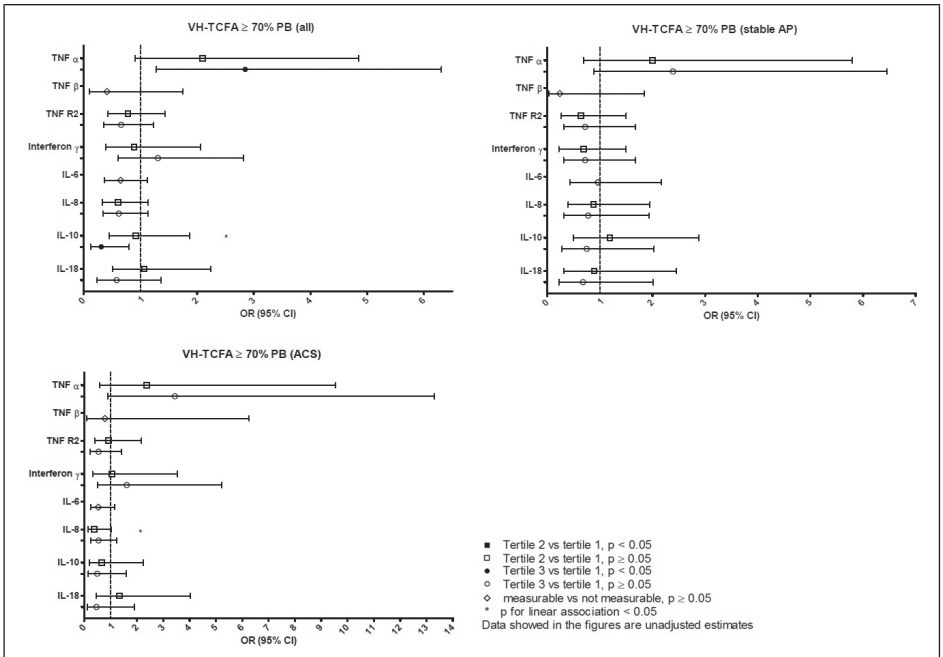

Figure 3. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in all patients, patients with stable AP and patients with ACS.

Biomarkers and MACE

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 reached the composite endpoint. Hazard ratios for the occurrence of MACE are shown in Figure 4 and supplemental tables 4a, b and c. Higher TNF R2 was associated with MACE in SAP patients (OR[95%CI]: 2.99 [1.10-8.13], per Ln (ng/mL) TNF R2) on univariable analysis; after multivariable adjustment, this association lost statistical significance. No significant associations could be demonstrated between any of the other biomarkers and MACE. Additional analysis of the composite of all-cause mortality or ACS (secondary endpoint) did not result in significant associations either.

DISCUSSION

This study examined whether circulating cytokine concentrations are associated with extent and composition of coronary atherosclerosis, as determined by IVUS and IVUS-VH in a non-culprit vessel, in patients with SAP or ACS undergoing coronary angiography. We also investigated whether these cytokines have prognostic value for cardiovascular

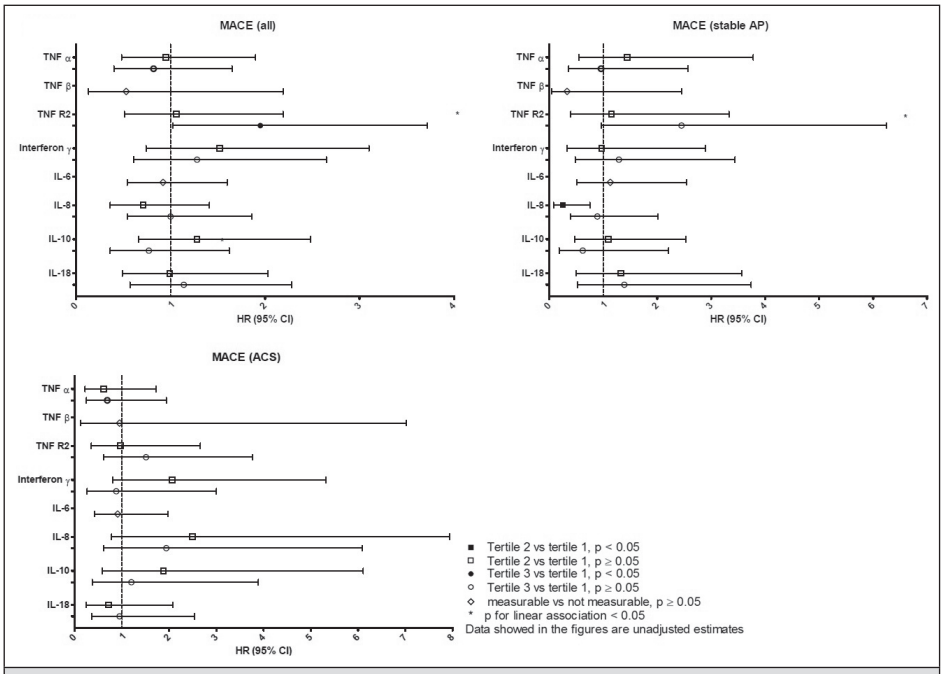

Figure 4. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in all patients, patients with stable AP and patients with ACS.

outcome. In patients with SAP, higher concentrations of TNF-α were associated with higher coronary plaque burden and with presence of VH-TCFA lesions, and displayed a tendency towards a positive association with presence of VH-TCFA lesion with a plaque burden ≥ 70%. Overall, higher concentrations of IL-10 were inversely associated with coronary plaque burden and with presence of VH-TCFA with a plaque burden ≥ 70%. These effects of IL-10 did not reach statistical significance in the separate groups. No associations were found between any of the studied cytokines and the occurrence of MACE.

Inflammation is known to play a major role in atherosclerosis. In a previous study in the current patient population, we have demonstrated an association between CRP and IVUS characteristics as well as incidence of MACE[15]. TNF-α is a proinflammatory cytokine that is secreted from activated innate immunity cells and is capable of inducing a cascade with a broad range of effects, including immunological activation, apoptosis, and procoagulative and antifibrinolytic actions, all of which can have an effect on the course of atherosclerosis [5, 16]. Experimental studies on the role of TNF-α in plaque development and stability in mice have rendered inconsistent results, some finding anti-atherogenic effects and others finding pro-atherogenic effects [5]. This discrepancy in results may be due to differences in underlying mechanisms of atherogenesis in different types of mouse models. A recent study [17] in human saphenous vein organ culture, to which a combination of TNF-α and LDL was applied, demonstrated phenotypic changes characteristic of the initial development of atherosclerotic plaques. Clinical studies on the role of TNF-α in cardiovascular disease have also rendered inconsistent results. A prior study found an increase of serum TNF-α in patients with MI and unstable angina pectoris compared to healthy subjects[18]. Ridker et al. [19] found that plasma concentrations of TNF-α are persistently elevated among post-MI patients at increased risk for recurrent coronary events. [20]. Furthermore, Naranjo et al. [21] found that TNF-α therapy was associated with a lower incidence of cardiovascular events in patients with rheumatoid arthritis, who are known to be at high cardiovascular risk. On the other hand, Cherneva et al. [22] and Sukhija et al. [23] examined the prognostic abilities of TNF-α in patients with known coronary artery disease, but did not find any associations between TNF-α and patient outcome. In the current study, we found that higher TNF-α level are associated with both extent of atherosclerosis and with plaque vulnerability in patients with SAP, which is in line with the presumed proinflammatory nature of this cytokine. On the other hand, we have recently demonstrated in the same study population [24] that presence of lesions with a high plaque burden, and presence of VH-TCFA lesions, are both independently associated with a higher MACE rate. However, higher TNF-α was not associated with the occurrence of MACE. Altogether, these findings imply that the deleterious effect of TNF-α does not translate into a higher MACE rate in the current study population. Possible explanations may include the fact that the magnitude of the

effect of TNF-α is small in the context of this multifactorial disease, or that the current study lacks statistical power to expose such an effect.

IL-10 is an anti-inflammatory cytokine that is produced by macrophages and lymphocytes [6]. This cytokine is capable of inhibiting many cellular processes that may play an important role in atherosclerotic lesion development and in the modulation of plaque composition [6, 25]. Mallat et al. [25] investigated atherosclerotic lesions in IL-10 deficient mice and showed increased infiltration of inflammatory cells, increased production of INF-γ, and decreased collagen content, which resulted in development of atheromatous lesions with signs of increased vulnerability. Several clinical studies have been performed on IL-10 and cardiovascular disease. Heeschen et al. [26] demonstrated that a reduced serum IL-10 level in patients with ACS is indicative of a poor prognosis. Most subsequent studies on the association of elevated circulating IL-10 levels with cardiovascular outcome have demonstrated positive associations with better prognosis [27-31]. In line with this, we found an inverse association between IL-10 and coronary plaque burden as well as between IL-10 and presence of large, vulnerable plaques (i.e., VH-TCFA lesions with a plaque burden ≥ 70%) in the overall study population. However, we did not find an association of IL-10 with presence of TCFA lesions in general. These results suggest that IL-10 may in particular be associated with lower extent of coronary atherosclerosis and slower growth of VH-TCFAs. In any case, these findings further support the hypothesis of a protective role of IL-10 in atherosclerosis. In a recent study performed in the same population[24], we have demonstrated that lesions with a high plaque burden, as well as VH-TCFA lesions with a plaque burden of ≥70%, are both independently associated with a higher MACE rate. While an inverse association was present of IL-10 with both plaque burden and with presence of VH-TCFA lesions with plaque burden >70% in the current study, an inverse association between IL-10 and MACE could not be demonstrated. Taken together, these results imply that the potential advantageous effect of IL-10 on plaque burden and large TCFA does not translate into a lower MACE rate. Again, the magnitude of the effect of IL-10 may be small, or statistical power may be insufficient to demonstrate the effect.

Since no associations could be demonstrated between the individual cytokines and MACE, clinical usefulness of this study may be debated. Nevertheless, we believe that our findings are informative, because they provide additional insights into the complex pathophysiologic relation between cytokines and cardiovascular disease. Moreover, we did not find any associations between several cytokines we examined and the extent or composition of atherosclerosis. Analysis of some of the biomarkers (TNF-β and IL-6) was complicated by the fact that over 50% of the measurements were too low to detect. Cytokine assays are generally known to display limitations in terms of % detectability [32, 33]. This makes clinical investigations into the pathophysiological role and the prognostic value of these biomarkers challenging. In line with this, few clinical studies have

been performed on circulating TNF-β. Furthermore, IL-6 is known to have large circadian variations, and a relatively short half-life of less than 6 hours [34] which also makes this marker difficult to investigate. Clinical studies on circulating TNFR2, INFγ, and IL-8 in patients with coronary artery disease are also limited in number. IL-18 has been examined more often, and has been suggested to be associated with the presence and severity of coronary atherosclerosis [35, 36]. In the present study, we could not demonstrate such an association.

Some aspects of this study warrant consideration. Our study population consisted of patients with SAP as well as patients with ACS. The group of patients with ACS is likely to be more heterogeneous, which may have influenced the findings. To account for this, we have performed the analyses separately in both groups. Furthermore, VH-IVUS imaging took place of a prespecified single target segment of a single non-culprit coronary artery, based on the assumption that such a non-stenotic segment adequately reflects coronary wall pathophysiology of the larger coronary tree. Although this assumption may be debated, previous studies evaluating IVUS have demonstrated that the coronary wall of comparable non-culprit, non-stenotic segments of a single vessel does reflect coronary disease burden at large and is associated with subsequent cardiovascular outcome [24, 37, 38]. Moreover, it is important to note that IVUS is formally not capable of detecting the most rupture prone of all plaque phenotypes, the TCFA [39, 40], because the spatial resolution of IVUS is insufficient for thin cap detection (23, 24). Nonetheless, a concept of VH-IVUS derived TCFA has been postulated for plaques with a plaque burden ≥ 40% and a confluent necrotic core ≥ 10% in direct contact with the lumen in at least three VH-IVUS frames (13, 23). Notably, we have recently demonstrated that such VH-IVUS derived TCFA lesions are strongly and independently predictive of the occurrence of major adverse cardiac events within the current study population [24].

In conclusion, in patients undergoing coronary angiography, higher circulating TNF-α was associated with higher plaque burden and with presence of VH-TCFA lesions in patients with SAP. Overall, lower circulating IL-10 was associated with higher plaque burden and with presence of VH-TCFA lesions with a plaque burden ≥ 70%. The latter effects did not reach statistical significance in the separate SAP and ACS groups. These cytokines were not associated with occurrence of MACE. These in-vivo findings illustrate that TNF-α and IL-10 appear to play a role in both extent and vulnerability of coronary atherosclerosis, which is in line with experimental studies. However, their clinical value in terms of risk stratification warrants further investigation.

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SUPPLEMENTAL TABLES

Supplemental table 1a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in all patients.

	Unadjusted model		Multivariable model*	
Segment plaque burden	beta (95%CI)	P	beta (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	reference		reference	
Tertile 2	2.39 (−0.10-4.88)	0.060	1.94 (−0.52-4.39)	0.12
Tertile 3	3.67 (1.10-6.23)	0.005	3.13 (0.63-5.62)	0.014
TNFbeta				
not measurable	reference		reference	
measurable	−0.88 (−4.78-3.03)	0.66	−1.39 (−5.25- −2.47)	0.48
TNFR2 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.34 (−0.96-3.64)	0.25	−0.56 (−2.89-1.76)	0.63
Tertile 3	0.48 (−1.92-2.88)	0.69	−1.73 (−4.29-0.82)	0.18
Ln (TNFR2)	0.61 (−1.98-3.20)	0.65	−2.43 (−5.15-0.29)	0.080
Interferon γ (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.29 (−1.02-3.61)	0.27	0.41 (−1.86-2.67)	0.73
Tertile 3	2.24 (−0.05-4.53)	0.055	0.51 (−1.98-2.99)	0.69
Ln (Interferon γ)	1.61 (−0.15-3.37)	0.072	0.11 (−1.71-1.92)	0.91
IL-6				
not measurable	reference		reference	
measurable	−1.40 (−3.36-0.56)	0.16	−0.70 (−2.74-1.35)	0.50
IL-8 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	−0.96 (−3.29-1.36)	0.42	−0.78 (−3.06-1.49)	0.50
Tertile 3	−0.89 (−3.27-1.50)	0.46	−1.63 (−4.08-0.82)	0.19
Ln (IL8)	−0.07 (−2.22-2.09)	0.95	−0.54 (−2.70-1.62)	0.63
IL-10 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.37 (−2.00-2.73)	0.76	0.63 (−1.73-3.00)	0.60
Tertile 3	−3.88 (−6.00- −1.76)	<0.001	−3.27 (−5.55- −0.99)	0.005
Ln (IL10)	−1.52 (−2.49- −0.55)	0.002	−1.25 (−2.26- −0.24)	0.016
IL-18 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.77 (−1.52-3.06)	0.51	1.04 (−1.24-3.33)	0.37
Tertile 3	−0.14 (−2.50-2.21)	0.91	0.14 (−2.15-2.42)	0.91
Ln (IL18)	−0.84 (−3.17-1.48)	0.48	−0.53 (−2.80-1.74)	0.65

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 1b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in patients with stable AP.

	Unadjusted model		Multivariable model*	
Segment plaque burden	beta (95%CI)	P	beta (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.86 (−2.58-4.30)	0.62	0.33 (−3.09-3.74)	0.85
Tertile 3	4.45 (0.99-7.91)	0.012	4.64 (1.11-8.16)	0.010
TNFbeta				
not measurable	reference		reference	
measurable	−1.94 (−6.61-2.73)	0.41	−1.63 (−6.27-3.00)	0.49
TNFR2 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.54 (−1.71-4.80)	0.35	−0.16 (−3.49-3.18)	0.93
Tertile 3	2.26 (−1.22-5.73)	0.20	0.40 (−3.48-4.29)	0.84
Ln (TNFR2)	2.90 (−0.94-6.74)	0.14	0.64 (−3.54-4.82)	0.76
Interferon γ (tertiles)				
Tertile 1	reference		reference	
Tertile 2	3.08 (−0.31-6.47)	0.075	2.57 (−0.92-6.05)	0.15
Tertile 3	1.60 (−1.79-4.99)	0.35	0.40 (−3.22-4.02)	0.83
Ln (Interferon γ)	1.39 (−1.01-3.80)	0.26	0.44 (−2.07-2.95)	0.73
IL-6				
not measurable	reference		reference	
measurable	0.44 (−2.68-3.57)	0.78	0.47 (−2.76-3.70)	0.78
IL-8 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.56 (−2.50-3.63)	0.72	0.17 (−2.90-3.23)	0.91
Tertile 3	0.57 (−3.04-4.17)	0.76	−0.18 (−3.87-3.50)	0.92
Ln (IL8)	2.03 (−1.11-5.16)	0.21	1.10 (−2.08-4.28)	0.50
IL-10 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.28 (−2.76-3.32)	0.86	0.34 (−2.65-3.33)	0.82
Tertile 3	−2.95 (−6.23-0.33)	0.078	−3.30 (−6.64-0.04)	0.053
Ln (IL10)	−1.03 (−3.02-0.95)	0.31	−1.34 (−3.34-0.66)	0.19
IL-18 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.07 (−3.33-3.47)	0.97	−0.34 (−3.71-3.02)	0.84
Tertile 3	0.99 (−2.30-4.29)	0.55	0.11 (−3.24-3.47)	0.95
Ln (IL18)	1.72 (−1.83-5.28)	0.34	0.99 (−2.57-4.56)	0.58

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 1c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with segment plaque burden in patients with ACS.

	Unadjusted model		Multivariable model*	
Segment plaque burden	beta (95%CI)	P	beta (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	reference		reference	
Tertile 2	3.76 (0.17-7.35)	0.040	2.98 (−0.65-6.61)	0.11
Tertile 3	2.10 (−1.63-5.84)	0.27	1.79 (−1.83-5.41)	0.33
TNFbeta				
not measurable	reference		reference	
measurable	−0.62 (−7.48-6.24)	0.86	−1.08 (−7.98-5.82)	0.76
TNFR2 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.01 (−2.26-4.27)	0.54	−1.19 (−4.48-2.10)	0.48
Tertile 3	−1.19 (−4.47-2.09)	0.48	−3.37 (−6.86-0.13)	0.059
Ln (TNFR2)	−1.18 (−4.67-2.30)	0.51	−4.53 (−8.17- −0.89)	0.015
Interferon γ (tertiles)				
Tertile 1	reference		reference	
Tertile 2	−0.37 (−3.47-2.74)	0.82	−0.96 (−4.01-2.08)	0.53
Tertile 3	2.40 (−0.87-5.67)	0.15	0.58 (−2.89-4.05)	0.74
Ln (Interferon γ)	1.09 (−1.51-3.70)	0.41	−0.22 (−2.89-2.46)	0.87
IL-6				
not measurable	reference		reference	
measurable	−1.58 (−4.22-1.07)	0.24	−1.49 (−4.18-1.20)	0.28
IL-8 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	−2.44 (−5.96-1.07)	0.17	−2.25 (−5.71-1.22)	0.20
Tertile 3	−1.27 (−4.58-2.03)	0.45	−2.77 (−6.12-0.59)	0.11
Ln (IL8)	−0.99 (−3.99-2.02)	0.52	−2.02 (−5.02-0.97)	0.19
IL-10 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	0.81 (−3.01-4.63)	0.68	1.31 (−2.65-5.28)	0.51
Tertile 3	−3.42 (−6.57- −0.27)	0.034	−3.12 (−6.24-0.01)	0.051
Ln (IL10)	−1.30 (−2.52- −0.08)	0.038	−1.27 (−2.48- −0.05)	0.041
IL-18 (tertiles)				
Tertile 1	reference		reference	
Tertile 2	1.40 (−1.72-4.52)	0.38	2.11 (−1.14-5.35)	0.20
Tertile 3	−0.93 (−4.23-2.37)	0.58	0.07 (−3.21-3.34)	0.97
Ln (IL18)	−2.30 (−5.35-0.75)	0.14	−1.52 (−4.55-1.51)	0.32

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 2a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in all patients.

	Unadjusted model		Multivariable model *	
VH-TCFA	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.13 (0.69-1.84)	0.63	1.12 (0.68-1.83)	0.67
Tertile 3	1.76 (1.10-2.81)	0.018	1.82 (1.13-2.93)	0.014
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.59 (0.29-1.23)	0.16	0.70 (0.33-1.47)	0.34
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.46-1.04)	0.079	0.68 (0.44-1.04)	0.078
Tertile 3	0.85 (0.57-1.28)	0.45	0.84 (0.54-1.30)	0.43
LN (TNFR2)	0.87 (0.55-1.37)	0.55	0.85 (0.52-1.40)	0.52
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.17 (0.75-1.84)	0.50	1.21 (0.76-1.91)	0.42
Tertile 3	1.12 (0.72-1.76)	0.62	1.22 (0.75-1.97)	0.43
LN (Interferon γ)	1.08 (0.76-1.52)	0.68	1.15 (0.79-1.66)	0.47
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.98 (0.69-1.38)	0.90	0.97 (0.67-1.41)	0.87
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.70 (0.46-1.05)	0.085	0.69 (0.46-1.06)	0.089
Tertile 3	0.81 (0.54-1.22)	0.81	0.77 (0.50-1.18)	0.23
LN (IL8)	0.91 (0.62-1.33)	0.63	0.87 (0.59-1.30)	0.50
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.95 (0.60-1.51)	0.84	0.95 (0.59-1.52)	0.83
Tertile 3	1.24 (0.80-1.95)	0.34	1.21 (0.75-1.94)	0.44
LN (IL10)	1.15 (0.95-1.39)	0.16	1.13 (0.92-1.39)	0.25
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.87 (0.55-1.36)	0.54	0.90 (0.57-1.43)	0.66
Tertile 3	0.77 (0.49-1.21)	0.25	0.76 (0.48-1.20)	0.24
LN (IL18)	0.90 (0.57-1.42)	0.64	0.91 (0.57-1.44)	0.67

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 2b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in patients with stable AP.

	Unadjusted model		Multivariable model *	
VH-TCFA	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.23 (0.59-2.56)	0.58	1.27 (0.60-2.66)	0.53
Tertile 3	2.30 (1.17-4.52)	0.015	2.31 (1.16-4.59)	0.017
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.52 (0.20-1.35)	0.18	0.52 (0.20-1.37)	0.19
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.37-1.30)	0.25	0.67 (0.35-1.29)	0.23
Tertile 3	1.21 (0.65-2.24)	0.55	1.14 (0.58-2.23)	0.71
LN (TNFR2)	1.44 (0.70-2.94)	0.32	1.38 (0.62-3.04)	0.43
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.02 (0.49-2.15)	0.95	0.96 (0.45-2.05)	0.91
Tertile 3	1.24 (0.62-2.48)	0.55	1.19 (0.57-2.50)	0.64
LN (Interferon γ)	1.23 (0.74-2.05)	0.43	1.23 (0.71-2.13)	0.45
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	1.03 (0.58-1.84)	0.92	0.95 (0.51-1.76)	0.87
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.23 (0.69-2.20)	0.48	1.27 (0.70-2.29)	0.44
Tertile 3	1.00 (0.52-1.92)	1.00	0.95 (0.48-1.85)	0.87
LN (IL8)	1.15 (0.64-2.05)	0.64	1.08 (0.59-1.97)	0.81
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.19 (0.64-2.22)	0.58	1.22 (0.65-2.30)	0.54
Tertile 3	1.10 (0.51-2.40)	0.81	1.06 (0.47-2.36)	0.90
LN (IL10)	1.41 (0.93-2.15)	0.11	1.39 (0.90-2.14)	0.14
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.91 (0.46-1.79)	0.78	0.88 (0.44-1.76)	0.72
Tertile 3	0.91 (0.46-1.81)	0.78	0.82 (0.41-1.68)	0.60
LN (IL18)	1.01 (0.48-2.13)	0.99	0.95 (0.44-2.04)	0.89

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 2c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA in patients with ACS.

	Unadjusted model		Multivariable model *	
VH-TCFA	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.05 (0.54-2.03)	0.89	0.89 (0.45-1.78)	0.74
Tertile 3	1.35 (0.70-2.64)	0.37	1.43 (0.72-2.84)	0.31
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.86 (0.27-2.76)	0.80	0.98 (0.29-3.34)	0.98
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.72 (0.42-1.25)	0.25	0.69 (0.39-1.22)	0.20
Tertile 3	0.66 (0.38-1.14)	0.14	0.62 (0.34-1.12)	0.11
LN (TNFR2)	0.63 (0.34-1.14)	0.13	0.59 (0.30-1.15)	0.12
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.35 (0.76-2.41)	0.30	1.38 (0.77-2.49)	0.28
Tertile 3	1.13 (0.61-2.10)	0.69	1.15 (0.60-2.21)	0.68
LN (Interferon γ)	1.06 (0.65-1.73)	0.83	1.05 (0.63-1.76)	0.86
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.83 (0.53-1.30)	0.42	0.96 (0.59-1.55)	0.86
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.37 (0.20-0.67)	0.001	0.40 (0.21-0.74)	0.004
Tertile 3	0.55 (0.32-0.95)	0.033	0.60 (0.33-1.08)	0.086
LN (IL8)	0.70 (0.42-1.17)	0.17	0.76 (0.44-1.30)	0.31
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.35-1.37)	0.29	0.76 (0.37-1.54)	0.44
Tertile 3	1.03 (0.56-1.90)	0.93	1.14 (0.61-2.14)	0.68
LN (IL10)	1.02 (0.81-1.28)	0.90	1.03 (0.82-1.31)	0.79
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.83 (0.45-1.52)	0.55	0.90 (0.48-1.69)	0.75
Tertile 3	0.66 (0.36-1.21)	0.18	0.65 (0.35-1.21)	0.17
LN (IL18)	0.82 (0.46-1.46)	0.50	0.82 (0.46-1.49)	0.52

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 3a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in all patients.

	Unadjusted model		Multivariable model*	
VH-TCFA≥ 70% PB	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	2.10 (0.91-4.85)	0.083	2.11 (0.91-4.93)	0.084
Tertile 3	2.85 (1.28-6.31)	0.01	2.78 (1.24-6.23)	0.013
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.41 (0.10-1.75)	0.23	0.41 (0.10-1.78)	0.24
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.78 (0.43-1.43)	0.42	0.67 (0.36-1.25)	0.20
Tertile 3	0.66 (0.35-1.23)	0.19	0.52 (0.26-1.04)	0.064
LN (TNFR2)	0.65 (0.32-1.30)	0.22	0.50 (0.23-1.09)	0.081
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.89 (0.39-2.06)	0.79	0.78 (0.34-1.83)	0.57
Tertile 3	1.31 (0.61-2.82)	0.49	0.93 (0.41-2.14)	0.87
LN (Interferon γ)	1.21 (0.66-2.21)	0.54	0.93 (0.48-1.80)	0.83
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.65 (0.37-1.12)	0.12	0.75 (0.42-1.36)	0.35
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.61 (0.33-1.14)	0.12	0.63 (0.34-1.18)	0.15
Tertile 3	0.62 (0.34-1.14)	0.12	0.64 (0.34-1.22)	0.17
LN (IL8)	0.57 (0.32-1.02)	0.059	0.57 (0.31-1.05)	0.069
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.92 (0.45-1.87)	0.81	0.97 (0.47-2.02)	0.94
Tertile 3	0.31 (0.12-0.80)	0.016	0.36 (0.13-0.97)	0.043
LN (IL10)	0.64 (0.42-0.97)	0.037	0.69 (0.44-1.08)	0.10
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.07 (0.51-2.24)	0.87	1.09 (0.51-2.32)	0.82
Tertile 3	0.58 (0.24-1.36)	0.21	0.59 (0.25-1.40)	0.23
LN (IL18)	0.49 (0.23-1.08)	0.077	0.51 (0.22-1.14)	0.10

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 3b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in patients with stable AP.

	Unadjusted model		Multivariable model*	
VH-TCFA≥ 70% PB	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	2.00 (0.69-5.79)	0.20	2.11 (0.72-6.18)	0.17
Tertile 3	2.39 (0.89-6.45)	0.086	2.48 (0.90-6.79)	0.078
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.24 (0.03-1.85)	0.17	0.24 (0.03-1.86)	0.17
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.64 (0.27-1.50)	0.30	0.63 (0.26-1.53)	0.31
Tertile 3	0.72 (0.31-1.67)	0.45	0.71 (0.28-1.77)	0.46
LN (TNFR2)	0.79 (0.29-2.15)	0.65	0.81 (0.27-2.42)	0.70
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.69 (0.22-2.19)	0.53	0.64 (0.20-2.05)	0.45
Tertile 3	0.90 (0.32-2.53)	0.85	0.83 (0.28-2.47)	0.73
LN (Interferon γ)	0.96 (0.44-2.09)	0.91	0.90 (0.38-2.12)	0.81
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.96 (0.43-2.17)	0.93	0.99 (0.42-2.33)	0.99
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.88 (0.40-1.95)	0.76	0.91 (0.41-2.04)	0.82
Tertile 3	0.78 (0.31-1.94)	0.59	0.79 (0.31-2.00)	0.62
LN (IL8)	0.85 (0.38-1.94)	0.70	0.87 (0.37-2.01)	0.74
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.19 (0.50-2.88)	0.69	1.23 (0.50-2.98)	0.66
Tertile 3	0.75 (0.28-2.03)	0.57	0.74 (0.27-2.05)	0.57
LN (IL10)	0.66 (0.32-1.36)	0.26	0.64 (0.30-1.36)	0.25
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.89 (0.32-2.45)	0.82	0.87 (0.31-2.42)	0.79
Tertile 3	0.68 (0.23-2.01)	0.48	0.63 (0.21-1.93)	0.42
LN (IL18)	0.55 (0.18-1.71)	0.30	0.53 (0.17-1.68)	0.28

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 3c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with presence of VH-TCFA with plaque burden ≥ 70% in patients with ACS.

	Unadjusted model		Multivariable model*	
VH-TCFA≥ 70% PB	OR (95%CI)	P	OR (95%CI)	P
TNFalpha (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	2.37 (0.59-9.53)	0.23	2.07 (0.50-8.65)	0.32
Tertile 3	3.44 (0.89-13.29)	0.073	3.57 (0.90-14.13)	0.070
TNFbeta				
not measurable	1 (reference)		1 (reference)	
measurable	0.78 (0.10-6.25)	0.82	0.86 (0.10-7.15)	0.89
TNFR2 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.92 (0.39-2.17)	0.86	0.66 (0.27-1.66)	0.38
Tertile 3	0.54 (0.21-1.42)	0.22	0.35 (0.12-1.03)	0.056
LN (TNFR2)	0.51 (0.19-1.39)	0.19	0.30 (0.09-0.97)	0.044
Interferon γ (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.04 (0.31-3.54)	0.95	1.02 (0.29-3.56)	0.98
Tertile 3	1.61 (0.50-5.22)	0.43	1.12 (0.32-3.86)	0.86
LN (Interferon γ)	1.40 (0.53-3.71)	0.50	1.02 (0.37-2.84)	0.97
IL-6				
not measurable	1 (reference)		1 (reference)	
measurable	0.53 (0.25-1.14)	0.10	0.60 (0.27-1.36)	0.22
IL-8 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.37 (0.14-1.01)	0.052	0.38 (0.13-1.07)	0.066
Tertile 3	0.54 (0.24-1.23)	0.14	0.50 (0.20-1.23)	0.13
LN (IL8)	0.42 (0.18-0.96)	0.039	0.38 (0.16-0.91)	0.029
IL-10 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	0.65 (0.19-2.23)	0.49	0.69 (0.19-2.50)	0.57
Tertile 3	0.49 (0.15-1.59)	0.24	0.53 (0.16-1.79)	0.31
LN (IL10)	0.69 (0.40-1.20)	0.19	0.71 (0.41-1.23)	0.22
IL-18 (tertiles)				
Tertile 1	1 (reference)		1 (reference)	
Tertile 2	1.33 (0.44-4.02)	0.61	1.37 (0.43-4.42)	0.60
Tertile 3	0.46 (0.11-1.90)	0.28	0.52 (0.12-2.21)	0.37
LN (IL18)	0.44 (0.14-1.35)	0.15	0.45 (0.13-1.54)	0.20

*adjusted for age, gender, indication for coronary angiography, diabetes, hypertension, and CRP

Supplemental table 4a. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in all patients.**

	Unadjusted model		Multivariable model*		Multivariable model#	
MACE	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
TNFalpha (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.95 (0.48-1.90)	0.89	0.88 (0.44-1.77)	0.73	0.96 (0.48-1.93)	0.91
Tertile 3	0.82 (0.40-1.65)	0.57	0.76 (0.37-1.54)	0.44	0.74 (0.36-1.51)	0.40
TNFbeta						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.53 (0.13-2.19)	0.38	0.51 (0.12-2.08)	0.34	0.54 (0.13-2.23)	0.40
TNFR2 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.06 (0.51-2.19)	0.88	0.88 (0.42-1.86)	0.75	1.01 (0.48-2.09)	0.99
Tertile 3	1.95 (1.02-3.72)	0.042	1.55 (0.77-3.09)	0.22	1.71 (0.88-3.32)	0.11
LN (TNFR2)	2.34 (1.20-4.55)	0.012	1.92 (0.92-3.99)	0.08	1.81 (0.91-3.57)	0.090
Interferon γ (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.52 (0.74-3.10)	0.25	1.38 (0.68-2.84)	0.38	1.47 (0.72-3.01)	0.29
Tertile 3	1.28 (0.61-2.65)	0.51	0.97 (0.45-2.09)	0.94	1.15 (0.55-2.42)	0.72
LN (Interferon γ)	1.15 (0.67-1.98)	0.62	0.93 (0.52-1.65)	0.79	1.08 (0.63-1.87)	0.78
IL-6						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.923 (0.54-1.60)	0.79	1.03 (0.58-1.81)	0.93	0.78 (0.43-1.40)	0.40
IL-8 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.71 (0.36-1.41)	0.33	0.71 (0.36-1.40)	0.32	0.66 (0.33-1.32)	0.24
Tertile 3	1.00 (0.54-1.86)	1.00	0.95 (0.50-1.80)	0.87	0.83 (0.43-1.58)	0.56
LN (IL8)	1.25 (0.69-2.27)	0.47	1.18 (0.64-2.17)	0.60	1.07 (0.58-1.97)	0.84
IL-10 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.28 (0.66-2.48)	0.47	1.31 (0.67-2.57)	0.43	1.12 (0.57-2.20)	0.75
Tertile 3	0.77 (0.36-1.62)	0.48	0.83 (0.38-1.81)	0.65	0.74 (0.35-1.57)	0.43
LN (IL10)	0.98 (0.72-1.32)	0.88	1.03 (0.75-1.42)	0.87	0.98 (0.71-1.34)	0.89
IL-18 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.99 (0.49-2.03)	0.98	0.98 (0.48-2.02)	0.96	1.10 (0.53-2.27)	0.81
Tertile 3	1.14 (0.57-2.28)	0.71	1.18 (0.59-2.36)	0.65	1.18 (0.58-2.37)	0.65
LN (IL18)	1.10 (0.54-2.21)	0.80	1.15 (0.56-2.36)	0.71	1.05 (0.53-2.06)	0.89

** MACE = major adverse cardiac events: all-cause mortality, acute coronary syndrome or unplanned coronary revascularization during 1-year follow-up (n=56)

*adjusted for age, gender and indication for coronary angiography

#additionally adjusted for diabetes mellitus, hypertension and CRP

Two separate models were constructed for adjustment because of limited number of endpoints.

Supplemental table 4b. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in patients with stable AP.**

	Unadjusted model		Multivariable model*		Multivariable model#	
MACE	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
TNFalpha (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.44 (0.55-3.78)	0.46	1.40 (0.53-3.70)	0.50	1.45 (0.55-3.83)	0.46
Tertile 3	0.96 (0.36-2.57)	0.93	0.95 (0.35-2.55)	0.91	0.81 (0.29-2.24)	0.68
TNFbeta						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.33 (0.05-2.46)	0.28	0.35 (0.05-2.55)	0.30	0.34 (0.05-2.47)	0.28
TNFR2 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.15 (0.40-3.33)	0.79	1.09 (0.37-3.18)	0.88	1.08 (0.37-3.11)	0.89
Tertile 3	2.45 (0.96-6.25)	0.062	2.38 (0.88-6.46)	0.087	2.07 (0.78-5.44)	0.14
LN (TNFR2)	2.99 (1.10-8.13)	0.031	2.80 (0.97-8.07)	0.057	2.29 (0.80-6.53)	0.12
Interferon γ (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.97 (0.33-2.89)	0.96	0.93 (0.31-2.76)	0.89	0.94 (0.31-2.82)	0.91
Tertile 3	1.29 (0.48-3.44)	0.61	1.13 (0.41-3.15)	0.82	1.17 (0.43-3.16)	0.76
LN (Interferon γ)	1.41 (0.68-2.91)	0.36	1.26 (0.59-2.69)	0.56	1.30 (0.62-2.71)	0.49
IL-6						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	1.13 (0.51-2.55)	0.76	1.19 (0.53-2.68)	0.67	0.87 (0.36-2.10)	0.76
IL-8 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.25 (0.08-0.75)	0.014	0.25 (0.08-0.74)	0.012	0.23 (0.07-0.69)	0.009
Tertile 3	0.89 (0.39-2.01)	0.78	0.87 (0.38-1.96)	0.73	0.71 (0.30-1.68)	0.44
LN (IL8)	1.03 (0.44-2.41)	0.94	0.98 (0.42-2.28)	0.95	0.81 (0.34-1.97)	0.65
IL-10 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.09 (0.47-2.53)	0.83	1.11 (0.48-2.57)	0.81	1.08 (0.47-2.51)	0.85
Tertile 3	0.62 (0.18-2.21)	0.47	0.60 (0.17-2.12)	0.42	0.50 (0.13-1.90)	0.31
LN (IL10)	1.28 (0.73-2.27)	0.39	1.26 (0.71-2.22)	0.43	1.17 (0.65-2.14)	0.60
IL-18 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.33 (0.50-3.57)	0.57	1.32 (0.49-3.55)	0.58	1.20 (0.44-3.27)	0.72
Tertile 3	1.39 (0.52-3.74)	0.51	1.32 (0.49-3.55)	0.58	1.15 (0.42-3.18)	0.78
LN (IL18)	1.78 (0.61-5.19)	0.29	1.70 (0.58-5.02)	0.33	1.49 (0.50-4.44)	0.48

** MACE = major adverse cardiac events: all-cause mortality, acute coronary syndrome or unplanned coronary revascularization during 1-year follow-up (n=56)

*adjusted for age, gender and indication for coronary angiography

additionally adjusted for diabetes mellitus, hypertension and CRP

Two separate models were constructed for adjustment because of limited number of endpoints.

Supplemental table 4c. Association of TNF-α, TNF-β, TNF R2, INFγ, IL-6, IL-8, IL-10 and IL-18 with occurrence of MACE in patients with ACS.**

	Unadjusted model		Multivariable model*		Multivariable model#	
MACE	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
TNFalpha (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.61 (0.22-1.72)	0.35	0.55 (0.20-1.55)	0.26	0.64 (0.22-1.83)	0.40
Tertile 3	0.69 (0.25-1.94)	0.48	0.61 (0.22-1.73)	0.35	0.62 (0.22-1.79)	0.38
TNFbeta						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.95 (0.13-7.02)	0.96	0.96 (0.13-7.09)	0.97	1.01 (0.14-7.52)	0.99
TNFR2 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.97 (0.35-2.66)	0.95	0.74 (0.26-2.10)	0.57	0.93 (0.33-2.59)	0.89
Tertile 3	1.51 (0.61-3.76)	0.37	1.01 (0.37-2.72)	0.99	1.27 (0.49-3.31)	0.63
LN (TNFR2)	1.95 (0.77-4.96)	0.16	1.39 (0.48-4.00)	0.54	1.41 (0.53-3.74)	0.49
Interferon γ (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	2.06 (0.80-5.32)	0.13	1.92 (0.74-4.97)	0.18	1.90 (0.73-4.94)	0.19
Tertile 3	0.88 (0.26-3.00)	0.83	0.68 (0.19-2.37)	0.54	0.77 (0.22-2.73)	0.69
LN (Interferon γ)	0.80 (0.35-1.83)	0.60	0.65 (0.28-1.51)	0.32	0.75 (0.32-1.78)	0.52
IL-6						
not measurable	1 (reference)		1 (reference)		1 (reference)	
measurable	0.91 (0.42-1.97)	0.82	0.91 (0.42-1.97)	0.81	0.73 (0.32-1.70)	0.47
IL-8 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	2.49 (0.78-7.94)	0.12	2.36 (0.74-7.58)	0.15	2.37 (0.74-7.59)	0.15
Tertile 3	1.94 (0.62-6.09)	0.26	1.48 (0.46-4.80)	0.51	1.56 (0.48-5.07)	0.46
LN (IL8)	1.70 (0.71-4.06)	0.23	1.38 (0.56-3.41)	0.49	1.43 (0.58-3.51)	0.43
IL-10 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	1.88 (0.58-6.11)	0.29	1.81 (0.55-5.97)	0.33	1.51 (0.45-5.04)	0.51
Tertile 3	1.20 (0.37-3.89)	0.76	1.20 (0.37-3.90)	0.77	1.10 (0.34-3.60)	0.88
LN (IL10)	0.95 (0.63-1.42)	0.80	0.96 (0.64-1.43)	0.84	0.95 (0.63-1.44)	0.81
IL-18 (tertiles)						
Tertile 1	1 (reference)		1 (reference)		1 (reference)	
Tertile 2	0.72 (0.25-2.08)	0.55	0.67 (0.23-1.96)	0.47	0.94 (0.31-2.81)	0.91
Tertile 3	0.95 (0.36-2.54)	0.92	1.04 (0.39-2.77)	0.94	1.09 (0.40-2.96)	0.87
LN (IL18)	0.77 (0.29-2.00)	0.58	0.82 (0.30-2.23)	0.70	0.76 (0.31-1.89)	0.56

** MACE = major adverse cardiac events: all-cause mortality, acute coronary syndrome or unplanned coronary revascularization during 1-year follow-up (n=56)

*adjusted for age, gender and indication for coronary angiography

#additionally adjusted for diabetes mellitus, hypertension and CRP

Two separate models were constructed for adjustment because of limited number of endpoints.