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ORIGINAL ARTICLE
Ischemic Heart Disease

Relationship Between Multiple Plasma Biomarkers and Vulnerable Plaque Determined by Virtual Histology Intravascular Ultrasound

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Background: The relationship between plasma biomarkers and vulnerable plaque is not well understood.

Methods and Results: The 188 patients who underwent 3-vessel virtual histology (VH) intravascular ultrasound (IVUS) with peripheral blood sampling were enrolled. Plasma levels of matrix metalloproteinase 2 and 9 (MMP-2, -9), tissue inhibitor of metalloproteinase-1, adiponectin, and macrophage migration inhibitory factor were measured. VH-IVUS-derived thin cap fibroatheroma (VH-TCFA) was defined as a necrotic core >10% of plaque area in the presence of >40% plaque burden. There were 38 patients with ruptured plaque and 150 patients without (107 patients with VH-TCFA, 43 patients without VH-TCFA) in culprit/target lesions. Among the biomarkers, only the MMP-9 level was significantly higher in patients with ruptured plaque (P=0.002). In the subgroup without ruptured plaque, significant differences in the levels of several biomarkers were not observed between patients with and without VH-TCFA. In both culprit/target and nonculprit/non-target vessels, the MMP-9 level showed a weak correlation with the total number of ruptured plaques (r=0.231, P=0.002).

Conclusions: Among the biomarkers tested in this study, the MMP-9 level was significantly higher in patients with ruptured plaque. However, measurement of several biomarkers, including MMP-9, was incapable of predicting the presence of VH-TCFA. (*Circ J* 2010; **74:** 332–336)

Key Words: Coronary disease; Plaque; Ultrasonics

Vulnerable plaque is more prone to rupture, resulting in thrombotic occlusion, so patients with vulnerable plaque have a high probability of adverse clinical cardiac events.[1] Efforts to detect the vulnerable plaque have been ongoing and some coronary imaging modalities and measurement of biomarker levels have become important tools in the early identification of vulnerable plaque and thus patients at risk of acute coronary syndrome (ACS). Among the coronary imaging modalities, intravascular ultrasound (IVUS) can detect plaque rupture in vivo,[2,3] and virtual histology (VH) IVUS can be used to characterize plaque type and to identify VH-IVUS-derived thin cap fibroatheromas (VH-TCFA).[4–9] It is known that matrix metalloproteinases-2 and -9 (MMP-2, -9), tissue inhibitor of metalloproteinase-1 (TIMP-1), adiponectin, and macrophage migration inhibitory factor (MIF) correlate with either plaque destabilization or stabilization.[10–14] However, there are only a few studies of the relationship between plasma biomarkers and plaque characteristics determined by imaging.[15] Therefore, we investigated whether levels of biomarkers that are known to be associated with plaque vulnerability, such as MMP-2, MMP-9, TIMP-1, adiponectin and MIF, can be used as predictors of ruptured plaque or VH-TCFA as determined by 3-vessel VH-IVUS study.

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Methods

Study Population

Three-vessel pre-intervention VH-IVUS was successfully performed in 212 nonconsecutive patients,[7] from among whom 188 patients [82 with ACS, 106 with stable angina pectoris

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Table 1. Baseline Clinical Characteristics

	All (n=188)	ACS (n=82)	SAP (n=106)	P value
Age (years)	60±11	61±11	60±10	0.5
Men	152 (81%)	73 (89%)	79 (75%)	0.02
DM	39 (21%)	16 (20%)	23 (22%)	0.9
Hypertension	82 (44%)	32 (39%)	50 (47%)	0.33
Smoker	77 (41%)	50 (61%)	27 (26%)	<0.001
TC (mg/dl)	177±36	181±39	174±34	0.17
HDL (mg/dl)	44±14	39±11	47±16	<0.001
LDL (mg/dl)	104±33	113±35	97±29	0.001
TG (mg/dl)	158±115	162±138	155±94	0.66
CRP (mg/dl)	0.4±0.8	0.5±0.8	0.3±0.7	0.11
MMP-2 (ng/ml)	181±42	176±43	185±42	0.14
MMP-9 (ng/ml)	57±50	73±64	45±31	<0.001
TIMP-1 (ng/ml)	69±17	69±18	70±17	0.70
Adiponectin (μg/ml)	3.3±2.4	3.2±2.3	3.3±2.2	0.70
MIF (ng/ml)	25±10	27±12	25±9	0.18

ACS, acute coronary syndrome; SAP, stable angina pectoris; DM, diabetes mellitus; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; CRP, C-reactive protein; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; MIF, macrophage migration inhibitory factor.

(SAP)] had given a peripheral venous blood sample before coronary intervention were enrolled in this study. A previous study had been performed to evaluate the systemic distribution of TCFAs in 3 major epicardial arteries of the patients with coronary artery disease and no VH-IVUS-related complications had occurred.[7] The patients with ACS included 31 with unstable angina pectoris, 21 with non ST-segment elevation myocardial infarction (MI), and 30 with ST-segment elevation MI. Acute MI was defined as continuous chest pain at rest with abnormal level of cardiac enzymes (creatinine kinase-MB or troponin T). SAP was defined as no change in the frequency, duration, or intensity of symptoms within 6 weeks before the intervention. The culprit lesion in ACS or the target lesion in SAP was identified by a combination of left ventricular wall motion abnormalities, ECG findings, angiographic lesion morphology, and scintigraphic defects. In patients with SAP who underwent multivessel intervention, the lesion with the worst diameter stenosis and more complex morphology in the territory of the scintigraphic reversible defects was selected as the target lesion for VH-IVUS analysis.

IVUS Imaging and Analysis

VH-IVUS examination of all 3 major epicardial arteries was performed before any intervention and after intracoronary administration of 0.2 mg nitroglycerin. The 2.9-Fr IVUS imaging catheter (Eagle Eye, Volcano Corp, Rancho Cordova, CA, USA) incorporated a 20 MHz phased-array transducer. In all 3 coronary arteries in each of the patients studied, the transducer was advanced into the distal coronary artery, and an imaging run was performed back to the aorto-ostial junction using a motorized transducer pullback system (0.5 mm/s).

Conventional gray-scale quantitative IVUS analyses of the areas of the external elastic membrane, lumen, plaque and media (external elastic membrane–lumen) were performed according to the criteria of the Clinical Expert Consensus Document on IVUS.[16] IVUS signs of plaque rupture were a cavity that communicated with the lumen and had an overlying residual fibrous cap fragment.[3,17] Planar VH-IVUS analysis was performed at the site of the minimal lumen area using pcVH software (Volcano Corp).[7] VH-IVUS analysis coded tissue as green (fibrotic), yellow-green (fibrofatty), white (dense calcium) or red (necrotic core). VH-IVUS analyses were reported in absolute amounts and as percentages (relative amounts) of plaque area.[4–7] VH-TCFA was defined as a necrotic core >10% of plaque area at the site of the minimal lumen area or largest area of necrotic core in at least 3 consecutive frames without overlying fibrous tissue in the presence of >40% plaque burden (plaque and media÷external elastic membrane).[5,7] Based on both the gray-scale and VH-IVUS findings, lesions were classified into 3 groups: ruptured plaque, VH-TCFA (without evidence of plaque rupture), and non-VH-TCFA plaque.

Blood Sampling and Measurements of Plasma Biomarkers

Blood was collected immediately before the first bolus injection of heparin was given, and was directly transferred into plastic tubes prepared with EDTA. Plasma was obtained after centrifugation at 3,000×g for 15 min at 4°C and aliquots were stored at –70°C until analysis. Plasma levels of MMP-2, MMP-9, TIMP-1, adiponectin and MIF were measured by the Quantikine® immunoassay (R&D Systems, Minneapolis, MN, USA), which uses a quantitative enzyme immunoassay technique.[18–20] In brief, a monoclonal antibody specific for each of MMP-2, -9, TIMP-1, MIF and adiponectin is precoated onto a microplate. Antigen standards and samples are pipetted into the wells and each biomarker is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for each biomarker is added to the wells and following a wash to remove any unbound antibody–enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of each biomarker bound in the initial step. The color development is stopped and color intensity is measured.[18]

Statistical Analysis

Statistical analysis was performed with SPSS 15.0 (Chicago, IL, USA). Continuous variables are expressed as means± standard deviation. All categorical variables are expressed as frequencies and percentages. Differences between continuous variables were determined using analysis of variance supplemented with the t-test. Categorical variables were tested by

Table 2. Gray-Scale and Virtual Histology IVUS Findings According to Characteristics of Plaque in the Culprit/Target Lesions

	Ruptured plaque (n=38)	Virtual histology IVUS-derived thin-cap fibroatheroma (n=150)	
		Yes (n=107)	No (n=43)
Gray-scale findings			
EEM area (mm^2)	18.3±3.7*	14.6±3.8	15.7±4.2
Lumen area (mm^2)	3.8±0.7	3.7±0.7	3.8±0.7
Plaque and media area (mm^2)	14.5±3.8*	10.9±3.8	11.9±3.9
Virtual histology findings			
Absolute area (mm^2)			
Fibrotic (green)	5.8±2.5*	3.7±2.2¶	5.4±2.7
Fibrofatty (yellow-green)	0.6±0.5	0.3±0.3¶	0.8±0.6
Dense calcium (white)	1.0±0.8	0.7±0.8	0.6±0.8
Necrotic core (red)	3.6±1.8*	2.8±1.3¶	1.6±1.0
Percentages (%)			
Fibrotic	53±14	48±13¶	64±14
Fibrofatty	6±6	3±3¶	9±7
Dense calcium	9±7	10±8	8±10
Necrotic core	32±13	39±10¶	19±9

*P<0.005 comparing ruptured and unruptured plaques; ¶P<0.001 comparing virtual histology IVUS-derived thin-cap fibroatheroma and non-virtual histology IVUS-derived thin-cap fibroatheroma.
IVUS, intravascular ultrasound; EEM, external elastic membrane.

Table 3. Lipid and Biomarker Levels According to Characteristics of Plaque in the Culprit/Target Lesions

	Ruptured plaque (n=38)	Virtual histology IVUS-derived thin-cap fibroatheroma		P value
		Yes (n=107)	No (n=43)	
TC (mg/dl)	185±42	175±34	174±34	0.27
HDL (mg/dl)	41±11	43±13	47±19	0.10
LDL (mg/dl)	113±37	104±32	96±29	0.08
TGs (mg/dl)	167±123	158±125	150±77	0.81
CRP (mg/dl)	0.6±1.3	0.3±0.6	0.3±0.5	0.28
MMP-2 (ng/ml)	170±43	185±40	180±48	0.17
MMP-9 (ng/ml)*	81±63	54±49	43±30	0.002
TIMP-1 (ng/ml)	67±14	70±19	70±16	0.60
Adiponectin (μg/ml)	2.6±2.0	3.5±2.5	3.3±2.3	0.14
MIF (ng/ml)	27±13	27±14	24±8	0.35

*P<0.05 comparing ruptured and unruptured plaques.
Abbreviations see in Tables 1,2.

chi-square test. Correlations between levels of plasma biomarkers and VH-IVUS parameters were tested by regression analysis. Multiple stepwise logistic regression analysis was performed to assess independent predictors for plaque rupture or VH-TCFA. A P-value <0.05 was considered statistically significant.

Results

Baseline clinical characteristics and plasma biomarker levels of the patients with ACS or SAP are listed in Table 1. Compared with the SAP patients, the level of high-density lipoprotein-cholesterol was significantly lower, and the levels of low-density lipoprotein-cholesterol and MMP-9 were significantly higher in ACS patients. In the culprit/target lesions of the overall group (n=188), ruptured plaques were observed in 38 patients and in the other 150 patients without ruptured plaque, 107 had VH-TCFA and 43 patients did not. Gray-scale and VH-IVUS findings for ruptured plaques, VH-TCFA and non-VH-TCFA plaques are shown in Table 2. Table 3 shows the comparison of plasma biomarkers levels and lipid profiles among the 3 groups. Compared with the patients without ruptured plaques, MMP-9 levels were significantly higher in the patients with ruptured plaques, but there were no significant differences between the 2 groups in the levels of high-sensitivity C-reactive protein (hs-CRP), MMP-2, adiponectin, TIMP-1 and MIF or in the lipid profiles. In multivariate logistic regression analysis, both ACS and MMP-9 were independent predictors for ruptured plaques [odds ratio (OR) 3.30, 95% confidence interval (CI) 1.47–7.41, P=0.004, and OR 1.01, 95%CI 1.00–1.01, P=0.04, respectively].

In the subgroup without ruptured plaque in the culprit/target lesions, the lipid profiles and levels of plasma biomarkers, including MMP-9, did not show statistically significant differences between the patients with and without VH-TCFA. Multivariate logistic regression analysis showed that ACS was the only independent predictor for VH-TCFA (OR 3.34, 95%CI 1.41–7.94, P=0.006). Although the levels of plasma

biomarkers were incapable of predicting the presence of VH-TCFA, there was a weak, but significant positive correlation between the absolute area of necrotic core and the MMP-9 level (r=0.252, P=0.002) and a negative correlation between the absolute area of necrotic core and the adiponectin level (r=−0.163, P=0.046). The percentage of necrotic core in the culprit/target lesions did not show any significant correlations with the levels of plasma biomarkers in the subgroup without ruptured plaque.

When the analysis was performed with both culprit/target and nonculprit/non-target vessels, there was a significant correlation between the total number of ruptured plaques and the level of MMP-9 (r=0.231, P=0.002). Significant differences in any biomarker level were not found between the patients with and without VH-TCFA.

Discussion

This 3-vessel VH-IVUS study of 188 patients showed that the plasma level of MMP-9 might increase in patients with multiple ruptured plaques, as well as in patients with ruptured plaque in the culprit/target lesions. Both ACS and the MMP-9 level were independent predictors of ruptured plaque in the culprit/target lesions. However, the presence of VH-TCFA in the culprit/target lesions or multiple VH-TCFAs detected by 3-vessel VH-IVUS study were not be predictive with the use of several biomarker assays, including MMP-9, in this study. The clinical presentation of ACS, not the level of the biomarkers, was the only independent predictor of VH-TCFA in the culprit/target lesions.

MMPs belong to a family of multidomain zinc-dependent endopeptidases that promote degradation of all protein and proteoglycan–core-protein components of the extracellular matrix.[21] Among the family of MMPs, MMP-2 and MMP-9 are found in the macrophages and smooth muscle cells covering the shoulder region of atherosclerotic plaque.[11] MMP-9 and MMP-2 are highly expressed in the vulnerable regions of atherosclerotic plaque and it has been suggested that they are causally involved in plaque rupture.[22] Other studies have shown that the level of MMP-2 activity is higher in stable lesions of carotid artery plaque,[10] and that the level of MMP-9 is increased in more unstable plaque.[10,20] Our finding that the MMP-9 level was higher in patients with ruptured plaque would support the evidence of an association between plaque vulnerability and MMP-9.[20,22] Blakenberg et al reported that a higher level of plasma MMP-9 was a predictor of cardiovascular mortality: the patients in the highest quartile of MMP-9 level (>72 ng/ml) had the highest probability of cardiovascular death.[19] In our study, the mean MMP-9 level in patients with ruptured plaque was 81 ng/ml. We could not find any relationship between plasma MMP-2 level and vulnerable plaque as determined by VH-IVUS study (ie, ruptured plaque or VH-TCFA). We could suppose that MMP-9 has a more significant role in plaque vulnerability than MMP-2, but more clinical studies are required to evaluate the exact role of MMP-2 in plaque vulnerability. Elevated MMP-9 levels have been observed in patients with coronary, carotid and peripheral artery disease, diabetes mellitus, as well as autoimmune diseases, multiple sclerosis and other inflammatory conditions.[19–21] In the present study there were no significant differences in the hs-CRP level between patients with and without VH-TCFA in the culprit/target lesions.

Adiponectin is an adipose-specific plasma protein and a lower level is observed in patients with coronary artery disease.[23] Using VH-IVUS, Otake et al investigated the relationship between adiponectin and coronary plaque components in ACS patients, and reported a negative correlation between the level of adiponectin and percentage of necrotic core in ACS patients (r=−0.29, P=0.04).[15] The weak negative correlation between adiponectin level and absolute necrotic core area in patients without ruptured plaque in the present study (r=−0.163, P=0.046) was similar to their result.

The acute-phase reactant, CRP, is a very sensitive, although nonspecific, marker of inflammation. Circulating levels of CRP may constitute an independent risk factor for cardiovascular disease.[24] CRP levels are considered to reflect the severity and progression of the atherosclerotic process in the vessel.[25] A recent IVUS study reported that the hs-CRP level has a significant correlation with the percentage of necrotic core in both culprit and nonculprit lesions of ACS patients.[15] However, we could not find any relationship between plaque characteristics and hs-CRP level. When lesions are analyzed post-rupture, after the necrotic core may have embolized, they most often appear "dark-green" and will be classified as fibrotic plaque rather than necrotic core in VH-IVUS analysis, and have a reduced calculated relative size of the necrotic core. Therefore, when the relationships between each plaque characteristic and plasma biomarker levels, including hs-CRP, were evaluated in the present study, ruptured plaques were excluded to avoid the possibility of incorrect VH-IVUS interpretation and this may partially explain the different results between studies.

Early detection of TCFA before rupture is clinically significant for the prevention of catastrophic events such as ACS or sudden death. Several methods (ie, imaging modalities or measurement of plasma biomarkers) have been tested and are used in clinical practice to identify TCFA. For the patient, a non-invasive method such as biomarker assay is more comfortable, but is less accurate, whereas invasive methods such as VH-IVUS study are more accurate for detecting TCFA, but are uncomfortable and inconvenient for the patient. If it was possible to replace invasive study with a biomarker assay for detection of TCFA, it would be the ideal situation from patient's viewpoint of preference and safety. In the present study, measurement of the levels of biomarkers such as MMP-9 appeared useful for screening for the presence of ruptured plaques, one of several fates of TCFA, but the presence of TCFA prior to rupture could not be identified by simple measurements of biomarker levels. This finding suggests that imaging modalities rather than biomarker level assay should be emphasized as the screening tool for early detection of TCFA in the current clinical situation. There are several reasons for the lack of a relationship between detecting VH-TCFA and biomarker level assay in the present study. First, a limited number of biomarkers were evaluated, so more studies are needed to discover more sensitive biomarkers for detecting TCFA (without rupture) as well as ruptured plaques. Second, the clinical impact and future outcomes of TCFA defined by VH-IVUS study need to be evaluated more.

Study Limitations

This study was a single center, retrospective study. In ACS patients with lesions and thrombus, pre-intervention VH-IVUS study was done after thrombus suction was achieved using a thrombectomy catheter alone without use of balloon dilation or occlusion. The relationship between the absolute amount of necrotic core and possible candidate VH-TCFAs was not evaluated in this study.

Conclusion

A higher level of MMP-9 may predict ruptured status of coronary plaques, but not VH-TCFA (without rupture). In the present study there were weak correlations between the levels of MMP-9 and adiponectin and the area of necrotic core in the culprit/target lesions.

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