

Original article

Relationship between tissue type plasminogen activator and coronary vulnerable plaque in patients with acute coronary syndrome: virtual histological study

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Keywords: acute coronary syndrome; tissue type plasminogen activator; vulnerable plaque; diagnostic technique; intravascular ultrasound

Background The association between vulnerability of plaque assessed with intravascular ultrasound (IVUS) and plasma levels of fibrinolytic biomarkers was determined in patients with acute coronary syndrome (ACS). However, few data are available on the relationship between the levels of tissue type plasminogen activator (t-PA) and virtual histological intravascular ultrasound (VH-IVUS) signs of plaque instability.

Methods Eighty-nine patients with ACS were enrolled in the study. Blood was collected to measure t-PA levels by liquid phase bead flow cytometry. Eighty-nine nonbifurcate lesions (identified by coronary angiography and ECG) were investigated using IVUS before catheterization. IVUS radiofrequency data obtained with a 20 MHz catheter were analyzed with IVUS virtual histological software. The areas of plaque and media were calculated and lesions were classified into two groups: VH-IVUS derived thin cap fibroatheroma (VH-TCFA) and non-VH-TCFA plaque.

Results Plasma t-PA level in the patients with TCFA was significantly lower than that with non-TCFA (1489 ± 715 pg/ml vs 2163 ± 1004 pg/ml). Decreased plasma levels of t-PA were associated with plaque vulnerability. Plasma levels of t-PA correlated negatively with plaque plus media and necrotic core in plaque in patients with ACS.

Conclusions t-PA is an independent risk factor and a powerful predictor of vulnerable plaques. Decreased levels of t-PA may reflect instability of atherosclerotic plaques and might therefore serve as noninvasive determinants of those at high risk for consequent adverse events.

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Cardiovascular disease places an enormous medical and economic burden on society as the cause of the majority of deaths worldwide. Most of these deaths are caused by the rupture of atherosclerotic plaques in the coronary arteries. The majority of acute coronary events are caused by the disintegration or erosion of vulnerable plaque (defined as plaques likely to cause atherothrombosis).¹ We know that vulnerable plaques are not the only factor for the development of acute coronary syndromes (ACS) – vulnerable blood (prone to thrombosis) plays an important role in the outcome.^{2,3} So it is important to develop a sensor that can detect a vulnerable plaque and indicate for the clinician the appropriate treatment. There are tools to aid clinical identification of patients at risk of ACS, such as imaging (e.g. coronary angiography) and biochemical measurements e.g. tissue type plasminogen activator (t-PA)). These tools can identify vulnerable plaque or vulnerable blood respectively. However, few investigations related the morphological changes of the lesion and the plasma levels of biomarkers involved in pathological changes of ACS. Intravascular ultrasound (IVUS) is the method to evaluate coronary plaque, lumen and vessel dimensions. Although conventional grey scale IVUS provides cross sectional morphometric detail and quantifies atherosclerotic plaque area and plaque burden, it has significant limitations in accurately assessing atheromatous plaque composition.

To some extent, these limitations have been removed by virtual histology IVUS (VH-IVUS).^{4,5} Accordingly, we investigated the relationship between plaque composition and signs of plaque instability with VH-IVUS and the levels of t-PA in the circulation.

METHODS

From November 2006 to October 2007, 89 patients with diagnosis of ACS *de novo* lesions by IVUS prior to treatment, enrolled in this study at Qingdao Municipal Hospital, Shandong, China. There were 54 patients with unstable angina and 35 patients with acute myocardial infarction. Patients with a bundle branch block, pacemakers, suspected myocarditis or pericarditis, fever,

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cardiogenic shock, or in whom it was impossible to localize the lesion with angiography had been excluded. Acute myocardial infarction was defined as continuous chest pain at rest with abnormal levels of cardiac enzymes (creatinine kinase-MB or troponin T). The lesion in ACS was identified by the combination of left ventricular wall motion abnormalities, electrocardiographic findings and angiographic lesion morphology. The lesion with the worst diameter stenosis was selected as the target lesion for VH-IVUS analysis. Total occlusions, bifurcate lesions, lesions with severe angulations and heavily calcified lesions were excluded from this study.

Coronary angiography was performed within 12 hours after the onset of symptoms and 3 to 5 hours after presentation at our hospital. After identification of the lesion and diagnostic IVUS, coronary interventions were performed. The medical history, including age, gender, cerebrovascular disease and preexisting coronary risk factors such as diabetes mellitus, hypertension, hypercholesterolemia and smoking, was recorded for each patient.

IVUS imaging and analysis

After completion of the diagnostic coronary angiography, the lesion was localized according to the angiographic signs of vessel morphology and electrocardiogram patterns. Before the IVUS procedure, 200 µg intracoronary nitroglycerine was administered to prevent vasospasm and a 2.9F IVUS imaging catheter (Eagle Eye; Volcano Corporation, USA) was advanced distally to the lesion and pulled back at 0.5 mm/s. Simultaneously, the images were recorded on videotapes for subsequent offline analysis.

IVUS images were analyzed independently by two experienced observers. Conventional grey scale quantitative IVUS analyses were performed according to criteria of the clinical expert consensus document on IVUS⁶ to include external elastic membrane (EEM), luminal and plaque areas. The EEM cross sectional area (defined as the area encompassed within the media-adventitia border) was measured in diastolic frames. The plaque cross sectional area, defined as the intima media area, was equated to the space between EEM and the lumen. The plaque burden on each cross section was calculated as $100 \times (\text{external elastic membrane cross sectional area} - \text{lumen cross sectional area}) / \text{external elastic membrane cross sectional area}$.

Planar VH-IVUS analysis was performed on the minimal luminal area.⁷ VH-IVUS analysis colour coded and classified tissue as green (fibrotic), yellow green (fatty fibrous), white (dense calcium) and red (necrotic core). VH-IVUS estimates were reported as percentages of plaque area. VH-IVUS delineated thin cap fibroatheroma (VH-TCFA) was defined as (A) necrotic core with at least 10% of plaque area at either the minimal luminal area site or the largest necrotic core site; (B) in at least 3

consecutive frames; (C) without evident overlying fibrous tissue and (D) in the presence of at least 40% plaque burden (Figure).^{5,8} According to VH-IVUS findings, lesions were classified into two groups: VH-TCFA plaque and non-VH-TCFA plaque.

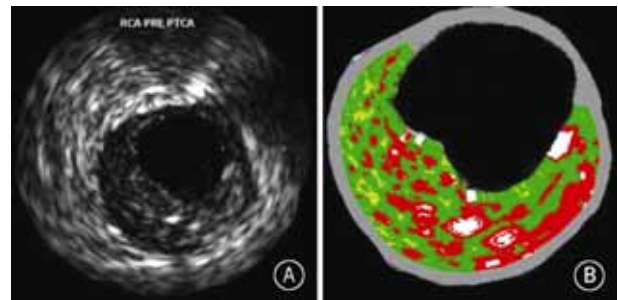


Figure. Intravascular ultrasound (IVUS) grey scale image (A) and VH-IVUS image (B) of the thin cap fibroatheroma (TCFA). Colour coded tissue shows as green (fibrotic), yellow-green (fatty fibrous), white (dense calcium), and red (necrotic core).

Blood sampling and measurements

Blood was collected immediately before the first heparin bolus was given and directly transferred into plastic tubes prepared with ethylenediamine tetraacetic acid. Plasma was obtained following centrifugation at $3000 \times g$ for 15 minutes at 4°C . Plasma aliquots were stored at -70°C until analysed. Plasma levels of t-PA were measured by liquid phase bead flow cytometry (BD PharMingen, USA).

Statistical analysis

Statistical analysis was performed with SPSS13.0. Continuous variables were expressed as means \pm standard deviation (SD). All categorical variables were 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 chi-square test. Correlations between t-PA and IVUS parameters were tested by regression analysis. A *P* value less than 0.05 was considered statistically significant.

RESULTS

The baseline clinical characteristics are listed in Table 1. A lesion was localized in the left anterior ascending, left circumflex or right coronary artery in 56%, 19% and 25% patients, respectively. Single vessel disease was present in 51% and double vessel disease was present in 31% of patients.

IVUS data

Table 2 lists grey scale IVUS findings of lesions, comparing lesion with TCFA and without TCFA. The minimum luminal, external elastic membrane and plaque plus media cross sectional areas were $(3.3 \pm 1.6) \text{ mm}^2$, $(14.3 \pm 2.6) \text{ mm}^2$ and $(11.1 \pm 2.7) \text{ mm}^2$ respectively, whereas the plaque burden was $(77 \pm 12) \%$ in TCFA group. In the other group, the minimum luminal, external elastic membrane and plaque plus media cross sectional

Table 1. Clinical characteristics of patients

Clinical characteristics	Lesions with	Lesions with	<i>P</i> value
	non-VH-TCFA (<i>n</i> =28)	VH-TCFA (<i>n</i> =61)	
Age (years)	60±13	61±9	0.7
Male (%)	18 (64)	37 (61)	0.5
Diabetes mellitus (%)	8 (29)	15 (25)	0.3
Hypertension (%)	15 (54)	35 (57)	0.6
Smoking (%)	14 (50)	33 (54)	0.5

VH-TCFA: virtual histological thin cap fibroatheroma.

Table 2. Gray scale intravascular ultrasound findings comparing plaques with VH-TCFA and non-VH-TCFA

Variable	non-VH-TCFA	VH-TCFA	<i>P</i> value
	(<i>n</i> =28)	(<i>n</i> =61)	
EEM CSA (mm ²)	13.9±3.7	14.3±2.6	0.5
Lumen CSA (mm ²)	4.9±2.2	3.3±1.6	0.003
Plaque CSA (mm ²)	9.0±2.6	11.1±2.7	0.008
Plaque burden (%)	66.0±13.9	77.2±12.0	0.003

VH-TCFA: virtual histological thin cap fibroatheroma; EEM: external elastic membrane; CSA: cross sectional area.

areas were (4.9±2.2) mm², (13.9±3.7) mm² and (9.0±2.6) mm² respectively and the plaque burden was (66±14)%. VH-TCFA group had greater plaque burden compared with the non-TCFA.

Planar VH-IVUS analysis on the minimum luminal showed that the percentage of necrotic core area was significantly greater in VH-TCFA group ((34±12)% vs (21±10)%), and the percentage of fibrotic plaque was significantly smaller in non-VH-TCFA group ((49±11) % vs (61±14)%) (Table 3).

Table 3. Virtual histological intravascular ultrasound findings comparing plaques with VH-TCFA and non-VH-TCFA

Variable	non-VH-TCFA	VH-TCFA	<i>P</i> value
	(<i>n</i> =28)	(<i>n</i> =61)	
Fibrotic (%)	61±14	49±11*	<0.05
Fatty fibrous (%)	9±7	7±5	0.3
Dense calcium (%)	9±8	10±9	0.4
Necrotic core (%)	21±10	34±12*	<0.05

**P*<0.05 comparing TCFA with non-VH-TCFAs. VH-TCFA: virtual histological thin cap fibroatheroma.

t-PA

Decreased levels of t-PA were found in the patients with TCFA, compared with t-PA levels in the patients without TCFA ((1489 ±715) pg/ml vs (2163 ± 1004) pg/ml, *P* < 0.05).

Correlation between t-PA and burden and instability of plaque and composition determined with VH-IVUS

For the VH-IVUS signs of plaque instability, lower levels of t-PA were measured in association with lesion with TCFA. In addition, the necrotic core of the lesion was associated with significantly decreased levels of t-PA ($r=-0.425$, $P<0.05$) in TCFA, as compared with non-TCFA. The plaque plus media and external elastic membrane areas of the lesion also correlated significantly with the plasma levels of t-PA ($r=-0.392$, $P<0.05$; $r=-0.411$, $P<0.05$).

DISCUSSION

In this study, we have shown a direct relationship

between coronary vulnerable plaque identified by VH-IVUS and the impaired fibrinolytic capacity. These findings suggest that ACS influences the balance of fibrinolytic system and show a link between endothelial dysfunction, vulnerable plaque and vulnerable blood.

Atherosclerosis is a diffuse, chronic inflammatory disorder involving vascular, metabolic and immune systems with various local and systemic manifestations.⁹ The patient vulnerable to cardiovascular disease (CVD) is likely harbouring not only vulnerable plaque but also vulnerable blood. Morphology of the lesion can be detected by coronary angiography (CAG) and IVUS. CAG has several inherent limitations and inaccuracies because it can assess only the arterial lumen and is unable to take account of arterial remodelling.¹⁰⁻¹² In contrast with angiography, IVUS is unique in assessing both the intraluminal plaque and the surrounding arterial wall morphology.^{13,14} However, coronary plaque composition, which determines the vulnerability of plaque, can not be assessed accurately using grey scale IVUS. Using VH-IVUS, composition of coronary plaque was determined and plaque was characterized as fibrotic, fatty fibrous, dense calcium or necrotic core.^{4,5,15} The TCFA delineated by VH-IVUS are referred as precursor lesions associated with rupture.

Biomarkers of vulnerable blood, including blood markers reflecting hypercoagulability, are one tool to identify high risk individuals for accurate diagnosis and prognostication to treat the patient effectively. The importance of the coagulation/fibrinolytic system is highlighted by several autopsic studies that show a high prevalence of old plaque disruptions without infarctions. A transient shift in the coagulation and anticoagulation balance is likely to result in an acute event. The prolonged presence of residual thrombus over a disrupted or eroded plaque will induce smooth muscle migration and produce new intima, leading to plaque expansion.¹⁶ Autopsic studies show that plaque growth is induced by episodic plaque disruption and thrombus formation.¹⁷ Therefore, an active fibrinolytic system may be able to prevent luminal thrombosis in some cases of plaque disruption.¹⁸ t-PA, as a crucial factor in fibrinolytic system, plays an important role in the balance between coagulative system and fibrinolytic system, which is mainly responsible for the dissolution of fibrin clots in the circulation, by converting inactive plasminogen to active plasmin. A rapid decline in release of active t-PA is associated with an increasing plaque burden and vulnerability. The reduction in acute fibrinolytic capacity reflects impairment of acute t-PA release that is likely to involve endothelial cell injury.¹⁹⁻²¹

In conclusion, we found a correlation between the reduced plasma levels of t-PA and vulnerable plaque with TCFA of the lesion in patients with ACS, as compared with those with non-TCFA. The decreased t-PA levels might reflect ongoing thrombus formation in the lesions

of these patients, resulting in quick advancement of the lesion. Therefore, detection of t-PA levels may be helpful in the guidance of antithrombotic treatment. Accordingly, decreased levels of the t-PA might serve as noninvasive marker of risk stratification and therapeutic monitoring of patients with ACS.

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