

Plaque Vulnerability Phenotype in Patients with Coronary Artery Disease

An Intravascular Ultrasound Radiofrequency Analysis

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Abstract: The relationship between plaque morphology and clinical presentation was examined. Lumen dimensions, atheroma morphology and composition were assessed by virtual histology intravascular ultrasound (VH-IVUS) in 1757 frames of coronary segments of interest of 17 patients with acute and chronic coronary artery disease, i.e., ST-elevation (STEMI) and non-ST-elevation (NSTEMI) myocardial infarction, unstable angina (UA) and stable angina (SA). Large plaque areas with distended elastic lamina (EEL), rich in fibrotic (FB) and fibro-fatty (FF) tissues associated with STEMI and UA. Variants of this phenotype consisting of large calcium deposits and reduced lumen area were prevalent in NSTEMI patients. SA patients consistently showed plaques with small areas, marked constrictive growth and low FF content. IVUS-derived plaque measures provided phenotypes of vulnerability and rupture that may help improving risk stratification of both symptomatic and asymptomatic patients.

1 INTRODUCTION

Coronary artery disease is still the main cause of death worldwide. This disease is characterized by impaired function of endothelial cells, which line the vessel luminal surface. The endothelial dysfunction promotes the adhesion of leukocytes and their migration into the vessel wall. Atherosclerosis is a pathological process of the vasculature causing a progressive thickening of artery intima. As atherosclerosis progresses the atheroma builds up in the vessel wall in consequence of abnormal transport of low density lipoproteins (LDL) in endothelial cells, inflammatory cell recruitment to the vessel wall and inefficient turnover of debris of modified and/or oxidized products of metabolism. Several events such as, cell apoptosis of macrophage-derived lipid-load cells, increased secretion of inflammatory molecules and proteases by activated cells in the intima, which damage the extracellular matrix, promote cell activation and smooth cell migration

from arterial media, contribute to plaque fibrosis and development of the necrotic core and calcified areas. The perpetuation of oxidative, inflammatory and proteolytic activity originates an inflamed plaque with a metabolically active fibrous cap. Eventually the plaque ruptures, which results in a clinical spectrum of presentations ranging from sudden cardiac death, myocardial infarction and unstable angina, to an asymptomatic event with plaque progression (Stone, 2011).

Intravascular ultrasound (IVUS) is a catheter based imaging modality providing two-dimensional visualization of the arterial wall. The analysis of the radiofrequency spectrum, usually called Virtual Histology IVUS (VH-IVUS), allows determining the fibrotic, fibro-fatty, necrotic and calcium contents in plaques (Vancraeynest, 2011); (Nair, 2007); (Fayad and Fuster, 2001).

In coronary segments of interest the VH-IVUS-derived plaque dimensions and composition data may help characterizing the high-risk and vulnerable atherosclerotic plaques. The relationship between

atheroma composition and arterial remodelling characteristics in patients with acute and non-acute coronary artery syndromes would dramatically improve risk stratification of both symptomatic and asymptomatic patients (Ramos et al, 2013); (Garcia-Garcia, 2012); (Calvert, 2011).

2 OBJECTIVES

The aim of this study was to examine the plaque morphological and histological characteristics obtained with VH-IVUS, and associate these features with clinical symptoms.

In particular, we investigated how vessel measures, indicative of plaque expansive and constrictive growth, were predicted by plaque composition changes.

It can be anticipated that plaque phenotype may be related to vulnerability and plaque rupture.

3 METHODS

3.1 Patients

Seventeen patients of both sexes with coronary artery disease (CAD) presenting to the Cardiology Service of Santa Marta Hospital (CHLC, Lisbon, Portugal) undergoing percutaneous coronary intervention (PCI) for troponine-positive acute coronary syndrome (ACS), such as, ST elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and absence of biochemical evidence of myocardial damage such as, unstable angina (UA) were prospectively eligible non-ACS such as, chronic stable angina were also included in the study.

ACS patients were assessed within 6 hours after onset of symptoms and before medication administration. Non-ACS patients were assessed before PCI. Demographics, risk factors, clinical history, and angiographic data were recorded for each patient. Biochemical tests were also carried out, which included creatinine kinase (CK), troponin T, N-terminal pro-brain natriuretic peptide (NT-proBNP) and C-reactive protein (CRP) determination (Table 1).

The Ethical Committee of the CHLC approved the study protocol. All participants gave written consent before enrolment.

3.2 VH-IVUS

The VH-IVUS acquisition was performed using an EagleEye catheter (20 MHz) at pullback speed of 0.5 mm/sec. For each coronary segment, vessel, lumen and atheroma measurements were obtained for every VH-IVUS frame (0.5 mm thickness) throughout the region of interest and lesion borders established using the leading edges of external elastic lamina (EEL) and the luminal contour.

Atheroma area (AAT) was determined as the difference between EEL and lumen areas. Atheroma volume was calculated as the differences between EEL and lumen areas across all evaluable slices. Plaque burden was calculated as plaque area divided by EEL area.

Table 1: Patients characterization. Results are medians and interquartile range (Q25 – Q75), unless otherwise specified. Abbreviations: ACS - Acute Coronary Syndrome; TIMI (Thrombolysis In Myocardial Infarction) grade flow 0 – no perfusion to 3 – complete perfusion; Multivessel ≥ 1 vessel affected.

Patient's Characterization	
Male sex (%)	66
Age (y)	66 (57 – 76)
Risk factors >2 (%)	67
Previous medication (%)	89
ACS (%)	41
Multivessel (%)	47
TIMI ≤ 2	94
CK (U/l)	125 (87 – 424)
Troponin T (U/l)	0.05 (0.01 – 1.87)
CRP (mg/l)	5.6 (1.0 – 8.2)
NT-proBNP (pg/ml)	162 (0.01 – 219)

IVUS data was recorded for the reconstruction of the radiofrequency backscatter information using In-Vision gold commercial software (Volcano Corporation, USA). Following spectral analysis, the areas and percentages of fibrotic (FB), fibro-fatty (FF), calcified (Ca) and necrotic core (NC) were calculated for each frame. A colour image of the plaque is formed using a colour code: green (FB); light green (FF); red (necrotic core); white (Ca).

3.3 Statistical Analysis

Statistical analysis was performed with SPSS V.21. IVUS data was evaluated taking into account all frames in every segment of interest and single cross-sections selected at larger stenosis, distal and proximal regions of the coronary segment.

The correlation between VH-IVUS derived measurements was calculated using Kendall's Tau

algorithm. Linear regression (stepwise selection of variables) was applied to estimate plaque composition in positive or negative growth. Discriminant analysis was used to correlate plaque morphology and composition in combination to clinical symptoms. Variables were transformed whenever appropriate. Continuous variables were compared by Mann-Whitney U statistic test.

A p-level <0.05 was considered statistically significant for all analysis.

4 RESULTS

4.1 Plaque Characterization

The coronary segments of each patient were evaluated by extracting selected frames in three distinct regions, i.e. distal, proximal and major stenosis region and by using the total number of frames recorded in each visualized segment (multi-frame analysis).

In the multi-frame approach a total of 1757 VH-IVUS frames were analysed (median = 56; IQ25-IQ75: 26-93 frames per coronary segment).

The results obtained using both approaches are summarized in Table 2. When plaque data was assessed using a reduced number of frames luminal dimensions were underestimated relative to multi-frame approach.

Table 2: Patients characteristics using selected frames and total number of frames. Significant differences (pair-test) for p<0.05. Abbreviations: EEL - external elastic lamina; AAT - atheroma area; PB -plaque burden; FB - fibrotic tissue; FF - fibro-fatty tissue; Ca - calcified tissue; NC - necrotic core.

Plaque characteristics				
		Analysis of 3 frames	Multi-frame analysis	p
	FB (%)	57 (50 – 69)	51 (45 – 61)	0.877
	FF (%)	12 (8 – 17)	11 (6 – 17)	0.234
	Ca (%)	11 (5 – 19)	10 (3 – 16)	0.017
	NC (%)	16 (10 – 21)	21 (14 – 25)	0.056
Lumen	diameter (mm)	2.2 (1.9 – 2.7)	2.6 (2.4 – 2.9)	0.005
	area (mm ²)	3.6 (2.9 – 5.1)	5.1 (4.6 – 6.7)	0.001
EEL	diameter (mm)	4.5 (4.2 – 4.9)	4.5 (4.0 – 4.8)	0.326
	area (mm ²)	17 (14 – 19)	16 (12 – 18)	0.215
	AAT (mm ²)	13 (10 – 15)	10 (7 – 12)	0.001
	PB (%)	77 (66 – 84)	60 (50 – 68)	<0.001

Consequently plaque area and plaque burden become overestimated as can be inferred from data listed in Table 2. Data for plaque composition using selected frames from each coronary segment depicted higher values of Ca tissue content (p=0.017), whereas NC content was marginally reduced (p=0.056).

4.2 Relationship between Plaque Morphology and Composition

Lumen and EEL dimensions, whether minimum or maximum diameters, area or volumes, were positively correlated (p<0.05). The NC content of the plaque failed to correlate with lumen, EEL or plaque dimensions.

Considering EEL area (or volume) as dependent variable in the regression model and plaque composition (FB, FF, Ca, and NC) as predictor variables, EEL was positively associated to plaques rich in FB, FF. The regression model became more significant when plaque area was considered as dependent variable. The model accuracy was above 68% and both the model fitted and variables retained were highly significant (p<0.001). The FB and FF contents were moderately useful to estimate plaque size (r=0.355, p<0.0001). The plaque outwards enlargement was related to FF increases in detriment of plaque FB content.

However, the plaque composition failed to associate with lumen dimensions (diameter, area or volume).

To further estimate the importance of plaque composition and morphology to the plaque phenotype having into account patient clinical presentation, i.e., STEMI, NSTEMI, UA and SA, the concordance correlation, not dependent on linear combinations, between area of EEL or plaque area and plaque components was studied.

More expansive plaques (large EEL area or plaque area) are rich in FF and FB and this pattern was associated to STEMI and UA patients. Results showed that the shared correlation between the atheroma area, FB and FF content accounted for 48% of the total variability, and enabled to discriminate STEMI and UA patients (Function 1, negative scores) from SA patients (Function 1, positive scores) (Figure 1). The plaque Ca content expressed 44% of the total variability identifying NSTEMI patients whereas the remaining 8% discriminated plaques with low NC content.

Both STEMI and UA patients showed plaques with significantly higher FF content (median and IQ of normalized area: STEMI - 14%, 8-20%; UA -

16%, 8-27%) when compared to NSTEMI and SA patients (median and IQ of normalized area: NSTEMI - 9%, 6-14%; SA - 6%, 3-12%) ($p < 0.001$). Plaques from STEMI and UA patients were also rich in FB content (normalized areas of approximately 57% in both groups, contrasting with NSTEMI and SA patients showing atheromas with lower content of FB (normalized areas of 37% and 47%, respectively) ($p < 0.001$).

These two plaque components were positively correlated with atheroma area, indicating that increased contents of FB and/or FF were consistent with large plaque areas. The atheroma area did not differ between ACS groups. However all ACS groups differ from SA, which showed the lowest plaque areas (median, IQ25-IQ75: SA - 7mm², 5-10mm²; STEMI - 12mm², 7-13mm²; NSTEMI - 11mm², 9-13mm²; UA - 11mm², 8-15mm²) ($p < 0.001$).

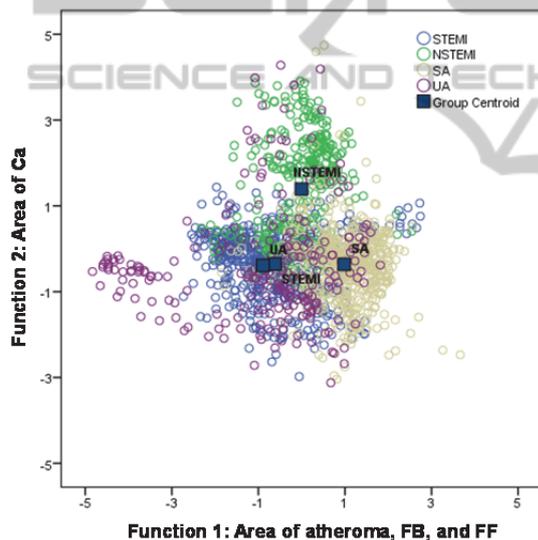


Figure 1: Scatterplot of the two first discriminant functions. The atheroma area, FB and FF correlated with function 1, whereas Ca was correlated with the discriminant function 2.

The Ca content of plaques clearly identified NSTEMI patients, as can be observed in Figure 1. In fact, plaques of NSTEMI patients showed the highest levels of calcified tissue (median, IQ25-IQ75: 28%, 12%-35%) which were highly significant when compared to the other groups of patients (median, IQ25-IQ75: STEMI - 6%, 2%-13%; UA - 6%, 2%-15%; SA - 14%, 3%-23%) ($p < 0.001$).

The highest correlation with the third discriminant function (which had a limited discriminating power) was observed for NC. The

limited importance of NC in group stratification, and therefore in phenotype identification is consistent with the similar variance of NC, although the plaque NC contents significantly differed between groups, as will be referred below.

The influence of plaque constriction was also evaluated in addition to plaque outward expansion. Both the discriminating models showed similar solutions. When the lumen area was added, three significant functions were obtained, of which the two first discriminating functions explained most of the variability (92%). The first discriminating function clearly correlated plaque FB and FF contents (51% of total variance explained) differentiating STEMI from UA patients whereas the second discriminating function correlated the atheroma size with Ca distinguishing NSTEMI from SA.

Thus, the results obtained were similar to the previous model, which only considered atheroma area and plaque structure.

The third function, although significant only explained a moderate 8% of the total variance and correlated the decrease of luminal area with the rise of NC content and helped distinguishing STEMI and UA patients. In fact UA showed larger luminal areas (median, IQ25-75: 9mm², 5-16 mm²) and diminished NC area (median, IQ25-75: 1.1mm², 0.4-2.2mm²) when compared to STEMI (median, IQ25-75: lumen area - 6mm², 3-10mm²; NC - 1.5mm², 0.8-2.3mm²). However the differences between these two groups for NC plaque content were as relevant as those observed for NSTEMI and SA patients ($p \leq 0.001$). The latest showed large necrotic areas when compared to UA and STEMI patients (median of normalized area NC > 23% vs NC < 17% for STEMI and UA).

Moreover, if plaque burden is included in the model, the plaque content relationship was maintained and equally explained by the two first functions as previously described. The third component of the model expresses minor variance associated with lumen area, which changes are opposite to plaque burden and NC content.

This reinforces the view that large luminal areas associate to expansive plaques and that NC may play a role in constrictive plaque phenotype, as observed in SA patients.

5 DISCUSSION

The structure of atheroma was assessed in depth and along the length of coronary segments of interest

using VH-IVUS modality. The plaque characteristics were studied by extracting data of selected cross-sections and of the total number of frames of the scanned region of the vessel.

The study showed that both vessel characteristics and morphology were underestimated when selecting few points along the vessel by report to a multiframe approach covering the whole length of the injured region of the coronary. The detailed evaluation of the atheroma structure paved the way to define plaque phenotypes describing different clinical presentations of CAD.

Large plaque areas with distended elastic lamina, rich in fibrotic and fibro-fatty tissues were associated with STEMI and UA. Variants of this phenotype consisting of large calcium deposits and reduced lumen area were prevalent in NSTEMI patients. SA patients consistently showed plaques with small areas, marked constrictive growth and low FF content.

These findings suggest that SA and NSTEMI were associated with more constrictive remodelling whereas STEMI and UA were associated to expansive remodelling. In addition, the phenotype associated to STEMI and UA patients, can be connected to plaque rupture and/or plaque instability.

In previous studies using VH IVUS the plaque instability had been associated to nonrestenotic thin-capped fibroatheroma and adverse outcomes (Calvert et al., 2011). Samady et al (2011) reported on the influence of shear stress in plaque constrictive and expansive remodelling was associated with the development of necrotic core and FB and FF content suggesting that the excessive expansive remodelling is indicative of plaque vulnerability.

The limited importance of necrotic core for plaque phenotypes and the association with luminal areas and plaque burden suggests that this plaque component may be involved in constrictive plaque growth, possibly having a limited value to plaque vulnerability occurring in ACS.

However, a thoroughly evaluation of the atheroma in terms of necrotic core depth and thin cap extension should be further addressed (Fayad and Fuster, 2001); (Goldstein, 2000). These features may help improving vulnerability phenotype definition.

6 CONCLUSIONS

Specific plaque phenotypes were associated to ACS and non-ACS.

Vessels with enlarged lumens and with plaques characterized by marked outward growth and high fibrotic and fibro-fatty contents were found in STEMI and SA patients. NSTEMI patients allied to the above plaque structure an important increase of calcified tissue and a plaque bi-directional growth, both outwards and inwards the vessel lumen.

A second plaque phenotype characterized by small constrictive and fibrotic plaques, with low fibro-fatty content was associated with SA patients

Therefore, IVUS-derived plaque measures provided phenotypes of plaque vulnerability and rupture that may help improving risk stratification of symptomatic patients.

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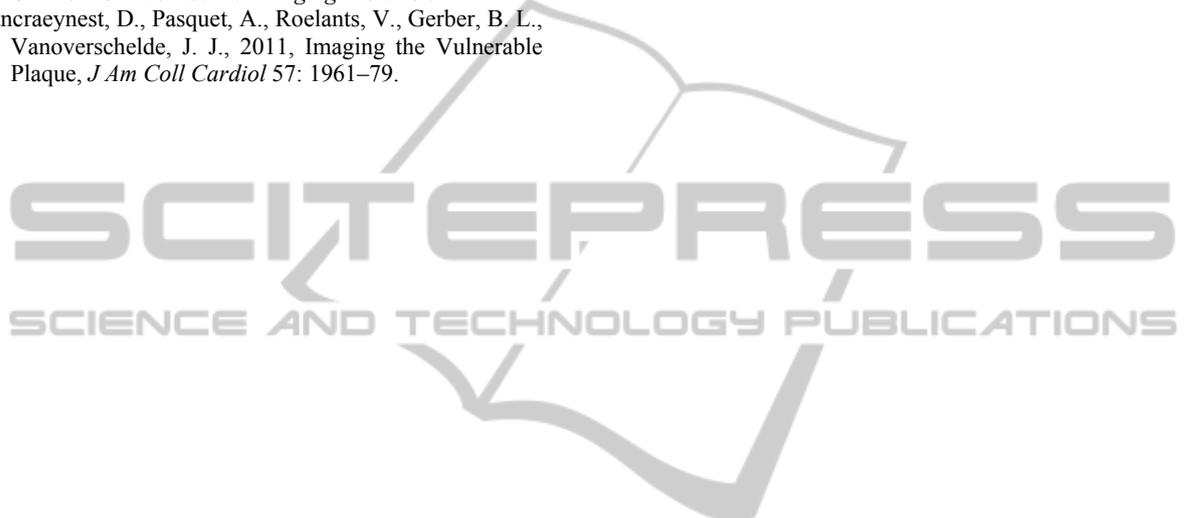
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