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Non-invasive endothelial function assessment using digital reactive hyperaemia correlates with three-dimensional intravascular ultrasound and virtual histology-derived plaque volume and plaque phenotype

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Abstract

Background and aim—To study relationships between endothelial dysfunction (ED) and coronary atherosclerosis derived from intravascular ultrasound (IVUS) and virtual histology (VH).

Methods—Endothelial dysfunction was examined by EndoPAT system (Itamar Medical) in 56 patients who underwent IVUS and VH (Volcano corp.). Reactive hyperaemia index (RHI) < 2 was used for definition of ED. IVUS sequences were divided into 5 mm-long non-overlapping and adjacent vessel segments. Plaque phenotype was determined for each frame and 5 mm vessel segment was labeled according to highest frame score (from 0 for “no lesion” to 5 for “thin cap fibroatheroma; TCFA”).

Results—IVUS-VH data were collected from 41 patients suitable for three-dimensional analysis. Patients with ED exhibited larger plaque burden than those without ED (0.46 ± 0.08 vs. 0.39 ± 0.07 , $p = 0.014$), smaller lumen area (8.59 ± 2.19 vs. 11.90 ± 3.50 , $p = 0.016$), higher plaque risk score (2.82 ± 1.18 vs. 1.84 ± 0.90 , $p = 0.012$), and higher number of TCFA frames (0.36 ± 0.22 vs. 0.22 ± 0.16 , $p = 0.038$). Relative amounts of fibrous tissue correlated positively with RHI ($p = 0.034$, $r = 0.33$). The numbers of fibroatheromas and calcified plaques correlated with RHI inversely ($r = 0.34$, $p = 0.031$ and $r = 0.32$, $p = 0.044$, respectively).

Conclusions—Endothelial dysfunction correlates with severity and phenotype of coronary lesions and can contribute to non-invasive detection of individuals with higher risk of cardiovascular events.

Keywords

intravascular ultrasound; endothelial dysfunction; thin cap fibroatheroma; prediction

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INTRODUCTION

Endothelial dysfunction (ED) plays an important role in the development of coronary atherosclerosis. It is defined as disability of endothelial cells to produce stimuli for vasodilatation (namely nitric oxide [NO]). The NO molecule has a more diverse function than only vasodilatation. NO serves as an inhibitor of leukocyte adhesion, thrombocyte adhesion, and proliferation of vascular smooth muscle cells [1]. Together, these functions can explain the substantial role of ED in the development of atherosclerosis.

Endothelial dysfunction can be easily tested non-invasively using different methods. We have chosen EndoPAT® (Itamar Medical Ltd., Caesarea, Israel) for our study. EndoPAT measures digital reactive hyperaemia [2]. EndoPAT uses peripheral artery tone (PAT) signal for non-invasively measuring arterial tone changes in peripheral arterial beds. The PAT signal is measured from the fingertip by recording finger arterial pulsatile volume changes.

We aimed to correlate ED with detailed examination of coronary atherosclerosis using geometrically correct three-dimensional (3D) coronary artery reconstruction done by fusion of coronary angiography and intravascular ultrasound (IVUS) with virtual histology (VH).

METHODS

Study population

Patient data were taken from a database of the clinical trial entitled “The prediction of extent and risk profile of coronary atherosclerosis and their changes during lipid-lowering therapy based on non-invasive techniques”, Clinical Trial Gov. NCT01773512. This study is still ongoing and its main purpose is to test the possibilities of non-invasive examinations in the prediction of coronary atherosclerosis extent and risk profile. All patients underwent IVUS and VH examination and testing of endothelial function by EndoPAT. The study included patients with stable angina pectoris, who had undergone coronary angiography for a typical chest pain. Exclusion criteria were indication for coronary artery bypass grafting (CABG), liver or renal dysfunction, and statin intolerance. Criteria for target vessels were:

- native artery with diameter stenosis between 20% and 50% by angiography with no indication for either percutaneous coronary intervention or CABG;
- plaque length > 20 mm by IVUS; plaque length was defined as an artery segment with plaque burden (PB) > 20% by IVUS.

In case of similar findings in more than one coronary artery during angiography, the artery with the longer plaque was selected for IVUS analysis to examine the most significant non-culprit plaque. Only one segment from each target artery was chosen for the study from each patient.

Measurement of endothelial function by EndoPAT

Changes in PAT signal are measured after ischaemia caused by 5 min of compression of the brachial artery by a cuff inflated to 60 mm Hg above the systolic pressure or 200 mm Hg for 5 min and then deflated to induce reactive hyperaemia. Result is expressed as the ratio of the

capillary perfusion in a finger of the compressed arm to the capillary perfusion in a finger of the non-compressed arm. This ratio is known as the reactive hyperaemia index (RHI) and its value of less than 1.67 is usually used as a criterion suggesting ED. However, this number was reached after correction for baseline vascular tone. Originally, an RHI value of 2.0 served as a threshold (Itamar medical web pages) [3]. We decided to use the original value because it corresponds with known findings from coronary physiology of coronary flow reserve (ability of coronary circulation to increase flow after stimulation), where a threshold value of 2.0 is also used [4]. RHI is known to correlate well with coronary flow reserve [5]. All subjects rested in bed in a quiet room for 15 min before the examination and they were asked to refrain from smoking, alcohol, and caffeine intake 12 h before procedure.

Catheterisation and IVUS imaging

Intravascular ultrasound was performed in the standard fashion using the IVUS phased-array probe (Eagle Eye 20 MHz 2.9 F monorail, Volcano Corporation, USA), IVUS console, gold standard software, with automatic pullback at 0.5 mm/s (research pullback, model R-100, Volcano Corporation, USA). After administration of 200 µg of intracoronary nitroglycerin, the IVUS catheter was inserted into the target vessel beyond the segment of interest and then pulled back to the aorto-ostial junction.

B-mode IVUS pullback image data were acquired at the Charles University in Prague, archived on DVDs, and transferred to the Iowa Institute for Biomedical Imaging for quantitative analysis. For each frame of all IVUS pullbacks, luminal and external elastic membrane surfaces were automatically segmented using a fully 3D LOGISMOS graph-based approach [6]. Automatically determined surfaces were reviewed and algorithmically refined by an expert cardiologist (TK) using an operator-guided computer-aided interface [7]. External elastic membrane (EEM) and lumen surfaces/contours served as the input for off-line VH computation using Volcano's research software, which is identical to that available on the Volcano IVUS console but allows VH computations based on user-supplied segmentation of lumen and external elastic membrane (Volcano Corporation, USA). Employing our previously reported approach [8], geometrically correct fully 3D representation of the vascular wall surfaces and VH-defined tissue characterisation was obtained via fusion of two-plane angiography and IVUS. This geometrically correct 3D model served as a basis for quantitative morphologic analyses and quantitative assessment of plaque composition in every pullback-sequence frame of the imaged vessel. Frame-based indices of plaque morphology and VH were computed and averaged for the whole pullback length in 5 mm.

Vessel and plaque measurement morphologic indices included: EEM cross-sectional area (CSA), lumen CSA, and PB ($\text{EEM CSA} - \text{lumen CSA} / \text{EEM CSA}$) [9].

Virtual histology data were used for plaque phenotype definitions. VH-IVUS classifies plaque into four components: fibrous — F, fibro-fatty — FF, dense calcification — DC, and consisting of necrotic core — NC. Using quantitative assessment of VH tissue types, phenotypes of all 5-mm vessel segments were classified into six categories according to the American Heart Association's Committee on Vascular Lesions [10]. These phenotypes are as follows:

- NL — no lesion (PB less than 40%);
- PIT — pathologic intimal thickening;
- FP — fibrous plaque;
- FcP — fibro-calcified plaque;
- ThCFA — thick cap fibroatheroma;
- TCFA — thin cap fibroatheroma.

For determination of the TCFA category, three adjacent R-wave-gated frames were considered. As can be seen from the flowchart (Fig. 1) the phenotypic classification is mainly based on the PB and the four VH-determined plaque components [11].

We have developed a plaque stage scoring system or plaque risk score (PRS) based on combined weighting of plaque phenotype presence in individual IVUS frame-based locations by assigning the following weights: TCFA ... 5 points, ThCFA ... 4 points, FcP ... 3 points, FP ... 2 points, PIT ... 1 point, NL ... 0 points. A plaque phenotype/the risk score frames was defined located in 5-mm vessel segment.

We also calculated the Liverpool plaque score (LPS), to allow comparisons with research of others, using the following formula: $2.149 + 0.68 \times \text{NC/DC} + 3.39 \times \text{MLA} + (5.1 \text{ if remodelling index was } > 1.05) + 3.7 \times \text{TCFA}$ adapted from Murray et al. [12], where MLA denotes minimal lumen diameter.

Analysis of changes in 5-mm vessel segments will be used for comparison between baseline and follow-up examination. For the purpose of comparison between baseline coronary atherosclerosis and baseline ED testing, we used averaged values of plaque morphological indices and PRS for whole pullback per patient.

Statistical analysis

Mean values $\pm$ standard deviations (or percentages) were calculated for all numerical variables. Differences of two numerical datasets were examined by Spearman and Pearson tests. For categorical versus numerical variables (like RHI > 2), statistical significance was calculated using t-test. R environment was employed for statistical computing. A p value of 0.05 denoted the threshold of statistical significance. Continuous parameters were normally distributed.

RESULTS

Study patients

From 56 patients enrolled in the trial, we used data from 41 patients with high quality IVUS/VH pullback suitable for 3D analysis (without noticeable pullback speed discontinuity, vessels free of severe calcification to avoid inconsistency of IVUS-VH plaque type determination in areas of acoustic shadowing), which have high-quality measurement of ED. Coronary arteries were as follows: left anterior descending artery 14 (34.2%), left circumflex artery 6 (14.6%), and right coronary artery 21 (51.2%).

We divided patients into two groups: with $RHI \geq 2$ and $RHI < 2$. We found two significant differences among demographic parameters between these two groups: body mass index (BMI) and use of calcium channel blockers. Higher occurrence (but statistically non-significant) of arterial hypertension, diabetes mellitus, and history of smoking were found in group with $RHI < 2$. These findings reflect impaired endothelial function in those groups of patients (Table 1).

Correlation of $RHI < 2$ with coronary atherosclerosis

We chose a cut-off value of 2.0 as is explained in the “Methods” and discussed in the “Discussion” section. Differences in coronary atherosclerosis indices between these groups are summarised in Table 2. The occurrence of different plaque phenotype according to RHI is shown in Figure 2.

Correlation of RHI as a continuous variable with coronary atherosclerosis

We searched also for correlation between RHI as a continuous variable and plaque features. Significant findings are summarised in Table 3. RHI positively correlated with the lumen area, adventitia area, numbers of frames with no lesion (meaning with $PB < 40\%$), and with relative amounts of fibrous tissue. It inversely correlated with numbers of frames labeled as fibrotic plaques, fibroatheromas with thick fibrous cap, and with the following combination of high-risk features (TCFA, $MLA < 4 \text{ mm}^2$, $PB > 70\%$).

DISCUSSION

The most important finding of this study is a significant-relation between non-invasively measured ED, which can be easily obtained in almost every general practitioner’s office, and the sophisticated evaluation of plaque volume and plaque phenotype in coronary arteries. This finding can improve the assessment of the risk for development of cardiac event in the future. Previously published trials demonstrated that some IVUS-derived indices like PB, MLA, or plaque phenotype are predictors of major cardiac events (MACE) [13, 14].

We also know that many of the MACE events occurred in patients with intermediate risk score based on traditional risk score [15] such as the Framingham risk score [16] or Score [17]. Therefore, our findings that ED correlates with PB, lumen area, and plaque phenotype can further improve these traditional scoring systems.

Sawada et al. [18] published results of a trial comparing ED and coronary atherosclerosis assessed by IVUS and VH. Unlike our study, they measured the ED by flow-mediated dilatation in a brachial artery. They examined 111 patients with stable angina pectoris (57.7%) and acute coronary syndromes (42.3%). Both types of plaques (culprit and non-culprit) were considered. Patients with ED had plaques with lower percentages of fibrous and fibro-fatty tissues, contained larger percentages of necrotic core and calcifications, and exhibited higher numbers of VH-derived TCFA. They did not find differences in plaque volume and plaque length between patient groups with and without ED.

Schoenenberger et al. [19] published a study that included 362 patients who underwent examination of endothelial function by digital reactive hyperaemia (EndoPAT) and IVUS

with VH. They divided patients into two groups based on RHI with a cut-off value of 1.67. They described a clear relationship between RHI below cut-off point and higher plaque volume, PB, higher necrotic core and calcification, lower amount of fibrous and fibro-fatty tissue. They did not see any relationships between RHI and lumen volume. Unlike our study, Schoenenberger et al. [19] included patients with acute coronary syndrome (12.4% of enrolled patients).

Our study is based on a substantially smaller number of patients compared to the two above-referenced trials. However, we used 3D geometrically correct quantitative analysis of IVUS and VH, assessed plaque phenotype, and calculated plaque score indices. By using such a sophisticated approach, we confirmed the previous results of correlations between low RHI and higher PB, and lower amounts of fibrous tissue inside plaques. We were unable to confirm the reported relationships between RHI and presence of necrotic core and calcification. These correlations did not reach statistical significance in our study. However, newly studied indices of plaque phenotype showed a more frequent occurrence of early lesion in patients with $RHI \geq 2$ and a more frequent occurrence of advanced lesions in patients with $RHI < 2$. These findings were further supported by the presence of higher PRS and higher LPS in patients with $RHI < 2$. Combinations of risk features, which predict MACE better than TCFA alone in the PROSPECT trial [20] (combined TCFA with $MLA < 4 \text{ mm}^2$ and with $PB > 70\%$), reached the highest levels of significance in patients with $RHI < 2$.

Interestingly, we found a positive correlation between RHI and lumen area (by using frame-based and also 5 mm segment-based approaches) and also with the adventitia area (frame-based only). Schoenenberger et al. [19] did not find correlation between the lumen volume and RHI (they did not compare RHI and vessel area). Sawada et al. [18] described the presence of more pronounced positive vessel remodelling in patients with ED. We can only speculate what the reasons are for these different findings. The correlation between $RHI \geq 2$ and higher lumen area can be explained simply by lower PB in those patients. However, such a correlation was not found in a larger patient cohort undergoing the same test (Schoenenberger et al. [19]). Furthermore, lumen volumes in the two patient groups were almost identical in that study. Despite the fact that our approach of 3D analysis yields more accurate volumetric measurements due to full consideration of vessel geometry, our findings may be affected by a relatively low number of patients in the study rather than by real existence of this phenomenon. However, future studies using digital reactive hyperaemia may focus on proving or disproving these findings.

The two aforementioned studies did not measure the vessel area surrounded by adventitia. Therefore our finding of significant correlation between $RHI \geq 2$ and the vessel area cannot be compared. Sawada et al. [18] found higher remodelling index values in patients with ED determined by brachial flow mediated dilatation. Considering that the remodelling index is an expression of comparison between the vessel location containing plaque and a plaque-free reference vessel location, no quantitative information about the absolute vessel size was available. Moreover, comparison of our findings with those of the Sawada et al. study [18] may be problematic because the correlation between brachial flow mediated dilatation and EndoPAT examinations is quite low ($r = 0.35$) [21]. We can only hypothesise that

nitroglycerin given to all patients before IVUS examination led to a more substantial vessel enlargement in patients with healthier endothelium — judged by RHI ≥ 2 .

Our findings together with results from Schoenenberger et al. [19] and Sawada et al. [18] suggest promising prospects of using ED testing for assessing advanced coronary atherosclerosis. Our work helps explain previously published findings of correlations between EndoPAT results and prediction of cardiovascular events, including death [22].

Another difference between our study and published trials comparing ED using EndoPAT and coronary atherosclerosis is the use of a different cut-off value (RHI = 2.0 instead of RHI = 1.67). The reason why we decided to use RHI = 2.0 was explained in the “Methods” section and was further justified by achieving better discrimination between low and high risk plaque features in our study when the RHI = 2.0 threshold was used. Many of our findings lost statistical power when the RHI = 1.67 cut-off value was employed. Based on the findings of our study, and the analogy with coronary circulation where doubled increase of flow is a proven cut-off point for detection of ischaemia and original finding of peripheral artery tone signal, we propose RHI < 2 as a probably better discriminant for correlation between RHI and coronary atherosclerosis.

Limitations of the study

The main limitation of our study was the low number of enrolled patients, which was the result of our strict inclusion criteria requiring high image quality of both IVUS and VH analysis and ED testing. Another limitation is examination of only one plaque per patient. On the other hand, the advanced and geometrically correct 3D reconstruction of coronary arteries and choosing the most significant can compensate for a lower number of enrolled subjects and only one plaque per patient. After dividing the patients into two groups according to RHI, we found significantly higher values of BMI and a higher number of patients taking calcium channel blockers in the group with RHI < 2 . Obesity is a known factor contributing to ED due to the increased chronic inflammatory status [23]. For this reason, our finding is not surprising. Furthermore, we would expect that drugs causing vasodilatation would be more frequently found in the group of patients with RHI ≥ 2 . This opposite situation in our cohort is probably caused by a relatively low number of patients enrolled in the study, who exhibited significant ED and for whom this vasodilation medication was unable to sufficiently improve the endothelial function assessed by RHI.

CONCLUSIONS

Digital reactive hyperaemia is a promising tool for the prediction of coronary plaques with higher risk phenotype. This finding can improve risk stratification of subjects with moderate risk of coronary atherosclerosis. However, this data must be confirmed in a larger trial. EndoPAT now has data not only for detection of coronary atherosclerosis, but also for prediction of residual risk of patients on statin therapy [24] and for prediction of future cardiac events [25]. Therefore, it should be used more frequently in patients with moderate risk of coronary atherosclerosis.

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Figure 1.

Determination of plaque phenotypes; PIT — pathologic intimal thickening; FcP — fibro-calcified plaque; FP — fibrous plaque; ThCFA — thick cap fibrous atheroma; TCFA — thin cap fibroatheroma

Figure 2.

The presence of endothelial dysfunction and percentage of different plaque phenotype in examined vessel segment; RHI — reactive hyperaemia index; PIT — pathologic intimal thickening; FcP — fibro-calcified plaque; FP — fibrous plaque; ThCFA — thick-cap fibrous atheroma; TCFA — thin-cap fibroatheroma. The comparisons without “p” value did not reach statistical significance

Table 1

Demography and medication

	RHI 2 (n = 11)	RHI < 2 (n = 30)	P
Age	63.0 ± 10.1	61.9 ± 12.7	0.78
Previous MI	7 (63.6%)	21 (70%)	1
Hyperlipidaemia	9 (81.8%)	27 (90%)	1
Hypertension	8 (72.7%)	28 (93.3%)	0.16
Ex-smoking	4 (36.4%)	19 (63.3%)	0.26
Active smoking	4 (36.4%)	6 (20%)	0.24
Diabetes mellitus *	1 (9.1%)	10 (30%)	0.23
Body mass index	25.3 ± 3.4	29.6 ± 3.0	0.003
Family history	5 (45.5%)	15 (50%)	1
Beta-blockers	7 (63.6%)	24 (80%)	0.4
Nitrates	0 (0%)	4 (13.3%)	0.57
Acetylsalicylic acid	10 (90.9%)	26 (86.7%)	0.57
ACE inhibitors	9 (81.8%)	27 (90%)	1
Statins	8 (72.7%)	26 (86.7%)	0.59
Calcium channel blockers	0 (0%)	12 (40%)	0.02

* All such patients had type II; RHI — reactive hyperaemia index; MI — myocardial infarction; ACE — angiotensin converting enzyme

Table 2

The presence of endothelial dysfunction and plaque morphological indices

Variables	RHI ≥ 2	RHI < 2	P
PB [%]	0.39 ± 0.07	0.46 ± 0.08	0.014
Lumen area [mm ²]	11.90 ± 3.50	8.59 ± 2.19	0.016
Plaque risk score	1.84 ± 0.90	2.82 ± 1.18	0.012
Liverpool plaque score	−0.31 ± 0.89	0.67 ± 0.94	0.009
TCFA *	0.22 ± 0.16	0.36 ± 0.22	0.038
MLA < 4 mm ² *	2.40 ± 4.74	12.13 ± 16.82	0.006
PB > 70% *	2.20 ± 3.85	6.06 ± 6.65	0.032
TCFA + MLA < 4 mm ²	0.80 ± 1.75	5.13 ± 6.67	0.002
TCFA + MLA < 4 mm ² + PB > 70% *	0.00 ± 0.00	2.35 ± 4.06	0.003
Fibroatheromas (TCFA + ThCFA) *	0.39 ± 0.19	0.60 ± 0.26	0.012

* Marked rows provide relative numbers of frames of specified properties; RHI — reactive hyperaemia index; TCFA — thin-cap fibroatheroma; MLA — minimal lumen area; PB — plaque burden

Table 3

Correlation between endothelial dysfunction and plaque volumetric indices and plaque phenotype

Variables	p	r
Lumen area	0.02	0.36
Adventitia area	0.009	0.4
Non lesion per cent	0.045	0.31
Fibrous plaque per cent	0.034	0.33
FcP plaque per cent	0.044	−0.32
ThCFA per cent	0.009	−0.4
TCFA + MLA < 4 mm ² + PB > 70% count	0.026	−0.35

FcP — fibro-calcified plaque; TCFA — thin cap fibroatheroma; ThCFA — thick-cap fibrous atheroma; MLA — minimal lumen area; PB — plaque burden