Coronary Endothelial Dysfunction Is Associated With Inflammation and Vasa Vasorum Proliferation in Patients With Early Atherosclerosis


Objective—Endothelial dysfunction is an early manifestation of atherosclerosis. Inflammation and vasa vasorum play a pivotal role in the pathophysiology of plaque initiation, development, and complications. Optical coherence tomography allows high-resolution imaging of tissue microstructure. Therefore, the aim of this study was to test the hypothesis that segments with endothelial dysfunction show macrophages and vasa vasorum in patients with early coronary artery disease.

Approach and Results—Optical coherence tomography images were obtained from 40 patients with mild coronary atherosclerosis who underwent coronary endothelial function assessment. Optical coherence tomography findings, including macrophages and microchannels, were evaluated in 76 coronary segments corresponding to those in endothelial response to acetylcholine. Coronary artery diameter change in response to acetylcholine was more severe in segments showing macrophages (−17.7±14.7% versus −6.3±13.9%; P<0.01) and microchannels (−15.9±15.9% versus −6.4±13.5%; P<0.01) than those without. There were increasing trends of the prevalence of macrophages and microchannels with endothelial dysfunction as stratified by quartiles of coronary artery diameter change (P<0.01 and P=0.02 for trend, respectively). In particular, segments with both macrophages and microchannels (n=12) tended to have worse endothelial function than those with macrophages alone (n=15) and microchannels alone (n=15; −22.1±14.6% versus −10.9±15.6% and −10.9±15.6%; P=0.07 and P=0.06, respectively).

Conclusions—Epicardial endothelial dysfunction was associated with optical coherence tomography–identified macrophages and microchannels in mild coronary atherosclerosis. The current study further supports the role of inflammation and vasa vasorum proliferation in the early stage of coronary atherosclerosis. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: coronary disease ■ inflammation ■ optical coherence tomography

Endothelial dysfunction is a key step in early lesion formation and also involved in plaque progression and the occurrence of atherosclerotic complications.1,3

Alteration in epicardial endothelial function has been considered to precede the development of morphological atherosclerotic change and to contribute to lesion development.4,5 Previous in vivo human imaging studies using coronary angiography6,7 and grayscale intravascular ultrasound (IVUS)8 did not detect significant relationship between endothelial function and morphological appearances of atherosclerosis.

However, microstructural manifestations of vasa vasorum neovascularization occur within a few weeks in the initial stage of the coronary atherosclerosis of experimental hypercholesterolemia.9 Moreover, from a pathobiological point of view, inflammatory mechanisms, critical to all stages of cardiovascular disease progression, play a causal role in the pathogenesis of vascular endothelial dysfunction.10 Local and systemic release of inflammatory cytokines promotes lipid-laden foam cell accumulation and impairs endothelium-dependent arterial vasodilation.11

We have previously demonstrated that coronary arterial segments with endothelial dysfunction are associated with specific plaque characteristics consistent with necrotic core12 or lipid core.13 Recently developed optical coherence tomography (OCT) allows in vivo visualization of coronary artery microstructure, including macrophages14,15 and vasa vasorum16,17 in advanced atherosclerotic plaques, and therefore, may have the potential for identification of specific plaque characteristics related to the early stage of atherosclerosis with endothelial dysfunction.
Therefore, the aim of this study was to test the hypothesis that segmental coronary endothelial dysfunction is associated with the presence of macrophages and intimal vasa vasorum in patients with early coronary artery disease.

Material and Methods

Material and methods are available in the online-only Data Supplement.

Results

Patients

After screening of 201 patients, a total of 42 patients were enrolled in the study. Patients with early coronary artery disease, defined as diameter stenosis <30% throughout entire coronary arteries on diagnostic coronary angiography, and vasoconstriction in epicardial coronary artery on endothelial function test were consecutively enrolled. Exclusion criteria were low ejection fraction (<45%), acute coronary syndrome, angioplasty, or bypass surgery within 6 months before the study, uncontrolled hypertension, valvular heart disease, significant endocrine, renal disorder, or pregnancy. From 42 patients, 84 coronary segments were identified. After excluding 8 segments because of poor image quality, 76 segments in 40 patients (2 segments in 36 subjects and 1 segment in 4 subjects) were analyzed. The clinical characteristics of the study subjects are described in Table 1. The comparisons of coronary artery function assessments, IVUS, and OCT findings between 2 groups stratified according to endothelial dysfunction are shown in Table 2. There was no correlation between plaque burden assessed by IVUS and the percent change of coronary artery diameter ($r^2<0.01; P=0.84$). The OCT imaging demonstrated 21 lipid-rich plaques (27%) among all segments. Thirteen of 23 (56%) segments with macrophage images and 7 of 27 (26%) segments with microchannels were identified in lipid-rich plaques. Compared with segments with normal endothelial function, those with endothelial dysfunction showed significantly higher prevalence of macrophage images ($P=0.03$) and higher, but nonsignificant, prevalence of microchannels ($P=0.07$). The maximum angle of macrophage arc was insignificantly greater in segments with endothelial dysfunction compared with those without (Table 2).

Endothelial Dysfunction in Segments With Macrophage Image and Microchannels

When segments were divided into 2 groups according to the presence or absence of macrophage images or microchannels, more severe endothelial dysfunction was observed in segments with macrophage images ($−17.7±14.7\%$ versus $−6.3±13.9\%$; $P<0.01$) and in those with microchannels.

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### Table 1. Demographics of Study Subjects (76 Segments From 40 Patients)

<table>
<thead>
<tr>
<th></th>
<th>Endothelial Dysfunction (n=38)</th>
<th>Normal Endothelial Function (n=38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57.0±11.1</td>
<td>52.0±11.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>10 (26.3)</td>
<td>10 (26.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.7±0.6</td>
<td>32.0±7.6</td>
<td>0.70</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0 (0)</td>
<td>4 (10.5)</td>
<td>...</td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 (47.4)</td>
<td>17 (44.7)</td>
<td>0.57</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>20 (52.6)</td>
<td>14 (36.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>Current smoking</td>
<td>5 (13.5)</td>
<td>3 (8.1)</td>
<td>0.45</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>18 (47.4)</td>
<td>22 (57.9)</td>
<td>0.56</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>7 (18.4)</td>
<td>12 (31.6)</td>
<td>0.25</td>
</tr>
<tr>
<td>ACE inhibitors/ARBs</td>
<td>5 (13.5)</td>
<td>7 (18.9)</td>
<td>0.68</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>17 (44.7)</td>
<td>18 (47.4)</td>
<td>0.86</td>
</tr>
<tr>
<td>Statins</td>
<td>20 (62.6)</td>
<td>13 (34.2)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

| Laboratory data          |                               |                                   |    |
| Total cholesterol, mg/dL | 185±33                        | 189±45                            | 0.72|
| LDL cholesterol, mg/dL   | 95±30                         | 100±40                            | 0.66|
| HDL cholesterol, mg/dL   | 66±20                         | 64±18                             | 0.88|
| Triglyceride, mg/dL      | 123±65                        | 125±57                            | 0.97|
| Glucose, mg/dL           | 98±13                         | 99±20                             | 0.96|
| Hemoglobin A1c, %        | 5.3±0.4                       | 5.4±0.4                           | 0.23|
| C-Reactive protein, mg/L | 3.3±2.7                       | 5.1±5.3                           | 0.29|

HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

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### Table 2. Segmental Comparison of Intravascular Ultrasound and Optical Coherence Tomography Results According to Endothelial Function

<table>
<thead>
<tr>
<th></th>
<th>Endothelial Dysfunction (n=38)</th>
<th>Normal Endothelial Function (n=38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary artery function assessments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% CAD change, response to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>$−18.9, (−29.2, −12.6)$</td>
<td>$0.2, (−5.4, 8.9)$</td>
<td>...</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>9.1 (2.1, 15.0)</td>
<td>14.0 (5.1, 21.5)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

| IVUS findings             |                               |                                   |    |
| Vessel area, mm²          | 14.2±4.6                      | 14.4±4.0                          | 0.85|
| Lumen area, mm²           | 10.7±3.3                      | 11.7±3.1                          | 0.20|
| Plaque area, mm²          | 4.5±1.7                       | 4.6±1.5                           | 0.78|
| Plaque burden, %          | 30.0±7.5                      | 30.0±7.3                          | 0.84|

| OCT findings              |                               |                                   |    |
| Lipid-rich plaque, n (%)  | 11 (29)                       | 10 (26)                           | 0.95|
| Macrophage image, n (%)   | 16 (42)                       | 7 (18)                            | 0.03|
| Maximum angle*, degree    | 37.9 (24.9, 49.2)             | 25.2 (14.1, 31.0)                 | 0.05|
| Length*, μm               | 700 (600, 940)                | 600 (400, 800)                    | 0.24|
| Microchannels, n (%)      | 18 (47)                       | 9 (24)                            | 0.07|
| Maximum number†, n        | 1.5 (1, 2)                    | 1 (1, 2)                          | 0.16|
| Length†, μm               | 800 (600, 850)                | 800 (600, 1300)                   | 0.38|

Measured in lesions with *macrophage image or †microchannels.
The prevalence of segments with macrophage image or microchannels tended to have worse endothelial function than those with microchannels alone (−22.1 ± 14.6% versus −10.9 ± 15.6%; P = 0.07 and P = 0.06, respectively).

Because of a substantial overlap between segments with macrophage images and those with microchannels, all segments were divided into 4 groups (Figure 4): those with both macrophage image and microchannels (n = 12), those with macrophage image alone (n = 11), those with microchannels alone (n = 15), and those without macrophage image or microchannels (n = 38). Segments with both characteristics tended to have worse endothelial function than those with macrophages alone and microchannels alone (−22.1 ± 14.6% versus −10.9 ± 15.6%; P = 0.07 and P = 0.06, respectively).

**Discussion**

The present study demonstrates the association of coronary endothelial dysfunction with OCT-identified macrophages and microchannels in patients with early coronary artery disease. The segments with macrophage image or microchannels showed significantly more severe epicardial endothelial dysfunction. In addition, segments with endothelial dysfunction showed significantly higher prevalence of macrophage image and microchannels, although they had similar extent of atherosclerosis as assessed by IVUS. Thus, the present study supports the role of inflammation and the vasa vasorum in early atherosclerosis in humans.

In the initial stage of atherosclerosis, the dysfunctional endothelium produces proinflammatory cytokines and reduces nitric oxide activity with anti-inflammatory properties, subsequently facilitating recruitment and transmigration of circulating monocytes into the intima followed by their differentiation into macrophages.18 Taking up oxidized lipoproteins, macrophages form the characteristics of foam cells, a hallmark of early atherosclerosis, which can be detected by OCT.19 These activated macrophages in turn secrete proinflammatory cytokines aggravating endothelial dysfunction.20,21 The present study extends our previous observations of the correlation between lipoprotein-associated phospholipase A2 levels in the coronary circulation and coronary endothelial dysfunction.22,23 In the study, the levels of lipoprotein-associated phospholipase A2, a protein produced by inflammatory cells, including macrophages, was shown to be correlated with plaque volume as assessed by IVUS.24 Given the potential of OCT to specifically identify macrophages in plaques, the present study provides the possibility to directly visualize functional activity of local inflammation of coronary plaques.

Pathology studies on early atherosclerosis demonstrated that even pathological intimal thickening is always vascularized.25,26 Furthermore, the intimal vasa vasorum originating from adventitial vasa vasora precede the development of epicardial endothelial dysfunction and the appearance of any atherosclerotic features in animal models.27,28 Hence, intimal vasa vasora have the potential to contribute to the development of endothelial dysfunction in both the conduit vessels and vasa vasorum, which results in reduction in oxygen supply to the coronary artery wall.27,28

There was a substantial overlap between segments with macrophage image and those with microchannels. More severe endothelial dysfunction in segments with both characteristics suggests an incremental effect of inflammation and intimal neovascularization in atherogenesis.29 Intimal neovascularization increases blood flow into the vascular wall and facilitates inflammatory cells to penetrate into plaque.30 Activated macrophages promote angiogenesis and facilitate further macrophage recruitment.31 More investigations are required to better elucidate the link between inflammation and intimal neovascularization and their association with endothelial dysfunction.

Recently, we have demonstrated that coronary endothelial dysfunction is associated with necrotic core as assessed by virtual histology IVUS32 or lipid core plaque by near-infrared spectroscopy.33 However, no association was found between endothelial function and OCT-identified lipid-rich plaque in the present study. These discrepancies may result from differences in stages of atherosclerosis, imaging modality applied to each study, and the definition of atheromatous plaques. Further comparative studies using different imaging techniques are needed to better understand the role of inflammation and the vasa vasorum in coronary atherosclerosis.
modalities are needed to elucidate more precise mechanisms of endothelial dysfunction.

Clinical Implications
A recent study has shown that interventions to improve endothelial function contribute to attenuate plaque progression.\footnote{Yoon MH, Reriani M, Mario G, Rihal C, Gulati R, Lennon R, Tilford JM, Lerman LO, Lerman A. Long-term endothelin receptor antagonism attenuates coronary plaque progression in patients with early atherosclerosis. \textit{Int J Cardiol.} 2013;168:1316–1321.} The present study suggests that OCT-identified microstructures, such as macrophages and vasa vasorum, could be a surrogate marker for the evaluation of new therapeutic strategies. Further studies are needed to demonstrate that inflammation and vasa vasorum imaging by OCT can be used to monitor the effectiveness of the therapy.

Study Limitations
We enrolled a relatively small number of patients and analyzed only the left anterior descending artery. Therefore, caution should be taken in interpreting and generalizing the results to all patient populations. In addition, the definition of macrophage image using visual assessment may introduce bias. However, the validation study demonstrated that macrophage accumulations had distinctive OCT findings and that OCT could evaluate accurately cap macrophages.\footnote{Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. \textit{Nature.} 1993;362:801–809.} This visual assessment of macrophage has been used in the previous studies\footnote{Quyyumi AA, Cannon RO III, Panza JA, Diodati JG, Epstein SE. Endothelial dysfunction in patients with chest pain and normal coronary arteries. \textit{Circulation.} 1992;86:1864–1871.} and also recommended by international working group.\footnote{Zeiher AM, Raurich AJ, Anderson T, Charbonneau F, Delagrange D, Creager MA, Selwyn AP, Ganz P. Systemic nature of endothelial dysfunction in atherosclerosis. \textit{Am J Cardiol.} 1995;75:71B–74B.} Finally, OCT has a limitation to evaluate a microstructure, such as vasa vasorum, located in deep site within plaque because of its shallow penetrating depth. Signal can be attenuated by macrophages, lipid pool, or necrotic core. In the present study, OCT images were acquired from early atherosclerosis, in which plaque burden at MLA site was comparable in both segments, and 2 independent examiners have evaluated OCT images to minimize bias.

Conclusions
The present study demonstrates that coronary segments with endothelial dysfunction are associated with relative abundance of macrophages and microchannels, which might have an important role in plaque initiation and progression. These observations can refine the current understanding of the link between epicardial coronary endothelial dysfunction and early structural changes in the vascular wall.

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Disclosures
None.

References
1. Anderson TJ, Gerhard MD, Meredith IT, Charbonneau F, Delagrange D, Creager MA, Selwyn AP, Ganz P. Systemic nature of endothelial dysfunction in atherosclerosis. \textit{Am J Cardiol.} 1995;75:71B–74B.


Significance

We tested the hypothesis that segments with endothelial dysfunction, an early manifestation of atherosclerosis, show macrophages and vasa vasorum detected by optical coherence tomography, high-resolution and novel imaging modality. A total of 76 segments were studied in 40 patients with endothelial dysfunction. Macrophages and microchannels identified by optical coherence tomography were evaluated according to epicardial endothelial function. Coronary artery diameter change in response to acetylcholine was more severe in segments showing macrophages and microchannels than those without. There were increasing trends of the prevalence of macrophages and microchannels with coronary endothelial dysfunction as stratified by quartiles of coronary artery diameter change. In particular, segments with both macrophages and microchannels tended to have worse endothelial function than those with macrophages alone and microchannels alone. Coronary endothelial dysfunction was associated with optical coherence tomography–identified macrophages and microchannels, suggesting that macrophages and vasa vasorum proliferation might play an important role in patients with early coronary atherosclerosis.
Coronary Endothelial Dysfunction Is Associated With Inflammation and Vasa Vasorum Proliferation in Patients With Early Atherosclerosis

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SUPPLEMENTAL MATERIAL

Material and Methods

Study population

Between February 2011 and November 2012, patients who were referred to coronary catheterization laboratory for evaluation of coronary artery disease and were found to have non-obstructive disease, and who had a coronary endothelial function study were screened. Among them, patients with early coronary artery disease, defined as diameter stenosis <30% throughout entire coronary arteries on diagnostic coronary angiography, and vasoconstriction in epicardial coronary artery on endothelial function test were consecutively enrolled. Patients with ejection fraction less than 45%, acute coronary syndrome, angioplasty or bypass surgery within 6 months prior to study, uncontrolled hypertension, valvular heart disease, significant endocrine, renal disorder, or pregnancy were excluded from enrollment. Long-acting nitrates or calcium channel blockers were withheld for 36-48 hours before the study to allow assessment of baseline coronary physiology. The study protocol was approved by the Mayo Clinic Institutional Review Board and informed consent was obtained from all of the patients.

Endothelial function assessment

Coronary angiography was performed according to standard techniques using femoral approach. Coronary endothelial function assessment was performed as previously described.\textsuperscript{1,4-6} Endothelium-dependent coronary vascular function was assessed by selective infusion of increasing concentrations of intracoronary acetylcholine for 3 min at each concentration (10\textsuperscript{-6}, 10\textsuperscript{-5} and 10\textsuperscript{-4} mol/L) into the left anterior descending artery (LAD). Hemodynamic data (heart
rate and mean blood pressure) and coronary angiography were obtained after each infusion. The infusion was terminated when the highest molar concentration of acetylcholine (10^{-4} \text{ mol/L}) was reached. Nitroglycerin (100 \text{ μg}) was then injected as an intracoronary bolus. The coronary artery diameter (CAD) at the corresponding segment of OCT in the proximal and middle LAD was quantitatively measured using QCA-CMS 6.0 (Medis, Leiden, the Netherlands) by a technician who was blinded to the clinical data and OCT analysis. The maximal effect of acetylcholine was expressed as percent change in CAD, which represents the degree of epicardial endothelial function. Endothelial dysfunction was defined as a decrease in percent change of epicardial CAD below the median value in response to the maximum dose of acetylcholine.\textsuperscript{7} Endothelium-independent epicardial coronary artery function was determined by the change in CAD in response to intracoronary nitroglycerin bolus.

**IVUS image acquisition and analysis**

The IVUS examination was performed as previously described.\textsuperscript{8} In brief, after intracoronary administration of 100-200 mg nitroglycerin, a 20-MHz, 2.9F phased-array IVUS catheter (Eagle Eye Gold, Volcano Corporation, Rancho Cordova, CA) was advanced into the LAD and automatic pullback at 0.5 mm/s was performed. The IVUS image was recorded on a DVD-Rom for later offline analysis. Offline volumetric reconstructions and analyses were performed by two experienced investigators in a blinded manner using Volcano Image Analysis Software V3.1 (Volcano Corporation). At the site of minimum lumen area in each coronary segment described below, quantitative analysis was performed and vessel and lumen area, plaque area (vessel area-lumen area) and plaque burden (plaque area/vessel area×100) were determined.\textsuperscript{9}
OCT image acquisition and analysis

For acquisition of OCT images, C7-XR OCT Intravascular Imaging System (St Jude Medical, St Paul, MN) was used. The intracoronary OCT technique has been described previously.\textsuperscript{10} Imaging catheter (Dragonfly, St Jude Medical, St. Paul, MN) was advanced into the mid distal segment of the LAD, and automatic pull-back at a speed of 20 mm/sec (100 frames/sec) was initiated in concordance with blood clearance by infusion of contrast media. All OCT images were digitally stored and analyzed offline using proprietary software (St Jude Medical). In order to co-register identical segments on OCT and coronary angiography, anatomical landmarks such as side branches were used. For segmental analysis, the length of each segment was defined as 20 mm and separated from the adjacent segment by at least 10 mm for matching to endothelial function data of the corresponding segment. Image review and analysis were performed by two independent examiners who were blind to clinical characteristics and the results of endothelial function assessment. Any discrepancies between two observers were resolved by consensus. Each segment was evaluated in terms of plaque type and additional 2 OCT-based characteristics including macrophage image and microchannels. Plaques were classified into 2 categories according to plaque type: lipid or fibrous.\textsuperscript{11,12} A lipid plaque has low signal region with diffuse border. A plaque with lipid occupying two or more quadrants of any cross-sectional area within the plaque was considered as a lipid-rich plaque. A fibrous plaque was defined as a lesion with homogeneous high backscattering region. Macrophage image was defined as signal-rich distinct or confluent punctate regions that exceed the intensity of background speckle noise, which are accompanied by high behind signal attenuation.\textsuperscript{12-14} In plaques with macrophage image, angles of macrophage arc were measured using a protractor centered on the lumen at every frame. Maximum angle and longitudinal length were recorded (Supplemental figure A).\textsuperscript{15}
Microchannels were defined as intraplaque signal-voiding tubular structures with a diameter of 50-300 μm which were sharply delineated and identified on more than three consecutive cross-sectional OCT images. Maximum number and longitudinal length of microchannels were measured (Supplemental figure B).\textsuperscript{16,17} Longitudinal length was measured on longitudinal view. Interobserver agreement for the presence or absence of macrophage image and microchannels were 0.84 (95%CI 0.69 to 0.99) and 0.82 (95% CI 0.66 to 0.97), respectively.

**Statistics**

Continuous variables are summarized as mean ± standard deviation or median [25th to 75th percentiles] as appropriate. Discrete variables are presented as frequency (percentage). Generalized estimating equations (GEE) was used to test for differences in baseline characteristics and IVUS and OCT findings between segments with and without endothelial dysfunction. GEE performed with gamma-log, binomial-logit, and an exchangeable structure in the correlation matrix for all baseline characteristics and with an independent structure for IVUS and OCT findings. GEE was necessary because of the clustered nature of ≥ 1 individual coronary segments measured from LAD, resulting in unknown correlations among measurements within these segment clusters. Similarly, generalized linear models with GEE adjustment was used to model % change in coronary artery diameter with the presence of individual OCT parameters, plus a 4-level group defined by the presence of macrophages, microchannels, or both. CAD response was grouped into quartiles and scored linearly. We tested for a trend in the prevalence of OCT characteristics across quartiles by using GEE in a generalized linear model with a logit link. All statistical tests were 2-sided and a p value < 0.05 was considered to be statistically significant. Statistical analysis was performed using SAS 9.3 software (SAS Institute, Cary, NC).
Supplemental Figure. Representative optical coherence tomography images. **A**, Macrophage image is shown as depict signal-rich distinct punctate regions with high signal attenuation (arrow heads). **B**, Microchannels are demonstrated as intraplaque signal-voiding tubular structures (arrows).
References


