Frequency and Spatial Distribution of Thin-Cap Fibroatheroma Assessed by 3-Vessel Intravascular Ultrasound and Optical Coherence Tomography —— An Ex Vivo Validation and an Initial In Vivo Feasibility Study ——

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**Background:** The precursor of plaque rupture is known as thin cap fibroatheroma (TCFA). In the present study, the feasibility and accuracy of optical coherence tomography (OCT) for detecting the frequency and spatial distribution of TCFA was investigated ex vivo, and a 3-vessel OCT analysis was conducted to assess the feasibility of this modality in vivo.

**Methods and Results:** In the ex vivo study, 108 coronary arterial segments from 38 human cadavers were examined by OCT, intravascular ultrasound (IVUS) and histology. The 3-vessel IVUS and OCT examinations were performed in 11 patients with acute coronary syndrome. By histological examination, 30 of 77 fibroatheromas were diagnosed as TCFAs, which showed a clear pattern of clustering in the proximal part of each coronary vessel. OCT accurately detected TCFA (sensitivity 90%, specificity 79%). The in vivo OCT study showed that 64% patients with acute coronary syndrome had 1 or more TCFAs.

**Conclusions:** TCFAs, clustering in the proximal segments of the 3 major epicardial coronary arteries, are a common finding in unselected autopsy subjects. OCT is a feasible and accurate modality for detecting TCFA both ex vivo and in vivo. (*Circ J* 2009; 73: 1086–1091)

**Key Words:** Coronary artery disease; Imaging; Intravascular ultrasound; Pathology

Recent intracoronary imaging studies using intravascular ultrasound (IVUS) have shown that plaque rupture may be present not only in the culprit lesion but also in non-culprit lesions. Multiple plaque ruptures have been reported in 10–79% of patients with acute coronary syndrome (ACS), suggesting more extensive plaque vulnerability as a result of systemic inflammation. The precursor of plaque rupture is known as thin cap fibroatheroma (TCFA), which is characterized by an avascular, hypocellular necrotic core with an overlying fibrous cap measuring <65 μm. Because the resolution of current IVUS systems is 100–150 μm, the frequency and geographical distribution of in vivo TCFA has not been investigated by IVUS. Optical coherence tomography (OCT) is a high-resolution (10–20 μm) imaging method that can provide an accurate measurement of the thickness of the fibrous cap and thus enable a diagnosis of TCFA. Accordingly, we hypothesized that OCT might enable accurate detection of frequency and spatial distribution of TCFA in vivo. To test this hypothesis, we first evaluated the frequency and distribution of TCFA by 3-vessel histological and OCT examinations in ex vivo human coronary artery specimens and second, we conducted 3-vessel IVUS and OCT analyses to evaluate the feasibility of this method in vivo.

**Methods**

**Ex Vivo Study**

We examined coronary arterial segments from 38 human cadavers (24 males, 14 females, mean age 74±7 years). Of these, 6 had symptomatic cardiovascular disease (16%). Hypercholesterolemia was defined as a total cholesterol level ≥240 mg/dl or medication use; hypertension was systolic blood pressure (BP) ≥140 mmHg, diastolic BP ≥90 mmHg, or use of an antihypertensive drug; diabetes mellitus was use of antidiabetic medications (insulin or oral hypoglycemic agents). The study protocol was approved by the Ethics Committee of Kawasaki Medical School, and written informed consent was given by each family.

Segments measuring approximately 5 cm length were obtained from the proximal site of the 3 major coronary
arteries, including the ostial segment, at autopsy within 3h of death. Surrounding soft tissues were also dissected from each specimen. Small arterial perforators and branches were ligated, and the distal end of each artery was occluded with a large cork. A 7F sheath was sewn into the proximal end of the artery to complete the closed system. Saline (0.9%), kept at 37°C, was infused through the side arm of the sheath. The pressure inside the coronary artery was maintained at a physiologic level (60–80 mmHg) with a sphygmomanometer connected to the infusion.

**OCT and IVUS Images**

An intravascular OCT catheter (ImageWire®; LightLab Imaging, Westford, MA, USA) and an IVUS catheter (Atlantis SR Pro® 2.5F, 40-MHz; Boston Scientific, Natick, MA, USA) were inserted sequentially through the diaphragm of the sheath. Serial OCT and IVUS images were obtained using an automatic pullback device. The OCT images were processed and analyzed using proprietary software from LightLab Imaging and the IVUS images were analyzed offline by commercially available image processing software (Netra 3D IVUS system, ScImage, Los Altos, CA, USA). The external elastic membrane (EEM), lumen, and plaque plus media cross-sectional area (CSA) were measured on the IVUS images. References were single cross-sectional frames with the largest lumen and the smallest plaque burden within 5 mm proximal and distal to an atherosclerotic lesion, and the mean value of these 2 reference measurements was calculated. Plaque burden was calculated as (plaque plus media CSA/EEM CSA)×100%. The remodeling index was calculated as lesion divided by the reference EEM CSA: positive remodeling was defined as remodeling index >1.05.16-18

**Histological Examination**

After OCT and IVUS imaging, each coronary artery was pressure-fixed in 10% neutral buffered formalin for 48h, followed by standard paraffin embedding. In every 400μm of the coronary arteries, 2 series of 4-μm thick sections were cut and stained with hematoxylin-eosin and elastica van Gieson technique, as previously reported.19

**Identification of TCFA**

Histological images were digitized. If necrotic core was present in ≥1 quadrant, the lesion was considered to be a fibroatheroma and if the fibrous cap was <65μm assessed, the lesion was defined as TCFA.20 If the thickness of the fibrous cap was ≥65μm, the lesion was defined as non-TCFA. The distance between each TCFA and the respective coronary ostium was measured. OCT cross-sectional images were then selected to correspond with the TCFA found by histological examination. In the OCT images, necrotic cores were characterized by signal-poor regions with diffuse borders, and fibrous caps were defined as a signal-rich layer from the coronary artery lumen to the inner border of the underlying necrotic core.11-13,20,21 The thickness of the fibrous cap was also measured by OCT, as previously reported.15 Assessment of the OCT images was strictly at the same sites identified as TCFAs by histological examination, located according to the distance from anatomical landmarks such as side branches or calcification.

**In Vivo Study**

We performed 3-vessel OCT examinations to evaluate the feasibility of detecting TCFA in 11 patients with ACS (8 men, 3 women; mean age 70±5 years). We excluded patients with acute myocardial infarction, chronic total occlusions, and a history of coronary artery bypass grafts because of the difficulties of performing OCT in these conditions. The institution’s Ethics Committee approved the study protocol, which complied with the Declaration of Helsinki, and written informed consent was given by all patients.

**Cardiac Catheterization Analysis**

Cardiac catheterization was performed by the conventional femoral approach, using a 7F sheath and catheters. All OCT and IVUS studies were performed before any intervention and after administration of 200μg nitroglycerin. The intravascular OCT catheter was advanced to the distal end of all 3 epicardial coronary arteries through a 3F occlusion balloon catheter (Helios®; LightLab Imaging). In order to remove blood from the imaging field, the occlusion balloon was inflated to 0.5 atm in the proximal end of each coronary artery and lactated Ringer’s solution was infused from the distal tip of the occlusion balloon catheter at 0.3–0.5 ml/s, as previously reported.22 The IVUS catheter was introduced to the distal coronary bed of the 3 coronary arteries. The culprit lesion was identified on the basis of coronary angiography find-
ings and percutaneous coronary intervention with stenting was performed in the usual manner at the culprit lesion. All angiograms were analyzed using standard methodology and the OCT and IVUS images were analyzed in the same manner as in the ex vivo study. In all images of plaque with an OCT-determined necrotic core, the fibrous cap was measured at its thinnest and TCFA was defined as a plaque with lipid content in ≥1 quadrant and the thinnest part of the fibrous cap measuring <65 μm.

Statistical Analysis
Discrete variables are presented as counts and percentages. Continuous variables are presented as mean values ± SD when indicated. Un-paired Student's t-test was used to differentiate between 2 sets of data with normal distribution. If normality tests failed, the Mann-Whitney U-test was used. Fisher's exact test was performed, as indicated. A 2-sided P<0.05, indicated statistical significance.

Results
Ex Vivo Study
We explored 108 major epicardial coronary arteries by histological examination and 77 fibroatheromas were detected: 30 were diagnosed as TCFA (39%). There were no significant differences in age, gender, or coronary risk factors (diabetes mellitus, hyperlipidemia, hypertension, current smoking) between cadavers with and without TCFA (P=0.109, P=0.206, P=0.095, P=0.567, P=0.238, P=0.584, respectively). Cadavers with TCFA had a significantly higher incidence of ischemic heart disease and hemodialysis than cadavers without TCFA (34% vs 0%, P=0.003, and 25% vs 0%, P=0.025, respectively).

Identification of TCFA
The total length of the coronary arteries examined by histology was 53±15 mm in the left anterior descending arteries (LAD), 54±13 mm in the left circumflex arteries (LCX), and 60±17 mm in the right coronary arteries (RCA). Frequency of TCFA as detected by histology in the LAD, LCX, and RCA was 23%, 28%, and 33%, respectively. The frequency of 30 TCFAs according to distance from each coronary ostium is shown in Figure 1. The results showed a clear clustering pattern of the lesions along the coronaries, with 21 (70%) TCFA located in the first 30 mm, whereas further along the vessels the frequency was significantly lower.

Table 1. IVUS and OCT Characteristics of TCFA and Non-TCFA

<table>
<thead>
<tr>
<th></th>
<th>TCFA (n=30)</th>
<th>Non-TCFA (n=47)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVUS findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEM CSA, mm²</td>
<td>14.7±6.2</td>
<td>12.6±3.9</td>
<td>0.064</td>
</tr>
<tr>
<td>Lumen CSA, mm²</td>
<td>6.4±3.0</td>
<td>5.4±1.9</td>
<td>0.094</td>
</tr>
<tr>
<td>Plaque plus media CSA, mm²</td>
<td>8.3±3.8</td>
<td>7.2±2.5</td>
<td>0.108</td>
</tr>
<tr>
<td>Lesion site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEM CSA, mm²</td>
<td>15.3±6.1</td>
<td>13.4±4.2</td>
<td>0.110</td>
</tr>
<tr>
<td>Lumen CSA, mm²</td>
<td>4.4±2.2</td>
<td>4.0±1.6</td>
<td>0.332</td>
</tr>
<tr>
<td>Plaque plus media CSA, mm²</td>
<td>10.9±4.3</td>
<td>9.4±3.2</td>
<td>0.086</td>
</tr>
<tr>
<td>Plaque burden, %</td>
<td>71±7</td>
<td>70±7</td>
<td>0.636</td>
</tr>
<tr>
<td>Remodeling index</td>
<td>1.06±0.11</td>
<td>1.08±0.15</td>
<td>0.463</td>
</tr>
<tr>
<td>Positive remodeling, n (%)</td>
<td>15 (50%)</td>
<td>29 (62%)</td>
<td>0.157</td>
</tr>
<tr>
<td>OCT findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCFA detected by OCT</td>
<td>27 (90%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness of the fibrous cap, μm</td>
<td>46±32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IVUS, intravascular ultrasound; OCT, optical coherence tomography; TCFA, thin cap fibroatheroma; EEM, external elastic membrane; CSA, cross-sectional area.
lower (9, 30%, P<0.01). Complete histological examination of all 3 coronary arteries was available in 32 patients (84%). Among 32 cadavers, 6 (19%) had 1 TCFA, 6 (19%) had 2 TCFA, and 4 (13%) had 3 TCFA.

**IVUS and OCT Assessment of TCFA**

Representative IVUS and OCT images of TCFA are shown in Figure 2. Table 1 shows comparison of IVUS and OCT characteristics between TCFA and non-TCFA. Quantitative IVUS measurements did not show significant differences between TCFA and non-TCFA. OCT showed a high sensitivity for detecting TCFA (sensitivity 90%, specificity 79%).

**In Vivo Study**

We explored 33 major epicardial coronary arteries in 11 patients with ACS and 12 TCFAs were detected in 7 patients (64%). The total length of the coronary arteries examined by OCT was 34±8 mm in the LAD, 28±8 mm in the LCX, and 37±13 mm in the RCA. Of the 12 TCFAs, 3 were detected at culprit sites (25%). Seven patients with TCFA had an incidence of hypercholesterolemia of 57%, hypertension of 71%, and diabetes mellitus of 57%. Frequency of TCFA as detected by OCT in the LAD, LCX, and RCA was 4 (33%), 3 (25%), and 5 (42%), respectively. Among 7 patients, 3 (43%) had 1 TCFA, 3 (43%) had 2 TCFA, and 1 (13%) had 3 TCFA. Table 2 shows the angiographic, IVUS and OCT characteristics of TCFA.

**Discussion**

Plaque rupture is considered to be a major cause of thrombotic occlusion of a coronary artery, which can in turn lead to ACS and even sudden cardiac death. Several investigators have noted the presence of more than 1 ruptured plaque in patients experiencing a cardiovascular event.8–12,23–28 TCFAs with a large avascular, hypocellular necrotic core seem particularly prone to rupture, resulting in thrombotic occlusion of the coronary artery.8–10 Thus, this study, which found that multiple TCFA occur in the coronary arteries of high-risk patients, concurs with previous reports.27 Furthermore, in a recent angiographic study, Wang et al analyzed 208 consecutive patients with ST-segment elevation myocardial infarction to determine the location of epicardial thrombosis and found that the occlusions tended to cluster within the proximal third of each coronary artery.28 Kolodgie et al also reported that over 50% of TCFAs occur in the proximal portions of the major coronary arteries.29 Therefore, the distribution of TCFA in our ex vivo study accords with that found by previous studies, with a clear pattern of clustering around the ostium, indicating non-uniform distribution of vulnerable plaque within the entire coronary tree.

At present, IVUS is widely used in interventional cardiology for evaluating the wall structure of coronary arteries.30,31 In addition, Virtual Histology™ IVUS (Volcano Therapeutics, Rancho Cordova, CA, USA) has the potential to provide useful information about TCFA in vivo.32,33 However, the resolution of conventional gray-scale IVUS is not sufficient for assessment of TCFA because the “thin” fibrous caps are less than the IVUS resolution (100–150 μm).34,35 The thickness of the fibrous cap of lipid-rich plaque, as compared with histological examination (including 37 of the 77 fibroatheromas (48%) examined in our previous study),6 the present study, OCT observers were not blinded to the results of histological examination. Although that could affect the results of OCT assessment of TCFA in the ex vivo study, that study revealed that OCT had high sensitivity and specificity for detection of TCFA. Furthermore, in our present in vivo clinical study, OCT with IVUS examination was feasible in evaluation of TCFA. Considering these results, OCT is well suited for evaluation of TCFA in which the relevant morphologic features are localized close to the luminal surface. On the other hand, necrotic cores were defined as signal-poor regions with diffuse borders in the OCT image in the present study. Though this definition was based on previous histological examination, the limited depth of OCT penetration (approximately 2.0 mm) may contribute to false-positive diagnoses of necrotic cores (ie, causing signal-poor regions in the deeper vessel wall to be misinterpreted as necrotic cores). Improved penetration of the OCT catheter would be required.

IVUS, but not OCT, could visualize the entire coronary arterial structure and thus provide an accurate evaluation of the degree of coronary arterial remodeling and whole plaque volume, which are important characteristics of the vulnerable plaque.36 The combination of OCT and IVUS could potentially enhance an accurate diagnosis of the vulnerable plaque and aid the assessment of the effect of anti-atherosclerotic drugs and, therefore, ensure a more comprehensive pathophysiologic approach towards natural history studies of vulnerable plaque such as TCFA.

**Study Limitations**

One limitation was the unblinded study design. It should be recognized that accurate comparison between OCT and histological examination definitely requires a learning curve and prone to observer bias. The fact that OCT observers were not blinded to the results of histological examination might have affected the results of OCT assessment of TCFA in the ex vivo study. Further blind study will be needed to evaluate OCT assessment of TCFA.

In the ex vivo study, OCT images were obtained in saline solution without the need for proximal balloon occlusion and continuous saline infusion, so the quality of the OCT images was better than those in the in vivo study. Furthermore, heart motion artifacts are a potential limitation in vivo.

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**Table 2. Angiographic, IVUS and OCT Characteristics of 12 TCFAs**

<table>
<thead>
<tr>
<th>TCFA site</th>
<th>OCT findings</th>
<th>IVUS findings</th>
<th>Angiographic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference site</td>
<td>EEM CSA, mm²</td>
<td>Lumen CSA, mm²</td>
<td>Plaque plus media CSA, mm²</td>
</tr>
<tr>
<td>EEM CSA, mm²</td>
<td>13.5±5.8</td>
<td>7.2±3.5</td>
<td>6.3±2.7</td>
</tr>
<tr>
<td>Remodeling index</td>
<td>0.9±0.21</td>
<td>0.8±0.21</td>
<td>0.8±0.21</td>
</tr>
<tr>
<td>Thickness of the fibrous cap, μm</td>
<td>45±20</td>
<td>45±20</td>
<td>45±20</td>
</tr>
</tbody>
</table>

Abbreviations see in Table 1.
Our study population was limited. Therefore, the EEM CSA and plaque plus media CSA values shown in Table 2 showed substantial differences, but did not achieve statistical significance between TCFAs and non-TCFA.

Because of the need for proximal occlusion, the ostial part of each coronary vessel could not be imaged and investigated in the in vivo study. Therefore, it did not show the distribution of TCFAs as was shown by the ex vivo study. Recently, Kataiwa et al. reported a non-occlusive image acquisition technique for OCT,59 and by using this technique, assessment of distribution of TCFAs may be possible in the clinical setting59.

Finally, because of the study design, our results do not show the natural course of unruptured TCFAs. Further follow-up study is needed to investigate whether TCFAs diagnosed by OCT will truly rupture in the future.

Conclusions

TCFAs, clustering in the proximal segments of the 3 major epicardial coronary arteries, are a not uncommon finding in unselected autopsy subjects. OCT is a feasible and accurate intracoronary imaging modality for detecting TCFAs in the coronary arteries, either ex vivo or in vivo. A larger, prospective IVUS and OCT analysis is needed to evaluate the natural history of TCFAs and to show the efficacy of pharmacological as well as mechanical interventions.

References


33. Hong MK, Mintz GS, Lee CW, Lee JW, Park JH, Park DW, et al. A


