Atherosclerotic Component of the Yellow Segment After Drug-Eluting Stent Implantation on Coronary Angioscopy — An Ex-Vivo Validation Study —

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Background: Coronary angioscopy (CAS) is used to comprehensively evaluate vascular responses after drug-eluting stent (DES) implantation. This study sought to evaluate the capability of CAS for evaluating DES strut coverage grade and color grade of the intima compared with histological images in coronary autopsy specimens.

Methods and Results: A total of 23 DES extracted from 11 autopsy hearts were imaged by CAS. All stent segments were graded as white or yellow according to the luminal surface color, and thrombus was evaluated according to a previous report. Neointimal coverage over the DES was graded as 0 (stent struts fully visible) to grade 3 (stent struts fully embedded and invisible). Of 76 segments, neointimal coverage was graded as 0 in 35 (46%), 1 in 22 (29%), 2 in 8 (11%), and 3 in 11 (14%). The neointimal thickness increased significantly with increasing neointimal coverage grade on angioscopy. Neointimal color was graded as white in 40 (53%) and yellow in 36 segments (47%). Histological analysis revealed that yellow neointima contained fibroatheroma, foam cells accumulation or superficial calcium deposition. A thrombus was identified in 13 segments. Thrombi adherent around the stent strut were partly intimal erythrocyte accumulation around the strut.

Conclusions: In-stent yellow segment had atherosclerotic components. CAS could evaluate vascular status comprehensively after DES implantation.

Key Words: Atherosclerosis; Coronary angioscopy; Vulnerable plaque

Coronary angioscopy (CAS) involves direct visualization of the color and superficial morphology of plaque and thrombus, using projected white light through thin, flexible glass fibers loaded into catheters. Several studies have shown that CAS identifies atherosclerosis as yellow-colored plaques, which have been regarded as high-risk plaques and correlated with future coronary events. CAS has also been used after drug-eluting stent (DES) implantation, because of its ability to comprehensively evaluate vascular responses, including the degree of neointimal coverage, with intimal color detected as yellow or white. A prospective study has revealed that a yellow intima after DES implantation is significantly associated with a higher risk of thrombus formation and target lesion revascularization, as assessed during long-term follow-up. Therefore, the presence of yellow intima after DES implantation has been considered as a surrogate marker of risk of DES failure. Furthermore, CAS is suitable for evaluating the degree of neointimal coverage after DES implantation. A previous study has revealed that incomplete neointimal coverage after DES implantation assessed by CAS was associated with subclinical thrombus formation; however, to date, a validation study comparing CAS findings to histological findings after DES implantation has not been carried out. Therefore, in the present study, we assessed the capability of CAS for evaluating DES strut coverage grade and color grade of the intima, compared with histological images after DES implantation in coronary autopsy specimens.

Methods

Study Subjects and Tissue Excision
A total of 23 DES (7 sirolimus-eluting stents [SES], 4
In the present study, CAS images were obtained using a 4.5F angioscopic catheter (Vecmova; FiberTech, Tokyo, Japan). The white balance was initially adjusted for color correction. The light power was adjusted to gain appropriately bright colored images without reflection. The coronary arteries were observed with simultaneous distal 0.9% saline flush delivery, in order to remove blood cells from the imaging field. CAS catheter was introduced and manually pulled back to identify the stent segment. All segments were graded as white or yellow according to the luminal surface color in a manner similar to that adopted in earlier reports. 4 Thrombus was defined as a coalescent, red superficial or protruding mass, adhering to the vessel surface, based on the criteria of the European Working Group on Coronary Angioscopy. 7 Neointimal coverage over the DES was classified into 4 grades: grade 0=stent struts fully visible; grade 1=stent struts bulging into the lumen and still transparently visible; grade 2=stent struts embedded into the neointima but seen translucently; grade 3=stent struts fully embedded and invisible on angioscopy. 3, 8

### Histological Study

After CAS examination, the stented segments were fixed in phosphate-buffered saline and subsequently, CAS examination was performed. The time interval between death and CAS examination did not exceed 12 h. The experimental and ex-vivo study protocols were approved by the Institutional Review Board of Hyogo College of Medicine, and written informed consent was obtained from the patients' relatives in all cases.

### CAS Imaging Procedure and Analysis

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### Table. Profiles of the Study Subjects and the Implanted Drug-Eluting Stents

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cause of death</th>
<th>Vessel</th>
<th>Stent diameter (mm) × length (mm)</th>
<th>Implant duration (months)</th>
<th>Neoatherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pneumonia</td>
<td>RCA</td>
<td>EES (3×18)</td>
<td>2</td>
<td>Foam cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LAD</td>
<td>SES (3×23)</td>
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<tr>
<td></td>
<td></td>
<td>LCX</td>
<td>EES (2.5×8)</td>
<td>2</td>
<td>None</td>
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<tr>
<td>2</td>
<td>Infective endocarditis</td>
<td>LAD</td>
<td>SES (3×28)</td>
<td>45</td>
<td>Calcification</td>
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<tr>
<td>3</td>
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<td>RCA</td>
<td>SES (3.0×18)</td>
<td>61</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>RCA</td>
<td>EES (3.0×15)</td>
<td>28</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>RCA</td>
<td>PES (3.0×12)</td>
<td>43</td>
<td>Foam cells, fibroatheroma</td>
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<tr>
<td></td>
<td></td>
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<td>SES (3.5×23)</td>
<td>61</td>
<td>Foam cells, fibroatheroma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LAD</td>
<td>SES (3.0×18)</td>
<td>61</td>
<td>Foam cells</td>
</tr>
<tr>
<td>4</td>
<td>AMI, cardiac tamponade</td>
<td>RCA</td>
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<tr>
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<td></td>
<td>RCA</td>
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</tr>
<tr>
<td>5</td>
<td>Thoracic aortic dissection</td>
<td>RCA</td>
<td>EES (2.5×20)</td>
<td>7</td>
<td>Foam cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LAD</td>
<td>EES (2.5×38)</td>
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<tr>
<td>6</td>
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<td>RCA</td>
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<tr>
<td>8</td>
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<tr>
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<td></td>
<td>LCX</td>
<td>EES (2.5×28)</td>
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<tr>
<td>9</td>
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<td></td>
<td></td>
<td>RCA</td>
<td>PES (3.0×16)</td>
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<td>Fibroatheroma, calcification</td>
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<tr>
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<td>EES (3.5×28)</td>
<td>20</td>
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<td></td>
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<td>Calcification</td>
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<td>PES (2.75×32)</td>
<td>35</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LCX</td>
<td>SES (2.5×13)</td>
<td>35</td>
<td>None</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; EES, everolimus-eluting stent; LAD, left anterior descending; LCX, left circumflex artery; PES, paclitaxel-eluting stent; RCA, right coronary artery; SES, sirolimus-eluting stent.
Statistical Analysis
Continuous data were tested by 1-way analysis of variance of the different categories (coverage grade), and the Mann-Whitney test was performed in cases of a significant difference. The incidence of calcification was compared among the groups using the chi-square test or Fisher’s exact test. All tests were performed using JMP10 (SAS Institute, Cary, NC, USA).

10% neutral buffered formalin for 48 h, then embedded in methyl methacrylate. Histological cross-sections were sliced 5-μm thick, at approximately 3–4 mm intervals, using a diamond blade. Each slice was stained with hematoxylin and eosin, Masson’s trichrome, and elastica van Gieson stain. Histological analysis was based on evaluation by a single pathologist (H.H.), who was blinded to the imaging results. Neoatherosclerosis was defined as fibroatheroma, foam cell accumulation, and superficial calcium deposition in the intima. A thrombus was defined as an organized collection of fibrin and erythrocytes in the lumen as illustrated by Lammie et al.9}

Figure 2. Representative images on coronary angioscopy (CAS) and corresponding histology. (Left) Angioscopic images, (Middle & Right) corresponding histological images. In the angioscopic images, the color of each segment was graded as yellow (A,D,G) or white (J). (A) CAS image of light-yellow segment with grade 3 intimal coverage. (B) Corresponding histological image of the lesion in (A) showing eccentric proliferation of the neointima (H&E, scale bar=500 μm). (C) Magnified image of the inset in (B) showing many foam cells accumulating on the luminal surface (H&E, scale bar=200 μm). (D) CAS image shows an intense-yellow segment covered with grade 3 intima. (E) Corresponding cross-sectional histological image of the lesion in (D) shows a fibrous cap overlying a large, lipid-rich necrotic core within the stent (H&E, scale bar=1 mm). (F) Higher magnification of the neointima within the stent struts shows lymph cells infiltrating around stent strut. *Stent strut. (H&E, scale bar=500 μm.) (G) CAS image of an intense-yellow segment with a sharp border and intimal coverage of grade 2. (H) Histological image of the same section of the lesion in (G) (H&E, scale bar=1 mm). (I) Magnified image of the inset in (H) showing sheet calcification in the neointima between stent struts (*)(H&E, scale bar=100 μm). (J) CAS image of a white segment with grade 3 intimal coverage. (K) Corresponding histological image of the lesion in (J) showing neointima proliferation on all stent struts (H&E, scale bar=500 μm). (L) Magnified image of the inset in (K) showing stable, homogeneous fibrous tissue without lipid deposition (H&E, scale bar=200 μm).
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Sponding histology are listed in Figure 5. Contrast, segments classified as white were composed of deposition (11 segments, 4 DES) within the neointima. In accumulation (11 segments, 6 DES), or superficial calcium such as fibroatheroma (4 segments, 3 DES), foam cell yellow by CAS contained neoatherosclerotic components. Histological analysis revealed that segments classified as 

Neointimal Color Grade

The main findings of the present study were as follows. (1) Neoatherosclerosis is considered to be one of the mechanisms leading to stent thrombosis after DES implantation. In fact, it has been reported that rupture of yellow plaques within the stented segment was detected by CAS, in lesions with late or very late stent thrombosis after DES implantation. In line with these results,

Thrombus on Angioscopy and Histology

The prevalence of foam cell accumulation was 29%, 25%, and 25%, calcification was 14%, 50%, and 8%, and fibroatheroma was 14%, 50%, and 0% in SES, PES, and EES, respectively. No significant difference was identified for each finding among the 3 DES.

Incidence of Neoatherosclerosis

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findings, we previously demonstrated ex vivo that of an intensive yellow segment after DES implantation on CAS imaging represented a large lipid-rich necrotic core, covered with a thin fibrous cap infiltrated by lipid-laden macrophages, with few smooth muscle cells on histology. Similarly, in the present study, yellow segments after DES implantation included neatherosclerosis. In segments with a yellow neointima, the main tissue components were fibroatheroma, macrophage accumulation, and calcification. We previously demonstrated that calcification has several angiographic color grades, and some superficial calcium plates appeared yellow on CAS in an ex-vivo histopathological validation study. Although the precise mechanism of the calcification after stent implantation has not been elucidated, a previous pathological study reported deposition of calcium within the intima after 1st-generation DES implantation. In our previous an ex-vivo imaging study, most superficial calcium deposits had a sharp border and most fibroatheroma had a diffuse border. In our current study, when yellow intima had a sharply defined border on CAS, it contained superficial intimal calcium deposition with high diagnostic accuracy (Figure 2G). This finding means it is possible to differentiate superficial calcium deposition from fibroatheroma and foam cell accumulation. In the current ex-vivo study, the color of the neointima was affected by underlying tissue morphology behind the stent strut when the neointimal coverage was sparse. Therefore, careful interpretation is required to evaluate yellow neointimal color grade by CAS, especially in the presence of visible stent strut.

Thrombus appearance around the stent strut on CAS was often intimal erythrocyte accumulation without intraluminal fibrin accumulation as evaluated by histology, reflecting intimal hemorrhage or thrombus absorption adherent to the stent strut. The eluted drug may affect delayed stent healing resulting in the thrombus remaining for longer. Plaque hemorrhage is an important mechanism of plaque progression in native coronary arteries, especially in advanced coronary atherosclerosis. Carotid plaque hemorrhage by magnetic resonance imaging predicts cerebrovascular events. In the present study, intimal erythrocyte accumulation was covered with fibrous tissue and endothelial cells, which may not directly lead to late stent thrombosis, but may be a cause of late stent restenosis or neatherosclerosis in the future.

Study Limitations
First, a positional discrepancy in the evaluated cross-sections between the angiographic and histological images may have influenced the results, although we took care to achieve optimal co-registration of the images. Second, the color of the segments was determined unaided visually, and therefore was quite subjective. Third, the angiographic images were taken in cadaver hearts, which may have affected the results. Fourth, we did not perform any immunohistochemical staining to identify macrophages.

Conclusions
In-stent yellow segments had atherosclerotic components, such as fibroatheroma formation, foam cell accumulation, and superficial calcium deposition. Furthermore, the yellow color reflected vulnerable components underneath the stent when neointimal coverage was sparse. CAS can comprehensively evaluate vascular status after DES implantation.

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References