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.1,2 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.3,4 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.5 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.6 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
paclitaxel-eluting stents [PES], and 12 everolimus-eluting stents [EES]) with implant durations of 1–69 (median 24) months taken from 11 cadavers were examined. All stented coronary arteries were dissected at autopsy within 6 h of death. Harvested coronary arteries were immediately stored in phosphate-buffered saline and subsequently, CAS examinations were 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**

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. 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.

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.

**Histological Study**

After CAS examination, the stented segments were fixed in

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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) x 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 (3x18)</td>
<td>2</td>
<td>Foam cells</td>
</tr>
<tr>
<td>1</td>
<td>Pneumonia</td>
<td>LAD</td>
<td>SES (3x23)</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Pneumonia</td>
<td>LCX</td>
<td>EES (2.5x8)</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Infective endocarditis</td>
<td>LAD</td>
<td>SES (3x28)</td>
<td>45</td>
<td>Calcification</td>
</tr>
<tr>
<td>3</td>
<td>Sepsis</td>
<td>RCA</td>
<td>SES (3.0x18)</td>
<td>61</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Sepsis</td>
<td>LAD</td>
<td>SES (3.5x23)</td>
<td>61</td>
<td>Foam cells, fibroatheroma</td>
</tr>
<tr>
<td>3</td>
<td>Sepsis</td>
<td>LAD</td>
<td>SES (3.0x18)</td>
<td>61</td>
<td>Foam cells</td>
</tr>
<tr>
<td>4</td>
<td>AMI, cardiac tamponade</td>
<td>RCA</td>
<td>EES (3.5x15)</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>AMI, cardiac tamponade</td>
<td>LAD</td>
<td>EES (3.5x18)</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Thoracic aortic dissection</td>
<td>RCA</td>
<td>EES (2.5x20)</td>
<td>7</td>
<td>Foam cells</td>
</tr>
<tr>
<td>5</td>
<td>Thoracic aortic dissection</td>
<td>LAD</td>
<td>EES (2.5x38)</td>
<td>7</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>Congestive heart failure, ischemic cardiomyopathy</td>
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<td>SES (3.5x28)</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>Thoracic aortic rupture</td>
<td>RCA</td>
<td>EES (2.5x12)</td>
<td>17</td>
<td>Foam cells</td>
</tr>
<tr>
<td>8</td>
<td>Thoracic aortic aneurysm</td>
<td>LAD</td>
<td>EES (3.0x18)</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>Ventricular fibrillation</td>
<td>LAD</td>
<td>EES (2.5x28)</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>Ventricular fibrillation</td>
<td>RCA</td>
<td>EES (3.5x12)</td>
<td>69</td>
<td>Calcification</td>
</tr>
<tr>
<td>9</td>
<td>Ventricular fibrillation</td>
<td>RCA</td>
<td>EES (3.5x16)</td>
<td>69</td>
<td>Fibroatheroma, calcification</td>
</tr>
<tr>
<td>10</td>
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<td>LAD</td>
<td>EES (2.75x28)</td>
<td>29</td>
<td>Calcification</td>
</tr>
<tr>
<td>11</td>
<td>AMI</td>
<td>LAD</td>
<td>PES (2.75x32)</td>
<td>35</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>AMI</td>
<td>LCX</td>
<td>SES (2.5x13)</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.
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.*

**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).

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*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 struts. *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 neo-intima 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).
sclerosis on CAS, the sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy for neoatherosclerosis were 96%, 76%, 67%, 98%, and 83%, respectively. Based on the histological analysis, false-positive diagnoses of CAS for neoatherosclerosis comprised neointima of low coverage grade (grade 0: n=8, grade 1: n=3, grade 2: n=1) with underlying plaque morphology of lipid-rich fibroatheroma or calcified plaque. Color grading of the neointima was affected by tissue morphology behind the stent strut when the neointimal coverage was sparse.

**Thrombus on Angioscopy and Histology**

Although thrombus was observed in 13 segments (17%) by CAS, histopathological analysis identified thrombus in only 5 segments; the other 8 segments contained intraplaque erythrocyte accumulation without fibrin around the stent strut (Figure 3).

**Incidence of Neoatherosclerosis**

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 DESs.

**Discussion**

The main findings of the present study were as follows. (1) Neointimal thickness increased significantly as neointimal coverage grade increased on angioscopy. (2) Histological analysis revealed that yellow neointima after DES implantation contained atherosclerotic components, including fibroatheroma, foam cell accumulation and superficial calcium deposition. (3) When intimal coverage was sparse, the yellow segments reflected fibroatheroma formation underneath the stent. (4) Thrombi adherent around the stent strut on CAS were often intimal erythrocyte accumulation on histology.

In native coronary arteries, yellow plaques are often observed as culprit lesions in acute coronary syndrome (ACS) patients, and are considered as vulnerable plaques that may lead to ACS events.\(^{12}\) Our ex-vivo study demonstrated dark-yellow plaques on CAS, including fibroatheroma formation with high sensitivity. On the other hand, white plaques, including fibrous plaques or thick fibrous caps, are considered as stable.\(^{10}\) Thus, the varying color of plaques reflects the internal components and the degree of plaque vulnerability.

CAS has also shown that there are 2 neointimal colors after coronary stent implantation: white and yellow. A prospective clinical study revealed that yellow intima after DES implantation significantly correlated with a higher risk of thrombus formation and target lesion revascularization during long-term follow-up.\(^{8}\) Therefore, yellow neointima could contain thrombogenic components such as a necrotic core. A previous pathological study demonstrated that the prevalence of neoatherosclerosis was higher after DES implantation, as compared with bare metal stent implantation.\(^{14}\) 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.\(^{12}\) In line with these results, a recent retrospective study of 204 patients demonstrated that yellow neointima on CAS was significantly associated with a higher risk of thrombosis after DES implantation.\(^{15}\) Therefore, close monitoring of CAS findings is necessary in this patient population.

**Figure 3.** (A) Coronary angioscopy (CAS) image of a dark yellow-red segment distributed around a stent strut with grade 1 intimal coverage. (B) Corresponding histological image from the same section of the lesion in (A) showing sheet calcification underneath the stent strut. Neointimal proliferation is sparse (H&E, scale bar=1mm). (C,D) Magnified image of the inset in (B) showing red blood cells around the stent strut (*) inside the neointima (H&E, scale bar=100µm).

### Results

A total of 80 histological segments were obtained for 23 DES. After co-registration of the segments and CAS imaging, 4 histological segments were excluded because of cutting artifacts (n=2) or co-registration difficulty (n=2). DES profiles are listed in the Table. CAS demonstrated neointimal coverage as grade 0 (n=35, 46%), grade 1 (n=22, 29%), grade 2 (n=8, 11%), and grade 3 (n=11, 14%). Neointimal color was graded as white in 40 segments (53%) and yellow in the remaining 36 segments (47%). A thrombus was identified in 13 segments by CAS.

**Neointimal Coverage Grade**

The relationship between neointimal coverage grade and neointimal thickness obtained by histology is shown in Figure 1. The median intimal thickness by histology of grades 0–3 was, respectively, 6µm (interquartile range [IQR], 0–20µm), 89µm (IQR, 38–90µm), 202µm (IQR, 118–418µm), and 429µm (IQR, 387–566µm; P<0.001). Neointimal thickness increased significantly with increasing neointimal coverage grade on angioscopy. The neointimal thickness was significantly larger in yellow intima than in white intima (170±294µm vs. 132±200µm, P=0.05).

**Neointimal Color Grade**

Histological analysis revealed that segments classified as yellow by CAS contained atherosclerotic components such as fibroatheroma (4 segments, 3 DES), foam cell accumulation (11 segments, 6 DES), or superficial calcium deposition (11 segments, 4 DES) within the neointima. In contrast, segments classified as white were composed of smooth muscle cells with extracellular matrix and collagen fibers. These features as recognized by CAS and corresponding histology are listed in Figure 2.

When a yellow segment was considered to be neoatherosclerosis on CAS, the sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy for neoatherosclerosis were 96%, 76%, 67%, 98%, and 83%, respectively. Based on the histological analysis, false-positive diagnoses of CAS for neoatherosclerosis comprised neointima of low coverage grade (grade 0: n=8, grade 1: n=3, grade 2: n=1) with underlying plaque morphology of lipid-rich fibroatheroma or calcified plaque. Color grading of the neointima was affected by tissue morphology behind the stent strut when the neointimal coverage was sparse.

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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.13 Similarly, in the present study, yellow segments after DES implantation included neoatherosclerosis. In segments with a yellow neoointima, the main tissue components were fibroatheroma, macrophage accumulation, and calcification. We previously demonstrated that calcification has several angioscopic color grades, and some superficial calcium plates appeared yellow on CAS in an ex-vivo histopathological validation study.10 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.14 In our previous an ex-vivo imaging study, most superficial calcium deposits had a sharp border and most fibroatheroma had a diffuse border.10 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 neoimal coverage was sparse. Therefore, careful interpretation is required to evaluate yellow neoimal color grade by CAS, especially in the presence of viable 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 clotted 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.15 Carotid plaque hemorrhage by magnetic resonance imaging predicts cerebrovascular events.16 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 neoatherosclerosis in the future.

**Study Limitations**

First, a positional discrepancy in the evaluated cross-sections between the angioscopic 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 angioscopic 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 neoimal coverage was sparse. CAS can comprehensively evaluate vascular status after DES implantation.

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**References**


