Histological Characteristics of Glistening Yellow Coronary Plaques Seen on Angioscopy
– With Special Reference to Vulnerable Plaques –
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Background: Glistening yellow coronary plaques (GY) seen on angioscopy are considered vulnerable to disruption. Collagen fiber (CF) is the main substance that protects coronary plaques against mechanical stress. Therefore, whether angioscopically defined vulnerable plaques correlate with those defined histologically was investigated.

Methods and Results: One hundred and thirty-two excised human coronary plaques were classified by angioscopy into 19 GY, 49 non-glistening yellow plaques (non-GY) and 64 white plaques, and their relation to CF density was examined. CF-dense (>15/100 μm), CF-loose (>5 and <15/100 μm), and CF-scanty (<5/100 μm) plaques were hypothesized to be stable, relatively stable, and vulnerable, respectively. Histologically the plaques were classified into non-lipid deposition, superficial lipid deposition and diffuse lipid deposition groups; the diffuse lipid deposition group was classified into necrotic core (NC) and non-NC types. Nineteen GY were composed of 4 with superficial lipid deposition, 4 with non-NC type of diffuse lipid deposition, and 11 with NC type. Sixteen (84%) of these were CF scanty. Forty-nine (100%) of non-GY and 57 (89%) of white plaques were CF dense or CF loose. The sensitivity, specificity and predictive value of GY in detecting histologically vulnerable plaques were 90%, 97% and 84%, respectively, indicating that GY represented histologically vulnerable plaques.

Conclusions: These pathohistological characteristics might indicate that GY, less-protected plaques against mechanical stress, are vulnerable plaques. (Circ J 2011; 75: 1913–1919)

Key Words: Angioscopy; Collagen fibers; Glistening yellow coronary plaques; Histology; Vulnerable plaques
Histological classification

- Non-necrotic core type
- Diffuse lipid deposition group
  - Necrotic core type
    - Non-calcified cap type
      - CF-dense subtype
      - CF-loose subtype
      - CF-scanty subtype
    - Calcified cap type
      - CF-dense subtype
      - CF-loose subtype
      - CF-scanty subtype

Non-glistening yellow plaques
  - A) Superficial lipid deposition group
  - B) Diffuse lipid deposition group
    - Regular subtype
      - CF-dense subtype
      - CF-loose subtype
      - CF-scanty subtype
    - Jelly-like subtype
      - Calcified cap subtype

White plaques
  - C) Non-lipid deposition group
    - Regular subtype
    - Jelly-like subtype
    - Calcified cap subtype

*Glistening yellow plaque. †Non-glistening yellow plaque. ‡White plaque. Each subtype was labeled with *, † or ‡ based on the data of Tables 2 and 3.
CF, collagen fiber.

Methods

Observation of Excised Human Coronary Arteries by Angioscopy

This in vitro study was performed with the approval of the Ethical Committees of Toho University Sakura Hospital (Sakura, Japan) where the autopsies were performed after obtaining informed consent from families.

Angioscopy The angioscopy system was composed of a light source (OTV-A, Olympus Corporation, Tokyo, Japan), a 5-F angioscope made of glass fibers (AF14, Olympus Company, Tokyo, Japan), a CCD camera (CLV-A, Olympus Company, Tokyo, Japan) and a DVD. Before observation, the white balance was adjusted for color correction. A fiberscope made of glass fibers was used because different to the new fiberscopes made of quartz fiber bundles, each glass fiber for light-guide was imbedded separately and uniformly among the grids of those for image-guide, radiating light uniformly onto the target, thus avoiding reflection by the target surface.

One to 2 h after autopsy, a Y connector was introduced into the proximal portion of the coronary artery, which was perfused with saline solution. An angioscope (as mentioned above) was slowly introduced into the coronary artery for observation. A guidewire was not used because it might damage the plaques.

The light of the angioscope was seen through the coronary wall, so the angioscope tip and accordingly the targeted plaque could be confirmed. Plaques and normal segments were defined as previously described.9,10

Plaque Color Measurement for Angioscopy Plaque images obtained by angioscopy were classified into white and yellow using an AquaCosmos image analyzer (C7746, Hamamatsu Photonics Co, Hamamatsu, Japan) as follows. A window was set on the appropriate portion of a plaque image. The color within the window was separated into 3 primary colors: red, green and blue. The plaque was defined as “white plaque” when the color intensity ratio (red/green/blue) was 1.0/0.9–1.1. The plaque was defined as “yellow plaque” when the color intensity ratio (red/green/blue) was 1.0/0.8–1.2/0.3–0.6.10

Yellow plaques were further classified into GY and non-GY. A GY was a plaque exhibiting yellow fluorescence-like color with a glistening portion usually located in the area of the plaque center. Yellow fluorescence-like color is a yellow color closely resembling the reflected light by yellow fluorescence paint. By the present image analyzer, GY showed a yellow plaque with an intense white-to-yellow portion located usually in its center area. As the fluorescence-like color could not be discriminated by the present analyzer, it was determined by the naked eye. Non-GY was defined as a yellow plaque that did not exhibit these changes.8

Histology

Immediately after observation of the plaque by angioscopy, its center was cut into 2 parts. One part was not fixed and was sliced and stained by Oil Red-O and methylene blue in combination (thus staining the lipids red and calcium black). This staining method does not lose lipids, calcium and interstitial fluid, and accordingly does not alter the original architecture including the fibrous cap thickness. The thickness of the thinnest portion was measured microscopically using the slices stained by Oil-Red O and methylene blue, and the thickness thus measured was defined as the fibrous cap thickness.

The remaining adjacent slice was fixed with formaldehyde and cut into successive slices 10 μm in thickness. Several slices were used to stain for CF by silver staining, and the remaining slices were used to stain for macrophage-foam cells (MF) by Ziel-Neelsen staining.

Classification of Plaques by Histology The plaques were classified into non-lipid deposition, superficial lipid deposition (lipid deposition confined to the superficial layer ≤300 μm from the plaque surface) and diffuse lipid deposition groups. They were also classified into NC and non-necrotic types. The NC type was further classified into calcified cap and non-calcified cap subtypes (Table 1).

Normal CF were those that were 5–15 μm in diameter and reddish brown in color. CF >15 μm in diameter were called “thickened” CF, and ones with a collapsed or thread-like configuration were defined as “degenerated” CF. The CF were considered to be absent if the normal or thickened CF did not appear.10

Based on non-degenerated CF density (the number of CF/100 μm) in the superficial layer or in the cap (fibrous cap), the plaques were classified into CF-dense (≥15/100 μm), CF-loose (≥5 and <15/100 μm) and CF-scanty (<5/100 μm) subtypes. As non-degenerated CF that contain collagen protect the plaques against disruption,13 the plaques having dense CF, those having loose CF, and those having scanty CF were hypothesized histologically to be stable, relatively vulnerable, and vulnerable, respectively.

The distribution of MF was also investigated because they destroy CF.4 Ceramide is a marker of macrophage and foam cells.3 This substance was stained purple by Ziel-Neelsen staining. It was difficult to differentiate between the macrophages and foam cells because both contain ceramide. Therefore they
Statistical Analysis
Data of CF density were tested by Fisher’s exact test. Fibrous cap thickness was expressed as the mean±standard deviation and was tested by Student’s t-test. P<0.05 was considered significant. Sensitivity, specificity and predictive value of angioscopy in detecting histologically vulnerable plaques were also examined.

**Results**
Forty-eight coronary arteries were excised from 28 cadavers (age, 60.1±5.4 years; cause of death: hepatocellular carcinoma in 7, coronary heart disease in 7, diabetes mellitus in 2, renal failure in 5, lung cancer in 2, cerebral infarction in 2, aortic dissection in 1, and cerebral bleeding in 2). Autopsy was performed 3.8±1.2 h after death. One hundred and thirty-two non-disrupted plaques were angioscopically classified into 19 GY, 49 non-GY, and 64 white plaques.

**GY**
GY were composed of 4 with superficial lipid deposition (Figure 1A-1–A-4), 4 with non-NC type of diffuse lipid deposition (Figure 1B-1–B-4), and 6 with non-calcified cap subtype (Figure 2A-1–A-4) and 5 with calcified cap subtype (Figure 2B-1–B-4) of NC type.

CF density in GY was smaller than those of white and non-GY. There were no significant differences in CF density between superficial lipid deposition type and diffuse lipid deposition type, between non-NC type and NC type, and between non-calcified cap subtype and calcified cap subtype of this group (Table 2).

Of these GY, 16 plaques (84.2%) were CF scanty and 3 (15.7%) were CF loose, and none was CF dense. Deposition of MF and calcium was observed in 17 (89.4%) and 18 (94.7%) of GY, respectively (Table 3).

**Non-GY**
Forty-nine non-GY were composed of 16 with superficial lipid deposition (Figure 3A-1–A-4), 21 with non-NC core type (Figure 3B-1–B-4), 11 with non-calcified cap subtype and one with calcified cap subtype of NC type.

CF density in non-GY was smaller than that of white but larger than that of GY. There were no significant differences in CF density between superficial lipid deposition type and diffuse lipid deposition type, between non-NC type and NC type, and between non-calcified cap subtype and calcified cap subtype of this group (Table 2).

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**White Plaques**
The non-lipid deposition group was angioscopically white. This group was classified into regular white (white and CF dense), jelly-like, and calcified cap subtypes. The jelly-like
Subtype was defined as a transparent plaque exhibiting jelly-like appearance (Table 1).

Sixty-four plaques were classified by angioscopy as being white (Tables 2 and 3). The CF density in the jelly-like subtype and calcified cap subtype was smaller than that of the regular subtype of this group (Table 2).

Of these, 50 had dense CF and did not contain lipids by histological examination (regular subtype; Figure 4A-1–A-4). Eight plaques were jelly-like and transparent in color on angioscopy (such a plaque has not been described in the literature). Histologically, CF were loose to scanty, transparent matter occupied the spaces between the CF, and lipids and MF were not present (jelly-like subtype; Figure 4B-1–B-4). The remaining 6 plaques had a calcified cap covered with lipid-free endothelia with a NC below (calcified cap subtype; Figure 4C-1–C-4).

The CF were dense in the regular subtype, scanty in one of the jelly-like subtype and did not exist in 6 of the calcified cap subtype. As compression using a finger tip did not disrupt this calcified cap subtype in a preliminary study, and slicing the plaques with a blade was somewhat difficult, the calcified cap subtype was indicated to be mechanically stable.

Taking into consideration that the lipid- and CF-deficient calcified cap subtype was mechanically stable, the sensitivity, specificity, and predictive value of angioscopy in detecting histologically vulnerable plaques were 90.4%, 97.2% and 84.2%, respectively.

Table 2. CF Density of Coronary Plaques

<table>
<thead>
<tr>
<th>Plaque Type</th>
<th>n</th>
<th>CF Density (100 μm)</th>
</tr>
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<tbody>
<tr>
<td>Glistening yellow plaques</td>
<td>19</td>
<td>2.6±2.4* ††</td>
</tr>
<tr>
<td>Superficial lipid deposition group</td>
<td>4</td>
<td>3.0±0.8</td>
</tr>
<tr>
<td>Diffuse lipid deposition group</td>
<td>15</td>
<td>2.5±3.8</td>
</tr>
<tr>
<td>Non-necrotic core type</td>
<td>4</td>
<td>5.2±5.9</td>
</tr>
<tr>
<td>Necrotic core type</td>
<td>11</td>
<td>1.7±2.4</td>
</tr>
<tr>
<td>Non-calcified cap subtype</td>
<td>6</td>
<td>1.0±1.3</td>
</tr>
<tr>
<td>Calcified cap subtype</td>
<td>5</td>
<td>2.6±3.3</td>
</tr>
<tr>
<td>Non-glistening yellow plaques</td>
<td>49</td>
<td>17.2±2.6*</td>
</tr>
<tr>
<td>Superficial lipid deposition group</td>
<td>16</td>
<td>16.5±3.2</td>
</tr>
<tr>
<td>Diffuse lipid deposition group</td>
<td>33</td>
<td>17.1±2.8</td>
</tr>
<tr>
<td>Non-necrotic core type</td>
<td>21</td>
<td>18.4±3.2</td>
</tr>
<tr>
<td>Necrotic core type</td>
<td>12</td>
<td>16.3±1.4††</td>
</tr>
<tr>
<td>Non-calcified cap subtype</td>
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<td>16.1±1.5</td>
</tr>
<tr>
<td>Calcified cap subtype</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>White plaques</td>
<td>64</td>
<td>18.3±8.2</td>
</tr>
<tr>
<td>CF-dense subtype</td>
<td>50</td>
<td>2246±1.9</td>
</tr>
<tr>
<td>Jelly-like subtype</td>
<td>8</td>
<td>8.8±3.2§§</td>
</tr>
<tr>
<td>Calcified cap subtype</td>
<td>6</td>
<td>0.2±0.4§§</td>
</tr>
</tbody>
</table>

*P<0.05, ††P<0.0001 vs. white plaque. †P<0.0001 vs. non-glistening yellow plaques. ‡P<0.01 vs. non-necrotic core type. ‡‡P<0.01 vs. CF-dense subtype. §P<0.01 vs. CF-dense subtype. §§P<0.01 vs. jelly-like subtype.

CF, collagen fiber; n, number of plaques.
Histology of Glistening Yellow Coronary Plaques

Fibrous Cap Thickness
Fibrous cap thickness of NC types of GY, non-GY, and white was 125.2±54.6 (n=11), 123.3±41.4 (n=12), and 120.0±44.1 μm, respectively. There were no significant differences in cap thickness among them.

Vulnerability of Plaques
The angioscopic GY were composed of 4 histological subtypes, and the majority of them were CF scanty, irrespective of the lipid deposition patterns or presence or absence of a NC, indicating that GY were histologically vulnerable. This finding suggests applicability of the glistening yellow color by angioscopy for identification of histological vulnerable plaques.

The majority of non-GY were CF dense, indicating that they were histologically stable.

The white plaques were histologically composed of regular, jelly-like and calcified cap subtypes. The regular subtype was considered to be stable because it was CF dense. In the calcified cap subtype, the cap was composed of a lipid and CF-free plate-like calcium layer and was covered directly with the endothelia. As the cap was resistant to mechanical interventions, this subtype was considered to be stable.

Discussion

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Erosion

Plaque erosion was observed in around 25% of patients with ACS, and sudden death not infrequently occurs in this group of patients. Although erosion can be detected by angioscopy and optical coherence tomography, exactly what kind of images represents the pre-stage of erosion by these and other imaging tools has been unclear. Kolodgie et al observed deposition of abundant proteoglycans and hyaluronan matrix at the site of coronary erosion. Angheloiu et al observed superficial foam cells in erosion-prone plaque. Although deposition of proteoglycans and hyaluronan were not examined, frequent deposition of MF in the superficial layer indicates that the superficial lipid deposition group and the calcified cap subtype of yellow plaques were a pre-stage of erosion.

Nature of Glistening Yellow Color

In the present study the GY contained lipids and calcium particles in their superficial layers. β-Carotene, which is present in yellow plaques, exhibits yellow-to-orange fluorescence when irradiated by white light. Calcium crystals reflected white light in a preliminary study. It is therefore conceivable that yellow-to-orange fluorescence elicited by β-carotene and light reflected by calcium particles combined to exhibit a glistening yellow color.

Fibrous Cap Thickness

Studies using various imaging modalities have been performed to characterize vulnerable coronary plaques. It has been proposed that a plaque having a thin fibrous cap with a large NC beneath is vulnerable to disruption, but without a definite prospective clinical study. Also, it was reported that there is a close relationship between angioscopic plaque color and fibrous cap thickness measured by histology or optical coherence tomography. The thickness of the fibrous cap was not different among the NC types of GY, non-GY and white in the present study, suggesting that composition of the fibrous cap not fibrous cap thickness is the major determinant of plaque vulnerability, that is, CF density, presence or absence of MF and calcium compounds.

Generally, the excised plaque is fixed with formaldehyde or glutaraldehyde, cut into slices, and stained with dyes for microscopic measurement of fibrous cap thickness. However, this method causes loss of lipids, calcium and interstitial fluids and alters original cap thickness, and therefore the original fibrous cap thickness can not be measured by microscopy. In contrast, staining of a raw plaque slice with Oil-Red O and methylene blue does not lose lipids, calcium and interstitial fluid, and therefore keeps the original fibrous cap thickness. A preliminary comparative study revealed significant differ-
ences in fibrous cap thickness between these two different staining methods. This difference might be the reason for differences between the present and other studies. Errors and misinterpretation might occur during measurement of fibrous cap thickness by optical coherence tomography. Further comparative studies on the relationships between fibrous cap thickness measured by optical coherence tomography and by histology using a raw specimen are required.

Fishbein pointed out that a plaque with a thin fibrous cap with a large lipid pool (NC) does not necessarily disrupt. Plaque thickness is therefore not the sole determinant of plaque vulnerability.

Thus, the majority of GY had a fibrous cap and superficial layer composed of sparse CF. These pathohistological characteristics might indicate that GY, less-protected plaques against mechanical stress, are vulnerable plaques.

Study Limitations

Yellow fluorescence-like color of GY was determined by the naked eye, not by an image analyzer, and therefore is rather subjective. More objective determination can be attained by a fluorescence image analyzer.

Conclusion

GY have been proposed as being vulnerable. As CF mainly protect the coronary plaques against mechanical stress, based on CF density the plaques with dense CF were classified as stable and those with scanty CF were classified as being histologically vulnerable, and their relationship to angiographic images was examined in vitro. Coronary plaques were classified by histology into 15 subtypes.

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Disclosures

There are no conflicts of interest to disclose. This study was carried out without financial support. None of the authors have any relationships with industry.

References