Characterization of Coronary Fibrin Thrombus in Patients With Acute Coronary Syndrome Using Dye-Staining Angioscopy

Yasumi Uchida, Yasuto Uchida, Takeshi Sakurai, Masahito Kanai, Seiichiro Shirai, Toshihiro Morita

Objective—Because fibrin is transparent and almost invisible by any conventional imaging methodologies, clinical examinations of coronary fibrin thrombus have been ignored, and little is known about its role in the genesis of acute coronary syndrome (ACS). The present study was performed to visualize coronary fibrin thrombus and to examine its role in ACS.

Methods and Results—Dye-staining coronary angioscopy using Evans blue dye, which selectively stains fibrin blue but does not stain blood corpuscles, was performed for observation of globular coronary thrombi in 111 ACS patients. The thrombi were aspirated for histological examination. The thrombi were classified by visual appearance into 8 transparent, 3 light-red, 2 frosty glass–like and membranous, 32 white, 8 brown, 34 red, and 19 red-and-white in a mosaic pattern. Transparent thrombi that were not visible by conventional angioscopy were visualized as a blue structure by dye-staining angioscopy, and they were observed in patients with unstable angina (UA) and non-ST elevation myocardial infarction (NSTEMI). The thrombi caused total or subtotal coronary occlusion. The aspirated thrombi were composed of fibrin alone by histology. Fibrin-rich thrombi were visualized using dye-staining angioscopy in 60% of 50 patients with UA/NSTEMI and in 29% of 61 patients with ST-elevation myocardial infarction. By histology of the aspirated thrombi, fibrin-rich thrombi were observed in 71% of 33 patients with UA/NSTEMI and in 28% of 35 patients with ST-elevation myocardial infarction.

Conclusion—Fibrin-rich coronary thrombi were frequently observed by both dye-staining angioscopy and histology in ACS patients. Rarely, fibrin itself formed a globular thrombus and caused coronary occlusion. (Arterioscler Thromb Vasc Biol. 2011;31:1452-1460.)

Key Words: Evans blue dye ■ coronary thrombus ■ dye-staining angioscopy ■ fibrin thrombus ■ platelet thrombus
NSTEMI and STEMI.

There were no significant (P<0.05) differences in any parameters between UA+NSTEMI and STEMI.

### Backgrounds of Patients With ACS

<table>
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<tr>
<th>No. of diseased (≥75% diameter stenosis) vessels</th>
<th>UA (n=27)</th>
<th>NSTEMI (n=23)</th>
<th>UA+NSTEMI (n=50)</th>
<th>STEMI (n=61)</th>
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<td>4</td>
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### Medicines before attack

<table>
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<th>UA (n=27)</th>
<th>NSTEMI (n=23)</th>
<th>UA+NSTEMI (n=50)</th>
<th>STEMI (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrites</td>
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<td>3</td>
<td>6</td>
<td>8</td>
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<tr>
<td>Calcium antagonist</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Diuretics</td>
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<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>AT1 blocker</td>
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<td>6</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
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<td>3</td>
<td>5</td>
</tr>
<tr>
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<td>1</td>
</tr>
<tr>
<td>No medicines</td>
<td>11</td>
<td>8</td>
<td>19</td>
<td>26</td>
</tr>
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</table>

LAD indicates left anterior descending artery; AT1, blocker; angiotensin receptor 1 blocker; LCx, left circumflex artery; RCA, right coronary artery. There were no significant (P<0.05) differences in any parameters between UA+NSTEMI and STEMI.

### Angioscopy System

The angioscopy system was composed of a light source (OTV-A, Olympus Corp, Tokyo, Japan), a 5-F angioscope (VecMover, Clinical Supply Company, Gifu, Japan), and a color chilled charge-coupled device camera (CSVEC-10, Clinical Supply Company). Before the angioscopy examination was carried out, the white balance of the camera was adjusted for color correction. The system has been approved for clinical use by the Japanese Ministry of Health, Labor and Welfare. Details of this system are described elsewhere.11

### Conventional Angioscopy Procedure

All patients received heparin (5000 IU IV) just before the procedure and nitroglycerin (200 μg IC) just before coronary angiography. After coronary angiography, an angioscope was introduced into the culprit coronary artery. The thin fiberscope that was incorporated into the angioscope was advanced into the artery so that the culprit coronary artery. The thin fiberscope tip was located just proximal to the thrombus. The balloon catheter was inflated again, and the thrombus was observed.

Thereafter, the fiberscope incorporated into the angioscope was advanced across the thrombus distally, usually through the space between the thrombus and the coronary wall, to observe the entire aspect of the thrombus. Subsequently, the angioscope was replaced by an aspiration catheter (Astray, Johnson and Johnson Co) to aspirate the thrombus.

Thrombi were defined semiquantitatively by dye-staining angioscopy as fibrin-rich, fibrin-poor, or intermediate when the blue surface area was approximately two thirds or more, one third or less, or between one third and two thirds of the entire surface of the proximal end (head) of the thrombus, respectively.

### Angioscopic Definition of Thrombi and Plaques

Thrombus and plaques were defined based on the angioscopy guidelines of the Japanese Association for Cardioangiography.12,13 The definitions are as follows: a thrombus is a mural or protruding mass, mobile or not mobile, adherent to the vessel wall at least at 1 portion but clearly separated from the vessel wall, with changes in shape, location, or both due to saline solution flushing or to guide wire and angioscope manipulation. In contrast, a plaque is defined as a mass protruding into the lumen, nonmobile, not separated from the adjacent vascular wall, that does not change shape or location on saline solution flushing or on guide wire and angioscope manipulation.

In the present study, thrombi that protruded into the lumen, namely globular thrombi, were examined.

### Classification of Thrombus Color

Thrombi were classified by color into transparent, frosty glass-like (white but semitransparent), red (red area approximately two thirds or more of the surface of the proximal end, ie, head, of the thrombus), light red (dilated red), white (white area approximately two thirds or more), white and red in a mosaic pattern (so-called mixed thrombus; one third to two thirds red), or brown.11–13 Neutral intraobserver agreement about these colors was 96%, 100%, 92%, 93%, 96%, 98%, and 90%, respectively, and neutral intraobserver agreement interobserver agreement was 92%, 96%, 100%, 94%, 92%, 91%, and 100%.

### Dye-Staining Angioscopy Procedure

Evans blue (EB) is a blue dye that was used clinically for cardiac output measurement many years ago. Since 1995, this dye has been clinically administered into the arterial system for dye-staining angioscopy. Beneficial effects of this dye in preventing coronary restenosis have been reported.14,15 Histological examinations in animals and patients revealed that this dye stains fibrin and damaged endothelial cells but not blood corpuscles.10,11–13

Discrimination of endothelial flap and globular thrombus was made according to the criteria described in the guidelines.10

After observation by conventional angioscopy, 1 mL of 2.5% EB solution was injected during balloon inflation into the artery through the flush channel of the angioscope to stain the thrombus, and then the balloon was deflated for blood flow restoration. One to 2 minutes later, the balloon was inflated again, and the thrombus was observed.

Thrombi were defined semiquantitatively as fibrin-rich, fibrin-poor, or intermediate when the blue surface area was approximately two thirds or more, one third or less, or between one third and two thirds of the entire surface of the proximal end (head) of the thrombus, respectively.

### Histology

The aspirated major thrombus was fixed with 10% formaldehyde solution. The center of the aspirated clot was cut into slices and stained by phosphotungstic acid hematoxylin stain for fibrin, by immunostaining with von Willebrand factor for platelets, and by Giemsa stain for both fibrin and platelets. When multiple clots were aspirated, the first clot of significant size that was aspirated.

By using slides of Giemsa stain, a thrombus was defined as fibrin-rich when its superficial layer was occupied by fibrin and as fibrin-poor when it was not. Fibrin-poor thrombus was defined as platelet-rich, red blood cell (RBC)-rich, or debris-rich when the superficial layer was occupied by platelet aggregates, RBCs, or plaque debris, respectively. Examination of the composition was limited to the superficial layer of the thrombus to correlate micro-
scopic changes to angioscopic appearance because angioscopic imaging was limited to thrombus surface.

Statistical Analysis
The data were tested using the Fisher exact test. A $P<0.05$ was considered significant.

Results
Dye-staining angioscopy was successful in all 111 patients. Globular thrombi were aspirated in 64 patients. In 4 patients, thrombi were aspirated but were small clusters and could not be used for histological examinations. Aspiration of thrombi failed in 18 patients, and aspiration therapy was not carried out because of coronary anatomy, distal movement of thrombi, or hemodynamic instability in 25 patients.

Transparent Thrombi
Figure 1A to 1C shows a patient with UA in whom the left anterior descending artery was totally occluded by angiography. However, a large residual lumen was observed and nothing was seen in the residual lumen by conventional angioscopy. Dye-staining angioscopy exposed a blue structure occupying the residual lumen, suggesting that the structure was a fibrin thrombus. By advancing the angioscope across the structure, it was revealed that no other thrombus, such as red thrombus, existed behind the structure, indicating that this structure caused total occlusion.

Figure 1D shows the aspirated structure same as that in Figure 1C. By histology, a small number of RBCs and platelets was observed in loose fibrin networks (Figure 1F).

Light-Red Thrombi and Frosty Glass–Like Membranous Thrombi
Light-red thrombi were observed in 3 patients with NSTEMI. They were somewhat transparent (Figure 2B). They were stained blue with EB (Figure 2C). By histology, a small number of platelets was observed in loose fibrin networks (Figure 2F).

Frosty glass–like membranous thrombi were observed in 2 patients with UA (Figure 3B). Except for disrupted plaque, other thrombi were not observed behind them by angioscopy. They were stained blue with EB (Figure 3C). By histology, they were composed of fibrin attached by a small number of platelets (Figure 3 and Table 2).

White Thrombi
Two types of white thrombi were observed by dye-staining angioscopy; those stained with EB (Figure 4B and 4C). The incidence of these 2 subtypes was not different (Table 2). The former type of thrombi were further classified into those uniformly stained with EB and those stained with EB in a mosaic pattern (Figure 4E and 4F). There was a tendency that these subtypes of white thrombi were more frequently observed in UA+NSTEMI patients than in STEMI patients (Table 2). The distal portion of these thrombi was often red, indicating the existence of RBCs. Thrombi were aspirated in 15 of 32 patients. By histology, fibrin was rich in the majority of these thrombi (Table 3).
Brown Thrombi
Brown thrombi were stained blue in 6 cases and not stained in 2. By histology, they were composed of plaque debris and fibrin or debris alone, i.e., debris thrombi. This type of thrombus was observed in STEMI patients but not in UA+NSTEMI patients (Figure 4E and 4F and Tables 2 and 3).

Red Thrombi
The majority of red thrombi were solid and globular. The solid red thrombi were not stained with EB. By histology, the thrombi that were not stained were fibrin-poor and RBC-rich, at least in their cortex, although a fibrin core was frequently observed (Figure 5B and 5C and Tables 2 and 3).

Mixed Thrombi (Red and White in a Mosaic Pattern)
Thrombi that were red and white in a mosaic pattern were observed in 6 patients with UA+NSTEMI and 13 patients with STEMI. The thrombi were stained in 10 cases and not stained in 9. By histology, fibrin was rich in the thrombi that were stained but was not in those not stained (Figure 5E and 5F and Tables 2 and 3).

Incidence of Fibrin-Rich Thrombus in Subtypes of ACS
By dye-staining angioscopy, fibrin-rich thrombus was observed in 60% of 50 UA+NSTEMI patients and in 29% of 61 STEMI patients. By histology, fibrin was rich in 71% of 33 UA+NSTEMI patients and in 28% of 35 STEMI patients who underwent successful thrombus aspiration therapy (Table 3).

Discussion
In the present study, fibrin-rich coronary thrombi were frequently observed in ACS patients. The incidence of fibrin-rich thrombi was greater in UA+NSTEMI patients than in STEMI patients.

In previous studies, fibrin that was formed by adding thrombin to fibrinogen was stained blue with EB. Histological examinations on thrombus revealed that fibrin was stained but blood corpuscles and plaque debris were not.10,11,13 EB was therefore used for identification of fibrin in the present study.

Transparent Fibrin Thrombus
Despite total occlusion by angiography, a residual lumen was observed by conventional angioscopy in the occluded coronary segment in a certain group of UA+NSTEMI patients. The residual lumen was stained blue with EB, and the aspirated clot was proven histologically to be a fibrin thrombus, indicating that a fibrin thrombus obstructed the residual lumen. Because fibrin is transparent, it is likely that the thrombus could not be visualized by conventional angioscopy.

It is generally considered that fibrin networks are formed by platelet aggregation and fibrin polymerization and that they trap the other blood corpuscles to form a thrombus.5 The present findings indicate that fibrin itself formed a globular

Table 2. Relationships Between Fibrin Thrombus Visualized by Dye-Staining Angioscopy and Subtypes of ACS

<table>
<thead>
<tr>
<th>Thrombus Color and Dye Staining</th>
<th>ACS</th>
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<td></td>
<td>UA</td>
<td>NSTEMI</td>
<td>UA+NSTEMI</td>
<td>STEMI</td>
</tr>
<tr>
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<td>3</td>
<td>8</td>
<td>0†</td>
</tr>
<tr>
<td>Fibrin-rich</td>
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<td>3</td>
<td>8</td>
<td>0†</td>
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<td>3</td>
<td>0</td>
</tr>
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<td>0</td>
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<td>Not stained</td>
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</tr>
<tr>
<td>Total</td>
<td>17 (62%)</td>
<td>13 (61%)</td>
<td>30 (60%)</td>
<td>18* (29%)</td>
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</table>

*P<0.05, †P<0.01 vs UA+NSTEMI.

n indicates number of thrombi; stained, stained with EB.
thrombus and obstructed the coronary lumen. Such a phenomenon has not been described previously. The possible mechanisms for this obstructive fibrin thrombus formation are as follows: (1) a very early stage of thrombus formation in which fibrin was formed in the presence of a small number of tiny platelet aggregates, or (2) a specific type of fibrin was formed. The reason this type of thrombus was confined to UA/NSTEMI patients remains unclear.

In a certain group of UA/NSTEMI patients, a light-red thrombus and a membranous, frosty glass–like thrombus was observed by conventional angioscopy. These types of thrombi were stained blue with EB. They were fibrin thrombi attached by a small number of platelets and WBCs, or a small number of RBCs histologically. It is not clear whether these types are an early stage of tight thrombus formation or are due to loose fibrin. Because no other clot was aspirated, the present findings indicate that these types of thrombi could obstruct the coronary lumen and cause ACS.

**White Thrombus**

White thrombi were more frequently observed in UA+NSTEMI patients than in STEMI patients. This result is in accordance with other reports. It is believed that residual blood flow washes the RBCs covering the surface of the thrombi, and platelet aggregates become exposed to exhibit a white color. It has been generally believed that white thrombi are platelet thrombi and predominant in UA/NSTEMI patients. The findings of the present study indicate that in contrast to this generally believed concept, fibrin-rich white thrombus is not rare and plays an important role in formation of thrombus in UA+NSTEMI patients.

**Red thrombus**

The occlusive red thrombi were solid red and were more frequently observed in STEMI patients, as was reported. The majority of them were not stained blue with EB. Histological examination revealed that the cortex of the

**Figure 2.** Light-red thrombus. A, Severe stenosis of the middle segment of the left anterior descending artery in a 62-year-old female with UA, 11 hours after the onset of attack (arrow). A light-red structure was observed in the stenotic portion (B, arrow). After EB injection, the structure was stained blue (C, arrow), suggesting that RBCs were trapped by a fibrin network. Arrowhead in C indicates a disrupted plaque seen through the structure. The aspirated thrombus (same as that in Figure 1C) was shown to be light-red by ex vivo angioscopy (D, arrow), composed of a loose fibrin network (E, arrow) by phosphotungstic acid hematoxylin stain, and composed of a small number of RBCs (F, arrowhead) and platelets (F, arrow) by immunostaining with von Willebrand factor. Scale bars=100 μm (E) and 20 μm (F).

**Figure 3.** Frosty glass–like thrombus and thrombus composed of fibrin and platelets. A, Subtotal occlusion of the proximal segment of left anterior descending artery in a 66-year-old male with UA, 10 hours after the onset of attack (arrow). A frosty glass–like structure occupied the obstructed segment through which the distal portion was seen (B, arrow). After EB injection, the frosty glass–like structure was stained blue, indicating that it was composed of fibrin (C, arrow). D, Total occlusion of left anterior descending artery in a 56-year-old male with NSTEMI, 8 hours after the onset of attack (arrow). White thrombi were observed in the occluded portion (E, arrows and arrowhead). White portions indicated by white arrows in E were stained blue with EB (F, arrows), indicating that they were composed mainly of fibrin, whereas the white portions indicated by arrowhead in E were not stained with the dye (F, arrowhead), indicating that these portions were composed mainly of platelets.
tightly structured thrombi was densely covered with RBCs but not covered or only sparsely covered with fibrin.

**Brown Thrombus**

A brown structure was observed in an angiographically obstructed coronary segment in STEMI patients. The structure was stained blue or not stained with EB. In a preliminary in vitro study using excised human coronary arteries, the plaque debris was brown, but it was not stained with EB. The brown structure observed in the present study was histologically a mixture of fibrin and plaque debris; fibrin was dominant in the former, and debris was dominant in the latter. Most likely, plaque debris was ejected from the disrupted lipid core and was wrapped by a fibrin network to form an occlusive thrombus, ie, debris thrombus, resulting in STEMI.

**Possible Mechanisms for Different Thrombus Formation in Subtypes of ACS**

Fibrin-rich thrombus was detected more frequently in patients with UA+NSTEMI than in those with STEMI by both dye-staining angioscopy and histology.

Recently, Silvain observed substantial fibrin in the coronary thrombi aspirated from STEMI patients, as in the present study.

It is known that hemodynamic, cellular, and plasma mechanisms determine fibrin network density and stability; platelets support the formation of a dense and stable fibrin network via interactions between the \(\alpha_{IIb}\beta_3\) integrin and fibrin network; highly procoagulant extravascular cells (eg, fibroblasts and smooth muscle cells) support the formation of dense fibrin networks that are resistant to fibrinolysis, whereas unstimulated intravascular cells (eg, endothelial cells) produce coarse fibrin networks that are susceptible to fibrinolysis; smoking causes sticky fibrin; polyphosphates that is secreted by platelets produces a tight fibrin network; with aging, fine fibrin networks are formed; fibrin networks are altered by RBCs or fibrinogen concentration; and inflammatory cytokines cause production of dense fibrin networks.

In addition to the above-mentioned mechanisms, coronary blood flow and thrombus age may determine thrombus composition.

Together, these differences are likely to have caused the ACS subtype-related variety of fibrin networks and accordingly different thrombi.

**Significance of Dye-Staining Angioscopy**

Studies on platelet aggregates and RBCs in the genesis of ACS have been intensively undertaken clinically using conventional angioscopy, intravascular ultrasound, or optical coherence tomography because platelet aggregates and RBCs are visible and can be imaged.

Because fibrin is not visible, plasma fibrin-D-dimer has been studied instead, and a relationship to severity of culprit coronary lesion became clear. Visualization of fibrin has been attempted in vitro using magnetic resonance imaging, but this method is still not applicable clinically. Conventional intravascular ultrasound and optical coherence tomography cannot image fibrin because fibrin is echolucent and optically transparent, although a new optical coherence tomography system has a potential use for fibrin imaging. Thus, there are no clinically available imaging modality other than dye-staining angioscopy that enable direct visualization of fibrin.

**Dye-Staining Angioscopy for Analysis of Thrombus Formation**

With the aid of dye-staining angioscopy, it was revealed that fibrin thrombus, which is not visible by any other imaging technologies, causes coronary occlusion. Also, it became evident that white thrombi, which have been believed to be composed of platelet aggregates, are often composed mainly of fibrin. Furthermore, dye-staining angioscopy revealed the existence of residual fibrin thrombi exhibiting fluffy coronary occlusion.
Dye-Staining Angioscopy for Selection of Therapeutic Modality

It is well known that platelet aggregates are susceptible to antiplatelet agents such as aspirin, clopidogrel, and eptifibatide and resistant to fibrinolytic agents such as tissue plasminogen activators, and that the reverse is true of fibrin. By analyzing by dye-staining angioscopy whether fibrin or platelets are dominant in the target thrombus, an effective therapeutic modality can be selected.


dye-staining angioscopy

Although an intra- and interobserver agreement system was used, classification of thrombus color was rather subjective because the color was determined by naked eyes in the present study and other studies.11,13,31,32

Quantitative classification of coronary plaques by intensity ratio of the 3 primary colors separated from the plaque color using color analyzer was established because plaque color is simply either white or yellow, and it was therefore easy to discriminate them by color wavelength.33

In contrast, quantitative classification of thrombus by color wavelength is more difficult because thrombus color is variable, ranging from white to red, yellow, or brown. Nevertheless, discrimination of thrombus by color wavelength will be established for objective and quantitative assessment of thrombus in future.

The time required for dye-staining angioscopy is 13 minutes or less, and a cardiologist who is skilled in conventional coronary interventions can easily perform this imaging technology within this amount of time. The dye can be injected through the flush channel of the angioscope, and then a fibrinolytic or an antiplatelet agent can be administered through the same flush channel into the coronary artery where a thrombus exists. When necessary, the angioscope can soon be replaced by a catheter for mechanical therapies, such as stent implantation and thrombus aspiration. Therefore, dye-staining angioscopy is recommended to explore whether or not fibrin is participating in phenomena unexplainable by other imaging modalities, such as angiography, intravascular ultrasound, and optical coherence tomography.

Conclusions

It is generally believed that coronary platelet aggregates play a key role in the genesis of ACS. Because they are visible, they have been intensively examined by various imaging modalities in ACS patients, and considerable knowledge has been accumulated. In contrast, because fibrin is transparent and almost invisible, clinical examinations of fibrin have been ignored, and its roles in coronary thrombus formation and accordingly in the genesis of ACS are not well understood.

Dye-staining coronary angioscopy using EB, which stains fibrin blue but does not stain the blood corpuscles, was performed in 111 ACS patients. Fibrin and platelet aggregates could be differentiated using this imaging modality.

Fibrin-rich thrombus was detected by angioscopy in 60% of 50 UA/H11001 NSTEMI patients and in 29% of 61 STEMI patients. By histology, fibrin dominant thrombus was observed in 71% of 33 UA/H11001 NSTEMI patients and in 28% of 35 STEMI patients who underwent successful thrombus aspiration therapy.

Fibrin not only formed a network to form a thrombus with blood corpuscles, but the fibrin itself formed a globular thrombus (fibrin thrombus) and caused total coronary occlusion. In contrast to the generally believed concept that white coronary thrombi are composed of platelet aggregates, white thrombi rich in fibrin were frequently observed in the present study. Thus, it was concluded that in addition to platelet
thrombi, fibrin thrombi play an important role in the genesis of ACS.

Because fibrin cannot be imaged by any other modalities, dye-staining angioscopy may help with clarification of roles of fibrin in the underlying mechanisms of coronary thrombus formation and with the selection of an effective therapeutic modality for treating ACS.

The present study is, to the best of our knowledge, the first in vivo visualization of coronary fibrin thrombus in humans.

**Study Limitations**

This study had two main limitations. First, angioscopy enabled observation of the surface but not the interior of the thrombus. Second, angiographic images were fish-eye images because they were obtained through a lens. Quantitative assessment of thrombus was therefore difficult.

**Disclosures**

None.

**References**


Characterization of Coronary Fibrin Thrombus in Patients With Acute Coronary Syndrome Using Dye-Staining Angioscopy
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Arterioscler Thromb Vasc Biol. 2011;31:1452-1460; originally published online March 17, 2011;
doi: 10.1161/ATVBAHA.110.221671
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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