

## Histological Characteristics of Plaque With Ultrasonic Attenuation: A Comparison Between Intravascular Ultrasound and Histology

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### Abstract

**Objectives.** Intermediate echogenic plaque without acoustic shadow on intravascular ultrasound (IVUS) imaging has been recognized as fibrous plaque. Such echogenic plaque with ultrasonic attenuation may have higher risk for distal flow disturbance (slow flow/no-reflow) during percutaneous coronary intervention. However, histological evaluation of plaque with ultrasonic attenuation has not been performed. This study evaluated the histological characteristics of plaque with ultrasonic attenuation assessed by IVUS.

**Methods.** By using IVUS, 36 samples of human cadaveric coronary arterial echogenic plaque (percentage plaque area > 40%) without calcium were selected, and classified into the attenuation group; plaque with ultrasonic attenuation, and the non-attenuation group; plaque without attenuation. These plaques were classified for fibrous, fibrofatty, calcium, and necrotic core areas by histological examination.

**Results.** True fibrous plaque was found in 91.7% of the non-attenuation group, but only 68.0% of the attenuation group ( $p < 0.01$ ). On the other hand, the percentage fibrofatty and necrotic core plaque areas in the attenuation group were significant larger than those in the non-attenuation group (fibrofatty:  $16.3 \pm 13.8\%$  vs  $2.7 \pm 3.1\%$ ,  $p < 0.01$ ; necrotic core:  $13.0 \pm 19.4\%$  vs  $3.9 \pm 8.0\%$ ,  $p = 0.03$ ).

**Conclusions.** Plaque with ultrasonic attenuation contains more fibrofatty tissue and necrotic core compared to fibrous plaque without attenuation.

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### Key Words

- Intravascular ultrasound (intracoronary imaging)
- Plaque (fibrofatty, necrotic core, histological analysis)

### INTRODUCTION

Intravascular ultrasound (IVUS) is widely used during percutaneous coronary intervention (PCI) in the cardiac catheterization laboratory. IVUS provides not only morphometric but also morphological lesion characteristics. According to previous

validation studies, intermediate echogenic plaque without acoustic shadow by IVUS imaging has been recognized as hard or fibrous plaque.<sup>1,2)</sup> Although the fibrous rather than the fatty type of atherosclerotic plaque seems more stable, several studies have shown that such echogenic fibrous plaque with ultrasonic attenuation may have para-

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doxically higher risk for distal flow disturbance (slow flow/no-reflow phenomenon) during PCI.<sup>3,4)</sup> However, the mechanism of slow flow/no-reflow caused by the fibrous plaque has not been examined. Therefore, this study evaluated the histological characteristics of the plaque with ultrasonic attenuation assessed by IVUS to estimate the possible mechanism of distal flow disturbance.

## SUBJECTS AND METHODS

One hundred and seven coronary arteries were harvested from the heart in a series of 36 human cadavers. Of these 36 cadavers, 6 had symptomatic cardiovascular disease (16.7%). The study protocol was approved by the ethics committee of Kawasaki Medical School, and written informed consent was obtained.

Hypercholesterolemia was defined as total cholesterol level  $\geq 240$  mg/dl or current medication for hypercholesterolemia. Hypertension was defined as systolic blood pressure  $\geq 140$  mmHg, diastolic blood pressure  $\geq 90$  mmHg, or current antihypertensive medication. Diabetic patients were receiving insulin or some kind of oral hypoglycemic agent. All blood samples and blood pressure data were obtained during hospitalization.

Samples about 5 cm long of the proximal site of the three major coronary arteries, including the left anterior descending coronary artery, left circumflex artery, and right coronary artery were obtained from the cadaver at autopsy within 3 hr of death. Surrounding soft tissues were dissected from each specimen. Small arterial perforators and their branches were tied off with sutures, and the distal end of the arteries was occluded with a large cork. A 7F sheath was sewn into the proximal end of the arteries to ensure complete closure. Saline (0.9%) solution at 37°C was infused through the side arm of the sheath. The pressure inside the coronary artery was maintained at the physiologic level (60 to 80 mmHg) with a syringomanometer connected to the infusion. An IVUS catheter (Atlantis SR Pro-2.5F, 40-MHz; Boston Scientific) was inserted sequentially through the diaphragm of the sheath. Serial IVUS images were obtained using an automatic pullback device at 0.5 mm/sec.

IVUS images were analyzed off-line by commercially available image processing software (Netra 3D IVUS system, ScImage). Cross-sectional areas (CSA) of the external elastic membrane (EEM), lumen, and plaque plus media (P + M) were mea-

sured. Percentage plaque area was calculated as  $(P + M \text{ CSA} / \text{EEM CSA}) \times 100\%$ . Lesions with percentage plaque area  $> 40\%$  were considered significant and selected for this study.<sup>5)</sup> By using IVUS, fibrous plaque lesions, assessed according to the standards of the American College of Cardiology, were collected and classified into two groups, the attenuation group; plaque with ultrasonic attenuation of more than 90 degrees of the plaque, and the non-attenuation group; plaque without attenuation.<sup>3)</sup> There were no significant differences in "fibrous" plaque with attenuation plaque between cadavers with (2 in 6) and without cardiovascular disease (7 in 30). The arc of attenuation was measured and classified into two groups, attenuation  $> 120^\circ$ , and attenuation  $\leq 120^\circ$ .

After IVUS imaging, each coronary artery was pressure-fixed in 10% neutral buffered-formalin. Following 48 hr of fixation, standard paraffin embedding was performed. For every 400  $\mu\text{m}$  of each coronary artery, three series of 4- $\mu\text{m}$  thick sections were cut and stained with hematoxylin and eosin, Masson's trichrome, and elastica van Gieson stains. The image slice with the attenuation assessed by IVUS was compared with the corresponding histological images. Experienced observers unaware of the IVUS image used quantitative computerized planimetry to analyze the fibrous, fibrofatty, calcium, and necrotic core areas. By using National Institutes of Health (NIH) image software, the measured areas were standardized by the total plaque area and expressed as percentage areas. Furthermore, the plaque histological characteristics of the segments within the ultrasonic attenuation were also investigated in the attenuation group.

## Statistical analysis

Statistical analysis was performed with StatView 5.0 version (SAS Institute). Continuous variables are reported as mean  $\pm$  SD. Student's *t*-test was used to differentiate two sets of data with normal distribution. If no significance was found by Student's *t*-test, then the Mann-Whitney *U*-test was performed. A *p* value  $< 0.05$  was considered statistically significant.

## RESULTS

### Baseline patient characteristics

Of these 36 patients, 20 fibrous plaques with attenuation from 9 patients and 19 fibrous plaques

**Table 1** Baseline patient characteristics

	Attenuation group (n=9)	Non-attenuation group (n=12)	p value
Age (yr, mean ± SD)	71 ± 14	63 ± 17	0.08
Male	55.6%	75.0%	0.40
Hypertension*	22.2%	33.3%	1.00
Diabetes mellitus	33.3%	33.3%	1.00
Hypercholesterolemia <sup>†</sup>	22.2%	16.6%	0.58
Current smoking	33.3%	33.3%	1.00

\*Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of an antihypertensive drug.

<sup>†</sup>Hypercholesterolemia was defined as total cholesterol level ≥240 mg/dl or medication use.

**Table 2** Histological analysis

	Attenuation group	Non-attenuation group	p value
EEM CSA (mm <sup>2</sup> )	8.51 ± 3.04	7.52 ± 1.93	0.37
Lumen CSA (mm <sup>2</sup> )	3.01 ± 1.54	2.70 ± 1.30	0.38
Plaque plus media CSA (mm <sup>2</sup> )	5.50 ± 2.29	4.85 ± 1.20	0.42
Plaque plus media CSA / EEM CSA (%)	64.8 ± 15.0	65.2 ± 9.5	0.79

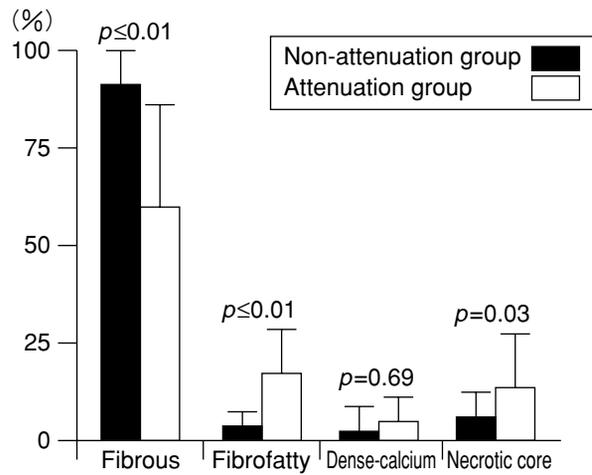
Values are mean ± SD.

EEM = external elastic membrane ; CSA = cross-sectional area.

without attenuation from 12 patients were selected. Baseline patient characteristics are listed in **Table 1**. There were no significant differences in age, sex, occurrence of diabetes, hypertension, hyperlipidemia, and current smoking between the two groups.

**Histological examination**

Morphometrical analysis from the histological specimens of the selected lesions is shown in **Table 2**. There were no significant differences in EEM, lumen, and P + M CSAs between the attenuation group and the non-attenuation group. As a result, percentage plaque area was similar between the two groups. **Fig. 1** shows the morphological analysis of the histological specimens. True fibrous plaque was found in 91.7% of the non-attenuation group, but only 68.0% of the attenuation group ( $p < 0.01$ ). On the other hand, percentage fibrofatty and necrotic core tissue areas in the attenuation group were significant larger than in the non-attenuation group



**Fig. 1** Histological analysis of the plaque with and without ultrasonic attenuation

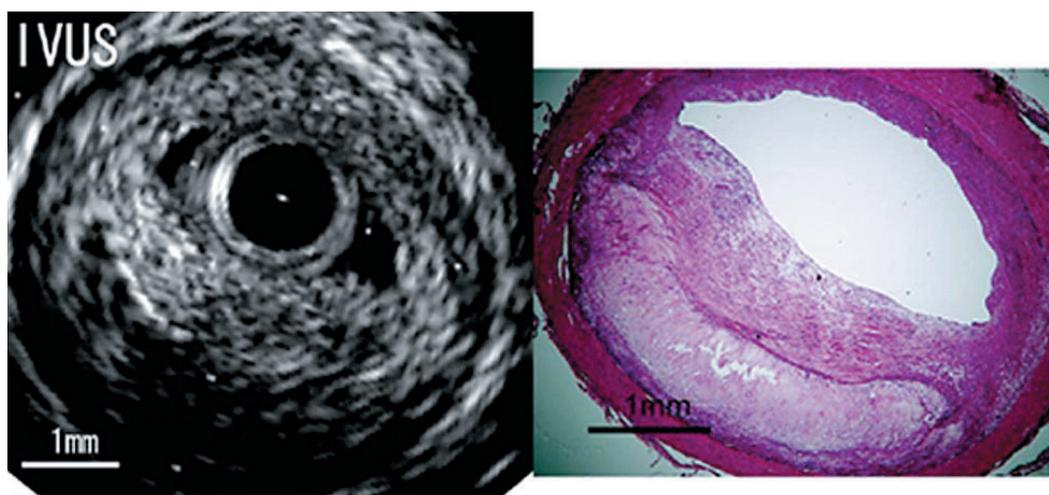
Percentage fibrofatty and necrotic core plaque areas in the attenuation group (white bars) were significant larger than in the non-attenuation group (black bars).

(fibrofatty :  $16.3 \pm 13.8\%$  vs  $2.7 \pm 3.1\%$ ,  $p < 0.01$ ; necrotic core :  $13.0 \pm 19.4\%$  vs  $3.9 \pm 8.0\%$ ,  $p = 0.03$ ). **Fig. 2** shows a representative IVUS image of the plaque with attenuation and the corresponding histological image. A large necrotic core can be seen in the attenuated part of the plaque. The percentage plaque burden area of the attenuation group was  $70.9 \pm 16.5\%$ .

A significantly large percentage plaque burden area (greater than 75%) occurred in 12 of 39 specimens (31%). Percentage fibrous plaque area in the attenuation  $> 120^\circ$  group was significantly smaller than that in the attenuation  $\leq 120^\circ$  group ( $75.8 \pm 20.8\%$  vs  $56.3 \pm 18.6\%$ ,  $p = 0.01$ ). On the other hand, percentage necrotic core plaque area in the attenuation  $> 120^\circ$  group was significantly larger than that in the attenuation  $\leq 120^\circ$  group ( $5.4 \pm 9.0\%$  vs  $24.4 \pm 25.3\%$ ,  $p = 0.03$ ).

**DISCUSSION**

The present study investigated the histological characteristics of plaque with ultrasonic attenuation assessed by IVUS. Histological examination revealed that fibrofatty plaque and necrotic core were more frequently found in echogenic plaque with attenuation. This study is the first report to evaluate histological characteristics of whole coronary sections with ultrasonic attenuation by IVUS. In general, lipid components are important as a cause of ultrasonic signal attenuation. The large



**Fig. 2** Representative intravascular ultrasound image of attenuation (*left*) and photograph of histological sample of plaque (*right*)

Large necrotic core tissue was identified as the attenuation area in the corresponding intravascular ultrasound image.

fibrofatty and necrotic components tend to attenuate the ultrasonic signal by scattering.<sup>3)</sup>

In abdominal ultrasonic imaging, pure cholesterol gallstone shows a strong acoustic shadow.<sup>6)</sup> Echogenic coronary plaque without acoustic shadow by IVUS has been recognized as “fibrous” plaque.<sup>1,2)</sup> However, several studies have shown that such echogenic “fibrous” plaque with ultrasonic attenuation may have higher risk for distal flow disturbance (slow flow/no-reflow phenomenon) during PCI.<sup>3,4)</sup> Furthermore, ultrasonic attenuation has not been nominated for a plaque qualitative classification in the existing IVUS guidelines.<sup>1,2)</sup>

Histopathologic examination of plaque with ultrasonic attenuation obtained by directional atherectomy (DCA) has been reported.<sup>3)</sup> The histological examination suggested that microcalcification with expansive positive arterial remodeling was associated with attenuation. However, in our present study, fatty tissue plaque area in the attenuation group was significantly larger than in the non-attenuation group. The calcification area in the attenuation group was not significantly different. Furthermore, the percentage necrotic core plaque area in the attenuation  $> 120^\circ$  group was significantly larger than that in the attenuation  $\leq 120^\circ$  group.

The limitation of the previous study is that the specimen obtained by DCA from acute coronary syndrome and stable angina pectoris patients does

not necessarily reflect the segments with attenuation. In that case, plaque with attenuation is detected in both acute coronary syndrome and stable angina pectoris. More importantly, the entire vessel structure could not be evaluated by this method. Thus, the methodological difference (DCA specimen vs postmortem examination) and possibly the small sample size ( $n = 6$ ) of the previous study might explain the discordant findings.<sup>3)</sup>

In general, necrotic core or fatty tissue (lipid pool/lipid core) protruding into the vessel lumen may cause intraluminal thrombus formation in patients with acute coronary syndrome.<sup>7)</sup> The presence of such lipid compounds is a predictor of the slow flow/no-reflow during PCI.<sup>8–11)</sup> In addition, atherosclerotic plaque with ultrasonic attenuation might cause transient coronary flow deterioration during PCI in patients with acute coronary syndrome.<sup>4)</sup> Thus, these lipid components might cause distal microembolization, resulting in the slow flow/no-reflow phenomenon during PCI.

Pre-procedural detection of plaque with ultrasound attenuation might be feasible for risk stratifying the regions with higher risk for slow flow during PCI. In addition, selective use of distal protection devices may be mandated for preventing serious complications during PCI.<sup>12,13)</sup>

### Study limitations

First, the histological examination might be

influenced by the shrinkage artifact in the processing of histological specimens, in spite of the use of pressure fixation.

Second, recent spectral analysis of IVUS radiofrequency (Virtual Histology) data has the potential to provide detailed quantitative information on plaque components. By using such IVUS Virtual Histology, it might be possible to evaluate lipid components with ultrasonic attenuation *in vivo*.

Third, because this study was a postmortem IVUS and histological examination, it is impossible

to elucidate the direct relationship between the plaque component with ultrasound attenuation and distal flow disturbance during PCI *in vivo*. Further study will be needed to investigate the relationship between the degree of coronary arterial stenosis assessed by coronary angiography and plaque attenuation assessed by IVUS.

Finally, this is a retrospective analysis of a small number of specimens from a single center. Thus the results need to be confirmed by a larger multicenter study.

## 要 約

### 超音波減衰を有するプラークの組織性状: 血管内エコーと組織の比較検討

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**目 的:** 血管内エコー上で音響陰影を伴わない中等度のエコー輝度のプラークはfibrousプラークと認識されている。Fibrousと思われるプラークで石灰化を疑わせる高輝度領域を伴わないにもかかわらず、病変後方のエコーが著しく減衰している像(attenuation)を有した例では、経皮的冠動脈形成術後にslow flow/no-reflowをきたしやすいとの報告が散見される。しかし、attenuationを有したプラークの組織性状を比較検討した報告は少ない。今回我々は、高周波血管内エコーでattenuationを有するプラークの組織性状を検討した。

**方 法:** 剖検36例から得られた冠動脈を対象に血管内エコー(Atlantis SR Pro-, 40-MHz)により観察を行った。プラーク面積率>40%以上でカルシウム病変を伴わない中等度のエコー輝度プラークを、attenuationを有するプラーク(attenuation群)、attenuationを有さないプラーク(non-attenuation群)の2群に分類した。血管内エコー後にattenuationを認めた箇所の組織性状を検討しfibrous, fibrofatty, calcium, necrotic coreの4つに分類した。

**結 果:** 組織性状の検討でfibrousはnon-attenuation群の91.7%に存在したが、attenuation群では68.0%のみであった( $p < 0.01$ )。一方でfibrofattyとnecrotic coreはattenuation群で有意に高値であった(fibrofatty:  $16.3 \pm 13.8\%$  vs  $2.7 \pm 3.1\%$ ,  $p < 0.01$ ; necrotic core:  $13.0 \pm 19.4\%$  vs  $3.9 \pm 8.0\%$ ,  $p = 0.03$ )。

**結 論:** 血管内エコー上でattenuationを有するプラークではfibrofattyおよびnecrotic coreを有意に多く検出した。

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