

In Vivo Quantitative Tissue Characterization of Angiographically Normal Coronary Lesions and the Relation With Risk Factors

— A Study Using Integrated Backscatter Intravascular Ultrasound —

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Background Autopsy studies have shown atherosclerotic changes in angiographically normal coronary lesions (ANCL), and conventional intravascular ultrasound shows intimal thickening in these lesions, but cannot differentiate the lipid core. Accurate characterization of ANCL is essential to prevent progression to coronary artery disease.

Methods and Results ANCL (n=120) were analyzed by integrated backscatter intravascular ultrasound (IB-IVUS) in 30 patients with stable angina pectoris. Of the 120 arterial segments analyzed by IB-IVUS, 78 (65%) showed lipid cores of $0.69 \pm 0.35 \text{ mm}^2$ with fibrous caps of $200 \pm 100 \mu\text{m}$ thick, 44 (37%) had intimal hyperplasia with a thickness of $350 \pm 100 \mu\text{m}$, and 65 (54%) showed fibrosis in the intimal wall without lipid core with a thickness of $450 \pm 150 \mu\text{m}$. The diabetes mellitus (DM) group (n=14) had significantly ($p < 0.05$) bigger lipid cores ($0.62 \pm 0.38 \text{ mm}^2$) and thinner intimal hyperplasia ($100 \pm 100 \mu\text{m}$) compared with the non-DM group ($0.31 \pm 0.33 \text{ mm}^2$, $150 \pm 150 \mu\text{m}$, respectively). The hypertension (HT) group (n=23) had significantly more intimal hyperplasia ($150 \pm 150 \mu\text{m}$) compared with the non-HT group ($50 \pm 100 \mu\text{m}$). Hyperlipidemia (n=16) or smoking (n=6) did not significantly affect tissue characteristics.

Conclusion IB-IVUS showed various types of plaque in ANCL and the plaque characteristics were affected by DM and HT. The results provide new clinical insight into the early stage of human coronary atherosclerosis. (Circ J 2005; 69: 543–549)

Key Words: Coronary disease; Imaging; Plaque; Tissue; Ultrasonics

Clinicopathological studies on adults have revealed that angiographically normal coronary lesions (ANCL) have atherosclerosis consisting of a lipid core with fibrous cap, intimal hyperplasia of smooth muscle cells and fibrosis.^{1–3} Also, the atherosclerotic lesions are much larger than suspected from the coronary angiography (CAG) findings.^{4,5} The discrepancy is explained by remodeling of coronary arteries with atherosclerosis⁶ and the difficulty in defining normal reference segments because of the diffuse nature of the disease.⁷ In the early stages of atherosclerosis, compensatory enlargement of the vessel prevents encroachment of the plaque on the lumen, thereby concealing the presence of a lesion on CAG. Although such lesions do not restrict blood flow, they represent an important substrate for the development of acute coronary syndromes and stable angina pectoris.^{8–10} The degree of risk for coronary

artery disease (CAD) varies among the risk factors such as diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL), smoking etc, suggesting that the tissue characteristics of ANCL may also differ according to the risk factor. For example, a recent conventional intravascular ultrasound (IVUS) study in ANCL reported that the size of both vessels and their lumens were smaller in patients with DM than in patients without DM!¹ Therefore, tissue characterization of ANCL is clinically important for understanding the pathogenesis of atherosclerosis and preventing its progression to CAD.

Many studies using conventional IVUS clearly show intimal thickening of ANCL;^{12,13} however, it is difficult to differentiate lipid cores and fibrous tissue using this method. Recently, it was reported that autoregressive classification techniques allow real-time analysis of IVUS data and coronary plaque tissue characterization could be predicted through analysis of IVUS radio-frequency (RF) data analysis!¹⁴ Also, we have developed a method that combines integrated backscatter (IB) with conventional 2-dimensional (2-D) echo and constructed 2-D color-coded maps that allowed tissue characterization of thrombus, lipid pool, intimal hyperplasia, fibrosis, mixed lesions and calcification!^{15,16} In the present study, we wanted to define the tissue

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Table 1 Characteristics of 30 Patients With Angina Pectoris Who Underwent Percutaneous Coronary Intervention

M/F	22/8
Mean age (years)	64±8
Medication, n (%)	
Aspirin	30 (100)
Ticlopidine	30 (100)
Nitrates	20 (67)
Calcium channel blocker	12 (40)
ACE inhibitor	10 (33)
-blocker	2 (7)
AT ₁ receptor antagonist	14 (47)
Statins	14 (47)
History of myocardial infarction	4 (13)
Multivessel disease	11 (37)
Previous coronary bypass graft surgery	0 (0)
Risk factor n (%)	
Smoking +/-	6 (20)/24 (80)
Diabetes mellitus (DM) +/-	14 (47)/16 (53)
HbA1c = DM +/- (%)	6.9±0.8/5.4±0.4*
Hypertension (HT) +/-	23 (77)/7 (23)
Systolic blood pressure = HT +/- (mmHg)	136±17/130±10
Diastolic blood pressure = HT +/- (mmHg)	72±10/70±9
Hyperlipidemia (HL)	16 (53)/14 (47)
Total cholesterol = HL +/- (mg/dl)	218±47/191±24
LDL cholesterol = HL +/- (mg/dl)	112±48/100±28
HDL cholesterol = HL +/- (mg/dl)	45±8/48±9

Data are number (%) of patients.

* $p < 0.05$ between + and - in each risk factor.

characteristics of ANCL in patients with stable angina pectoris using IB-IVUS and to evaluate the relation between the imaging data and risk factors.

Methods

Study Patients

Subjects were 30 patients with stable angina pectoris (Table 1). Stable angina was defined as the presence of chest pain on effort (unchanged over the previous 2 months), a positive stress test by ECG and/or Tl scintigraphy, and significant diameter narrowing of more than 75% in at least 1 major coronary artery on CAG. There were 11 patients with multivessel coronary artery disease and 19 with single-vessel disease. All patients underwent percutaneous coronary intervention (PCI) targeting the angina-related coronary arteries. Four patients had an old myocardial infarction. Patients with acute coronary syndrome within the previous 4 weeks, an ejection fraction <30%, secondary causes of hypercholesterolemia or severe hypertriglyceridemia (>400 mg/dl) were excluded.

Risk factors for CAD were also evaluated. DM was defined as medication-dependent, including oral hypoglycemic drugs and insulin, or hemoglobin (Hb)A1c $\geq 6.5\%$ or DM type based on a 75-g oral glucose tolerance test. HT was defined as medication-dependent or systolic blood pressure ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg. HL was defined as medication-dependent or serum cholesterol >220 mg/dl. Smoking status was defined as positive if patients were still smoking or had stopped smoking within 6 months of entering the study. The number of patients with risk factors and the concomitant medications are summarized in Table 1. The protocol was approved by the institutional ethics committee, and informed consent for these studies was obtained from each patient.

Coronary Angiography, Conventional IVUS and IB-IVUS

To exclude the effect of coronary spasm, all patients

Table 2 Correlation Coefficients Between Various Parameters of Angiographically Normal Coronary Lesions by Conventional IVUS

	EEM CSA	Lumen CSA	Atheroma CSA
EEM CSA	–	0.69	0.81
Lumen CSA	0.69	–	NS
Atheroma CSA	0.81	NS	–
Plaque burden	0.32	0.45	0.62
Eccentricity index	0.18	NS	0.35

Parameters were tested for significance by Pearson's correlation coefficient. $p < 0.05$ was considered to be statistically significant.

CSA, cross-sectional area; EEM, external elastic membrane; IVUS, intravascular ultrasound.

received an intracoronary injection of nitroglycerin before CAG. Right and left CAG was carried out in 3 and 6 projections and the best projection was selected for analysis. Quantitative coronary angiography (QCA) was performed with a computer-assisted CAG analysis system (Cardio 500 Kontron Elektronik). The angiographic images were reviewed by 2 independent observers experienced in angiographic interpretation and unaware of the clinical data. ANCL were defined with QCA as vessels with <10% narrowing of the lumen diameter.

Conventional and IB-IVUS analyses were performed in 4 different slices of ANCL with lumen diameters of 2.5–3.5 mm, each slice separated from the others by at least 10 mm. With 4 slices imaged in each of the 30 patients before PCI, a total of 120 IVUS cross-sections of ANCL were analyzed. Qualitative and quantitative conventional IVUS analyses were performed according to the criteria of the ACC Clinical Expert Consensus document on IVUS!⁷ Lumen cross-sectional area (CSA), external elastic membrane (EEM) CSA, atheroma CSA (EEM CSA–lumen CSA), atheroma eccentricity index {(maximal atheroma thickness–minimal atheroma thickness)/maximal atheroma thickness}, and plaque burden (atheroma CSA÷EEM CSA) were determined with use of the measurement software incorporated in the IVUS system. Sites with intimal thickness ≥ 0.5 mm were defined as atherosclerotic!¹³

Integrated Backscatter System Presets and Data Acquisition

Conventional IVUS images and IB signals were acquired using a commercially available IVUS imaging system to characterize the coronary arterial tissue using a 40 MHz IVUS catheter (Clear View, Boston Scientific, Natick, MA, USA). We used an analog-digital converter (Wavepro 960, LeCroy), which enabled acquisition, storage and retrieval of signals that were digitized at 2 GHz with 8-bit resolution. Off-line analyses of the acquired RF signals were performed on previously stored data from the built-in memory (Compact Flash, San Disk) using software that we described in an earlier report!¹⁶ Color-coded construction was performed by computer software (Noesys, Fortner Research LLC). In the present study, we used 256 vector lines per image (1.4 grade/line) and set 40 regions of interest (ROIs) for each 50- μ m depth on each vector line (total, 10,240 ROIs/image). The tissue IB values were calibrated by subtracting the IB values from the IB value of stainless steel placed at a distance of 1.5 mm from the catheter!¹⁶ It has been reported that the average attenuation using a 40 MHz frequency catheter is 5.9 dB/mm!^{18,19} Therefore, we corrected each IB value by adding 0.59 dB/0.1 mm when the ROI was located 1.5 mm further from the catheter and

by subtracting 0.59 dB/0.1 mm when the ROI was located 1.5 mm closer to the catheter.

Construction of Color-Coded Maps Using IB Values and Conventional 2-D IVUS

Based on the 5 categories used in our previous clinico-pathological study (category 1, thrombus [collections of erythrocytes embedded in a net of platelets]: $-88 < IB \leq -80$; category 2, intimal hyperplasia [smooth muscle cells occupying $>50\%$ of the sample area] or lipid core [extracellular lipid, macrophages and/or foam cells] in the intima and media: $-73 < IB \leq -63$; category 3, fibrous tissue: $-63 < IB \leq -55$; category 4, mixed lesion [mixed mineral deposits, extracellular lipid and fibrous tissues]: $-55 < IB \leq -30$; category 5, calcification: $-30 < IB \leq -23$),⁶ 2-D color-coded maps of tissue characteristics were constructed in 120 IVUS cross-sections of ANCL. In general, lipid cores (category 2) are located under fibrous caps consisting of fibrous tissue (category 3) and/or mixed lesion (category 4) from lumen was defined as a lipid core, whereas the direct presence of a ROI with category 2 from lumen was defined as intimal hyperplasia.⁶ In the present study, we observed the histology of an additional 30 plaques from human coronary arteries and the pathological data confirmed our imaging defini-

tions. The quantitative IB-IVUS analysis was performed by experienced operators using a manual method. The thickness of the fibrous caps was determined by averaging the thickness of 3 different site of the fibrous cap at their thinnest site. The area of each lipid core was traced and measured using a planimeter (Ushikawa X-PLAN 360d).

Reproducibility of Data

We determined the intra- and interobserver variabilities of the thickness of each characteristic tissue and the area of lipid core in 20 randomly selected recordings that were measured twice by the same observer and once each by 2 independent observers experienced in IB-IVUS interpretation and unaware of the clinical data. The respective variabilities of the area of lipid core were $4.2 \pm 2.8\%$ and $5.3 \pm 3.3\%$. Intraobserver and interobserver correlations of the thickness of each characteristic tissue were $r=0.92$ ($p < 0.01$) and $r=0.91$ ($p < 0.01$), and those for the area of lipid core were $r=0.91$ ($p < 0.01$) and $r=0.90$ ($p < 0.01$).

Statistical Analyses

Values are reported as the mean \pm standard deviation. Correlations between each conventional and IB-IVUS parameter were tested for significance by Pearson's correlation coefficient. Testing for significant differences of laboratory, conventional and IB-IVUS parameters between 2 groups was performed with a Mann-Whitney U test. Multiple regression analysis was used to compare patients with

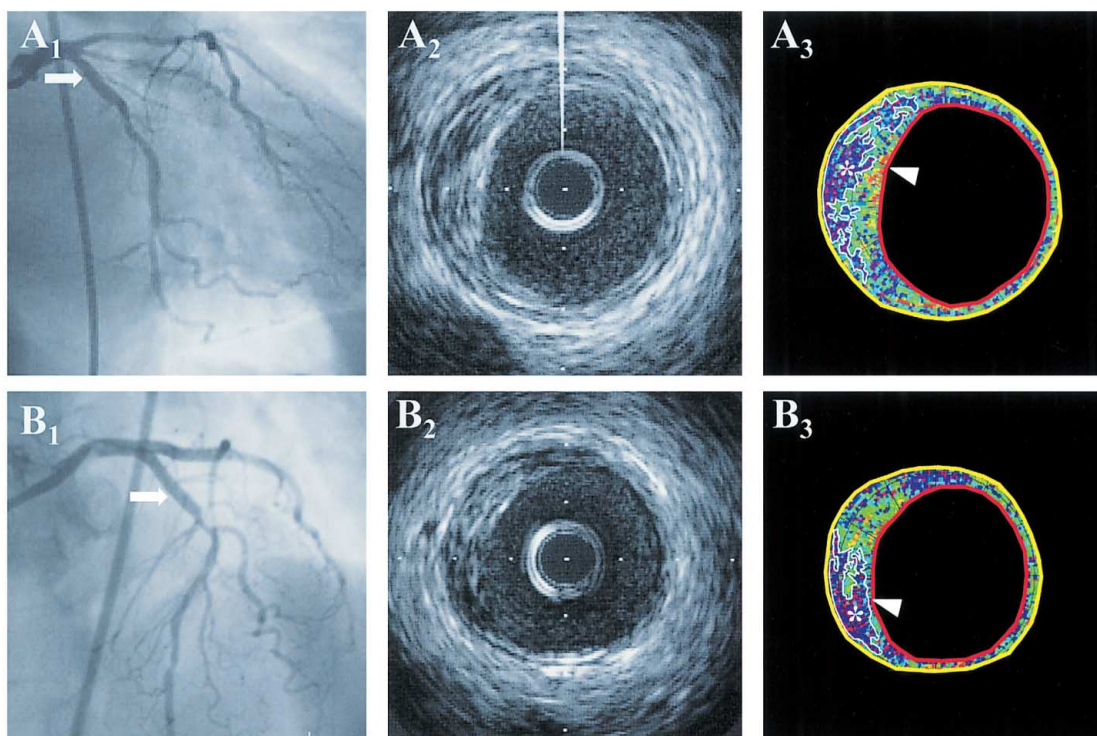


Fig 1. Color-coded map of the coronary arterial plaques by IB-IVUS in angiographically normal coronary lesions (ANCL). A1: ANCL (arrow). A2: conventional IVUS image of the ANCL in A1. A3: Color-coded map by IB-IVUS of the same segment as in A2. Note small lipid cores (*blue) (0.67 mm^2) with thick fibrous cap ($200 \mu\text{m}$) consisting of fibrous tissue (green) and mixed lesion (yellow) indicated by arrowheads. B1: another ANCL (arrow). B2: conventional IVUS image of the ANCL in B1. B3: color-coded map by IB-IVUS of the same segment as in B2. White circle line: area of lipid core. Note small lipid cores (*blue) (0.44 mm^2) with thin fibrous cap ($50 \mu\text{m}$) consisting of fibrous tissue (green) indicated by arrowheads. Blue: intimal hyperplasia or lipid core in the intima ($-73 < IB \leq -63$); Green: fibrosis ($-63 < IB \leq -55$); Yellow: mixed lesion ($-55 < IB \leq -30$); Red: calcification ($-30 < IB \leq -23$) (See Methods section for definitions of fibrous cap, lipid core, fibrosis and mixed lesion).

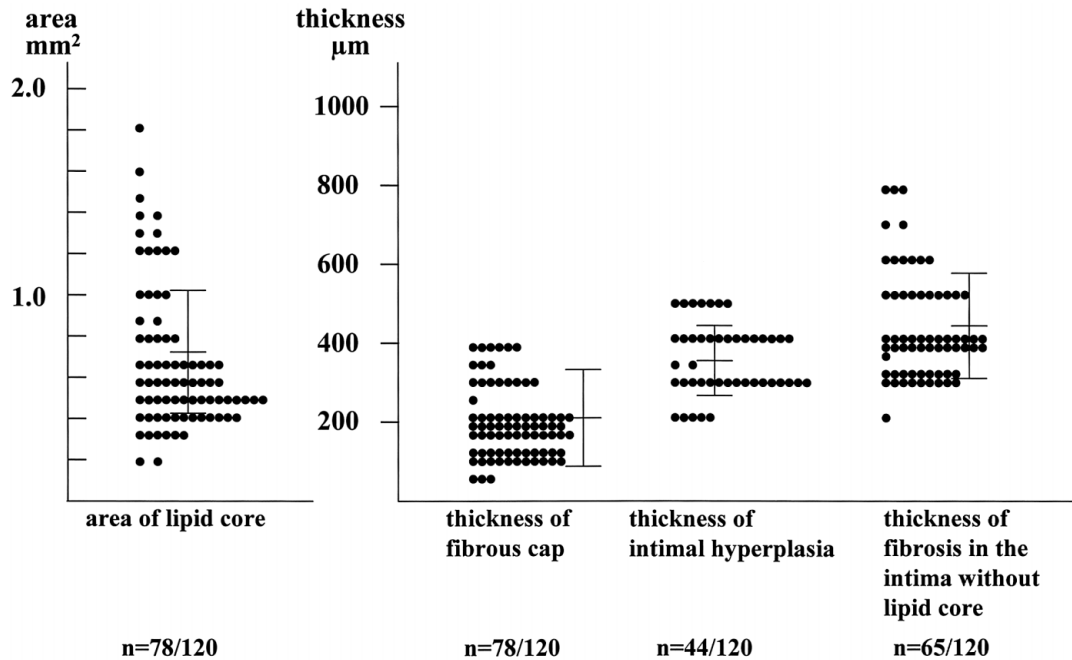


Fig 2. Area or thickness of various tissue types in angiographically normal coronary lesions. Note the variability in each parameter.

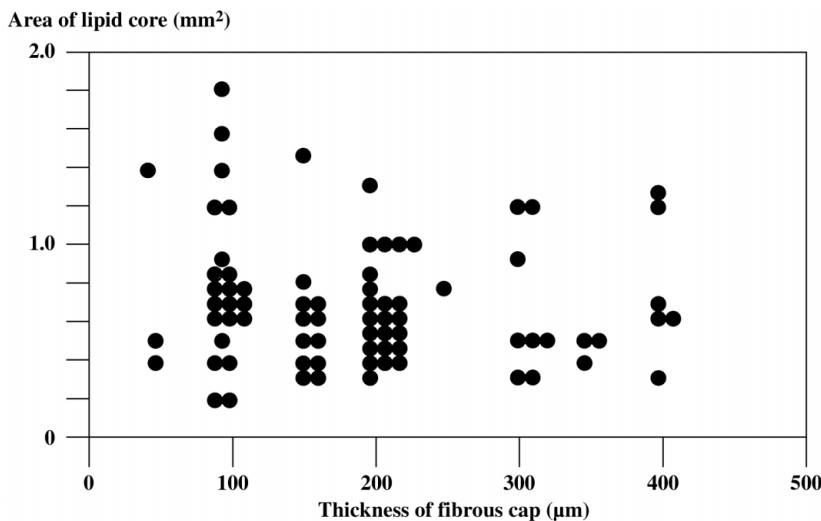


Fig 3. Relation between area of lipid core and thickness of fibrous cap in angiographically normal coronary lesions. Note the lack of a relation between the area of lipid core and thickness of fibrous cap.

and without risk factors. A chi-square test was used to compare categorical data. A value of $p < 0.05$ was considered statistically significant.

Results

Conventional IVUS Findings of ANCL

We examined 120 cross-sections with IVUS in 30 patients with ANCL: 48 were located in the left anterior descending coronary artery, 40 in the left circumflex coronary artery and 32 in the right coronary artery. The average angiographic lumen diameter was 2.93 ± 0.55 mm. With conventional IVUS, EEM CSA, lumen CSA, atheroma CSA, plaque burden and atheroma eccentricity index were 14.5 ± 4.0 mm², 7.7 ± 2.4 mm², 6.9 ± 3.0 mm², $45.6 \pm 13.3\%$ and 0.51 ± 0.21 , respectively. Intimal thickening (≥ 0.5 mm), that is atherosclerosis, was detected in each of the 120 cross-sections by conventional IVUS.

As shown in Table 2, there was a good correlation between EEM CSA and lumen CSA or atheroma CSA in ANCL. However, lumen CSA did not correlate with atheroma CSA.

IB-IVUS Findings in ANCL

Seventy-eight of the 120 cross-sections (65%) evaluated by IB-IVUS showed lipid cores of 0.69 ± 0.35 mm² with fibrous caps of 200 ± 100 µm, 44 (37%) had intimal hyperplasia with a thickness of 350 ± 100 µm, and 65 (54%) had fibrosis in the intimal wall without lipid core with a thickness of 450 ± 150 µm (Figs 1, 2). Also, 19 cross-sections (16%) contained calcification. Plaques with moderate lipid core (1–2 mm² in area) and thin fibrous cap (≤ 100 µm in thickness) were detected in 6 of the 120 ANCL IVUS cross-sections (5%) (Fig 3).

Lipid core and fibrosis in the intimal wall without lipid core were observed locally in the vessel wall, but intimal

Table 3 Correlation Coefficients Between Conventional IVUS and IB-IVUS Findings

IB-IVUS findings	Conventional IVUS findings				
	EEM CSA	Atheroma CSA	Plaque burden	Eccentricity index	Lumen CSA
Area of lipid core	NS	0.43	0.49	0.28	NS
Thickness of fibrous cap	NS	NS	NS	NS	NS
Thickness of fibrosis in the intima without lipid core	0.51	0.60	0.64	0.32	NS
Thickness of intimal hyperplasia	0.50	0.60	0.55	NS	NS

Parameters were tested for significance by Pearson's correlation coefficient. $p < 0.05$ was considered to be statistically significant. CSA, cross-sectional area; EEM, external elastic membrane; IB, integrated backscatter; IVUS, intravascular ultrasound.

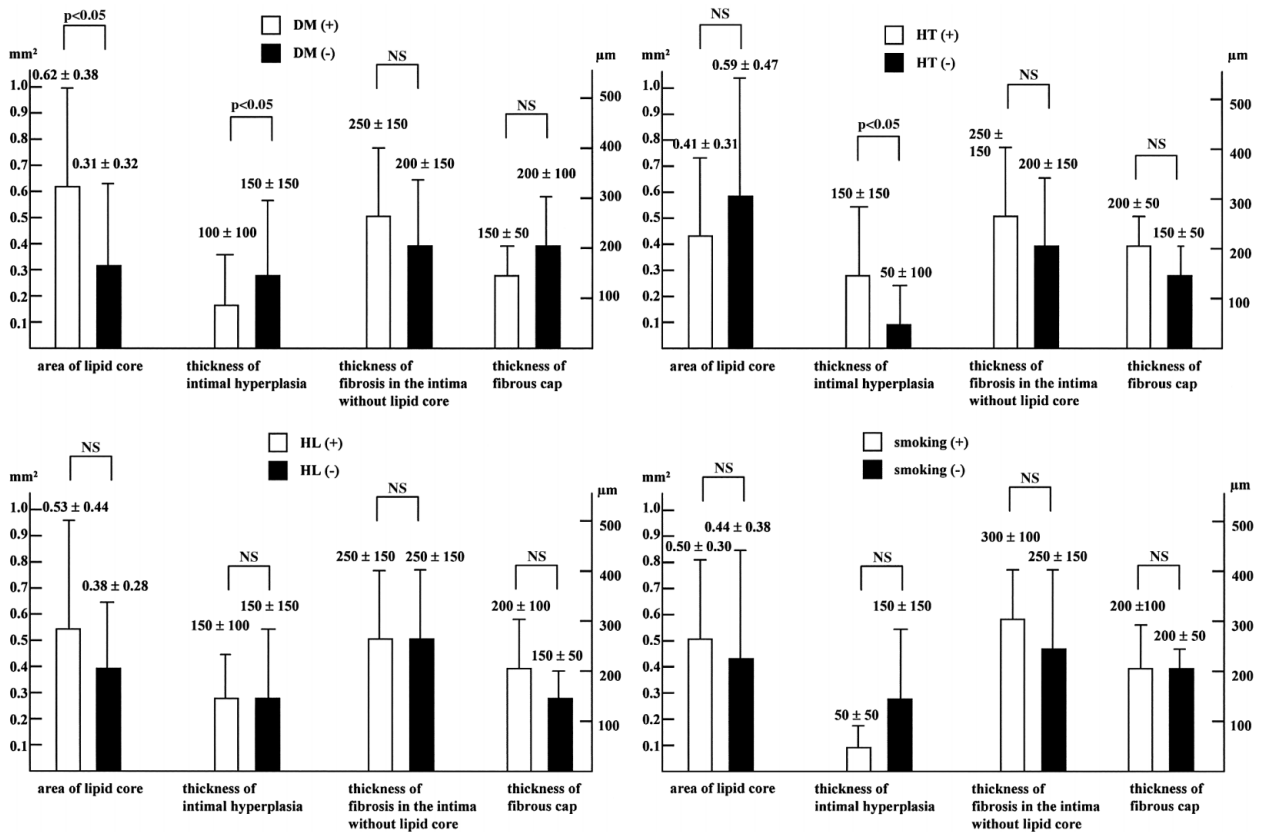


Fig 4. Tissue characteristics in patients with and without coronary risk factors. Note larger area of lipid core and thinner thickness of intimal hyperplasia in the patients with diabetes mellitus (DM), compared with those without DM. Also, thicker intimal hyperplasia is seen in the patients with hypertension (HT) than in those without HT. HL, hyperlipidemia.

hyperplasia was diffuse.

Correlation Between IB-IVUS and Conventional IVUS Findings

As shown in Table 3, the area of lipid core, thickness of intimal hyperplasia and fibrosis without lipid core were significantly correlated with atheroma CSA or plaque burden (Table 3). However, the thickness of the fibrous cap showed no relation to any other IB-IVUS or conventional IVUS parameter such as the area of lipid core, atheroma CSA etc.

EEM CSA was significantly correlated with either the thickness of fibrosis in the intimal wall without lipid core or intimal hyperplasia, but not with the area of lipid core (Table 3). The atheroma eccentricity index was significantly correlated with fibrosis without lipid core and the area of lipid core, but not with the thickness of intimal

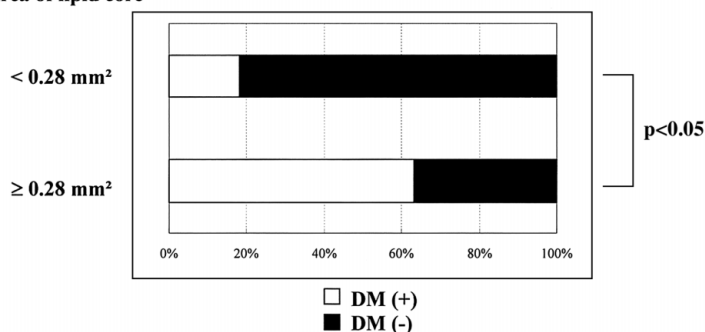
hyperplasia (Table 3).

Relation Between IB-IVUS Findings and Risk Factors

The 30 patients were divided into subgroups based on the presence or absence of each of the following risk factors: DM, HT, HL and smoking (Table 1). The IB-IVUS parameters were compared between the groups with and without each risk factor (Fig 4). The group with DM had significantly larger lipid cores (DM: $0.62 \pm 0.38 \text{ mm}^2$; Non-DM: $0.31 \pm 0.32 \text{ mm}^2$) and thinner intimal hyperplasia (DM: $100 \pm 100 \mu\text{m}$; non-DM: $150 \pm 150 \mu\text{m}$), and the group with HT had significantly thicker intimal hyperplasia (HT: $150 \pm 150 \mu\text{m}$; non-HT: $50 \pm 100 \mu\text{m}$) (Fig 4). There were no significant effects of HL or smoking on the tissue characteristics of ANCL.

In addition to stratifying patients based on risk factors, patients were also stratified based on the IB-IVUS findings.

Area of lipid core



Thickness of intimal hyperplasia

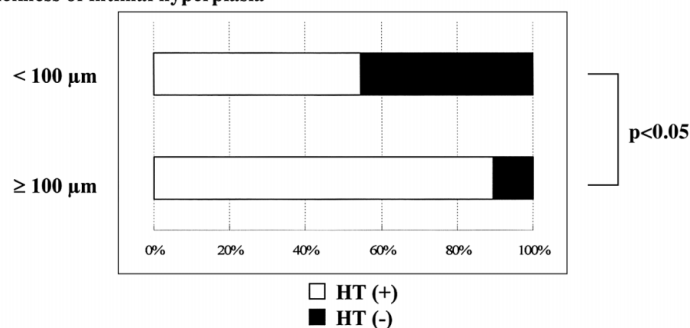


Fig 5. Frequency of patients with risk factors classified by tissue characteristics. Note that most patients with a lipid core area $>0.28 \text{ mm}^2$ had diabetes mellitus (DM). Conversely, most patients with lipid core area $<0.28 \text{ mm}^2$ had no DM. The difference was significant. Also, most patients with intimal hyperplasia thickness $>100 \mu\text{m}$ had hypertension (HT), but approximately half of the patients with intimal hyperplasia thickness $<100 \mu\text{m}$ had no HT. The difference was significant.

There were 12 of 19 patients with a lipid core area of more than 0.28 mm^2 who had DM and 9 of 11 patients with a lipid core area under 0.28 mm^2 who did not have DM (Fig 5). The difference was significant. There was no significant difference in lipid core area between patients with and without HT, HL or smoking. Seventeen of 19 patients with intimal hyperplasia of more than $100 \mu\text{m}$ had HT and 5 of 11 patients with intimal hyperplasia under $100 \mu\text{m}$ did not have HT (Fig 5). The difference was significant. There was no significant difference in intimal hyperplasia thickness between patients with and without DM, HL or smoking. In addition, neither the thickness of the fibrosis in the intimal wall without lipid core nor the thickness of fibrous cap showed a significant difference when comparing the 2 groups with and without each of the 4 risk factors.

Discussion

Conventional IVUS Findings of ANCL

Previous conventional IVUS studies in ANCL in adults have shown significant atheroma CSA in most of the cases, and a correlation between atheroma CSA and EEM CSA, but not lumen CSA. Thus, vessel enlargement because of remodeling compensates for the growth in plaque area in the early stages of atherosclerosis. These findings were confirmed in the present study.

Tissue Characterization of ANCL by IB-IVUS

The present IB-IVUS analyses of 120 ANCL cross-sections in patients with angina pectoris showed: (1) a lipid core with fibrous cap, intimal hyperplasia and fibrosis in the intimal wall without lipid core in approximately two-thirds, one-third and one-half of the sections, respectively; (2) the area of the lipid core and thickness of the fibrous cap were variable in each section (Fig 3). This is the first clinical demonstration of the tissue characteristics of early stage coronary plaques.

Another important finding in the present study was that

the area of the lipid core, thickness of the intimal hyperplasia and fibrosis in the intimal wall without lipid core significantly correlated with atheroma CSA or plaque burden, indicating that each influences the progression of plaque in ANCL.

Furthermore, the thickness of the fibrous cap was not significantly related to any parameters of IB-IVUS or conventional IVUS, which suggests that the thickness of the fibrous cap is determined by factors that cannot be identified by IVUS. It is possible that activated lymphocytes and various cytokines, such as the matrix metalloproteinase family, in the fibrous cap may play an important role.²⁰ The present study showed the presence of plaques with moderate-sized lipid cores and thin fibrous caps in 5% of ANCL. In a previous clinicopathological study, vulnerable plaque, which induces acute coronary syndrome following plaque rupture and thrombosis, was defined as a plaque with a large lipid core and thin fibrous cap;²¹ however, the precise size of a "large" lipid core has not been defined because of methodological limitations. Therefore, it is unclear whether the aforementioned plaques identified in the present study are vulnerable plaques.

Moreover, the atheroma eccentricity index was significantly correlated with EEM CSA or atheroma CSA, which suggests that plaque development is localized, rather than diffuse, in the vessel wall, followed by outward expansion. Also, the atheroma eccentricity index was correlated with the area of the lipid core or fibrosis in the intimal wall without lipid core, but not with the thickness of intimal hyperplasia, a finding explained by the localized formation of the lipid core and fibrosis in the intimal wall compared to the more diffuse process of intimal hyperplasia.

Tissue Characterization of ANCL and Coronary Risk Factors

The risk factors related to coronary atherosclerosis are well known, but the relationship between the tissue characteristics of ANCL and coronary risk factors has not been

previously examined. In the present IB-IVUS study, the area of lipid core was larger in patients with DM than without DM, but not in the patients with HT or HL. This may reflect that HbA1c was significantly higher in the patients with DM, but there was no significant difference in systemic blood pressure or serum lipids between the patients with and without HT or HL because of treatment with anti-HT or anti-HL drugs etc. The thickness of intimal hyperplasia was significantly greater in patients with HT, which confirms previous work in animal models and human studies that show intimal hyperplasia as the initial response of the vessel to HT^{22,23}. Since the development of various anti-HT and anti-HL drugs, control of HT and HL is easier compared with control of DM, as confirmed in the present study. Because DM is difficult to control with medication, it becomes a more important risk factor for coronary heart disease²⁴. This would explain the finding of a larger lipid core in ANCL in patients with DM.

Study Limitations

First, the performance of the quantitative IB-IVUS analysis. Although the interobserver and intraobserver reproducibility was sufficient, a method of computerized automatic assessment needs to be developed to enable a rigorous analysis. Second, because the ANCL were obtained from patients with angina pectoris, the findings do not necessarily reflect what would be found in healthy patients. Third, many patients had more than 1 risk factor and the interaction between risk factors was not evaluated. Fourth, IB-IVUS is limited by the size of the ROI (ie, 50µm depth). Finally, the present study consisted of a small number of patients and there were no follow-up measurements performed in the same patients. Therefore, large-scale and long-term follow-up studies of ANCL, which analyze the process of progression of coronary plaques and the relationship with coronary events, are needed.

Conclusion

ANCL have various types of plaque and the tissue characteristics appear to be significantly influenced by both DM and HT. This information may be useful for preventing the progression of coronary atherosclerosis.

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