Plaque Tissue Components Obtained from De Novo Lesions may Predict Restenosis after Directional Coronary Atherectomy

Kentaro Arakawa,1 * Hatsue Ishibashi-Ueda,2 Hiroyuki Hao,3 Yoshihiko Ikeda,2 and Atsushi Kawamura1

Background: A part of coronary stenotic lesions treated with directional coronary atherectomy (DCA) occur restenosis several months later. Specimens obtained by first DCA, present the histology of culplit lesions and may predict restenosis after PCI.

Methods: The study group comprised 76 patients (male/female 65/11, age 61 ± 11 years). Restenosis, defined as > 50% stenosis diameter by quantitative cineangiography, was present in 26 patients. The other 50 patients (< 50% stenosis) constitute the “no restenosis” group. Inflammatory cells and other atheroma components were planimetrically quantified as a percentage of total tissue area.

Results: As regards lymphocytes, neutrophils and smooth muscle cells, the grade of amount of cells did not differ between restenosis group and no restenosis group. The amount of obtained arterial media was similar, too. However, the area occupied by macrophages or calcified fragments was significantly larger in restenosis group than no restenosis group. And there was a tendency toward larger area occupied by cholesterol gruel, thrombus and myxomatous extracellular matrix (ECM) in restenosis group.

Conclusion: Rich macrophages infiltration, calcified fragments, cholesterol rich gruel and myxomatous ECM from primary lesions can be predictors of restenosis after DCA, suggesting a possible role in restenotic process after PCI.

Key words: directional coronary athelectomy, restenosis, percutaneous coronary intervention, atherosclerosis, inflammation

INTRODUCTION

The mechanisms involved in the process of restenosis after percutaneous coronary intervention are postulated to include elastic recoil, smooth muscle cells (SMCs) proliferation with extracellular matrix (ECM) production, and remodeling.1-5)

Directional coronary atherectomy (DCA) is one method of percutaneous coronary intervention (PCI) to remove atherosclerotic lesions from the coronary artery wall. Although the specimens obtained by DCA may not reflect the whole situation of coronary arteries, we can observe the histology of culpit lesions, which consist of multiple thin fragments. Atherectomy specimens in de novo lesions reflect the heterogeneity of features that are found in plaques.

Recently, a lot of reports related to DCA specimens have thrown lights on pathophysiology of coronary arteries. As regards to restenosis in de novo lesions, increased macrophages and lymphocytes, higher BTEB-2 expression to stellate SMCs and elevated immunoreactivity to C-reactive protein (CRP) etc. are reported to be indepen-
dent predictors of restenosis after DCA.\textsuperscript{6–10} To test the hypothesis that the specimens obtained from coronary arteries can predict restenosis after PCI, we quantified plaque tissue components and correlated with angiographic restenosis after DCA in patients with stable angina and acute coronary syndrome.

**Methods**

**Study patients**

From January 1993 to March 2006, 172 consecutive DCA procedures were performed at the cardiac catheterization laboratories in National Cardiovascular Center, Osaka, Japan. Patients were required to meet the following criteria, (1) successful DCA of the culprit lesion (> 20% reduction in diameter stenosis and residual diameter stenosis < 50%), (2) without stenting, (3) no previous coronary intervention at the site of the culprit lesion, (4) enough large specimens to evaluate (> 1.5 mm\(^2\)) (5) follow-up angiography 1 to 6 months after DCA. 76 patients met the inclusion criteria and constitute the study population. There were 65 men and 11 women, with a mean age of 61 ± 11 years. Restenosis, defined as > 50% stenosis diameter by quantitative cineangiography, was present in 26 patients. The remainder of the 50 patients constitute the “no restenosis” group.

**Histological analysis of atherectomy specimen**

DCA specimens retrieved from culprit lesions were immediately fixed in 10% buffered formalin solution for 6 hours at 4°C and embedded in paraffin. Sections (4 μm thick) were stained with hematoxylin-eosin, masson's trichrome and elastica van Gieson. Moreover, sub-serial sections were examined immunohistochemistry if necessary. The primary monoclonal antibodies used were anti-CD68 (DAKO, Japan) for macrophages, anti-alpha-smooth muscle actin (α-SMA; DAKO, Japan) for SMCs and UCHL-1 (DAKO, Japan) for T-lymphocytes, respectively. Briefly we performed, after deparaffinization, pretreatment of tissues with heat-induced epitope retrieval and blocked endogenous peroxidase activity in 3% hydrogen peroxidase in methanol for 10 minutes. Then the sections were incubated with antibodies. Intervening washes with phosphate-buffered saline were followed by incubation with Envision + (DAKO Japan) for 30 minutes. After further washes, the sections were incubated with 0.05% 3,3’ diaminobenzidine containing hydrogen peroxide and counterstained with Meyer's hematoxylin. As the negative control for immunostaining, normal mouse immunoglobulin G was used instead of the primary anti-body. Human liver obtained at autopsy was used as positive control.

In the quantitative analysis, immunopositive areas of each cell, such as macrophages, T-lymphocytes, neutrophils or SMCs, and calcified fragments were measured as a percentage of the total tissue area. Areas were counted by a planimetry software (Win roof, Mitani, Japan). Fibrin thrombus and cholesterol gruel were also evaluated as the same method (Fig. 1). The occupied area percentage of each inflammatory cell (lymphocytes, neutrophils and macrophages), cholesterol gruel and SMCs (α-SMA positive) to total area were calculated. We compared cases as 4 grades; (0), (1+), (2+) and (3+), indicating no cell, < 10%, from 10% to 50% and > 50%, respectively.

The presence of deep arterial wall components (media, adventitia) was analyzed, too. Extracellular matrix (ECM) was classified into two patterns, namely, hyalinized tissue dominant and myxomatous tissue dominant. The former is sclerotic tissue, composed of dense collagen tissue with low cellularity, while the latter is hypercellular tissue, composed of loose connective tissue matrix containing numerous stellate cells, which were positive to α-SMA (Fig. 2).

Macrophages and T-lymphocytes in the fibrous cap were evaluated in the same planimetrically quantitative analysis manner.

**Quantitative measurement of coronary stenosis**

The coronary angiograms were recorded, and quantitative coronary analysis was performed by QCA-CMS Ver.5 (Goodman, Nagoya, Japan). Measurements included the minimal lumen diameter (MLD) of the treated coronary segment, the reference diameter, lesion length and percent diameter stenosis. Acute gain was calculated; MLD immediately after DCA minus MLD immediately before DCA.

**Statistical analysis**

Data are expressed as mean ± standard deviation. Continuous variables were compared using the unpaired \( t \) test. Categorical variables were compared using Fisher’s exact test. With respect to histological evaluation except deep wall components and ECM, Scheffe’s method was used to perform multiple comparisons between 2 groups. Values of \( p < 0.05 \) were considered significant.

**Results**

**Patients and angiography**

The clinical baseline characteristics of 76 patients included in this study are shown in Table 1. And angio-
Fig. 1 A representative immunohistochemical staining for CD68 in DCA specimen obtained from a coronary artery, which presented restenosis after 6 months. The area occupied by macrophages is more than 50% (left; ×40, right; ×400 original magnification).

Fig. 2 Left; hyalinized ECM composed of dense collagen tissue (blue part) with low cellularity and organized thrombus (red part). Right; myxomatous ECM composed of a loose connective tissue matrix containing numerous stellate cells (Masson’s trichrome staining, ×40, original magnification respectively).
graphic characteristics are shown, too. All lesions were located in relatively large proximal coronary segments. There was a trend toward longer lesion and smaller post-MLD in restenosis group, but these did not reach statistical significance. The other baseline clinical and angiographic characteristics were similar between the 2 groups, including acute gain after DCA.

**Histologic findings and immunohistochemical findings**

The percentage of total area occupied by each inflammatory cell (lymphocytes, neutrophils and macrophages), cholesterol gruel and SMCs (α-SMA positive) were divided into 4 grades; (0), (1+), (2+) and (3+), indicating no cell, < 10%, from 10% to 50% and > 50%, respectively. As regards lymphocytes, neutrophils and SMCs, the grades of occupied area did not differ between restenosis group and no restenosis group. However, the area infiltrated by macrophages was significantly larger in restenosis group than those of no restenosis group (p = 0.028). And there was a tendency toward larger area occupied by cholesterol gruel in restenosis group (Table 2).

Then, the presence of fibrin thrombus and calcified fragments was also quantified according to the percentage of total tissue area occupied by them; (0), (1+), (2+) and (3+), indicating no fragment, < 10%, from 10% to 20%, and > 20%, respectively. No significant difference in the incidence of fibrin thrombus formation was found between the 2 groups. Calcified fragments were detected in 12 patients of restenosis group, (2+) in 6 patients and (1+) in 6 patients, meanwhile seventy percent of no restenosis group showed no calcified fragments (p = 0.012, Table 2).

All samples included materials from the intima, some included the media but none showed the presence of the adventitia. The presence of media did not differ between no restenosis group and restenosis group.

As regards ECM, there was a tendency of increased myxomatous tissue in restenosis group. Hyalinized ECM was prominent in no restenosis group, but with no statistical significance (Table 2).

The presence of lymphocytes and macrophages in the fibrous cap was also analyzed. Although no significant difference in the presence of lymphocytes was found between the 2 groups, foamy macrophages were detected in

---

**Table 1** Baseline clinical characteristics and coronary angiographic findings

<table>
<thead>
<tr>
<th></th>
<th>Restenosis (n = 26)</th>
<th>No-Restenosis (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (yr)</td>
<td>61.7 ± 2.1</td>
<td>60.7 ± 1.5</td>
<td>0.7055</td>
</tr>
<tr>
<td>Gender (male n (%))</td>
<td>23 (88%)</td>
<td>42 (84%)</td>
<td>0.5942</td>
</tr>
<tr>
<td>Stable AP/ACS n (%)</td>
<td>17/9 (65/35%)</td>
<td>26/24 (52/58%)</td>
<td>0.261</td>
</tr>
<tr>
<td>Coronary Risk Factors n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>16 (62%)</td>
<td>31 (62%)</td>
<td>0.9687</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>17 (65%)</td>
<td>29 (98%)</td>
<td>0.5304</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>17 (65%)</td>
<td>31 (62%)</td>
<td>0.7712</td>
</tr>
<tr>
<td>Smoking</td>
<td>19 (73%)</td>
<td>34 (68%)</td>
<td>0.6458</td>
</tr>
<tr>
<td>Family History of CAD</td>
<td>6 (23%)</td>
<td>15 (30%)</td>
<td>0.5180</td>
</tr>
<tr>
<td>Drugs n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>25 (96%)</td>
<td>46 (92%)</td>
<td>0.4698</td>
</tr>
<tr>
<td>Statins</td>
<td>16 (32%)</td>
<td>11 (21%)</td>
<td>0.7712</td>
</tr>
<tr>
<td>RCA/LAD/LCX n (%)</td>
<td>3/21/2 (12/81/7%)</td>
<td>1/48/1 (2/96/2%)</td>
<td>0.1039</td>
</tr>
<tr>
<td>Reference diameter (mm)</td>
<td>3.30 ± 0.12</td>
<td>3.48 ± 0.09</td>
<td>0.1180</td>
</tr>
<tr>
<td>Pre-minimum lesion diameter (mm)</td>
<td>0.62 ± 0.16</td>
<td>0.90 ± 0.11</td>
<td>0.1617</td>
</tr>
<tr>
<td>Post-minimum lesion diameter (mm)</td>
<td>2.78 ± 0.89</td>
<td>3.81 ± 0.62</td>
<td>0.0846</td>
</tr>
<tr>
<td>Pre percent stenosis (%)</td>
<td>80.3 ± 2.0</td>
<td>80.3 ± 1.4</td>
<td>0.9973</td>
</tr>
<tr>
<td>Post percent stenosis (%)</td>
<td>12.6 ± 1.5</td>
<td>13.3 ± 2.1</td>
<td>0.7670</td>
</tr>
<tr>
<td>Acute gain (mm)</td>
<td>2.17 ± 0.13</td>
<td>2.36 ± 0.17</td>
<td>0.2193</td>
</tr>
<tr>
<td>Lesion length (mm)</td>
<td>8.80 ± 1.89</td>
<td>6.45 ± 0.74</td>
<td>0.0782</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median value (25th to 75th percentile range) or n (%). AP, angina pectoris; ACS, acute coronary syndrome; CAD, coronary artery disease; yr, years; RCA, right coronary artery; LAD, left coronary artery; LCX left circumflex.
18 patients (69%) of restenosis group, with increased tendency than in patients of no restenosis group (Table 2).

**DISCUSSION**

This study shows that the extent of macrophages infiltration in the initial culprit lesion, as documented by immunohistochemistry, and calcified fragments of the atherectomy specimens, are positively associated with coronary restenosis during long-term follow-up after DCA. Additionally, myxomatous ECM and cholesterol gruel may lead to restenosis after DCA.

**Inflammatory cells**

Experimental and human studies have suggested that inflammatory responses to PCI play an important role in neointimal growth.8-10 In this study, three inflammatory variables were quantified in the patients’ atherectomy tissue: the extent of infiltration of T lymphocytes, which have important immune-regulatory functions in the inflammatory response,11, 12 macrophages with secretory effector functions and phagocytic reaction to lipid as a foam cell in plaque inflammation, and neutrophils which infiltrate early after endothelial denudation and releases oxygen radicals and proteases.13, 14

Our findings indicate that initial plaque inflammation detected by macrophages infiltration contributes to the severity of restenosis despite proliferation of SMCs. And this is consistent with the previous report.9 This phenomenon may be explained by the fact that after DCA, a substantial amount of the initial atherosclerotic plaque may still persist; hence the persistence of smoldering inflammatory process at the site of intervention cannot be dismissed. Otherwise, Libby et al postulated that angioplasty may induce a change in macrophage phenotype from a resting to an activated state that could be involved in the restenosis process.13 Macrophage exhibits an early and sustained DNA synthesis in both the intima and the media layers over the first 2 weeks.17 Macrophage-derived metalloproteinases correlated with SMCs migration from the media into the intima in the rat.18, 19 Moreover they induce structural disruption of the arterial wall, which triggers thrombosis, the cause of occlusion and the majority of acute vascular events.20 So, the regulation of inflammatory cells, especially macrophages, is important to prevent coronary restenosis and acute coronary syndrome.

**Calcification**

The directional atherectomy device is not capable resecting heavily calcified plaque so that only small calcified plaque fragments were typically seen. However, our

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Histological analysis of atherectomy specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Restenosis (n = 26)</td>
</tr>
<tr>
<td>The occupied area grade of total area</td>
<td></td>
</tr>
<tr>
<td>Grade 0/1/2/3 n (%)</td>
<td></td>
</tr>
<tr>
<td>Inflammatory cells PMNs</td>
<td>10/12/3/1 (38/46/12/4%)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3/19/3/1 (12/73/12/4 %)</td>
</tr>
<tr>
<td>Mφs</td>
<td>2/13/10/1 (8/50/38/4%)</td>
</tr>
<tr>
<td>Cholesterol gruel</td>
<td>1/16/8/1 (4/61/31/4%)</td>
</tr>
<tr>
<td>α-SMA positive cells</td>
<td>1/12/9/4 (4/46/35/15%)</td>
</tr>
<tr>
<td>Fibrin thrombus</td>
<td>6/12/6/2 (23/46/23/8 %)</td>
</tr>
<tr>
<td>Calcification</td>
<td>14/6/6/0 (54/23/23/0 %)</td>
</tr>
<tr>
<td>The presence of media n (%)</td>
<td></td>
</tr>
<tr>
<td>12 (46%)</td>
<td>26 (52%)</td>
</tr>
<tr>
<td>ECM myxomatous/hyalinized n (%)</td>
<td>14/12 (54/46%)</td>
</tr>
<tr>
<td>The presence of inflammatory cells in the fibrous cap n (%)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>15 (57%)</td>
</tr>
<tr>
<td>Mφs</td>
<td>18 (69%)</td>
</tr>
<tr>
<td>ECM, extracellular matrix; α-SMA positive cells, alpha smooth muscle actin positive cells; PMNs, polymorphonucler cells; Mφs, macrophages</td>
<td></td>
</tr>
</tbody>
</table>
results demonstrated that the presence of calcification in obtained sample predicted coronary restenosis after DCA. When PCI was performed to the culprit lesion with calcification, the injury of arterial wall often extend to the medium, followed by thrombus formation, macrophage accumulation and neointimal hyperplasia. In case of DCA, acute gain of calcified lesion after DCA would be limited.

Media

Deep arterial wall excision among DCA was not uncommon; arterial media were present in generally 50% of athrectomy specimens. Deep arterial wall excision is not generally associated with an increased incidence of restenosis. However, this was not supported by the results of another study in which the presence of internal elastic lamina and media in higher percentage of patients of restenosis. It was compatible with the fact that medial SMCs stimulated phenotypic modulation strictly from contractile state to stellate cells. But, it could be postulated that deeper dissection ensuring more complete removal of the atheroma and less residual stenosis after DCA, contribute the larger acute luminal gain in patients without restenosis. This beneficial effect in the form of a larger and smoother lumen after atherectomy appeared to overshadow any adverse event that could occur due to exposure of the media proving a stimulus for greater platelet aggregation and subsequent smooth muscle proliferation.

ECM

The ECM of the vessel wall is a system of acellular substances, providing the connective tissue scaffolding for cellular elements. The ECM consists of varying concentrations of proteoglycans (versican, biglycan, and decorin), hyaluronan, and collagen (types I and III). The ECM modulates important events within the developing neointima, including cell proliferation, migration, growth factor expression, and remodeling. And ECM accounts for > 50% of the volume of neointimal restenosis lesions.

In autopsy coronary arteries, post-PCI lesions at healing stage show the accumulation not only of collagen type I, decorin and biglycan but also of versican and hyaluronan at least for 18 months, suggesting the requirement of a long time for complete healing. But, it is reported that versican and hyaluronan decreases with an increase in collagen type III content as time goes by.

In the present study, even in de novo lesions, myxomatous ECM was frequently found, which was usually found in restenosis lesion after PCI, and rich proteoglycans, suggesting the possibility of previous plaque rupture before PCI. The present study with the result of a tendency of increased myxomatous tissue in restenosis group, suggests that myxomatous ECM shows ongoing intimal proliferation and potency to restenosis. Moreover, in myxomatous ECM, we observed elevated immunoreactivity to matrix metalloproteinases-1, 2 and 9, which may play an prominent role in plaque destabilization and coronary restenosis after PCI.

SMCs

In this study, SMCs content was similar in the two groups (restenosis/no restenosis), suggesting that the severity of vascular narrowing after coronary atherectomy was not related to the intimal SMC content in the culprit lesion.

The previous analysis of the intima revealed that α-SMA positive cells were quite numerous; however, they had lost smooth muscle myosin heavy chains (SM-MHCs) expression very importantly and smoothelin expression almost completely. These features support the assumption that in all the situations examined intimal SMCs have acquired the myofibroblastic phenotype. SM-MHCs/α-SMA, ie, as a value representative of SMC differentiation level, was lower in stable plaques and erosions with > 75% cross-sectional luminal narrowing compared with mildly stenotic plaques. It means that SMCs of the culprit lesions with the high degree of lesions severity in our study are differentiated from those in mildly stenotic lesions, suggesting that deep dissection evoked by PCI caused phenotypic modulation of medial SMCs to synthetic state and their proliferation.

Cholesterol gruel

There was a tendency toward larger area occupied by cholesterol gruel in restenosis group. It may depend on necrotic core size. Plaque size cannot be evaluated by DCA. However, larger plaque size may include larger necrotic core. The previous studies reported linear associations between plasma cholesterol level and coronary restenosis. One of the studies provides evidence for the critical role of cholesterol dependent oxidant stress in the pathophysiology of restenosis after PTCA. In our study, cholesterol gruel may induce coronary restenosis in the same mechanism.

Even in the current era of drug-eluting stents, restenosis at the proximal edge was the cause of about two thirds the cases of restenosis, possibly due to plaque compression and distribution along the vessel rather than intimal hyperplasia. Given the possible role of plaque shifting at the edges of a stent in causing restenosis, debulking could be added to the local drug effect in complex le-
sions.

**STUDY LIMITATIONS**

The lesions we can perform DCA are limited to relatively large proximal coronary segments. Loss of expression of α-actin in SMCs is associated with a phenotypic change from a contractile to a “synthetic” phenotype, and specific markers of activated SMCs were not evaluated in every sample. The weight of retrieved samples was not measured correctly, then we did not show the data.

**CONCLUSION**

In de novo lesions, atherectomy specimens reflect the heterogeneity of features that are found in plaques. Macrophages infiltration, calcified fragments, cholesterol rich gruel and myxomatous ECM might be predictors of restenosis after directional coronary atherectomy, suggesting a possible role of them in restenotic process after PCI even in de novo lesion.

**DISCLOSURES**

none

**REFERENCES**


19) Michelle P, Colleen I, Reidy MA. Inhibition of matrix


