

下の通りである。簡単のため、スピンエコー法の 90, 180 度ラジオ波 (RF) パルスや反転パルスにより、スピンは瞬時に理論的フリップ角で期待されるとおりの理想的運動をすと仮定した。

スピンエコー法:

m_0 を熱平衡状態の縦磁化, T_1 を縦緩和時間, TR を繰り返し時間, TE をエコー時間とすると, 90 度パルス直前の縦磁化は次の式で与えられる。

$$m_0 \left\{ 1 - \left(2 - e^{-\frac{0.5TE}{T_1}} \right) e^{-\frac{TR-0.5TE}{T_1}} \right\}$$

したがって, 信号強度は次の式で与えられる⁶⁾。

$$m_0 e^{-\frac{TE}{T_2}} \left(1 + e^{-\frac{TR}{T_1}} - 2e^{-\frac{TR-0.5TE}{T_1}} \right)$$

ここで, T_2 は横緩和時間を示す。

高速スピンエコー法:

T_a を 180 度パルス間の時間, etl を 180 度パルスの数 (echo train length) とすると, 最後の 180 度パルス直前の縦磁化は次の式で与えられる ($T_a \ll T_1$ のとき, この縦磁化は小さな値となる)。

$$m_{a1} = m_0 \left\{ 1 + (-1)^{etl} e^{-\frac{(etl-0.5)T_a}{T_1}} - 2 \frac{1 + (-1)^{etl} e^{-\frac{(etl-1)T_a}{T_1}}}{1 + e^{-\frac{T_a}{T_1}}} e^{-\frac{T_a}{T_1}} \right\}$$

これから, 90 度パルス直前の縦磁化は次の式で与えられる。

$$m_0 \left\{ 1 - \left(1 + \frac{m_{a1}}{m_0} \right) e^{-\frac{TR-(etl-0.5)T_a}{T_1}} \right\}$$

したがって, 信号強度は次の式で与えられる⁷⁾。

$$m_0 e^{-\frac{TE_{etl}}{T_2}} \left\{ 1 - (-1)^{etl} e^{-\frac{TR}{T_1}} - 2 \frac{1 - (-1)^{etl} e^{-\frac{etl T_a}{T_1}}}{1 + e^{-\frac{T_a}{T_1}}} e^{-\frac{TR-(etl-0.5)T_a}{T_1}} \right\}$$

ここで, TE_{etl} は k 空間の中心に対応する実効エコー時間である。

スピンエコー法を用いた反転回復法:

TI を反転時間とすると, 反転パルス直前の縦磁化は次の式で与えられる。

$$m_0 \left\{ 1 - \left(2 - e^{-\frac{0.5TE}{T_1}} \right) e^{-\frac{TR-TI-0.5TE}{T_1}} \right\}$$

したがって, このシーケンスの信号強度は次の

式で与えられる⁶⁾。

$$m_0 e^{-\frac{TE}{T_2}} \left[1 - \left\{ 2 - \left(2 - e^{-\frac{0.5TE}{T_1}} \right) e^{-\frac{TR-TI-0.5TE}{T_1}} \right\} e^{-\frac{TI}{T_1}} \right]$$

高速スピンエコー法を用いた反転回復法:

高速スピンエコー法の最後の 180 度パルス直前の縦磁化 m_{a1} を用いると, 反転パルス直前の縦磁化は次の式で与えられる。

$$m_{a2} = m_0 \left\{ 1 - \left(1 + \frac{m_{a1}}{m_0} \right) e^{-\frac{TR-TI-(etl-0.5)T_a}{T_1}} \right\}$$

反転パルス後, 時間 TI における縦磁化は, この m_{a2} を用いて次の式で与えられ, これが信号強度に比例する⁷⁾。

$$m_z = m_0 \left\{ 1 - \left(1 + \frac{m_{a2}}{m_0} \right) e^{-\frac{TI}{T_1}} \right\}$$

FLASH:

$T2^*$ を自由誘導減衰時間, FA をフリップ角とすると, 信号強度は次の式で与えられる¹⁾。

$$\frac{\sin(FA) \left(1 - e^{-\frac{TR}{T_1}} \right) e^{-\frac{TE}{T_2^*}}}{1 - \cos(FA) e^{-\frac{TR}{T_1}}}$$

balanced SSFP:

TR/2 をエコー時間とすると, 信号強度は次の式で与えられる⁸⁾。

$$\frac{m_0 \sin(FA) \left(1 - e^{-\frac{TR}{T_1}} \right) e^{-\frac{0.5TR}{T_2^*}}}{1 - \cos(FA) \left(e^{-\frac{TR}{T_1}} - e^{-\frac{TR}{T_2}} \right) - e^{-\frac{TR}{T_1}} e^{-\frac{TR}{T_2^*}}}$$

MPRAGE:

このシーケンスは, 3D で撮像する反転回復法の turbo-FLASH⁹⁾ シーケンスと考えることができる。TR をイメージング用 RF パルスの繰り返し時間, m を k 空間の中心を何回目の RF パルスで撮るか, $a = \cos(FA) \exp(-TR/T_1)$ とすると, MPRAGE シーケンスの信号強度は次の式で与えられる (Appendix 参照)。

$$m_0 \sin(FA) \left(a^{m-1} \left(1 - \left(1 + \frac{m_{01}}{m_0} \right) e^{-\frac{TR}{T_1}} \right) + \frac{(1-a^{m-1}) \left(1 - e^{-\frac{TR}{T_1}} \right)}{1-a} \right) e^{-\frac{TE}{T_2}}$$

ここで、 m_{01} は定常状態となったときの反転パルス直前の縦磁化で、TD をイメージング用 RF パルスの照射終了後から次の反転パルスまでの時間、 mtx を位相エンコード方向のマトリクス数として、次の式で表される。

$$m_0 \frac{(e^{\frac{TR}{T_1}} - a(e^{\frac{TR}{T_1}} - 1) - e^{-\frac{TR}{T_1}}) - a^{m+1}(a(1 - e^{-\frac{TR}{T_1}}) + e^{-\frac{TR}{T_1}} - e^{-\frac{TR}{T_1}})}{(1-a)(e^{\frac{TR}{T_1}} + a^{m+1}e^{-\frac{TR}{T_1}})}$$

拡散強調画像：

スピンエコー型エコープランナー法を用いて、拡散強調の程度を示す b 値や motion probing gradient の方向を変えるなどの目的で繰り返して撮像する場合を考える。その繰り返し時間を TR とすると、信号強度は次の式で与えられる。

$$m_0 \left(1 + e^{-\frac{TR}{T_1}} - 2e^{-\frac{TR-0.5TE}{T_1}} \right) e^{-bADC - \frac{TE}{T_2}}$$

ここで b は b 値、ADC は apparent diffusion coefficient である。

これらの関数に基づいて、撮像条件（独立変数）を与えたときに、信号強度と縦緩和時間の関係がどうなるかをグラフで表すことが可能である。また、画像としてはどのようなコントラストになるかを、単純なモデル画像を作成して表現することができる。ここでは、そのモデル画像として、脳と頸動脈プラークイメージングを取り上げた。

米国 Wolfram 社製 webMathematica 3.0 を用い、上に述べた様々な撮像条件や組織のパラメータをユーザがパソコンのマウスを用いて簡単に変化させて入力することができるようにし、それに応じて、ほぼリアルタイムで、信号強度が変化する様子をグラフや画像で表示することができるように

表 1. 組織パラメータ

組織	パラメータ*	デフォルト値**	設定可能な値の範囲
脳灰白質	PD	830	100-1000
	T1	1400 ms	500-2000 ms
	T2	90 ms	10-100 ms
	ADC	$0.7 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$
脳白質	PD	700	100-1000
	T1	900 ms	500-2000 ms
	T2	70 ms	10-90 ms
	ADC	$0.7 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$
脳脊髄液	PD	1000	500-1000
	T1	4500 ms	3000-5000 ms
	T2	1500 ms	50-2000 ms
	ADC	$3 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$
病的脳組織	PD	850	100-1000
	T1	1600 ms	100-4000 ms
	T2	110 ms	1-300 ms
	ADC	$0.6 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$
動脈壁中膜	PD	800	100-1000
	T1	1500 ms	500-2000 ms
	T2	60 ms	20-100 ms
	ADC	$1.6 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$
脂質コア	PD	700	100-1000
	T1	900 ms	100-1500 ms
	T2	40 ms	1-100 ms
	ADC	$0.36 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$
プラーク内出血巣	PD	700	100-1000
	T1	600 ms	100-1000 ms
	T2	40 ms	2-100 ms
	ADC	$0.4 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$
線維性皮膜	PD	800	100-1000
	T1	1400 ms	500-2000 ms
	T2	60 ms	20-100 ms
	ADC	$1.6 \mu\text{m}^2 \cdot \text{ms}^{-1}$	$0.1-3.5 \mu\text{m}^2 \cdot \text{ms}^{-1}$

*PD は m_0 に対応する proton density の略で、液体の水を 1000 としている。T1 は縦緩和時間、T2 は横緩和時間、ADC は apparent diffusion coefficient である。

** 静磁場強度 3 T を想定し、これまで報告されている値^{10,21)}を参考にした。

プログラムをコーディングした。モデル画像では、パラメータを様々に変えて画像のコントラストがどの様に変化するかを視覚的に評価できるようにした。パーソナルコンピュータで作成したページを、米国 Apache Software Foundation の Apache Tomcat 6.0 を用いてウェブ上に公開した。公開の制限はせず、誰でもアクセス可能なものとした。このウェブページで設定されている、静磁場強度 3 T を想定しての組織緩和時間などのパラメータのデフォルトの値、および必要に応じて変化させることができる値の範囲を表 1 に示す。

3. 結 果

簡単なマウス操作により、MRI の撮像条件を変えたときに信号強度がどうなるかを、短時間に計算して結果を表示することができる、動的にインタラクティブなウェブページを作成することができた²²⁾。ウェブページに表示された例として、図 1 に MPRAGE の信号強度と緩和時間の関係をグラフで表したものを示す。信号強度は、熱平衡の縦磁化の大きさを 1 としたときのものである (T_2^* 減衰は無視している)。負の値は縦磁化が反転している状態を示す。横軸 T_1 は縦緩和時間で

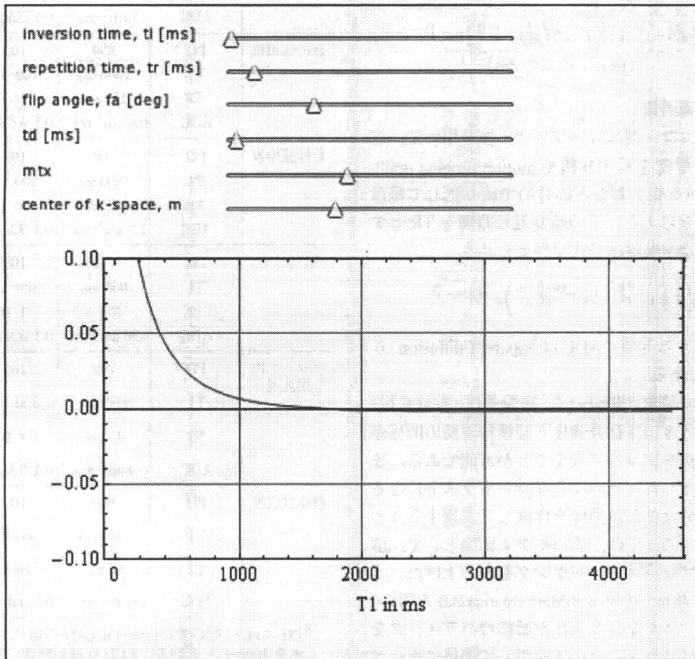


図 1. MPRAGE の信号強度と縦緩和時間の関係

縦軸は、熱平衡の縦磁化の大きさを 1 としたときの信号強度、横軸の T_1 は縦緩和時間である。実際のウェブページでは、三角形の小さなボタンをマウスで左右に動かすと撮像条件を変えられる。時間の単位はミリ秒 (ms) である。

ある。実際のウェブページでは、三角形の小さなボタンをマウスで左右に動かすと撮像条件を変えることができる。ウィンドウとレベルも変えることができる。マウスでそのボタンをクリックする際、各パラメータの数値がそのボタンの近くに表示される。時間はミリ秒単位である。ti は反転時間、tr はイメージング用 RF パルスの繰り返し時間、fa はフリップ角 (単位は度)、td はイメージング用 RF パルスの最後から次の反転パルスまでの時間、mtx は位相エンコード方向のマトリックス数、m は k-空間の中心を何回目の RF パルスで撮るかを示す。

図 2 に、高速スピネコー法を用いた反転回復法シーケンスである Fluid Attenuated Inversion Recovery (FLAIR) 法における信号強度画像を示す。図 1 に示した例と同様に、組織のパラメータと撮像条件は三角形の小さなボタンをマウスで左右に動かすことにより変えることができるように

なっている。

4. 考 察

このツールでは、緩和時間などの組織構成要素の MRI 特性を与えれば、様々な撮像条件において信号強度がどうなるかを見ることが可能で、撮像条件の最適化をする際の参考になると考えられる。多くの保健医療機関では、臨床の現場でインターネットに接続するための条件は整っていると思われ、このツールは手軽に撮像条件をチェックする上で有用であろう。見た目のコントラストはウィンドウ幅とレベルで変わってしまうため実際の見え方と直接に比較することは難しいが、コントラストの変化は捉えられるので、目的とする対象のコントラストを改善するのに役立つのではないかと考える。例えば、FLAIR シーケンスを最適化する場合、脳脊髄液の信号をゼロにするような撮像条件として、TR, etl, TI などのパラメー

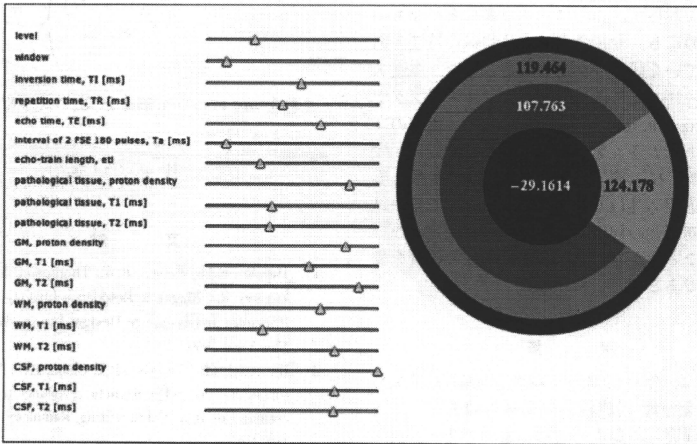


図 2. FLAIR シーケンスにおける信号強度画像

脳の軸位断模式図で、中心部が脳脊髄液 (CSF) を入れた脳室、脳室に接して白質 (WM)、その外部が灰白質 (GM)、右に楔状に病的組織、一番外側は脳脊髄液を表す。図中の数字は信号強度計算結果を示す。数字はウェブ上ではカラーで示され、組織のパラメータと撮像条件は三角形の小さなボタンをマウスで左右に動かすことにより変えられるようになっている。TI は縦緩和時間、T2 は横緩和時間を示す。撮像パラメータは、TI が反転時間、TR が繰り返し時間、TE がエコー時間、Ta は高速スピネコー法での 180° パルスの間隔、etl は 180° パルスの数である。

タの無数の組み合わせが可能であるが、そのすべてをボランティアで撮像して評価するのは不可能である。最も望ましいコントラストが得られるまでパラメータを変化させて試行錯誤するために多大な労力を必要とし、真に最適化されたかどうかの確認も難しい。本ツールを使えば、実際に撮像することなく、目的とするコントラストとなる撮像パラメータを推定できる。従って、その推定された撮像パラメータの近傍のみの値を用いて実際に撮像し評価をすることにより、効率的に求める撮像条件を得ることができる。実際のMRI装置では、RFパルスの不完全性や撮像スライス内の位置によるフリップ角の変化などにより、信号強度が我々の採用した理論式通りにならないので、推定されたパラメータは、そのまま使うことはできないが、ファントムやボランティアによる撮像で最適化をする際の撮像条件の目安として有用と考える。

一方、独立変数である生体組織の特性が十分には知られておらず、このツールを真に有用なものとするためにも、今後さらに生体組織の特性を明らかにしていく研究が必要である。また、高速スピネコー法のマルチスライスのイメージングでは magnetization transfer (MT) 効果が、画像のコントラストに大きく影響する²³⁾。この影響は、今回のツールでは、適宜に組織の緩和時間を変えることで対応する他はないが、MTの影響で見かけの緩和時間がどの程度変化するかは、測定しないと分からない面がある。この点は今後の改善を要する問題である。

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Appendix

turbo-FLASH シーケンスにおいて、 m_0 を熱平衡状態の縦磁化、 T_I を反転時間、 T_1 を縦緩和時間、 TR をイメージング用 RF パルスの繰り返し時間、

$a \equiv \cos(\text{FA})e^{-\frac{TR}{T_1}}$ とすると、 m 回目の RF パルス直前の縦磁化は次の式で与えられる⁶⁾。

$$m_0 \left(a^{m-1} \left(1 - \left(1 + \frac{m_0}{m_0} \right) e^{-\frac{TR}{T_1}} \right) + \frac{(1-a^{m-1})(1-e^{-\frac{TR}{T_1}})}{1-a} \right)$$

ここで、 m_0 は定常状態となったときの反転パルス直前の縦磁化で、 mtx を位相エンコード方向のマトリックス数 (これは単位シーケンス当たりのイメージング用の RF パルスの総数に等しい)、 TD をイメージング用 RF パルスによる信号採取が全て終了してから次の反転パルスまでの時間とすると、次の式が成り立つ。

$$m_0 \left[1 - \left(1 - a^{mtx} \left(1 - \left(1 + \frac{m_0}{m_0} \right) e^{-\frac{TR}{T_1}} \right) \right) \frac{(1-a^{mtx})(1-e^{-\frac{TR}{T_1}})}{1-a} \right] e^{-\frac{TR}{T_1}} = m_{01}$$

これを m_{01} について解くと、次の式が得られる。

$$m_{01} = m_0 \frac{\left(e^{\frac{TR}{T_1}} - a \left(e^{\frac{TR}{T_1}} - 1 \right) - e^{-\frac{TR}{T_1}} \right) - a^{mtx} \left(a \left(1 - e^{-\frac{TR}{T_1}} \right) + e^{-\frac{TR}{T_1}} - e^{-\frac{TR}{T_1}} \right)}{(1-a) \left(e^{\frac{TR}{T_1}} + a^{mtx} e^{-\frac{TR}{T_1}} \right)}$$

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Assessment of necrotic core with intraplaque hemorrhage in atherosclerotic carotid artery plaque by MR imaging with 3D gradient-echo sequence in patients with high-grade stenosis

Clinical article

TOMOHITO HISHIKAWA, M.D.,¹ KOJI IIHARA, M.D.,¹ NAOAKI YAMADA, M.D.,²
HATSUE ISHIBASHI-UEDA, M.D.,³ AND SUSUMU MIYAMOTO, M.D.¹

Departments of ¹Neurosurgery, ²Radiology, and ³Pathology, National Cardiovascular Center, Osaka, Japan

Object. The aim of this study was to assess the histopathological differences between advanced atherosclerotic carotid artery (CA) plaques with signal hyperintensity on T1-weighted MR images and those without, focusing on necrotic core size and intraplaque hemorrhage (IPH).

Methods. Thirty-five patients scheduled for carotid endarterectomy underwent preoperative CA MR imaging using 3D inversion-recovery-based T1-weighted imaging (magnetization-prepared rapid acquisition gradient-echo [MPRAGE]). The signal intensity of the CA plaque on MPRAGE sequences was classified as “high” when the intensity was more than 200% that of adjacent muscle. A total of 96 axial MR images obtained in 35 patients were compared with corresponding histological sections from 36 excised specimens. The area of the necrotic core in histological sections was compared between specimens with and without high signal intensity on MPRAGE sequences. The IPH was histopathologically graded according to the size of the area positive for glycophorin A as revealed by immunohistochemical staining. The difference between plaques with and without high signal intensity was investigated with respect to the degree of IPH. The relationship of the severity of IPH to size of the necrotic core was also evaluated.

Results. The area of the necrotic core in plaques with high signal intensity on MPRAGE sequences was significantly larger than that in plaques without high signal intensity (median 51.2% [interquartile range 43.3–66.8%] vs 49.0% [33.2–57.6%], $p = 0.029$). Carotid artery plaques with high signal intensity had significantly more severe IPH than plaques with lower signal intensity ($p < 0.0001$). The severity of IPH was significantly associated with the size of the necrotic core ($p < 0.0001$).

Conclusions. Atherosclerotic CA plaques with high signal intensity on MPRAGE sequences had large necrotic cores with IPH in patients with high-grade stenosis; MPRAGE is useful for the evaluation of CA plaque progression. (DOI: 10.3171/2010.3.JNS091057)

KEY WORDS • carotid artery stenosis • intraplaque hemorrhage • MR imaging • necrotic core

THE prophylactic effectiveness of CEA in patients with more than a certain degree of arterial stenosis has been proven in several randomized multicenter trials.^{7,8,11,21} Although the degree of lumen narrowing is a significant risk factor in stroke, the role of vulnerable CA plaque has recently been emphasized,²⁰ and some reports have demonstrated that episodes of cerebral ischemia in patients with CA lesions are not restricted to cases with severe stenosis.^{3,10,24} The type of vulnerable plaque that is most prone to rupture is characterized by a large necrotic core, a thin fibrous cap (< 65 μ m thick), and widespread macrophage infiltration within the fibrous cap.³¹ In addition, the role of IPH in the vulnerability of CA plaques has been emphasized by many investigators.^{6,17,26,28,29,34}

Magnetic resonance imaging is well suited for CA plaque evaluation in a clinical setting because it is noninvasive and widely available, provides excellent soft tissue contrast, and does not involve ionizing radiation. In response to reports demonstrating the close association of IPH with cerebral ischemic events,²⁶ many authors have recently emphasized the value of IPH detection by MR imaging and have demonstrated the clinical usefulness of this noninvasive modality.^{1,6,13,17,28,29,33}

Our institute has routinely performed CA plaque MR imaging using 3D inversion-recovery-based T1-weighted sequences (MPRAGE) before interventions for CA stenosis. Yamada et al.³³ demonstrated that CA plaque with high signal intensity on MPRAGE sequences was significantly associated with previous ipsilateral ischemic events in patients with moderate and severe stenosis. In that report,

This article contains some figures that are displayed in color online but in black and white in the print edition.

Abbreviations used in this paper: CA = carotid artery; CEA = carotid endarterectomy; ICA = internal carotid artery; IPH = intraplaque hemorrhage; IQR = interquartile range; MPRAGE = magnetization-prepared rapid acquisition gradient echo.

Assessment of carotid artery plaque by MPRAGE

CA plaque signal intensity on greater than 200% that of adjacent muscles was defined as high and the chi values for interobserver and intraobserver agreement were 0.729 and 0.792, respectively (good agreement).³³ Taking the close association of MPRAGE signal intensity (using a threshold of 200% of the intensity of adjacent muscles) with symptomatology into consideration, we sought to determine the histopathological differences between CA plaques with and without high signal intensity as the next stage of investigation. The aim of this study was to retrospectively assess the vulnerability of CA plaques, focusing on differences in necrotic core area and degree of IPH between plaques that are associated with high signal intensity on MPRAGE sequences and those that are associated with lower signal intensity (< 200% of the intensity of adjacent muscle tissue).

Methods

Study Population

Thirty-five patients (32 men, 3 women) with a mean (\pm SD) age of 69.0 ± 7.8 years who were scheduled for CEA at our institute between May 2006 and March 2007 were included in this study. Patient characteristics were examined retrospectively through a review of the relevant medical records. A total of 36 CEAs for CA stenosis (1 patient had bilateral CA stenosis) were performed, and 36 endarterectomy specimens were analyzed. Twenty patients (57.1%) had a history of ipsilateral ischemic events, including cerebral infarction, transient ischemic attack, and retinal ischemia, within the previous 6 months, and their lesions were defined as symptomatic. The degree of CA stenosis was measured using digital subtraction angiography (17 lesions), 3D CT angiography (17 lesions), or contrast-enhanced MR angiography (2 lesions) according to the method used in the North American Symptomatic Carotid Endarterectomy Trial (NASCET).⁹ The criteria for CEA were 70% stenosis or greater for symptomatic cases and 75% stenosis or greater for asymptomatic cases.¹² All 36 endarterectomy specimens were circumferentially removed with the plaque intact.

Magnetic Resonance Imaging Protocol

Imaging was performed using standard neck array and spine array coils in a Magnetom Sonata 1.5-T system (Siemens). Plaque imaging was performed using MPRAGE in the transaxial section with null blood condition (effective inversion time 660 msec; TR 1500 msec) and the water excitation technique to suppress fat signals.³³ Other scanning parameters were as follows: TE 5.0 msec; FOV 180×180 mm; matrix 256×204 ; section thickness 1.25 mm; 56 partitions, covering 70 mm around the CA bifurcation; and data acquisition time 5 minutes. The mean interval between MR imaging and CEA was 17.9 ± 20.4 days. In symptomatic patients, the mean duration of time from last symptoms to MR imaging was 16.2 ± 27.0 days.

Image Review and Criteria

The MR images were reviewed by an experienced radiologist (N.Y.) blinded to clinical and pathological data.

The signal intensity of plaques on MPRAGE relative to the signal intensity in adjacent muscle (typically the sternocleidomastoid muscle)—referred to in this paper as the relative MPRAGE signal intensity—was calculated in each image at 5-mm intervals extending rostrally along the ICA from the CA bifurcation. Any section of plaque that displayed a signal intensity that was more than 200% that of the adjacent muscle tissue was categorized as having high signal intensity.

Endarterectomy Specimen Processing and Criteria

In symptomatic cases, the mean value of the time from most recent symptoms to CEA was 20.3 ± 28.1 days. The endarterectomy specimens were immediately fixed in Histochoice fixative (Amresco, Inc.) for 48 hours and decalcified with EDTA. Subsequently, they were divided into 5-mm blocks (starting at the CA bifurcation and extending rostrally along the ICA) and embedded in paraffin. From each 5-mm block, a 3- μ m section was obtained and stained with H & E and Masson trichrome for histological evaluation. In addition, immunohistochemical testing for glycoporphin A was performed to identify IPH (1:200 dilution; Dako). Each section was histopathologically evaluated by an experienced histopathologist (H.I.U.) who was unaware of the MR imaging results. Atheromatous plaques were defined as areas of protrusion into the vascular lumen due to atherosclerosis that were bounded by an internal elastic layer and included fibrous caps. Necrotic core was defined as a core area of atheromatous plaques that consisted of necrotic macrophages, cholesterol crystals, and sometimes hemorrhage. The proportion of the necrotic core area to the total plaque area (NC proportion) was measured using a computer-based morphometric system (WinRoof, Mitani Co.). As an index of the degree of IPH (IPH score), the ratio of the glycoporphin A-positive area to the total plaque area was calculated and the sample was graded according to the following scale: a ratio ≥ 0.40 corresponded to a score of 3; a ratio ≥ 0.20 and < 0.40 , a score of 2; a ratio < 0.20 , a score of 1.

Correlation of MR Imaging and Histological Specimens

The MR images and histological sections were independently reviewed and evaluated. The relative distance from the CA bifurcation was used as a landmark to match the histological data with the MR images in the longitudinal direction of the artery. Morphological features such as lumen size and shape were also used as a reference landmark.

Statistical Analysis

Quantitative variables are presented as the medians and IQRs. The correlation between the relative MPRAGE signal intensity and the NC proportion or IPH score was assessed by nonparametric analysis of the Spearman rank correlation test. The Mann-Whitney U-test was used to compare the NC proportion in samples with high signal intensity on MPRAGE sequences to the proportion in samples with lower signal intensity. Contingency tables displaying IPH scores and MPRAGE signal intensity

(high vs not high) were generated and analyzed by means of the chi-square test to determine a trend. The relationship of IPH score to NC proportion was assessed through nonparametric analysis using the Kruskal-Wallis test. All statistical analyses were performed with StatView (SAS Institute). Differences were considered to be significant when p values were < 0.05 .

Results

A total of 96 histological sections corresponding to MR images from 36 endarterectomy specimens were evaluated in this study. Table 1 shows the baseline characteristics of patients, including demographic variables and risk factors.

Correlation Between Size of Necrotic Core and MPRAGE Signal Intensity

Figure 1 shows a scatterplot of the relative MPRAGE signal intensity and the NC proportion. A significant positive correlation was found between the relative MPRAGE signal intensity and the NC proportion ($p = 0.0032$). The NC proportions in plaques with high signal intensity were significantly larger than those in plaques without high signal intensity (median 51.2% [IQR 43.3–66.8%] vs 49.0% [IQR 33.2–57.6%], $p = 0.029$; Fig. 2).

Relationship Between IPH and MPRAGE Signal Intensity

Figure 3 shows a scatterplot of the relative MPRAGE signal intensity and the IPH score. There was a significant positive correlation between the relative MPRAGE signal intensity and the IPH score ($p < 0.0001$). High signal intensity was seen in images corresponding to 14.3, 25.0, and 64.3% of specimens with IPH scores of 1, 2, and 3, respectively. Higher IPH score was significantly associated with high signal intensity on MPRAGE sequences ($p < 0.0001$; Table 2).

Relationship Between IPH and Necrotic Core

The median values (IQRs) for NC proportion were 32.7% (25.3–47.8%), 45.4% (40.5–54.8%), and 53.1% (47.4–65.6%) for samples with IPH scores of 1, 2, and 3, respectively. Higher IPH scores were significantly associated with larger necrotic cores ($p < 0.0001$; Fig. 4).

Representative Examples

Figures 5 and 6 show the representative appearance of MPRAGE images and histological findings from corresponding specimens.

Discussion

Role of IPH in Plaque Progression

Naghavi et al.¹⁹ proposed the concept of vulnerable patients, along with criteria for defining vulnerable plaques, based on autopsy studies of coronary arteries. Among their criteria, IPH has recently been emphasized in pathological studies as an important factor in CA plaque progression.^{14,15,32} It has been reported that

TABLE 1: Summary of demographic and clinical characteristics in 35 patients*

Characteristic	Value
symptomatic lesions	20 (57)
mean degree of stenosis (%)	80 \pm 10
mean age in yrs	69 \pm 8
female sex	3 (9)
hypertension	24 (69)
diabetes mellitus	15 (43)
hyperlipidemia	19 (54)
cigarette smoking	20 (57)

* Values represent numbers of patients (%) unless otherwise indicated. Means are presented \pm SDs.

necrotic core enlargement is crucial for plaque rupture and that increased free cholesterol within the necrotic core is closely associated with lesion instability.³² Concerning the derivation of free cholesterol, it is generally accepted that apoptotic macrophages are an efficient source of free cholesterol in plaques,²⁷ although Kolodgie and colleagues^{15,32} have demonstrated new lines of evidence suggesting that erythrocyte membranes contribute a significant amount of the free cholesterol in coronary plaques. Nuotio et al.²² also reported that IPH could play a role in the vulnerability of symptomatic CA plaques by contributing to lipid accumulation and inducing adipophilin (an adipose differentiation-related protein) and further increasing lipid accumulation by preventing lipid efflux. From a symptomatological point of view, Lusby et al.¹⁶ demonstrated that a history of multiple hemorrhage (hemorrhages at various ages) was seen in 81% of patients with symptoms of cerebral ischemia associated with atherosclerotic CA stenosis.

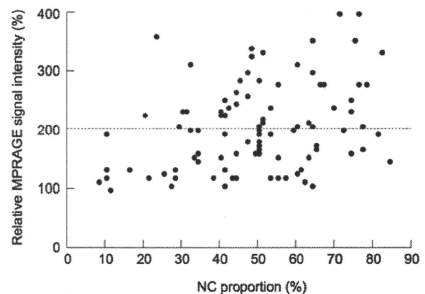


FIG. 1. Scatterplot showing the signal intensity of plaques on MPRAGE sequences relative to the signal intensity in adjacent muscle (relative MPRAGE signal intensity) and the proportion of the necrotic core (NC) area to the total plaque area (NC proportion) as established by pathological examination of specimens. The broken horizontal line indicates the level of 200% MPRAGE proportion, the threshold for "high" signal intensity in this study.

Assessment of carotid artery plaque by MPRAGE

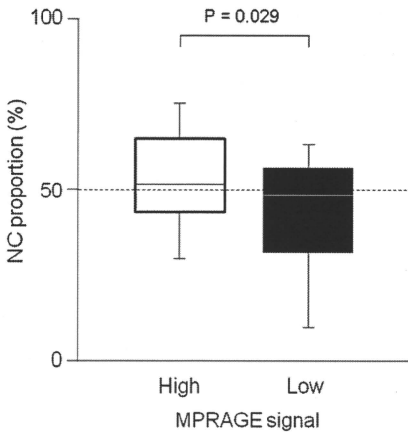


Fig. 2. Box and whisker plot showing the relationship of the NC proportion to MPRAGE signal intensity. The NC proportion in specimens corresponding to imaging studies that showed high signal intensity on MPRAGE sequences was significantly larger than that in specimens that showed lower signal intensity ($p = 0.029$, Mann-Whitney U-test). The broken horizontal line is at the level of 50% NC proportion.

The present study demonstrated a significant association of IPH with necrotic core in patients with high-grade CA stenosis. Therefore, the use of a noninvasive and objective modality to detect IPH and to predict subsequent

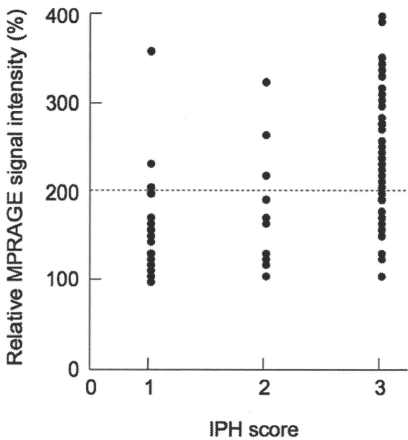


Fig. 3. Scatterplot showing the relative MPRAGE signal intensity and IPH score (1–3). The broken horizontal line is at the 200% threshold for high signal intensity.

TABLE 2: Correlation between high signal intensity on MPRAGE sequences in 96 axial MR images and IPH score in corresponding histological sections*

IPH Score	No. of MR Images	Relative Signal Intensity >200%		p Value
		Yes	No	
1	28	4 (14.3)	24 (85.7)	<0.0001
2	12	3 (25.0)	9 (75.0)	
3	56	36 (64.3)	20 (35.7)	

* Signal intensity in CA plaque was assessed relative to that of adjacent muscle tissue, using 200% as the threshold for "high" signal intensity on MPRAGE sequences. Values represent numbers of images, expressed as a percentage of the images in the given score category in parentheses.

necrotic core expansion in CA plaque could play an important role in assessing plaque vulnerability and in managing patients with CA stenosis in a clinical setting.

Assessment of Carotid Plaque by MPRAGE Sequence

Moody et al.¹⁷ used a T1-weighted magnetization-prepared 3D gradient echo sequence, a technique identical to that used in MPRAGE, to detect complicated plaque by detecting IPH in an *in vivo* study of CA plaques in symptomatic patients. Bitar et al.⁴ recently reported that MR imaging of IPH using a 3D spoiled gradient-echo sequence resulted in strong agreement between imaging and histologic findings. Yamada et al.³³ disclosed significant associations between cerebral ischemic events and MPRAGE hyperintensity according to stenosis severity using 200% of the signal intensity of adjacent muscle as the threshold for high signal intensity and reported the percentages of patients with high signal intensity were 21, 54, and 65% for mild (0–29%), moderate (30–69%), and severe (70–99%) stenosis, respectively. The present study demonstrated that the NC proportion in plaques with high signal intensity on MPRAGE sequences was significantly larger than that in plaques with lower signal intensity, and the severity of IPH was significantly correlated with signal intensity. The hemorrhage-rich larger necrotic core is a pathological basis for the high signal intensity on MPRAGE in these plaques, which has previously been shown to be significantly associated with symptomatology.³³ Also, in the present study, 58% of CAs examined (21 of 36) had high signal intensity on MPRAGE sequences and 58% of the endarterectomy sections (56 of 96) exhibited the highest degree of IPH (score of 3 in our pathological examination). Together with the data from Yamada et al.,³³ the data from this study shows, through the association between MPRAGE signal hyperintensity and high-grade CA stenosis, that IPH has a high prevalence in advanced CA atherosclerotic plaques; our data also raise the possibility that IPH is a major mechanism underlying the progression of stenosis through its stages of severity as well as the expansion of the necrotic core.

The majority of previous reports have used multiple contrast techniques (T1-weighted, T2-weighted, proton-density-weighted, and 3D time-of-flight MR imaging)

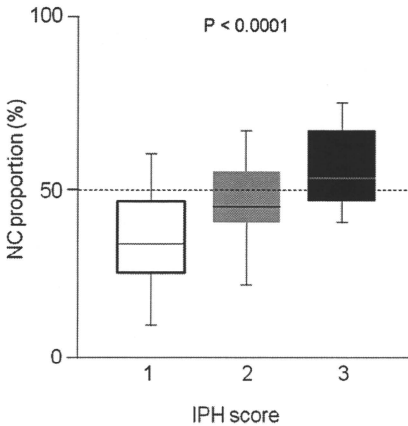


Fig. 4. Box and whisker plot showing the relation of IPH score to NC proportion. Higher IPH scores were significantly associated with higher NC proportion, according to the Kruskal-Wallis test ($p < 0.0001$). The broken horizontal line is at the level of 50% NC proportion.

to identify necrotic cores and IPH.^{6,13,28,29,34} Saam et al.²⁵ demonstrated the accuracy and reproducibility of using multicontrast imaging to quantify CA plaque components. On the other hand, the usefulness of single-contrast imaging using a 3D gradient-echo sequence has also been demonstrated.^{14,17,18,33} This sequence is often employed to identify IPH using a T1-weighted signal alone. In a study comparing the performance of a gradient-echo sequence with that of a spin-echo sequence, the gradient-echo sequence had a higher detection rate for IPH as well as much better interobserver agreement and better image quality.⁵ Likewise, Ota et al.²³ reported that the MPRAGE sequence at 3 T had the highest diagnostic capability for the detection and quantification of IPH, in a study comparing it with the fast spin echo and spoiled gradient echo sequences. In the present study, the area over which MPRAGE signal hyperintensity was present in CA plaque was not directly compared with the area of the necrotic core as measured through histopathological analysis. Nevertheless, our data reveal that the areas of high signal intensity on MPRAGE sequences coincide with the hemorrhage-rich large necrotic core. As the interpretation of MPRAGE signal intensity is simple and objective, requiring only a short image acquisition time (5 minutes), this 3D gradient-echo sequence technique is useful in a clinical setting.

As the practice of CA stent placement has rapidly become more prevalent, distal embolic complications during the procedure have become an important clinical issue. A report on the use of a filter protection device during CA stent placement in Japan revealed that disturbed blood flow presumably due to the blockage of the filter pores by plaque was observed in 40.0% of cases (slow flow in

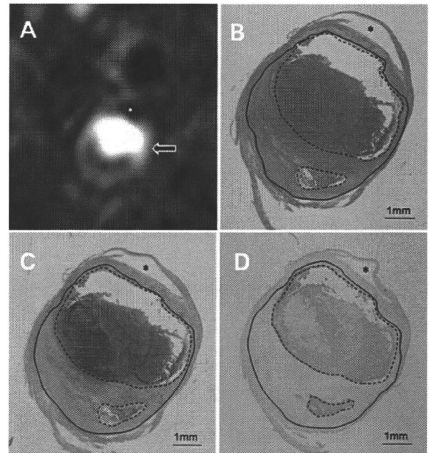


Fig. 5. An example of plaque with high signal intensity. **A:** An MPRAGE image showing the plaque (arrow). **B** and **C:** Photomicrographs of histological sections showing a large amount of necrotic core with IPH. **H & E (B)** and **Masson trichrome (C)**. **D:** Photomicrograph showing that the necrotic core is strongly positive for glycoprotein A. Asterisks indicate the ICA lumen. The total plaque area is delineated by a solid line and the necrotic core area by a dotted line. Bar = 1 mm.

26.7%, no flow in 13.3%).³⁰ Angeneli et al.² collected the embolized debris caught in such a filter and performed histopathological analysis on it; they reported that the collected debris consisted predominantly of thrombotic material, foam cells, and cholesterol clefts. The use of MPRAGE for CA plaque analysis before CA stent placement could be useful in predicting the risk of embolic complication and blood flow disturbances and could aid in selecting suitable distal protection devices.

Study Limitations

First, we used immunohistochemical staining for glycoprotein A as a marker of IPH. Glycoprotein A is a protein specific to erythrocytes that facilitates anion exchange; it is also an indicator of previous hemorrhage.¹⁵ Although high signal intensity on MPRAGE sequences was associated with the detection of previous hemorrhages by glycoprotein A, the signal hyperintensity may not be related to fresh thrombi or hemorrhages. In fact, in this study, a few endarterectomy sections with fresh thrombi revealed only lower-intensity signals. Because fresh thrombi play a crucial role in the pathogenesis of stroke,²⁶ it is important to detect them and to accurately classify IPH; thus, if MPRAGE is to become a useful technique, we must develop sequences that can detect fresh thrombi and also measure the age of IPH lesions. Second, because the patients included in this study had been selected as candidates for CEA based on the criteria of our institute,¹²

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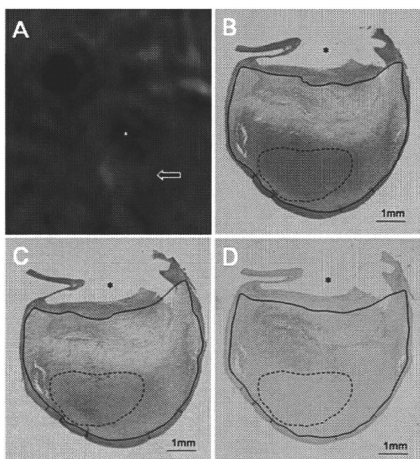


Fig. 6. An example of plaque with lower signal intensity. **A:** An MPRAGE image showing the plaque (arrow). **B and C:** Photomicrographs of ICA sections show the plaque is mainly consisted of fibrous tissue and focal calcification. H & E (**B**) and Masson trichrome (**C**). **D:** Photomicrograph showing that the plaque is only lightly stained for glycoporphin A. Asterisks indicate the ICA lumen. The total plaque area is delineated by a solid line and the necrotic area by a dotted line. Bar = 1 mm.

pathological examination and MR imaging analysis in this study were restricted to patients with high-grade stenosis. Yet the relative risk of ipsilateral ischemia according to MPRAGE signal intensity was reported to be the highest in the moderate-stenosis group.³³ In future studies of MPRAGE, it would be better to investigate the pathological differences between patients with moderate stenosis and high signal intensity and those with moderate stenosis and lower signal intensity.

Conclusions

We demonstrated that in CA plaque with high-grade stenosis, high signal intensity on MPRAGE sequences (> 200% increase relative to signal intensity in adjacent muscle tissue) indicates larger necrotic cores and more severe degrees of IPH than lower signal intensity. We also found that IPH is closely associated with necrotic core expansion in advanced CA plaque. These results indicate that MPRAGE, a T1-weighted imaging technique, can accurately reveal plaque progression in conjunction with IPH in patients with high-grade stenosis.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: T Hishikawa, K Iihara. Acquisition of data: N Yamada, H Ishibashi-Ueda. Reviewed final version of the manuscript and approved it for submission: T Hishikawa, K Iihara, N Yamada, H Ishibashi-Ueda, S Miyamoto. Study supervision: S Miyamoto.

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Address correspondence to: Tomohito Hishikawa, M.D., Department of Neurological Surgery, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama City, Okayama, 700-8558 Japan. email: t-hishi@md.okayama-u.ac.jp.

► **Original Article** ◀

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 culprit 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 atherectomy, 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 culprit 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 independent

¹ Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, Suita, Osaka, Japan

² Division of Pathology, National Cardiovascular Center, Suita, Osaka, Japan

³ Division of Surgical Pathology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

Received: May 3, 2009 Accepted: May 11, 2010
Address for reprint requests to Kentaro Arakawa, MD: Division of Cardiology, Fujisawa Municipal Hospital, 2-5-1, Fujisawa, Fujisawa, Kanagawa 251-8550, Japan
Tel: +81-466-25-3111, Fax: +81-466-25-3545
Email: hiroking@gamma.ocn.ne.jp

dent predictors of restenosis after DCA.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 mm²) (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- α -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. Intervending 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-

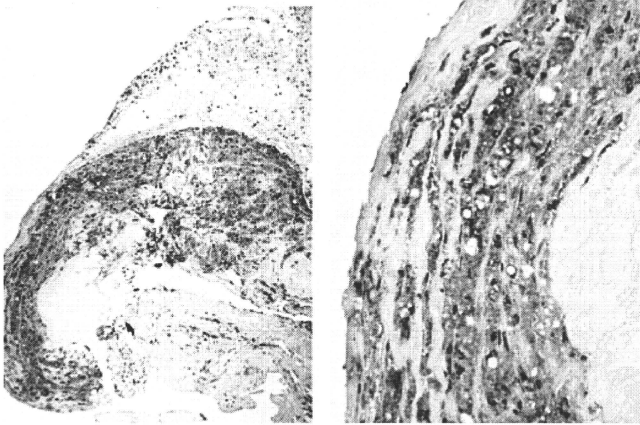


Fig. 1 A representative immunohistochemical staining for CD68 in DCA specimen obtained from a coronary artery, which presented restenosis after 6 months. The area occupied by macrophages is more than 50% (left; $\times 40$, right; $\times 400$ original magnification).

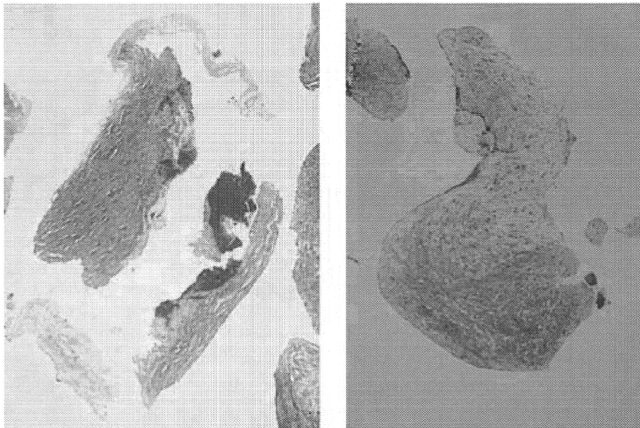


Fig. 2 Left; hyalinized ECM composed of dense collagen tissue (blue part) with low cellularity and organized thrombus (red part). Right; myxomatous ECM composed of a loose connective tissue matrix containing numerous stellate cells (Masson's trichrome staining, $\times 40$, original magnification respectively).

Table 1 Baseline clinical characteristics and coronary angiographic findings

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

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

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 2 Histological analysis of atherectomy specimen

	Restenosis (n = 26)	No-Restenosis (n = 50)	p value
The occupied area grade of total area			
Grade 0/1/2/3 n (%)			
Inflammatory cells PMNs	10/12/3/1 (38/46/12/4%)	29/17/3/1 (58/34/6/2%)	0.420
Lymphocytes	3/19/3/1 (12/73/12/4 %)	18/24/6/2 (36/48/12/4%)	0.106
Mφs	2/13/10/1 (8/50/38/4%)	12/28/8/1 (24/56/18/2%)	0.028
Cholesterol gruel	1/16/8/1 (4/61/31/4%)	12/26/9/3(24/52/18/6%)	0.063
α-SMA positive cells	1/12/9/4 (4/46/35/15%)	3/21/19/7 (6/42/38/14%)	0.959
Fibrin thrombus	6/12/6/2 (23/46/23/8 %)	10/23/14/3(20/46/28/6%)	0.958
Calcification	14/6/6/0 (54/23/23/0 %)	35/14/1/0(70/28/2/0%)	0.012
The presence of media n (%)	12 (46%)	26 (52%)	0.629
ECM myxomatous/hyalinized n (%)	14/12 (54/46%)	16/34 (32/68%)	0.065
The presence of inflammatory cells in the fibrous cap n (%)			
Lymphocytes	15 (57%)	25 (50%)	0.523
Mφs	18 (69%)	23 (46%)	0.051

ECM, extracellular matrix; α-SMA positive cells, alpha smooth muscle actin positive cells; PMNs , polymorphonucler cells; Mφs, macrophages

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.¹⁵ 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 after angioplasty 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

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 atherectomy specimens. Deep arterial wall excision is not generally associated with an increased incidence of restenosis.^{21, 22} 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.²³ 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.²⁴ The ECM consists of varying concentrations of proteoglycans (versican, biglycan, and decorin), hyaluronan, and collagen (types I and III).²⁵ The ECM modulates important events within the developing neointima, including cell proliferation, migration, growth factor expression, and remodeling.²⁶ And ECM accounts for > 50% of the volume of neointimal restenosis lesions.²⁶ 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.²⁷

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 rup-

ture 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 a 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.²⁸ 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.²⁹ 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.³⁰ 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

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