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Background and Purpose—Thrombosis on atherosclerotic lesions in the large extracranial arteries is the main cause of embolization in the distal cerebral circulation and thus is involved in the pathogenesis of ischemic stroke. The assessment of biological characteristics of lesions that are predictive of thrombotic complications might help in stratification of the risk for stroke but is currently imperfect.

Methods—We compared the performance of ^{111}In-platelet scintigraphy with blood pool subtraction, ultrasound-based tissue texture analyses, and transcranial Doppler techniques in their ability to predict the occurrence of superficial thrombosis or the presence of a lipid pool in carotid artery plaque specimens removed at the time of carotid endarterectomy in 22 patients with unilateral carotid artery stenosis of >70%.

Results—Positivity at ^{111}In-platelet scintigraphy was present in 8 patients and correctly identified the presence of thrombosis superimposed on a complicated plaque. Neither tissue texture analysis nor emboli detection by transcranial Doppler, performed in 12 patients, significantly identified plaque thrombosis. None of the techniques used were able to detect the presence of a significant lipid pool inside the plaque.

Conclusions—Indium-platelet scintigraphy is an accurate noninvasive diagnostic tool to detect thrombotic complications in carotid plaques. Prospective studies should assess its ultimate value in risk stratification, possibly to guide the decision of whether to perform endarterectomy in selected patient categories. (***Stroke***. 2001;32:719-727.)

Key Words atherosclerosis ◾ carotid stenosis ◾ platelets ◾ stroke assessment ◾ thrombosis

Atherosclerosis in the extracranial arteries has long been recognized as the most frequent pathological substrate of acute ischemic stroke.[1] Although as many as 30% of persons over age 50 years have evidence of carotid artery disease,[2] only some carotid stenoses carry an appreciable risk of stroke.[1,3,4] Stenosis severity is one parameter that can help to identify such a risk. The North American Symptomatic Carotid Endarterectomy Trial (NASCET)[5] and the European Carotid Surgery Trialists' Collaborative Study[6] have indicated the opportunity of carotid endarterectomy for patients with carotid stenoses of ≥70% in diameter at angiography, with a previous recent transient ischemic attack or stroke in the dependent territory. At the present time, however, endarterectomy is not mandatory in symptomatic patients with ipsilateral stenosis of <70% in diameter[7–9] and in those with asymptomatic severe stenosis,[10,11] who amount to about one third of those who currently undergo the procedure.[12] The uncertainty in the assignment of patients to surgery or medical therapy reflects at least in part the variable fate of the carotid artery plaque, which may "unstabilize" in many cases despite being diametrically mild or may remain "stable" in many cases despite being severe. There is therefore a need for techniques that can increase prediction of the fate of carotid lesions.[13] This need cannot be matched by the increasing diagnostic accuracy in stenosis severity estimation with ultrasonography,[2] because it is likely that stability depends little on severity and more on additional parameters that reflect the pathobiology of the plaque.[14,15]

In the coronary arteries, thrombosis on a fissured[16,17] or superficially eroded[18,19] plaque is currently considered the immediate precursor of acute ischemic syndromes. The positive results of stroke prevention studies with antiplatelet agents[20] and, more recently, anticoagulants[21,22] provide an argument that thrombosis on a plaque is in most cases the link between extracranial artery atherosclerosis and stroke.[16] Therefore, techniques able to detect fissured plaques or superimposed thrombosis should expand the ability to stratify the risk of clinical complications and potentially guide therapeutic decisions.

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TABLE 1.   Clinical Characteristics of Patients Recruited

Patient	Gender	Age, y	Symptoms	Angiography, %	Echo-Doppler Analysis, %	Therapy
Study 1						
1	M	74	TIA	Left ICA (75)	Left ICA (>70)	Lysine acetyl-salicylate 160 mg/d
2	M	71	Asymptomatic	Right ICA (75)	Right ICA (70)	ASA 100 mg/d
3	M	65	TIA	Right ICA (75)	Right ICA (75)	ASA 324 mg/d
4	F	48	TIA	Right ICA (75)	Right ICA (80)	ASA 100 mg/d, nifedipine
5	F	65	Asymptomatic	Right ICA (90)	Right ICA (>75)	Ticlopidine 250 mg BID, captopril, amiloride
6	M	69	TIA	Right ICA (90)	Right ICA (90)	Lysine acetyl-salicylate 160 mg/d
7	M	54	TIA	Right ICA (90)	Right ICA (>75)	ASA 100 mg/d
8	M	65	TIA	Right ICA (75)	Right ICA (>75)	Lysine acetyl-salicylate 160 mg/d
9	M	73	Asymptomatic	Right ICA (85)	Right ICA (80)	ASA 324 mg/d, nifedipine, buflomedil
10	M	68	TIA	Left ICA (90)	Left ICA (90)	ASA 100 mg/d
11	F	70	TIA	Right ICA (75)	Right ICA (75)	Lysine acetyl-salicylate 160 mg/d
12	F	59	Asymptomatic	Right ICA (80)	Right ICA (80)	ASA 324 mg/d
Study 2						
13	M	77	Asymptomatic	Left ICA (90)	Left ICA (90)	ASA 100 mg/d, propafenone, felodipine
14	F	72	Asymptomatic	Left ICA (75)	Left ICA (>75)	Lysine acetyl-salicylate 160 mg/d
15	F	73	TIA	Left ICA (80)	Left ICA (>70)	ASA 324 mg/d
16	M	67	Asymptomatic	Left ICA (90)	Left ICA (90)	ASA 100 mg/d, doxazosine
17	M	52	TIA	Left ICA (90)	Left ICA (>75)	Lysine acetyl-salicylate 160 mg/d
18	M	79	Asymptomatic	Right ICA (75)	Right ICA (>70)	Ticlopidine 250 mg/d
19	M	72	Asymptomatic	Left ICA (80)	Left ICA (>75)	ASA 100 mg/d
20	M	70	TIA	Right ICA (80)	Right ICA (>75)	ASA 324 mg/d
21	M	75	TIA	Left ICA (90)	Left ICA (>75)	ASA 324 mg/d
22	M	56	Asymptomatic	Right ICA (80)	Right ICA (>75)	ASA 100 mg/d

ICA indicates internal carotid artery; ASA, acetylsalicylic acid.

Thrombosis superimposed on a plaque in a superficial vessel can be detected with nuclear medicine techniques. Tracers that bind to developing thrombi include radiolabeled platelets, fibrinogen, fibrin fragments, immunoglobulins, and peptides directed against platelet and fibrin components.[23] Radiolabeled platelets are theoretically the best tool to image the platelet-rich thrombus developing on a lesion, because such thrombi may develop in high-shear regions even in the absence of notable fibrin deposition.[17] Here, ^{111}In-platelet scintigraphy has emerged as the technique of choice.[24,25] However, initial enthusiasm with this technique has been tempered by a low signal-to-noise ratio with the circulating blood pool and the lack of documentation of the underlying pathology.[23]

We sought to define the potential value of platelet scintigraphy in the detection of carotid artery thrombosis. We used a blood pool subtraction procedure to maximize the scintigraphic signal (due to platelet deposition) over the blood pool noise and thus studied a series of patients who were candidates for endarterectomy to compare, for the first time, scintigraphic results with plaque histology and to seek correlations with thrombosis. In parallel, we performed a comparative evaluation of ultrasound-based analyses aimed at evaluation of plaque texture and the detection of peripheral embolization by transcranial Doppler.

Subjects and Methods

Study Population
Twenty-two patients (16 men and 6 women), aged 48 to 77 years (median age 65 years), were recruited at the Neurosurgery Division of Pisa University Hospital from June 1996 to May 2000, and candidates for carotid endarterectomy were selected if they fulfilled the inclusion criterion of the presence of a unilateral >70%-diameter carotid artery stenosis on angiography. Exclusion criteria consisted of general contraindications to the procedure, such as severe ischemic heart disease, cancer, anemia, systemic infectious or inflammatory diseases, or interventions that were carried out soon after diagnosis and thus prevented study set-up. The clinical characteristics of the 22 patients recruited are summarized in Table 1. Twelve of the patients underwent a full protocol for comparative evaluations of noninvasive techniques with respect to histologically detected thrombosis (study 1), and 10 patients underwent a confirmatory study of the predictive value of platelet scintigraphy only (study 2). Twenty patients were treated with low-dose acetyl salicylic acid or lysine acetyl-salicylate (100 to 324 mg/d) at the time of surgery, whereas 2 were on ticlopidine (Table 1). All symptomatic patients had a cardiological work-up (ECG and 2-dimensional color Doppler echocardiogram) to exclude the contribution of cardioembolism to the reported symptoms.

Study Protocol
The 12 patients in study 1 underwent presurgical and postsurgical procedures as described in Figure 1. These included preoperative ex vivo separation and ^{111}In labeling of autologous platelets, reinjection, and performance 48 hours later of planar scintigraphy of the neck
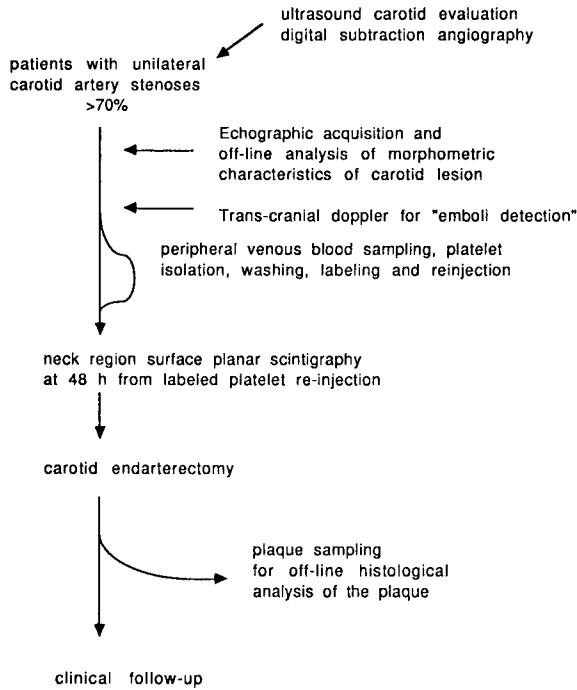
Figure 1. Flow chart of the study design.

region. This was immediately followed, during the same session, by (1) scintigraphic acquisition after imaging of the blood pool with ^{99m}Tc-human serum albumin, (2) a complete ultrasonographic evaluation of the carotid artery region, with derivatization of videodensitometric parameters of plaque texture, and (3) a transcranial Doppler evaluation of cerebromicroembolization. At the time of surgery, performed within 1 week after platelet scintigraphy, the carotid endarterectomy specimen was saved for a subsequent histological evaluation. The 10 additional patients in study 2 underwent the same procedure with the exception of ultrasound and Doppler studies. Study protocols were approved by the Pisa University Hospital Ethical Committee.

Angiography
Digital-subtraction carotid angiography was obtained in the 15 days preceding patient recruitment through the intravenous injection of a nonionic contrast medium (iopamidol). Carotid artery stenosis was evaluated according to the NASCET criteria as the ratio of luminal diameter (on 2 views) at the point of the greatest stenosis and the normal portion of the artery beyond the carotid bulb.[5]

Ultrasound Quantitation of Carotid Stenosis
Color-flow duplex investigation of the carotid arteries was performed with a Diasonics Spectra apparatus equipped with a 7.5-MHz transducer. The highest peak of systolic velocity in the stenotic area was recorded. Evaluation of the stenotic area was performed on the basis of conventional criteria[26,27] and with a curve of conversion from a linear index to stenosis area, where a diameter reduction of 50% corresponds to a 75% area reduction.[28]

Platelet Labeling
Platelet labeling was performed according to an established protocol.[29] Peripheral venous blood anticoagulated with acid-citrate dextrose (blood-anticoagulant ratio of 6:1) was centrifuged at 150g for 10 minutes at 37°C to obtain platelet-rich plasma (PRP). This was subsequently separated into platelet-poor plasma (PPP) and a platelet pellet through additional centrifugation at 1000g for 10 minutes at 37°C in the presence of prostacyclin for human use (1 μmol/L final concentration; Flolan; Glaxo-Welcome). The platelet pellet was then

sterilely resuspended twice in PBS without calcium and magnesium, pH 7.4, to a final count of 200 000/μL (platelet washing). Labeling was performed with the addition of 55 Mbq of ^{111}In-oxine (Byk Gulden Italia) for 30 minutes at 37°C. After the addition of more prostacyclin (1 μmol/L), the platelet suspension was centrifuged at 1000g for 10 minutes at 37°C to wash out unbound indium, and the final resuspension of platelet pellet was performed in autologous PPP. Labeling efficiency, calculated from indium bound to platelets (B) and unbound (A) as B/(A+B)×100, was always >70%.[29] The labeled platelet suspension was reinjected through an antecubital vein within 5 minutes of the final preparation. Injected dose, calculated from triplicate counting of a small aliquot of injected suspension in a γ-counter as the weight of the injected dose (in mg) multiplied by the average counts per minute per milligram of the counted aliquots, was 37±10 Mbq, corresponding to a whole body exposure of ∼6 mSv.

Subtraction Carotid Imaging With ^{111}In-Labeled Platelets and ^{99m}Tc-Labeled Human Serum Albumin
Scintigraphy of the neck region was performed 48 to 72 hours after the injection of ^{111}In-platelets, with patients lying supine. Planar scintigraphic images of the neck and upper chest were acquired with a General Electric Starcam 3000 XC γ-camera fitted with a medium-energy parallel-hole collimator and set at the 247-keV ^{111}In photopeak. By keeping the patient still, dynamic images (1 image/s for 1 minute) were also acquired immediately after the intravenous injection of 555 Mbq of ^{99m}Tc-labeled human serum albumin (Albutec; Sorin Biomedica), centering the ^{99m}Tc photopeak at 140 keV to detect carotid arteries. Soon, static blood pool images were acquired in the same position. A total of 500 000 counts were collected for both tracers.

A normalization ratio (In$_{ref}$/Tc$_{ref}$) was calculated for each patient according to the counts obtained from regions of interest (ROIs) drawn in the ^{111}In and ^{99m}Tc images over a reference area taken on the ipsilateral subclavian artery, where platelet deposition was expected to be negligible and where no hot spots at the presubtraction acquisition were found. This In$_{ref}$/Tc$_{ref}$ ratio describes the relative activity of circulating platelets in the blood pool; for any dimension of the ROI, ^{111}In in the blood pool (In$_{bp}$) can be calculated as

$$In_{bp}=(In_{ref}/Tc_{ref})Tc_{bp}$$

Thus, a subtraction image, taking into account the radioactivity due to circulating ^{111}In-labeled platelets, is obtained by subtracting, in a pixel-by-pixel manner, In$_{bp}$ from In$_{tot}$ (In$_{tot}$−In$_{bp}$). The resulting image now detects purely the deposition of radiolabeled platelets on the atherosclerotic carotid plaque complicated by thrombosis. Principles for this technique have been previously described.[30]

After image acquisition, elaborations and subtractions were performed offline by 2 experienced nuclear medicine physicians (G.M., R.C.B.) who were blinded to other results. Images were evaluated qualitatively (presence or absence of "hot spots" on carotid scintigrams after blood pool subtraction) and quantitatively by calculating a scintigraphic thrombus/blood index ("scintigraphic index"). In patients where a hot spot was detected visually, the index was obtained as the mean activity per pixel of the ROI placed on the hot spot (in the subtracted image) divided by the mean activity per pixel of the same ROI purely attributable to indium in the blood pool (In$_{bp}$), as defined earlier. When no hot spots were visually detected on the carotid subtraction image, the index was made equal to 0.

Transcranial Doppler Emboli Detection
Embolizations in the intracranial circulation ("emboli detection") were detected with a DWL Multidop X-TCO7 apparatus with a pulsed-wave 2-MHz probe according to previously described principles.[31,32] Acquisitions were performed for 1 hour in each patient.

Videodensitometric Analysis of Carotid Artery Plaque With Ultrasonic Parametric Imaging
To obtain tissue characterization parameters, echo-tomography images were acquired in a caudocranial direction at the site of the

plaque, with the same apparatus described for the ultrasound assessment of the stenosis. In each patient, a total of 7 to 10 longitudinal and transverse scans of the carotid axis, for a total of 47 images, spaced 0.5 to 2.5 cm, were obtained at the plaque level. The anatomic bifurcation was the anatomic reference point. Selected frames were digitized offline with an array processor–based computer for medical image processing. Images that corresponded to the carotid plaque were selected and converted into frames of 512×512 pixels of 256 gray levels each (0 black, 255 white). For each selected frame, ≥ 1 ROI was chosen to encompass a region that ranged in size from 0 to 255 pixels within a plaque. For each ROI, a histogram of the echo gray-level distribution was generated by plotting the gray level value on the abscissa and the frequency of occurrence on the ordinate. Several variables (first-order algorithms) were used to quantitatively describe the shape of the histogram, as described previously.[33] The mean gray level describes the average gray value distribution and allows the objective ultrasonographic assessment of characters described visually as "softness", "fibrosity," and "calcification."[34] In addition, an attempt was made to relate the spatial distribution to dependence among gray levels in an ROI. To quantify the spatial interrelationship of the tonal distribution of echograms, algorithms were applied to an ROI that encompassed the entire plaque (rather than specific, smaller regions within the plaque, as for first-order algorithms). A co-occurrence matrix is used to estimate the probability that a couple of pixels, with their own gray levels and separated by a displacement vector, occur in the considered ROI. Entropy reflects the coarseness of the image, as its value increases when homogeneity is reduced (ie, when co-occurrence matrix elements tend to be equal and the diagonal concentration lowers). Entropy values were obtained horizontally through scanning along the carotid axis and vertically through scanning perpendicular to the main axis. Detailed descriptions of the mathematics involved have been previously published.[34]

Carotid Endarterectomy

Endarterectomy was performed through a lateral neck incision within 1 week from the scintigraphic acquisition by a skilled neurosurgeon (G.P.). After exposure of the carotid bifurcation and an assessment of the upper limit of the plaque, the common, internal, and external carotid arteries were clamped. The wall of the common carotid artery was incised, and the plaque was excised. Attention was paid to the detection of pressure in the internal carotid artery after clamping as an index of a good function of intracranial collateral circles.

Histology

Each endarterectomy sample was embedded in paraffin and stained with both hematoxylin-eosin and Mallory trichrome stain. Histological diagnosis was performed by an expert pathologist (G.P.) who was blinded to other study results. Based on a previously defined grading system,[35] each sample was classified according to the 2 criteria of (1) the presence or absence of thrombosis or intimal hemorrhage (with presence of any of the 2 components being evidence of plaque fissuring or erosion) and (2) the presence or absence of extensive lipid deposition (with plaques classified as lipidic or fibrolipidic allocated into one group and those classified as fibrous and calcific allocated into another group).

Statistical Analysis

Quantitative data were described as mean±SD and compared by the Student's *t* test for unpaired data. To assess the correspondence with histology, all quantitative characteristics were dichotomized (positivity/negativity or presence/absence), taking the median as the cutoff value (dependent [*y*] variables). They were analyzed for their coincidence with the histology of the lesion (presence/absence or thrombosis-hemorrhage, and presence/absence of extensive lipid deposition) (independent [*x*] variables). All characteristics were analyzed in a 2×2 statistical matrix by the Fisher's exact test, giving absolute probability values that relationships could be due to chance. Bonferroni's correction was introduced to account for the number of

**TABLE 2. Results of ^{111}In Platelet Scintigraphy With Blood Pool Subtraction**

Patient	Endoarterectomy Site	Scintigraphic Index	Histology Plaque Type	Thrombosis
Study 1				
1	L	0	Fibrous	No
2	R	0	Fibrous	No
3	R	87	Fibrous	Yes
4	R	134	Fibrolipidic	Yes
5	R	267	Fibrous	Yes
6	R	220	Fibrous	Yes
7	R	146	Fibrolipidic	Yes
8	R	0	Fibrolipidic	No
9	R	70	Fibrolipidic	Yes
10	L	0	Fibrous	No
11	R	0	Fibrous	No
12	R	0	Fibrous	No
Study 2				
13	L	0	Fibrous	No
14	L	0	Fibrous	No
15	L	86	Fibrolipidic	Yes
16	L	0	Fibrolipidic	No
17	L	0	Fibrous	No
18	D	0	Fibrolipidic	Yes
19	L	0	Fibrous	No
20	D	0	Fibrous	No
21	L	193	Fibrolipidic	Yes
22	D	0	Fibrous	No

L indicates left; R, right; scintigraphic index, see text for details.

repeated tests. A probability level of <0.02 was therefore conservatively considered significant.

Results

Six of the 12 patients of study 1 had a clear-cut hot spot on ^{111}In-platelet carotid scintigraphy after blood pool subtraction. The scintigraphic index in these patients ranged between 70 and 267 (Table 2). In all these cases, evidence of plaque thrombosis-hemorrhage was present at histology (Table 2). On the other hand, when no hot spots were present at the carotid subtraction image, in no case was luminal thrombosis evident at the histological examination of the endarterectomy specimen (Table 2). Representative examples of these situations are shown in Figure 2. Histology revealed the presence of a quantitatively important lipid core (fibrolipidic plaques) in 3 of the 6 patients with thrombosis, whereas the nature of the plaque was determined to be fibrous in the remaining 3. In the additional series of patients in study 2, 2 additional hot spots were detected, and these again corresponded to the presence of thrombosis at histology. In this last series, 1 case of thrombosis at histology was not accompanied by positivity on platelet scintigraphy (Table 2). Overall, in the 22 patients studied for this purpose, specificity of platelet scintigraphy
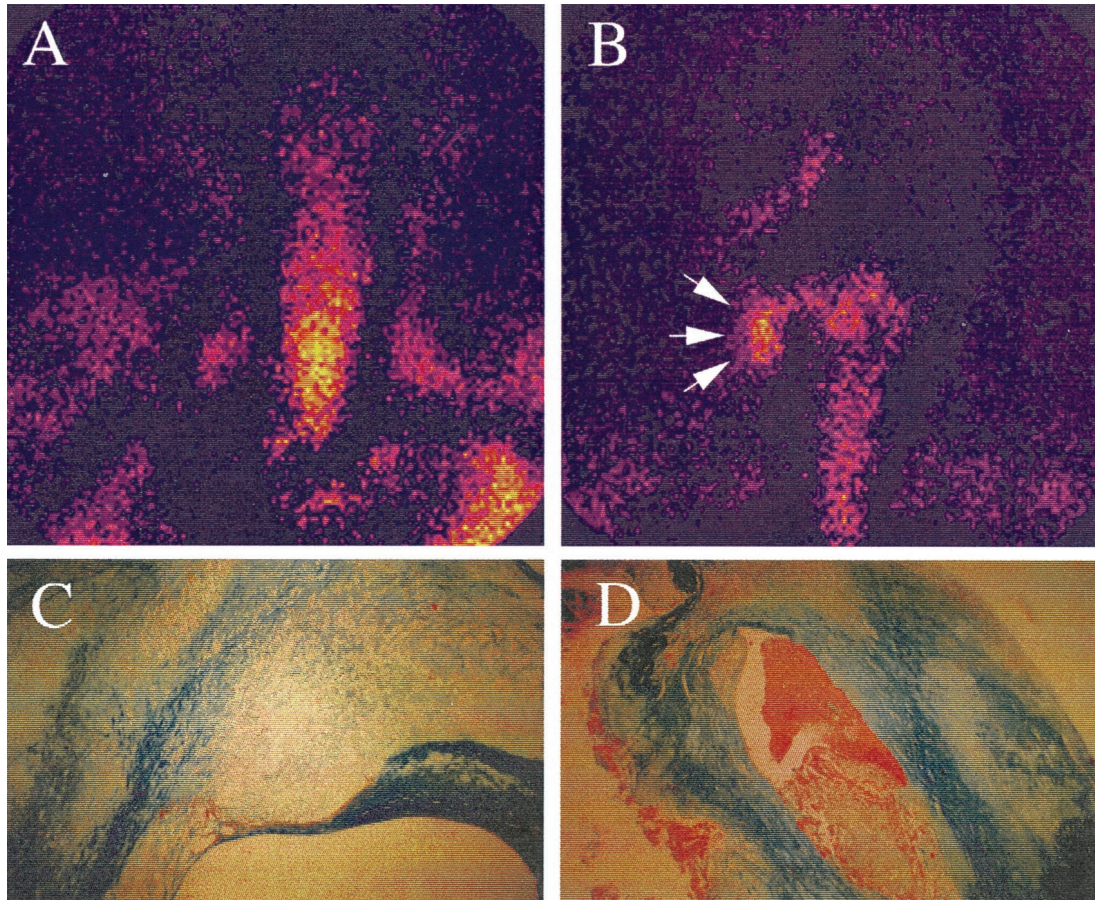
Figure 2. A comparison of examples of the correspondence between a scintigraphically silent carotid lesion (without significant ^{111}In platelet uptake) (A, left) and a lesion with significant ^{111}In uptake (B, right; region marked by arrows). C and D, Corresponding histological appearance, as a "fibrous" lesion, with no fissuring or thrombotic complications in 1 case (C) and a "complicated" plaque, with evidence of luminal thrombosis, in the other (D). A and C, From a 65-year-old male patient (patient 8), with an angiographically diagnosed 75% stenosis on the right internal carotid artery. B and D, From a 48-year-old female patient (patient 4) with a quantitatively similar 75% stenosis on the right internal carotid artery. Despite the similarity of the initial angiographic evaluation, the second patient shows clear-cut uptake of ^{111}In-labeled platelets, indicating active platelet deposition on the lesion, to which histological evidence of thrombotic complication corresponds.

for the detection of carotid plaque thrombosis was 100% and sensitivity was 89%. We found no obvious explanation for the discrepancy between platelet scintigraphy and the histological diagnosis of thrombosis in the only patient with plaque thrombosis and negative scintigraphy (patient 18, Table 2).

Transcranial Doppler emboli detection revealed the occurrence of microembolization in 3 of 12 patients. In 2 of these, there was evidence of thrombosis-hemorrhage at histology. Three patients with negative emboli detection at transcranial Doppler examination had evidence of mural thrombosis at histology (Table 3).

Videodensitometric analysis of carotid artery plaque by ultrasonic parametric imaging showed no statistically significant difference in plaque echogenicity (mean gray levels) between plaques histologically proved to be fibrous (mean$\pm$SD 35$\pm$22) and those classified as fibrolipidic (mean$\pm$SD 55$\pm$21). Mean gray levels analysis showed close-to-significance higher values in plaques histologically described as having thrombosis-hemorrhage (53.3$\pm$16.2)

compared with plaques described as not having thrombosis-hemorrhage (24.8$\pm$19.3) (*P*=0.026) (Table 3).

Vertical dishomogeneity was not statistically different between fibrous plaques (6.62$\pm$1.59) and fibrolipidic plaques (6.09$\pm$2.08). Also, no difference in this parameter was found with regard to plaques without thrombosis-hemorrhage (6.95$\pm$0.90) compared with those with thrombosis-hemorrhage (6.08$\pm$2.08) (*P*=NS) (data in Table 3).

Horizontal dishomogeneity also did not differ between fibrous plaques (6.16$\pm$1.09) and fibrolipidic plaques (6.39$\pm$1.71), as well as between plaques without thrombosis-hemorrhage (6.68$\pm$0.69) and plaques with thrombosis-hemorrhage (5.85$\pm$1.44) (data in Table 3).

When quantitative data were reduced to dichotomic variables as being either below or above the median value, none of these parameters were significantly coincident in 2$\times$2 contingency tables with either the histology diagnosis of thrombosis (Table 4) or the presence of a lipid core inside the plaque (data not shown). Similarly, no predictive value of emboli detection, with respect to the 2 histological parameters

TABLE 3.   Results of Alternative Noninvasive Ultrasound-Based Evaluations of Carotid Atherosclerosis

| Patient | Transcranial Doppler Emboli Detection, hits/h | Echographic Index | | | Histology | |
		Plaque Echogenicity Mean Gray Level	2 *y* Entropy (Vertical Dishomogeneity)	2 *x* Entropy (Horizontal Dishomogeneity)	Plaque Type	Thrombosis
Study 1						
1	6	56, 7	7, 72	6, 32	Fibrous	No
2	0	29, 1	8, 06	7, 26	Fibrous	No
3	30	60, 7	3, 50	4, 04	Fibrous	Yes
4	0	36, 4	6, 43	6, 40	Fibrolipidic	Yes
5	0	34, 0	6, 12	6, 38	Fibrous	Yes
6	0	60, 8	8, 60	5, 50	Fibrous	Yes
7	2	51, 0	3, 86	4, 68	Fibrolipidic	Yes
8	0	Missing	Missing	Missing	Fibrolipidic	No
9	0	76, 6	7, 97	8, 09	Fibrolipidic	Yes
10	0	14, 0	6, 29	7, 50	Fibrous	No
11	0	9, 1	6, 00	5, 81	Fibrous	No
12	0	15, 0	6, 70	6, 50	Fibrous	No
Study 2						
13					Fibrous	No
14					Fibrous	No
15					Fibrolipidic	Yes
16					Fibrolipidic	No
17					Fibrous	No
18					Fibrolipidic	Yes
19					Fibrous	No
20					Fibrous	No
21					Fibrolipidic	Yes
22					Fibrous	No

considered, was found. However, there was a highly significant coincidence between the positivity of carotid subtraction scintigraphy and the diagnosis of thrombosis-hemorrhage at histology (Table 4), although the results of subtraction scintigraphy did not predict the fibrous/fibrolipidic nature of the plaque at histology.

Discussion

The results of the present study demonstrate that ^{111}In-labeled platelet scintigraphy with blood pool subtraction is an accurate method to detect thrombotic complications of carotid artery plaques, having, for the first time, the histology of the

TABLE 4.   Contingency Table Results, 1 *x* Variable (Thrombosis-Hemorrhage at Histology) vs 6 *y* Variables

y Variable	n	Contingency Coefficient	Fisher's Exact *P* Value
Scintigraphy	12	0.707	0.0011
Emboli detection	12	0.189	0.5909
Plaque echogenicity	11	0.423	0.2424
Plaque vertical dishomogeneity	11	0.100	>0.9999
Plaque horizontal dishomogeneity	11	0.258	0.5671
Lipid/nonlipid histology	12	0.333	0.3030

plaque as the gold standard. This technique proves to be much better than any of the other ultrasound-based methods used to assess either the occurrence of distal embolization (emboli detection by transcranial Doppler) or plaque texture parameters, as obtained through videodensitometric analysis of plaque ultrasound imaging. Our study also demonstrates that neither platelet scintigraphy nor the ultrasound-based methods used here are able to yield significant correlations with the presence of a discrete lipid pool inside the plaque at histology. However, because of the relation of carotid plaque fissuring and thrombosis with clinical complications[16] that occur through distal embolization to the cerebral circulation,[36–38] this study suggests the potential usefulness of platelet scintigraphy to identify plaques at risk of such clinical complications.

At variance from previous studies that investigated the ability to detect carotid artery stenoses with indium-platelet scintigraphy with blood pool subtraction,[39,40] our study is the first to attempt a correlation with histology. This was carried out through the implementation of an accurate protocol of platelet labeling and carotid imaging immediately before the surgical removal of the plaque. Thus, we avoided the potential uncertainty in the definition of "ulceration" or "plaque complications" based on carotid

ultrasound images, as used in the 2 largest previous studies of the relationship of these noninvasive echographic parameters with results of platelet carotid scintigraphy.[39,40] Contrary to all previous studies, we used histology to identify the presence of mural thrombosis and plaque hemorrhage (2 markers of plaque fissuring) as the standard to which platelet scintigraphic results were compared. Our results thus confirm the findings by Moriwaki et al, who described the correlation of positivity at platelet scintigraphy with surface characteristics of complicated lesions,[40] but differ from those in which lesion complications were not inferred indirectly from echographic indices, the reliability of which is open to question.[27,41]

The virtually complete superimposition of positive results at platelet scintigraphy and evidence of thrombosis-complicated plaque fissuring at histology in our study is striking. A positive outcome of the present study may have been favored (or determined) by at least 2 methodological factors. The first is the injection of a relatively high dose of radioactivity ($\approx$1 mCi, implying an even higher amount of radioactivity to be used at the beginning of the ex vivo radiolabeling), still compatible, however, with an acceptable patient exposure. To this purpose, we estimated exposure to be in the same order of magnitude of other conventional nuclear medicine examinations. The second factor is the careful control of procedural details, ensuring an optimal functionality of injected platelets, as assessed by the evaluation of platelet function parameters and the response to aggregating agents after the labeling procedure, procedural details that were carefully optimized.[29] Patient selection also may have favorably influenced the outcome of the almost complete correspondence between scintigraphic and histological results. All of our patients were a priori judged candidates for endarterectomy on the basis of the angiographic findings of a significant ($>$70%) unilateral stenosis, and the majority of them (12 of 22), by being symptomatic, met the recognized international criteria for a clear indication for surgery.[5,6] Moriwaki et al[40] previously showed a significant, albeit weak, correlation between the degree of stenosis and scintigraphic evidence of platelet deposition. The fact that all of our patients had a unilateral stenosis of $\geq$70% on both angiography and echo-Doppler analysis indicates that patient selection likely contributed to a high prevalence of scintigraphic positivity in the small patient population studied. Regardless of the reasons, the technique appears to be, in our hands, not only extremely specific in the detection of mural thrombosis or intraplaque hemorrhage but also surprisingly ($\approx$90%) sensitive.

It is interesting to note that the 10 asymptomatic patients who were enrolled in this study indeed had equivocal indications for endarterectomy. Two of them had evidence of platelet deposition at carotid scintigraphy, and both of them had evidence of mural thrombosis on the plaque surface. Thus, platelet scintigraphy results, because they correlated so strongly with the histology of a lesion "at risk," might well theoretically guide therapeutic decisions in patients similar to these 10 subjects in whom surgical treatment is debatable. The fact that all of our patients

were studied on antiplatelet drugs (aspirin or ticlopidine), which, by reducing platelet function, may reduce surface platelet deposition, is compatible with the partial inefficacy of aspirin in the prevention of arterial thrombosis and stroke[20] and contributes to ensure the transferability of the additional information provided by platelet scintigraphy into current medical practice.

Our study failed to demonstrate any relationship of histology (as evaluating both the presence of thrombosis and the presence of a detectable lipid pool inside the plaque) and a series of ultrasound-based parameters aimed at detecting microembolization into the cerebral circulation by transcranial Doppler (emboli detection) and analyzing the gray levels of the plaque at videodensitometry of echo images. Emboli detection by transcranial Doppler is an interesting noninvasive, simple-to-perform, and therefore increasingly popular technique to improve risk stratification by detecting signals of embolization into the cerebral circulation.[31,32,42–45] Three of 12 patients of our complete protocol (study 1) underwent the detection of peripheral embolization, and 2 of them had histological evidence of thrombosis. Although the patients that we studied with this technique are few, one possible interpretation of the results is the incomplete overlapping of carotid thrombosis and peripheral embolization. Indeed, on the one hand, the source of embolization may be outside of the carotids, and on the other hand, thrombosis, when present, may be nonemboligenic. Practical problems in the reliability of the technique in detecting the target events may further complicate this evaluation.[32,42]

We also found discouraging results in the application of the performed videodensitometric analyses in an attempt to predict plaque histology. Although these analyses are better at the prediction of the presence of "soft," "lipid-rich,"[34] and therefore possibly vulnerable[15] plaques, and not of thrombosis, this was not the case in our series, with an almost random distribution of blindly measured videodensitometric parameters between fibrolipidic and fibrous plaques. Another consideration is that this technique would still be only indirectly related to the risk of fissuring and thrombosis. Its application to the specific goal of predicting mural thrombosis in the single subject would thus appear unlikely. Alternative ultrasonic parameters aimed at objective assessment of surface irregularities rather than structural features of the plaque might prove more useful clinically,[40,46] but this needs to be practically tested.

The clinical transferability of our findings with platelet scintigraphy has to be viewed in perspective of the other techniques that are available to image thrombosis and atherosclerosis.[23] Rival techniques for thrombosis imaging involve radiolabeled fibrinogen, fibronectin, and plasminogen activators (now mostly considered unpractical because of the risk of injecting nonautologous material); immunoglobulins against antigenic epitopes of activated platelets or fibrin; and peptides able to bind platelet activation receptors (for a review, see Vallabhajosula and Fuster[23]). For the imaging of atherosclerosis, refinement of echo-Doppler techniques, electron beam computed tomography, and magnetic resonance angiography appear to be

the most promising.[23] All of these techniques, with the notable exception of ultrasounds, are relatively "unavailable." Scintigraphy with radiolabeled platelets has probably been the most widely used functional imaging technique for atherosclerotic complications, because it uses a physiological cellular element as the carrier of the tracer and has clear pathophysiological background. The technique, however, lacks full validation. The present demonstration of the high specificity and sensitivity of platelet scintigraphy in the detection of mural thrombosis on a carotid artery plaque appears promising to start a thorough evaluation of the clinical potential of the technique in stratification of the thromboembolic risk in patients with carotid artery stenosis.

References

1. Busuttil RW, Baker JD, Davidson RK, Machleder HI. Carotid arterial stenosis: hemodynamic significance and clinical course. *JAMA*. 1981; 245:1438–1445.
2. Barnett HJM, Eliasziw M, Meldrum HE. Drugs and surgery in the prevention of ischemic stroke. *N Engl J Med*. 1995;332:238–246.
3. Sundt TMJ, Whisnant JP, Houser OW, Fode NC. Prospective study of the effectiveness and durability of carotid endarterectomy. *Mayo Clin Proc*. 1990;65:625–632.
4. Matchar DB. Decision making in the face of uncertainty: the case of carotid endarterectomy. *Mayo Clin Proc*. 1990;65:756–762.
5. North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Engl J Med*. 1991;325: 445–453.
6. European Carotid Surgery Trialists' Collaborative Group. MCR European Carotid Surgery Trial: interim results for symptomatic patients with severe (70–99%) or with mild (0–29%) carotid stenosis. *Lancet*. 1991;337:1235–1243.
7. European Carotid Surgery Trialists' Collaborative Group. Endarterectomy for moderate symptomatic carotid stenosis: interim results from the MRC European Carotid Surgery Trial. *Lancet*. 1996;347: 1591–1593.
8. Barnett HJM, Taylor DW, Eliasziw M, Fox AJ, Ferguson GG, Haynes RB, Rankin RN, Clagett GP, Hachinski VC, Sackett DL, Thorpe KE, Meldrum HE, for the North American Symptomatic Carotid Endarterectomy Trial Collaborators. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. *N Engl J Med*. 1998;339:1415–1425.
9. Chassin MR. Appropriate use of carotid endarterectomy. *N Engl J Med*. 1998;339:1468–1469.
10. Asymptomatic Carotid Atherosclerosis Study Group. Carotid endarterectomy for patients with asymptomatic internal carotid artery stenosis. *JAMA*. 1995;273:1421–1428.
11. National Medical Advisory Committee. *The Management of Patients With Stroke*. Edinburgh, UK: HM Stationery Office; 1993.
12. Fode N, Sundt TJ, Robertson J, Peerless S, Shields C. Multicenter retrospective review of results and complications of carotid endarterectomy in 1981. *Stroke*. 1986;17:370–376.
13. Warlow C. Endarterectomy for asymptomatic carotid stenosis? Lancet 1995;345:1254–1255.
14. Davies MJ, Bland JM, Hangartner JRW, Angelini A, Thomas AC. Factors influencing the presence or absence of acute coronary artery thrombi in sudden ischemic death. *Eur Heart J*. 1989;10:203–208.
15. Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Med J*. 1993; 69:377–381.
16. Falk E, Fernandez-Ortiz A. Role of thrombosis in atherosclerosis and its complications. *Am J Cardiol*. 1995;75:5B–11B.
17. Falk E, Shah P, Fuster V. Coronary plaque disruption. *Circulation*. 1995;92:657–671.
18. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation*. 1994;89:36–44.
19. Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J, Virmani R. Coronary plaque erosion without rupture into a lipid core: a frequent cause of coronary thrombosis in sudden coronary death. *Circulation*. 1996;93:1354–1363.
20. Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy, I: prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ*. 1994;308:81–106.
21. Smith P, Arnesen H, Holme I. The effect of warfarin on mortality and reinfarction after myocardial infarction. *N Engl J Med*. 1990;323: 147–151.
22. Anticoagulants in the Secondary Prevention of Events in Coronary Thrombosis (ASPECT) Research Group. Effect of long term oral anticoagulant treatment on mortality and cardiovascular morbidity after myocardial infarction. *Lancet*. 1994;343:499–503.
23. Vallabhajosula S, Fuster V. Atherosclerosis: imaging techniques and the evolving role of nuclear medicine. *J Nucl Med*. 1997;38: 1788–1796.
24. Kritz H, Underwood SR, Sinzinger H. Imaging of atherosclerosis (part II). *Wien Klin Wochenshr*. 1996;108:87–97.
25. Smyth JV, Dodd PDF, Walker MG. Indium-111 platelet scintigraphy in vascular disease. *Br J Surg*. 1995;82:588–595.
26. Beach K, Lawrence R, Philips D, Primozich J, Strandness D. The systolic velocity criterion in quantifying internal carotid artery stenosis. *J Vasc Technol*. 1989;13:65–68.
27. Bornstein N, Norris J. The value of carotid Doppler ultrasound in asymptomatic extracranial disease. *Can J Neurol Sci*. 1988;15: 378–383.
28. Alexandrov A, Bladin C, Maggisano R, Norris J. Measuring carotid stenosis: time for a reappraisal. *Stroke*. 1993;24:1292–1296.
29. Giannessi D, Bernini W, Puccetti P, De Caterina R. Platelet radiolabeling with [111]Indium for in vivo studies: a methodological reappraisal. *Nucl Med Biol*. 1995;22:399–403.
30. Bergmann SR, Lerch RA, Mathias CJ. The detection of acute coronary thrombosis in patients with In-111 labeled platelets. *J Nucl Med*. 1983;24:59.
31. Markus HS, Droste D, Brown MM. Ultrasonic detection of cerebral emboli in carotid stenosis. *Lancet*. 1993;341:1606.
32. Markus HS, Harrison MJ. Microembolic signal detection using ultrasound. *Stroke*. 1995;26:1517–1519.
33. Pinamonti B, Picano E, Ferdeghini E. Quantitative texture analysis in two-dimensional echocardiography: application to the diagnosis of myocardial amyloidosis. *J Am Coll Cardiol*. 1989;14:666–669.
34. Mazzone A, Urbani M, Picano E, Paterni M, Borgatti E, De Fabritiis A, Landini L. In vivo ultrasonic parametric imaging of carotid atherosclerotic plaque by videodensitometric technique. *Angiology*. 1995;46:663–672.
35. Robbins S, Cotran R. *The Pathological Basis of Disease*: Philadelphia, Pa: WB Saunders; 1979:593–642.
36. Awad I, Modic M, Little JR, Furlan AJ, Weinstein M. Focal parenchymal lesions in transient ischemic attacks: correlation of computed tomography and magnetic resonance imaging. *Stroke*. 1986;17: 399–403.

37. DeBono DP, Warlow CP. Potential source of emboli in patients with presumed transient cerebral or retinal ischemia. *Lancet*. 1981;1: 343–345.

38. Sitzer M, Muller W, Siebler M, Hort W, Kniemeyer HW, Jancke L, Steinmetz H. Plaque ulceration and lumen thrombus are the main source of cerebral microemboli in high-grade internal carotid artery stenosis. *Stroke*. 1995;26:1231–1233.

39. Minar E, Ehringer H, Dudzak R, Schofl R, Jung M, Koppensteiner R, Ahmadi R, Kretschmer G. Indium-111-labeled platelet scintigraphy in carotid atherosclerosis. *Stroke*. 1989;20:27–33.

40. Moriwaki H, Matsumoto M, Handa N, Isaka Y, Hashikawa K, Oku N, Nakamura M, Kamada T, Nishimura T. Functional and anatomic evaluation of carotid atherosclerosis: combined study of indium-111 platelet scintigraphy and B-mode ultrasonography. *Arterioscler Thromb*. 1995;15:2234–2240.

41. Eliasziw M, Rankin RN, Fox AJ, Haynes RB, Barnett HJ. Accuracy and prognostic consequences of ultrasonography in identifying severe carotid artery stenosis. North American Symptomatic Carotid Endarterectomy Trial (NASCET) Group. *Stroke*. 1995;26:1747–1752.

42. Grosset DG, Georgiadis D, Hillis WS, Lees KR. Clinical significance of cerebral emboli signals. *Lancet*. 1993;342:559.

43. Markus H, Loh A, Brown M. Computerized detection of cerebral emboli and discrimination from artifact using Doppler ultrasound. *Stroke*. 1993;24:1667–1672.

44. Markus HS, Droste TW, Brown MM. Detection of asymptomatic cerebral embolic signals with Doppler ultrasound. *Lancet*. 1994;343: 1011–1012.

45. Georgiadis D, König M, Zunker P, Nabavi D, Stögbauer F, Ringelstein EB. Microembolic signals in patients referred for echocardiography. *Stroke*. 1995;26:525. Letter.

46. Urbani MP, Picano E, Parenti G, Mazzarisi A, Fiori L, Paterni M, Pelosi G, Landini L. In vivo radiofrequency-based ultrasonic tissue characterization of the atherosclerotic plaque. *Stroke*. 1993;24: 1507–1512.