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Inflammation in Carotid Atherosclerotic Plaque: A Dynamic Contrast-enhanced MR Imaging Study

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Abstract

Purpose—To prospectively evaluate if there is an association between plaque enhancement at magnetic resonance (MR) imaging and proinflammatory cardiovascular risk factors and plaque content.

Materials and Methods—This study was performed with informed consent, HIPAA compliance, and institutional review board approval. Contrast agent dynamics within carotid plaques were measured in 30 patients (29 men, one woman; mean age, 67.7 years $\pm$ 10.7 [standard deviation]) who were scheduled to undergo carotid endarterectomy. Measurements were based on kinetic modeling of images obtained at 15-second intervals during which a gadolinium-based contrast agent was injected. The time-varying signal intensities within the plaques were used to estimate the fractional plasma volume (v_p) and transfer constant (K^{trans}) of contrast material into the extracellular space. Pearson correlation coefficients were computed between blinded MR measurements and histologic measurements of plaque composition, including macrophages, neovasculature, necrotic core, calcification, loose matrix, and dense fibrous tissue. Correlation coefficients or mean differences were computed regarding clinical markers of cardiovascular risk.

Results—Analyzable MR images and histologic results were obtained in 27 patients. Measurements of K^{trans} correlated with macrophage ($r = 0.75$, $P < .001$), neovasculature ($r = 0.71$, $P < .001$), and loose matrix ($r = 0.50$, $P = .01$) content. Measurements of v_p correlated with macrophage ($r = 0.54$, $P = .004$), neovasculature ($r = 0.68$, $P < .001$), and loose matrix ($r = 0.42$, $P = .03$) content. For clinical parameters, significant associations were correlated with K^{trans} only, with decreased high-density lipoprotein levels ($r = -0.66$, $P < .001$) and elevated K^{trans} measurements in smokers compared with nonsmokers (mean, 0.134 min⁻¹ vs 0.074 min⁻¹, respectively; $P = .01$).

Conclusion—The correlations between K^{trans} and histologic markers of inflammation suggest that K^{trans} is a quantitative and non-invasive marker of plaque inflammation, which is further supported by the correlation of K^{trans} with proinflammatory cardiovascular risk factors, decreased high-density lipoprotein levels, and smoking.

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See Materials and Methods for pertinent disclosures.

Keywords

Abbreviations: HDL = high-density lipoprotein; K^{trans} = transfer constant; v_p = fractional plasma volume

Contrast material enhancement in carotid atherosclerotic plaques has been observed in several recent investigations with magnetic resonance (MR) imaging after the injection of clinically available gadolinium-based contrast agents (1–5). Strong contrast enhancement suggests the presence of a vascular supply to the plaque and increased endothelial permeability that facilitates the entry of the contrast agent from the blood plasma. Because neovasculature growth into the plaque and increased endothelial permeability are associated with plaque inflammation (6–10), plaque enhancement has been argued to be a sign of plaque inflammation.

Such a link has considerable clinical potential because inflammation has been linked to an increased risk of clinical vascular events (11,12). Plaque inflammation may have multiple effects that weaken plaque structural integrity, including inhibition of collagen production (13) and dissolution of the fibrous matrix by means of matrix metalloproteinases (14–16). If plaque enhancement is a sign of inflammation, then contrast material-enhanced MR imaging may be a tool for detecting plaque inflammation prior to fibrous cap disruption. An association between plaque enhancement and histologically confirmed plaque inflammation, however, has yet to be demonstrated.

Advances in Knowledge

- After the injection of gadolinium-based contrast agents, carotid atherosclerotic plaques exhibited variable contrast enhancement rates in dynamic MR imaging sequences.
- The rate of contrast enhancement is significantly correlated with plaque neovasculature ($P < .001$), macrophage ($P < .001$), and loose matrix ($P = .01$) content.
- The rate of contrast enhancement is significantly associated with smoking ($P = .01$) and low serum levels of high-density lipoprotein ($P < .001$).

Furthermore, the potential to extract quantitative measurements of plaque enhancement and thus obtain a continuous marker of inflammation has not been fully explored. In fact, actual parameters demonstrating blood supply and permeability can be extracted by using kinetic modeling of dynamic contrast-enhanced MR imaging (17). In the standard kinetic model with a vascular component, contrast enhancement is characterized by a parameter for fractional plasma volume (v_p) and by a transfer constant (K^{trans}) that reflects blood supply, vessel permeability, and the extracellular space. The use of kinetic modeling with dynamic contrast-enhanced MR imaging to indicate blood supply and permeability is firmly established in oncology (18). Measurements of v_p by using dynamic contrast-enhanced MR imaging have been previously shown to correlate strongly with histologically determined neovasculature content in carotid atherosclerotic plaques (1). Because the neovasculature appears to serve as a key portal for the entry of inflammatory cells into the atherosclerotic plaques (both in humans [6–8] and in mouse models of atherosclerosis [9,10]), v_p was suggested to be associated with inflammation. This association, however, has not been tested. Also untested is whether K^{trans} , with its additional dependence on permeability, is associated with specific plaque characteristics, including inflammation.

In this study, we hypothesized that a higher density of macrophages in carotid atherosclerotic plaques will yield higher values of K^{trans} and v_p , thereby demonstrating increased neovasculature content and permeability. Thus, the purpose of our study was to prospectively

evaluate if there is an association between plaque enhancement at MR imaging and proinflammatory cardiovascular risk factors and plaque content.

Materials and Methods

This study was funded in part by a grant from AstraZeneca, London, England. This grant provided salary support for one author (W.S.K.) and paid for immunohistochemical results. Data and text for this study remained in the sole control of the authors.

Study Sample

A total of 30 consecutive patients (29 men, one woman; mean age, 67.7 years $\pm$ 10.7 [standard deviation]; age range, 48–83 years) who were scheduled to undergo elective carotid endarterectomy (15 left side, 15 right side), who exhibited no contraindications to MR imaging, and who agreed to participate in this study were recruited from March 2000 to August 2003. Patients underwent MR imaging, which included a dynamic contrast-enhanced MR imaging protocol, and informed consent was obtained from all patients. The study was compliant with the Health Insurance Portability and Accountability Act and was approved by the institutional review board. MR imaging was conducted within 1 week prior to endarterectomy, and the plaque was removed intact for histologic analysis. For each patient, clinical characteristics were recorded by the vascular surgeon (T.S.H.) and included age, body mass index, total cholesterol level, low-density lipoprotein levels, high-density lipoprotein (HDL) levels, blood pressure, diabetes, hypertension, smoking (or the time interval since quitting), and history of recent (within 90 days) symptoms of cerebral ischemia within the distribution of the index carotid artery.

Dynamic Contrast-enhanced MR Imaging

Estimates of v_p and K^{trans} were obtained by applying a kinetic model of contrast agent concentrations to time-varying signal intensities that were observed by using dynamic contrast-enhanced MR imaging of the atherosclerotic carotid arteries. Dynamic contrast-enhanced MR images were generated with a 1.5-T MR imager (Signa Horizon EchoSpeed 5.8; GE Medical Systems, Milwaukee, Wis) by using a transverse two-dimensional spoiled gradient-recalled-echo sequence without cardiac gating. Both carotid arteries were depicted. Relevant imaging parameters were 100/3.5 (repetition time msec/echo time msec); flip angle, 60°; section thickness, 3 mm; intersection gap, 1 mm; field of view, 16 $\times$ 12 cm; and matrix, 256 $\times$ 144. Data were simultaneously acquired at between five and seven locations, were centered on the carotid bifurcation, and were collected at 10 time points, with a separation interval of 15 seconds between time points. Total imaging time was approximately 2 minutes 30 seconds. Coincident with the acquisition of the second image in the sequence, 0.1 mmol (0.2 mL) of a gadolinium-based contrast agent (Omniscan; GE Medical Systems) per kilogram body weight was injected at a rate of 2 mL/sec by using a power injector. A spatial saturation band was applied to induce a T1-dependent blood signal, which resulted in dark blood on the images prior to contrast agent arrival.

We quantified contrast agent dynamics by using the generalized kinetic model (17) for dynamic contrast-enhanced MR imaging. This model included an intravascular contribution and neglected the reflux of contrast agent from the plaque into the blood plasma. This kinetic model assumes that the total concentration of contrast agent in a tissue is determined by the concentration in two compartments—one in the intravascular space (with v_p) and one in the extravascular extracellular space. A third (intracellular) compartment is assumed to exhibit no uptake of contrast agent. K^{trans} dictates the rate at which the contrast agent enters the extravascular extracellular space. Overall contrast agent dynamics are dictated by the differential equation $C_t' = v_p C_p' + K^{trans} C_t - K^{trans} C_t$, where C_t and C_p are the total concentration of

contrast agent in the tissue and blood plasma, respectively. Measurements of C_t and C_p over time—assumed to be linearly proportional to the change in signal intensity of the contrast agent in the tissue and blood (19)—are sufficient to solve for v_p and K^{trans} .

This model was applied to two consecutive image locations per patient. These locations were chosen in the transverse plane with maximal plaque thickness (W.S.K., with 6 years of experience in carotid MR imaging). By using a custom computer analysis package for motion correction and kinetic analysis (20), one author (W.S.K.) drew the vessel wall boundaries by hand on the image that was obtained at the time of contrast agent arrival in the carotid artery lumen.

Identification of the outer wall boundary was facilitated by the phenomenon of hyperenhancement of the outer rim of the plaque, which was likely caused by contrast agent perfusion of the adventitial vasa vasorum (21). Identification of the carotid artery lumen was facilitated by its transition from the region with the lowest signal intensity to the region with the highest signal intensity on contrast agent arrival. In addition, 8–10 pixels were selected within the jugular vein (which had more consistent contrast enhancement than the highly stenosed carotid artery lumen) on each image to establish the plasma concentration of the contrast agent.

Average signal intensity changes were then computed within the plaque to estimate C_t and within the jugular vein to estimate C_p . Kinetic modeling of these signal intensity changes yielded values for v_p (recorded as the percentage of total plaque volume) and K^{trans} (recorded as inverse minutes) for each plaque.

Histologic Methods

After endarterectomy, the excised plaque was fixed in neutral-buffered formalin, embedded in paraffin, and then sectioned to obtain matched cross sections for quantitative comparison with dynamic contrast-enhanced MR images. Sections that were 10 μ m thick were obtained at 0.5–1.0-mm intervals and were stained with hematoxylineosin. The sections that best matched the two image locations per patient were identified (W.S.K., M.S.F.) on the basis of their position relative to the carotid bifurcation and the overall shape of the lumen and plaque, without reference to the histologic composition of the cross sections. Matching was accomplished prior to quantitative analysis of the histologic slides or MR images, which were then analyzed independently.

Double-label immunohistochemical analysis was performed at each location (22). Slides were incubated overnight at 4°C with HAM-56 (Dako, Carpinteria, Calif), a monoclonal antibody for macrophages and endothelial cells. Next, 30-minute incubations were performed with a biotinylated antimouse secondary antibody and avidin-biotin-alkaline phosphatase conjugate (ABC-alkphos; Vector, Burlingame, Calif). The chromagen (New Fuchsin; Dako) yielded a red reaction product. After washing, Ulex lectin (Vector), which binds to endothelial cells, was applied for 60 minutes at room temperature, and 30-minute incubations were performed with biotinylated anti-Ulex and avidin-biotin-peroxidase conjugate (ABC Elite; Vector). The second chromagen, 3'-diaminobenzidine with nickel chloride, yielded a black reaction product. This technique facilitated quantification of red-staining macrophages while masking HAM-56 cross-labeled endothelial cells with the black-staining Ulex lectin.

From the hematoxylineosin-stained sections, areas of necrotic core, calcification, loose matrix (proteoglycan-rich extracellular matrix), and intraplaque hemorrhage were measured by a medical technologist (M.S.F., 16 years of experience in carotid pathologic analysis) who used a manual drawing technique and the histologic classification criteria established by the American Heart Association Committee on Vascular Lesions (23,24). The remaining areas

were classified as fibrous tissue. Plaque neovascularity and macrophage content were measured on high-resolution digital images of the immunohistochemical sections by using morphometric analysis software (Image-Pro Plus; Media Cybernetics, Silver Spring, Md). The total lumen area of the plaque neovascularity was measured by manually tracing the black-stained neovascular boundaries; macrophage content was measured by quantifying red immunostaining. The total areas of each component were normalized to account for plaque shrinkage in formalin by dividing by the total plaque area and were expressed as percentages.

Data Analysis

All measurements were made serially after completion of all imaging and surgical procedures, allowing for observation of typical image and histologic features. The resulting data were analyzed for associations between MR variables (v_p and K^{trans}) and histologic variables (necrotic core, calcification, loose matrix, intraplaque hemorrhage, dense fibrous tissue, neovascularity, and macrophages). Associations between MR variables and clinical risk factors (age, body mass index, total cholesterol level, low-density lipoprotein level, HDL level, blood pressure, diabetes, hypertension, smoking, and recent symptoms) were also evaluated. To obtain sufficient numbers for statistical testing, former smokers and nonsmokers were merged into a single group. Finally, we tested for associations between our histologic marker of inflammation (macrophages) and clinical risk factors.

Statistical analyses were completed by a biostatistician (N.P.) who had 30 years of experience. Associations between two continuous variables were computed as a Pearson correlation coefficient (r) and were visualized by using scatter plots, with v_p or K^{trans} as the dependent variable. The Pearson correlation was chosen because of its close relationship with linear regression and because we anticipated a linear relationship between MR variables and histologic variables, both of which are volumetric in nature.

The statistical significance of the correlation (compared with $r = 0$) was calculated as (and is equivalent to) an F test for zero slope in a simple linear regression analysis for which one variable is regressed on the other (25). To assess the dependence of v_p and K^{trans} on histologic variables, a multiple regression analysis was performed. The F test from analysis of variance tables of two nested regression models was used to compare a model with one predictor variable (eg, K^{trans} vs macrophages) with a model with an additional predictor variable (eg, K^{trans} vs macrophages and necrotic core) (26). The null hypothesis of the F test in this example is that, when controlling for macrophage content, an additional histologic variable (eg, loose matrix) does not independently add to the prediction of K^{trans} .

For categorical clinical variables, the differences in the mean values of v_p and K^{trans} were evaluated between subgroups of study patients by using a two-sided unequal variance t test. To evaluate the assumption of normal distributions, the Shapiro-Wilk test for continuous variables was applied in order to ensure that no statistically significant departures from a normal distribution were detected. Statistical significance for all tests was defined at $P < .05$.

Results

Of the 30 patients who were originally enrolled, three were subsequently excluded from histologic comparisons either because the prescribed MR imaging planes were proximal to the location of the lesion (one patient) or because the specimens were damaged during surgery or histologic processing (two patients). Analyses were performed in the remaining 27 patients (26 men, one woman; mean age, 67.3 years $\pm$ 11.1 [standard deviation]; age range, 48–83 years).

Comparison of Contrast Enhancement and Plaque Composition

A range of plaque enhancement patterns was observed on dynamic contrast-enhanced MR images, with some patients exhibiting no perceivable increase in signal intensity over time and others exhibiting an initial and abrupt increase in signal intensity that was followed by a continued slower rise (Fig 1). In all cases, the kinetic model closely tracked the temporal behavior of signal intensity. Histologic analyses showed regions that had large (>0.02 mm) or densely packed (more than five per high-power field) neovessels in several plaque specimens, as well as large clusters of macrophages, which are often associated with the neovasculature (Fig 2).

Strong correlations between K^{trans} and macrophage and neovasculature content were found (Table 1). Moderate correlation between K^{trans} and loose matrix content was also observed. Similar but lower correlations were observed between v_p and the same three histologic measurements. In both cases, the elevated correlations involving loose matrix content were primarily the result of two influential observations with more than 30% loose matrix content (Figs 3, 4). Both v_p and K^{trans} exhibited trends toward lower values in heavily calcified plaques. Slightly negative but nonsignificant ($P > .1$) correlations were found between both v_p and K^{trans} and necrotic core, and virtually no correlation was found with fibrous tissue, although K^{trans} exhibited borderline significance ($P = .054$) after the elimination of one outlying measurement.

For multiple regression analysis (Table 2), the association of v_p with neovasculature content remained statistically significant ($P \leq .004$) in analyses that controlled for other histologic variables (loose matrix, necrotic core, calcium, hemorrhage, dense fibrous tissue, or macrophages). On the other hand, none of the other histologic variables were significantly associated with v_p in models that controlled for neovasculature ($P \geq .11$). The multivariate extension of the correlation coefficient (ie, the coefficient of determination [R]) demonstrated a slight increase from 0.68 to a maximum of 0.72 when any of the other histologic variables was individually added to the regression model of v_p versus neovasculature.

In multiple regression analyses of K^{trans} , the strong association between K^{trans} and macrophages remained statistically significant ($P < .001$) when controlling for other histologic variables (Table 3). Additionally, loose matrix ($P = .008$) and neovasculature ($P = .002$) were each significantly associated with K^{trans} , even when controlling for macrophages. R increased modestly from 0.75 to 0.82 for loose matrix and from 0.75 to 0.84 for neovasculature when each of these two histologic variables was added to the regression model. Again, the statistically significant contribution of loose matrix to the prediction of K^{trans} is primarily due to the inclusion of two observations with very large values for the percentage of loose matrix area.

Correlation of Clinical Cardiovascular Risk Factors with Contrast Enhancement and Inflammation

For continuous risk factors (Table 4), the only statistically significant association was a negative correlation between HDL levels and K^{trans} ($P < .001$). For discrete risk factors (Table 5), the only significant difference was an elevated K^{trans} value for smokers compared with nonsmokers ($P = .01$). Notably, a history of recent cerebral ischemic symptoms was not associated with a significant difference for any parameter (all $P > .3$). Also of note, the actual plaque macrophage content exhibited a similar correlation with HDL levels ($r = -0.43$, $P = .04$), was elevated in current smokers versus nonsmokers (1.27% vs 0.58%, respectively; $P = .01$), and was not significantly different between symptomatic and asymptomatic patients (1.12% vs 0.92%, respectively; $P = .5$).

Discussion

The results of this study further elucidate the pathways of plaque enhancement during gadolinium-enhanced MR imaging of the carotid arteries. Kinetic modeling of enhancement dynamics observed by using dynamic contrast-enhanced MR imaging demonstrates (a) that the initial entry of contrast agent into the plaque—quantified by v_p —is consistent with the amount of neovasculature within the plaque and (b) that the delivery of the contrast agent into the extravascular extracellular space—quantified by K^{trans} —is consistent with a combination of neovasculature supply and permeability. For all the histologic factors that were analyzed, the dominant mediator of plaque permeability, and hence of K^{trans} , appears to be macrophages. The link between K^{trans} and macrophage content remained strong, even when controlling for other histologic factors. This suggests that K^{trans} is an independent marker of carotid atherosclerotic plaque inflammatory content.

Plaque components other than macrophages and neovasculature were also associated with plaque enhancement parameters. Loose matrix had a significant correlation with K^{trans} ($P = .01$) and v_p ($P = .03$) that was primarily caused by data from two plaques that had substantial loose matrix content. Eliminating these two plaques from the analysis showed virtually no correlation between K^{trans} and loose matrix for the remaining plaques. Nevertheless, we believe that this finding is correct on the basis of the previously reported strong contrast enhancement of the loose matrix (27).

Additionally, both necrotic core and calcification exhibited slightly negative correlations with contrast enhancement parameters. Such negative associations are expected, given that other study results have shown minimal entry of contrast agents into necrotic regions (2,3). In these studies, researchers also showed that fibrous tissue exhibited the highest level of contrast enhancement. Surprisingly, the fractional area of fibrous tissue in the current study exhibited no apparent association with v_p and only a slight trend with K^{trans} when an otherwise influential point was deleted. This result does not contradict the results of previous studies but merely implies that the rate of contrast enhancement in fibrous regions is caused by inflammatory activity rather than by the volume of fibrous tissue.

Further evidence of the link between K^{trans} and macrophage content is their mutual association with two major cardiovascular risk factors: low HDL levels and smoking. Low HDL levels have been shown to be strongly associated with cardiovascular disease risk (28), whereas higher HDL levels may have an antiinflammatory effect by promoting cholesterol efflux, thereby inhibiting low-density lipoprotein oxidation and impairing monocyte recruitment into the artery wall through the inhibition of endothelial cell adhesion (29).

Likewise, smoking has been associated with increased lesion macrophage content in developing atherosclerotic lesions, as was seen at autopsy in young patients in the Pathobiological Determinants of Atherosclerosis in Youth study (30), and with elevated levels of plasma markers of inflammation, including C-reactive protein (31,32) and the soluble CD40/CD40 ligand complex (33). Results of the present study provide further direct evidence for an antiinflammatory effect of HDL and a proinflammatory effect of smoking by demonstrating corresponding associations with both carotid artery plaque macrophage content and the dynamic contrast-enhanced MR imaging correlate of inflammation, K^{trans} .

Related Studies

Weiss et al (5) previously showed a possible link between observed hyper-enhancement of the wall and plaque inflammation on the basis of serum markers, although a study weakness acknowledged by the authors was the lack of a histologic comparison. Wasserman et al (3) and Yuan et al (2) both proposed that patchy contrast enhancement in advanced plaques might be

indicative of increased inflammatory activity. In a follow-up study (4), long-term kinetics of fibrous tissue enhancement were evaluated, but early rapid wash-in was not evaluated. In another study that examined early wash-in kinetics, Kerwin et al (1) showed a strong quantitative association between v_p and neovasculature and reported a correlation coefficient of 0.80, which is similar to the value reported in the current study. None of these previous studies, however, established the direct quantitative link between an MR imaging-derived measure and the inflammatory cell content of plaque, as is demonstrated in our study.

Although other experimental techniques have been suggested for the detection of inflammation in patients with atherosclerosis, dynamic contrast-enhanced MR imaging has several advantages in such investigations. Ultrasmall particles of superparamagnetic iron oxides have been shown to accumulate in macrophages within atherosclerotic plaques and to lead to characteristic losses in signal intensity on MR images (34). Superparamagnetic iron oxides, however, require multiple imaging sessions over periods of 24 hours or more and, unlike dynamic contrast-enhanced MR imaging, superparamagnetic iron oxides have not been shown to provide quantitative information regarding inflammation. Alternatively, the use of a catheter equipped with a temperature probe has been used to detect elevated plaque temperatures that are associated with the inflammatory process (35), but catheters are highly invasive and are not suitable for either general screening or serial studies. On the other hand, dynamic contrast-enhanced MR imaging is a minimally invasive quantitative technique that is well established in oncology (18) and has already been shown to have the capacity to facilitate the identification of inflammation in other disease states (36,37).

Limitations

Although K^{trans} appears to be a quantitative indicator of macrophages in this study, one important distinction must be made. K^{trans} does not directly measure macrophages; instead, it likely represents an aggregate of multiple consequences of the inflammatory process—namely, increased neovasculature content and permeability. Thus, changes in macrophage content can only be inferred from changes in K^{trans} .

Another potential limitation of this study is that it was conducted entirely on large advanced plaques in patients who underwent endarterectomy. Obtaining histologic validation necessitated the study of this group. Whether these findings are applicable to early- and intermediate-stage lesions is uncertain.

A final limiting factor in this study is that several technical constraints may have limited the accuracy of the kinetic model. First, the time separation of 15 seconds between images is relatively long for such examinations but was required to obtain the necessary spatial resolution. Additionally, measurements of the change in longitudinal relaxation rate (R1) are considered to more accurately indicate the concentration of contrast agent than are changes in signal intensity, as was used in our study (19). However, in vivo measurements of R1 in carotid plaques have not been demonstrated. New imaging approaches may therefore lead to improved characterization of v_p and K^{trans} .

Implications

Our study results show that contrast-enhanced MR imaging can be used to gauge macrophage content in advanced carotid atherosclerotic plaques, thereby adding to previous evidence that contrast-enhanced MR imaging identifies neovasculature content (1,2) and improves separation of fibrous and necrotic plaque regions (3,4,27). If the results of further studies establish these factors (particularly K^{trans}) and predict a higher risk for both plaque progression and clinical ischemic events, then several possible applications of contrast-enhanced MR imaging emerge.

The ability to quantitatively assess the inflammatory status of a plaque by using noninvasive imaging will be of tremendous value for studies evaluating the effectiveness of novel therapies intended to inhibit plaque inflammation. If, after these studies are performed, K^{trans} emerges as an independent risk factor, it could be used to identify patients with a moderate degree of carotid artery stenosis who might benefit from closer monitoring, more aggressive medical therapy, or perhaps even earlier surgical intervention.

The additional confirmation provided by this study—that major cardiovascular risk factors are associated with plaque inflammation measured both at histologic analysis and dynamic contrast-enhanced MR imaging—suggests that dynamic contrast-enhanced MR imaging may ultimately allow for the non-invasive detection of plaque inflammation in vivo.

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Figure 1.

Precontrast MR image (upper left) and dynamic contrast-enhanced MR images at bolus arrival (upper right) and 75 seconds later (lower left). On precontrast image, contours of the lumen (inner circle) and outer wall (outer circle) are identified. At 75 seconds, the outer contour has been placed just outside enhancing rim of adventitia (arrows). Strong contrast enhancement of the lumen and jugular vein (J) is seen at $t = 0$ sec. Graph shows increase in signal intensity (relative to precontrast signal intensity) at 15-second intervals. Kinetic model is fit to data (line).

Figure 2.

Histologic identification of macrophages and endothelial cells. Double-labelled immunohistochemical slides show macrophages (red reaction product) and endothelial cells (black reaction product) at (a) low power and (b) higher power (magnification, $\times 200$). (c) Macrophage and neovascular areas were quantified by using an image analysis program that yielded pseudocolor images for endothelial cells (green) and macrophages (red).

Figure 3. Scatter plots of v_p measured by using dynamic contrast-enhanced MR imaging versus histologic measurements exhibit significant or nearly significant correlations. Regression lines are drawn.

Figure 4. Scatter plots of K^{trans} measured by using dynamic contrast-enhanced MR imaging versus histologic measurements exhibit significant or nearly significant correlations. Regression lines are drawn.

Table 1
 Plaque Composition versus Dynamic Contrast-enhanced MR Imaging Parameters

Plaque Component	Mean *	v_p Value		K^{trans} Value	
		<i>r</i> Value	<i>P</i> Value [†]	<i>r</i> Value	<i>P</i> Value [‡]
Necrotic core	21.5 ± 14.7	−0.26	NS	−0.05	NS
Calcification	10.0 ± 13.1	−0.34	.09	−0.33	.10
Hemorrhage	13.3 ± 14.1	−0.01	NS	0.26	NS
Loose matrix	7.0 ± 11.0	0.42 (−0.09 [‡])	.03 (NS [‡])	0.50 (−0.16 [‡])	.01 (NS [‡])
Fibrous tissue	61.5 ± 21.0	0.18	NS	−0.02 (0.39 [§])	NS (.054 [§])
Neovasculature	1.3 ± 1.5	0.68	< .001	0.71	< .001
Macrophages	1.0 ± 0.7	0.54	.004	0.75	< .001

* Data are the percentage of plaque area ± standard deviation.

[†] NS = not significant ($P > .1$).

[‡] After elimination of two influential outlier points.

[§] After elimination of one influential outlier point.

Table 2

Multivariate Regression Parameters for v_p Based on Models including Neovascularity and One Additional Plaque Component

Plaque Component	Regression Parameters			Parameters for Neovascularity			<i>R</i> Value*
	<i>r</i> Value	Standard Error	<i>P</i> Value	<i>r</i> Value	Standard Error	<i>P</i> Value	
Necrotic core	−0.078	0.063	.2	2.7	0.6	−.001	0.70
Calcification	−0.052	0.077	.5	2.6	0.7	−.001	0.68
Hemorrhage	−0.030	0.067	.7	2.8	0.6	−.001	0.68
Loose matrix	−0.007	0.111	.9	2.8	0.8	.002	0.68
Fibrous tissue	0.057	0.044	.2	2.8	0.6	−.001	0.70
Macrophages	2.39	1.45	.11	2.2	0.7	.004	0.72
Neovascularity alone	...	...	...	2.7	0.6	−.001	0.68

* Coefficient of determination (Pearson correlation in univariate regression).

Table 3

Multivariate Regression Parameters for K^{trans} Based on Models including Macrophages and One Additional Plaque Component

Plaque Component	Regression Parameters			Parameters for Macrophages			<i>R</i> Value *
	<i>r</i> Value	Standard Error	<i>P</i> Value	<i>r</i> Value	Standard Error	<i>P</i> Value	
Necrotic core	−0.0004	0.0006	.6	0.073	0.013	< .001	0.75
Calcification	−0.0010	0.0007	.2	0.069	0.013	< .001	0.77
Hemorrhage	0.0003	0.0007	.7	0.071	0.014	< .001	0.75
Loose matrix	0.0022 (0.0002 [†])	0.0008 (0.0015 [†])	.008 (.9 [†])	0.066 (0.066 [†])	0.012 (0.13 [†])	< .001 (< .001 [†])	0.82 (0.74 [†])
Fibrous tissue	0	0.0005	.9	0.073	0.014	< .001	0.75
Neovasculature	0.0206	0.0059	.002	0.051	0.013	< .001	0.84
Macrophages alone	...	...	...	0.073	0.013	< .001	0.75

* Coefficient of determination (Pearson correlation in univariate regression).

[†] After elimination of two influential outlier points.

Table 4
Correlations of Continuous Clinical Variables versus MR Measurement and Macrophages

Parameter	No. of Patients	Mean [*]	v_p Value	K^{trans}	Macrophages
Age (y)	27	67.3 ± 11.1	-0.08	-0.04	-0.27
Body mass index (kg/m ²)	23	27.0 ± 4.3	-0.30	-0.23	0.02
Total cholesterol (mmol/L)	23	4.9 ± 1.3	0.01	-0.15	-0.14
Low-density lipoprotein (mmol/L)	19	2.6 ± 0.8	0.06	0.13	0.03
HDL (mmol/L)	22	1.1 ± 0.3	-0.34	-0.66 [†]	-0.43 [‡]
Systolic blood pressure (mmHg)	26	144.8 ± 27.7	0.02	0.09	0.13
Diastolic blood pressure (mmHg)	26	73.9 ± 13.7	0.25	0.03	0.01

* Data are the mean ± standard deviation.

[†] $P < .001$.

[‡] $P = .04$.

Table 5
Categoric Clinical Variables versus MR Variables and Macrophages

Parameter	No. of Patients	v_p Value	K^{trans}	Macrophages
Symptomatic *	13	9.11 ± 5.93	0.127 ± 0.072	1.12 ± 0.42
Asymptomatic	13	10.30 ± 6.79	0.097 ± 0.064	0.92 ± 0.57
Hypertension	18	10.17 ± 7.38	0.115 ± 0.075	1.18 ± 0.52
No hypertension	8	8.66 ± 2.53	0.105 ± 0.055	0.67 ± 0.25
Diabetes	5	12.23 ± 8.08	0.094 ± 0.048	1.09 ± 0.87
No diabetes	21	9.10 ± 5.84	0.116 ± 0.073	1.01 ± 0.43
Smokers	15	11.01 ± 7.31	0.134 ± 0.078 [†]	1.27 ± 0.56
Nonsmokers [‡]	10	7.92 ± 4.41	0.074 ± 0.031 [†]	0.58 ± 0.11

Note.—Unless otherwise indicated, data are the mean ± standard deviation.

* Patients had a history of stroke or transient ischemic attack appropriate to the distribution of the index carotid artery within the previous 90 days.

[†] $P = .01$.

[‡] All previous smokers had quit more than 1 year prior to the examination and were classified as nonsmokers.