Measurement of carotid plaque: a tool for patient management, genetic research and evaluation of new therapies

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Introduction
There are now a number of ways to evaluate preclinical atherosclerosis, including measurement of coronary calcium by electron-beam CT (EBCT), measurement of carotid intima-media thickness (IMT), and other approaches. Such measurements are useful for risk stratification, and for evaluating response to therapy.

Since 1992, we have been using measurement of carotid plaque in our patients referred for vascular prevention, to evaluate the extent of atherosclerosis, and to assess their response to preventive therapy with multiple risk factor interventions.

In the past 10 years the measurement of plaque has evolved from 2-dimensional measurement of plaque (cross-sectional area of plaques viewed in a longitudinal plane) to 3-dimensional measurement of plaque volume, and also to quantitative evaluation of plaque surface characteristics (plaque roughness).

Measurement of carotid plaque is useful not only in the management of patients, but also in genetic research, and in evaluation of new therapies.

Using carotid plaque measurements for patient management:
We began using ultrasound to screen for carotid stenosis, in patients referred for transient ischemic attacks in our Stroke Prevention Clinic, in 1980. With the advent of duplex scanners, it became apparent that in addition to being able to evaluate the severity of stenosis by measuring carotid velocity with Doppler, we could see plaques in the carotid arteries. I asked my technologists to make composite drawings of the carotid arteries, drawing in on a template the location and size of the plaques they saw. We did this annually in patients referred to our Hypertension Clinic, as well as those referred to our Stroke Prevention Clinic. Over a few years it became apparent that some patients were manifesting regression of plaque, while others were progressing, and we began to use these images to gain an impression of whether their treatment was working. However, the composite drawings did not represent precise quantities, and this was unsatisfactory. When this concern was expressed to the senior technologist, Maria DiCicco RVT, she asked if I would prefer measurements, telling me that the ATL Mark 8 duplex scanner had built into it software for tracing around the perimeter of objects to measure their cross-sectional area. She began making measurements of carotid plaque for research purposes in 1992, and since 1997 we have measured carotid plaque area at baseline and annually in all our patients.

Method for 2-D plaque area measurement:
The operator begins by performing a routine Doppler examination of the extracranial carotid arteries to evaluate severity of stenosis. Then a B-mode scan is performed, to identify all plaques that can be seen between the clavicle and the angle of the jaw. Each plaque is isolated in a longitudinal view, choosing the plane in which the plaque is biggest. The view chosen is magnified, the perimeter of the plaque is traced with a cursor on the screen, and the cross-sectional area of that plaque is measured. The operator goes on to measure each plaque in turn, in its own view, and then the total of cross-sectional areas of all plaques seen is summed to give total plaque area (TPA). The intra-observer reliability of repeat measurement is 0.94; inter-observer reliability between two technologists using two different machines was 0.89; the junior technologist with the lower-resolution machine (an ATL Mark 9 vs an ATL HDI 5000 with Sono-scan compound imaging) systematically measured less plaque. Figure 1 below shows an example of measurement of a long plaque in the common carotid artery.
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Figure 1. Measurement of carotid plaque area. Each plaque seen in the internal, common or external carotid artery on either side is isolated in a longitudinal view, choosing the plane in which it is biggest; the perimeter of the plaque is then traced to obtain plaque area; total plaque area is the sum of areas of all plaques seen. (Reprinted with permission of Lippincott, Williams and Wilkins Publishing from Stroke 2002 (Spence JD et al. Carotid Plaque Area: A Tool for Targeting and Evaluating Vascular Preventive Therapy. Stroke 2002; 33:2916-2922.)

Total Plaque Area as a predictor of risk:
We reported in 2002 that TPA was a powerful predictor of risk. We followed 1,686 patients for up to 5 years (mean followup of 2.5 years) after baseline measurement of TPA. Patients in the top quartile of baseline plaque area had a 3.4-fold higher risk of stroke, death or myocardial infarction compared to the lowest quartile, after adjustment for age, sex, systolic blood pressure, total cholesterol, pack-years of smoking, diabetes, plasma homocysteine, and treatment of hypertension and lipids. This is substantially more than the risk of patients with high coronary calcium scores (relative risk 1.8), and somewhat higher than the risk of patients in the top quintile of carotid IMT, after adjustment for a smaller panel of risk factors: O’Leary et al reported that patients in the top quintile of IMT had a relative risk of 3.15 compared to those in the lowest quintile. Furthermore, IMT is a stronger predictor of stroke than of myocardial infarction, whereas TPA is a stronger predictor of myocardial infarction than of stroke. A likely explanation for these differences is that IMT represents mainly hypertensive medial hypertrophy, whereas TPA quantifies plaque.

Figure 2 below shows the Kaplan-Meir survival curves by quartile of baseline plaque area.
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Figure 2. Survival free of stroke, death or myocardial infarction by quartile of baseline total plaque area, after adjustment for age, sex, systolic blood pressure, total cholesterol, pack-years of smoking, diabetes, plasma homocysteine, and treatment of hypertension and lipids (Reprinted with permission of Lippincott, Williams and Wilkins Publishing from Stroke 2002 (Spence JD et al. Carotid Plaque Area: A Tool for Targeting and Evaluating Vascular Preventive Therapy. Stroke 2002; 33:2916-2922.)

Plaque progression:
In our clinic, with intensive multiple risk factor intervention including Mediterranean diet, effective control of hypertension, lipid-lowering therapy, angiotensin receptor antagonists, smoking cessation, B vitamins to lower homocysteine and advice on exercise, we see regression of plaque in the first year in approximately a third of patients; stable plaque in about 20%, and progression in nearly half. Those with progression of plaque despite treatment have twice the risk of those with stable plaque or regression. Figure 3 below shows the event-free survival by status of plaque progression or regression.

Figure 3. Plaque progression as a predictor of vascular outcome.
Patients with progression of plaque in the first year (by more than 0.05 cm², which was the median rate of progression) had twice the risk of stroke, death or myocardial infarction compared with patients whose plaque was stable or regressing, after adjustment for age, sex, blood pressure, cholesterol, smoking, homocysteine and diabetes. (Reprinted with permission of Lippincott, Williams and Wilkins Publishing from Stroke 2002 (Spence JD et al Carotid Plaque Area: A Tool for Targeting and Evaluating Vascular Preventive Therapy. Stroke 2002; 33:2916-2922.)
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In our clinic, the therapeutic goal is to achieve plaque regression, or at least to halt the progression of plaque. To me, trying to treat atherosclerosis without measuring plaque would be like trying to treat hypertension without measuring the blood pressure. In patients whose plaque is regressing in two successive years, we can be confident that the treatment is working; such patients may require less frequent followup in clinic, allowing us more time to spend with patients whose plaque is progressing. In the latter group we have to try harder: this may mean addressing new risk factors such as Lp(a), borderline hypothyroidism, verifying that the vitamin therapy for homocysteine is effective, increasing doses of statins, adding fibrates or niacin, reinforcing advice about diet and smoking cessation, and other interventions. In patients whose plaque is progressing despite effective control of all known and putative risk factors, we consider genetic testing. If the family is large enough, a linkage study would be feasible.

Sex differences:

We have shown that at any age, women have less plaque than do men; in contrast women have more apparent stenosis as measured by Doppler velocities. This suggests that there may be sex differences in remodeling related to sex hormones. Figure 4 shows the distribution of TPA by age groups in men and women in our clinic.

![Graph showing sex differences in carotid plaque area by age group](image)

**Figure 4. At any age, women have less carotid plaque than do men.**
Adapted from reference 9.

Genetic research

There are important biological differences between IMT and plaque that must be kept in mind when considering response to therapy, or genetic influences. In multiple regression, the proportion of explained variance accounted for by traditional risk factors (the $R^2$) is only 15-17% for IMT, whereas for TPA it is 52%; for carotid stenosis measured by Doppler velocities it is only 13%.

Table 1 below shows a multiple regression model using plaque area (transformed by a cubic root transform to obtain a normal distribution) as the dependent variable. The $R^2$ is 0.52, and each risk factor is a significant predictor of plaque area. The largest beta (proportion of explained variance) is age, which accounts for almost half of baseline plaque area.
Table 1. Multiple regression with plaque area as dependent variable, age, sex, systolic pressure, pack-years of smoking, cholesterol, and treatment for hypertension or hyperlipidemia as predictors.

Model Summary

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<td>.519</td>
<td>.514</td>
<td>.280</td>
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a Predictors: (Constant), PKYRS, HTHYP1, SEX, Cholesterol, LIPMED1, SYSBP1, AGE  
b Dependent Variable: CUBPLAQ1**

Coefficients

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<th>Standardized Coefficients</th>
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Dependent Variable: Cube root transform of plaque area at baseline to achieve normality **

*lipid levels are weakly predictive, probably because of treatment; it is for that reason that the variable LIPMED1, representing treatment of lipids, is a significant predictor, as is HTHYP, which represents history of or treatment of hypertension. SYSBP1 = baseline systolic pressure; PKYRS = pack-years of smoking.

When this regression model is run, and the predicted plaque area as well as the residual scores (the distance off the regression line) are saved for each individual, the plot below (Figure 4) represents a way of separating our population into three groups: those whose carotid plaque area is about as much as would be expected from the traditional risk factors (explained cases), those who have much more plaque than would be predicted (unexplained atherosclerosis), and those who appear to be protected by some (probably genetic) factor (Protected cases). Figure 4 below shows a plot in which the 90th percentile and 10th percentile of residual scores were used to define unexplained and protected cases respectively.
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Figure 5. Unexplained Atherosclerosis: a quantitative trait.
The trait is quantified by computing the residual score for each individual, in a multiple regression model in which plaque area is the dependent variable. Independent variables are: age, sex, systolic blood pressure, pack-years of smoking, treatment of hyperlipidemia, and treatment of hypertension (with p<0.05 for each variable). The residual score is the distance away from the regression line and represents the quantitative trait of “unexplained atherosclerosis”. For the individuals marked in solid triangles, there is excess “unexplained atherosclerosis” (top 10th percentile of residual scores). These are the people whose atherosclerosis is least likely to be explained by the traditional risk factors. The individuals indicated by gray circles are considered to have about as much atherosclerosis as would be predicted by their Framingham risk factors. Those indicated by solid squares have less atherosclerosis than would be predicted (bottom 10th percentile of residual scores) and thus appear to be “protected” from atherosclerosis. (Reproduced by permission of Bentham Science Publishers from Spence JD, Hegele RA. Non-invasive assessment of atherosclerosis risk. Current Drug Targets - Cardiovascular and Haematological Disorders 2004;4:125-8.)

We used plasma homocysteine to test the hypothesis that patients in the top 10% of residual scores in the model (i.e. those whose atherosclerosis is least explained by age, sex, blood pressure, cholesterol and smoking, represented by the red triangles squares in the figure) have new causes of atherosclerosis. Compared to 20% of the Canadian urban population and 20.1% of the volunteers in the original stress study, and with 33% of patients in the premature atherosclerosis clinic, 47% of patients with unexplained atherosclerosis in our model had plasma homocysteine >14 µmol/L.

We have used this trait to study genetic influences on atherosclerosis in several genetic association studies. We showed that plasma homocysteine, but not MTHFR genotype, predicted plaque area independently (i.e. after controlling in multiple regression for age, sex, systolic pressure, lipids and smoking).
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We found that infection-susceptible alleles of Mannose-binding lectin, a protein involved in resistance to infection with Chlamydia pneumoniae, are associated with unexplained atherosclerosis. Because age accounts for nearly half of the explained variance in baseline plaque, and age is neither genetic nor treatable, regression models using rate of progression of plaque are a more sensitive approach to discovery of new therapeutic targets. We have found that a polymorphism of mannose binding lectin was independently associated with both baseline plaque area, and with rate of progression of plaque.

Because of the biological differences between IMT and plaque, genetic influences are differentially assessed by these traits. Whereas IMT has been associated with polymorphisms of the ACE and angiotensin genes, we and others have not found an association with carotid plaque. Similarly, we found that different polymorphisms of PPARγ were associated with IMT and carotid plaque.

Because the residual score in multiple regression represents a quantitative trait, it is ideally suited to linkage studies. We have begun such studies in collaboration with Dr. Daniel Gaudet and others in large kindreds in our Premature Atherosclerosis Clinic, and in a group of vascular patients in the Saguenay-Lac St.Jean district of Quebec, an area that is enriched in monogenic disorders because of a founder effect.

Measurement of 3-D plaque volume:
Beginning in 1993, Dr. Aaron Fenster’s Imaging Research Group at the Robarts Research Institute, with our collaboration, has been developing methods for measurement of 3-dimensional plaque volume. This work has progressed substantially in the past few years. Plaque volume can be measured accurately (compared to known volumes) and reliably, with intra-observer reliability of 94%, and inter-observer reliability of 93%.

At present the method is manual, requiring tracing of the cross-sectional area of plaque in cross-sectional views at intervals of 1-2 mm, with the slices stacked up for measurement of plaque volume (disk segmentation). Fixing the volume measured to a standard distance above and below the carotid bifurcation reduces variability.

Figure 5 below shows the disk segmentation procedure, fixed to the bifurcation.
Measurement of carotid plaque

**Procedure for determining plaque volumes from 3D ultrasound images.**  a) An approximate axis of the vessel is selected in a longitudinal view (coloured line) and the vessel wall and lumen boundary are outlined (yellow). b) Using the surfaces generated by the vessel contours and the 3D ultrasound image, the position of the bifurcation (BF, yellow arrow) is determined and marked. The axis of the vessels is selected based on the bifurcation point and marked along a distance along which the plaque volume can be measured (coloured line). This axis will be used as a reference for distance measurements. c) All plaques within the measurable distance are outlined, different colours being used for each separate plaque to aid in identification. d) Volumes are calculated for each plaque, and surfaces of the vessel wall and plaques are generated in order to better visualize the plaques in relation to the carotid arteries.

**Figure 6. Comparison of plaque outlines and volumes for a patient on atorvastatin therapy.**  
 a) 3D ultrasound image showing a plaque outline in a transverse view for the baseline time point. b) Vessel wall (yellow) and plaques (red and blue) surfaces created with the contours in a); the total plaque volume was 347 mm$^3$. c) Plaque and vessel wall outlines at 3 month follow up. d) Vessel wall and plaque surfaces for the 3 month follow up using the outlines in c); the total plaque volume was 289 mm$^3$. e) Plot of plaque burden (plaque volume between the bifurcation and the given distance from the bifurcation) versus distance from the bifurcation showing where the plaque volume is changing in relation to the bifurcation point.
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Registration of images, and semi-automated measurement of plaque volume, with automated segmentation of the lumen, are in development in Dr. Fenster’s laboratory.

**Plaque surface roughness:**
One approach to identifying vulnerable plaque is detection of ulceration. The figures below show ulcers on 3-D ultrasound images from a 77-year old man with 70% stenosis of the left carotid artery, and microemboli on transcranial Doppler embolus detection\textsuperscript{23}. Microemboli are associated with increased risk of stroke\textsuperscript{24}.

Figure 6. Longitudinal view of the common carotid and origin of the internal; pulsation artifact is present in longitudinal views, but because ~ 30 slices per second are recorded, not a problem in cross-sectional views shown below, in which spicules and ulcers are clearly seen\textsuperscript{23}. Adapted from reference 24.
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The amount of ulceration can be quantified, as shown in the figures below. This quantity, plaque surface roughness, promises to be not only a tool for risk stratification, but also a therapeutic target.

![Figure 7. Views of two different carotid arteries with plaque. Each surface value indicates the local mean Gaussian curvature of all surface points within a 1.0 mm radius of the corresponding surface location. The values of the mean Gaussian curvature have been colour-coded and “painted” onto the segmented surface of the vessel lumen. Reproduced by permission of Bentham Science Publishers from Fenster A, et al. 3D Ultrasound Imaging of the Carotid Arteries. Curr Drug Targets - Cardiovasc Hematol Disorders 2004;4:161-75.)](image)

Evaluation of new therapies
New therapies are being developed that are anti-atherosclerotic, but which do not affect intermediate targets such as plasma lipids, or blood pressure. In order to develop such drugs it will be necessary to measure atherosclerosis, not only to demonstrate efficacy for proof of principle, but even for dose-finding studies. Examples of such drugs include ACAT inhibitors\textsuperscript{26,27}, and the synthetic version of the ApoA1 Milano dimer\textsuperscript{28,29}.

Sample sizes and duration of therapy required for other methods of measuring atherosclerosis are substantially larger than with 3-D plaque volume. In large part this is because plaque progresses along the vessel wall, in the axis of flow, 2.4 times faster than it thickens\textsuperscript{2}. It also progresses and regresses circumferentially. Thus each dimension of measurement, from 1-dimensional measurement of intima-media thickness, to 2-D plaque area, to 3-D plaque volume, reduces the sample size required by \sim an order of magnitude.
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Bots et al.\textsuperscript{30} in a recent meta-analysis indicated that 468 patients per group would be required for a parallel clinical trial with an effect size of 30\% over 2 years, or 30 per group for a 100\% effect size over 3 years. In the REVERSAL trial\textsuperscript{31}, 250 patients per group were followed for 18 months to show a difference in progression of coronary plaque volume, comparing 80mg atorvastatin vs 40 mg pravastatin.

These sample sizes and durations of therapy are much greater than those required using measurement of carotid plaque. 2-D plaque area requires sample sizes of 150 per group for a 30\% effect size over two years\textsuperscript{2}, while 3-D plaque volume can show significant changes in 20 patients per group in 3 months with an effect size of 100\%\textsuperscript{32}. To show an effect that is 25\% as great as that of atorvastatin 80mg would require 86 patients per group followed 3 months, or 22 per group followed for 6 months.

![Graph showing change in plaque volume](image)

Figure 8. In a double-blind randomized study of atorvastatin 80mg daily vs placebo for three months, in 21 patients randomized to placebo vs 17 to atorvastatin, plaque regressed significantly on atorvastatin p<0.0001\textsuperscript{32}.

The recent paper by Corti et al.\textsuperscript{33} describing changes in vessel wall area measured by MRI in patients on statin therapy, raises the interesting and important issue of how best to measure effects of anti-atherosclerotic therapies. Although there was a reduction of vessel wall area over 12 months with statin therapy, no difference was shown between simvastatin 80mg and 20mg daily over 18 months of followup. This may have been due to the small sample size of 51 subjects, but it also was probably in part due to the method of measurement. Vessel wall area includes not only plaque, which is mainly in the intima, and likely to change with treatment, but also a large component of noise in the sense that the media and adventitia will change much less (if at all) with lipid lowering therapy.
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It is important not only in genetic research, but also in studies of effects of therapy on atherosclerosis, to understand that the various ways of measuring atherosclerosis give biologically distinct phenotypes. Traditional risk factors account for 52% of variance ($R^2$) of carotid plaque area, but only 13% of carotid stenosis measured by Doppler velocities, and only 15-17% of Intima-media thickness (IMT). This probably is determined by what is measured. IMT, for example, represents approximately 80% media and only 20% intima; it thus represents mainly hypertensive medial hypertrophy, and including it in a measurement of “atherosclerosis” introduces the noise of medial hypertrophy that is due mainly to blood pressure. For this reason the sample sizes required to measure treatment effects are relatively large. Bots et al report in a meta-analysis that for maximum carotid IMT sample sizes should be from 468 per group for a parallel clinical trial with an effect size of 30% over 2 years, to 30 per group for a 100% effect size over 3 years. Newer automated methods for measurement of IMT can achieve smaller sample sizes and study durations.

It should also be understood that sensitivity of measurements to effects of therapy is also highly dependent on the location and method of the measurement. The most important reason why IMT is so insensitive to effects of therapy is that it represents only a measurement of thickness, but plaques in the carotids grow along the artery 2.4 times faster than they thicken. Furthermore, IMT is often deliberately measured in a part of the distal common carotid with no plaque. Most carotid plaques are focal, with a proximal and distal end, which can progress or regress, and this change can be captured by measuring plaque area. Plaque volume is even more sensitive to effects of treatment, because it captures change in circumferential growth or regression of plaque in addition to change in thickness and length of plaque. Each dimension of measurement reduces the product of sample size x duration (and thus the cost of studies) by approximately an order of magnitude. As described above, we have recently shown that a sample of only 20 patients per group was sufficient to detect regression of plaque with high-dose atorvastatin vs. placebo in 3 months. Measurement of coronary plaque volume by intravascular ultrasound (IVUS) is not only invasive and much more costly, it is much less sensitive to effects of therapy. The reason is that coronary plaques, as they are diffuse, do not have a proximal and distal end; change in coronary plaque volume is thus reduced to a change in thickness. Therefore sample sizes and duration of therapy required to show effects of treatment with IVUS are substantially larger: 654 patients were followed 18 months to show a difference between high-dose atorvastatin and pravastatin.

Thus, if investigators wish to measure effects of therapy on atherosclerosis, this can be done in much smaller samples and over much shorter durations by measuring carotid plaque volume, than by other available methods.

Conclusions:

Plaque measurement is an important tool for risk stratification, and evaluation of patient’s responses to therapy. In clinical practice it informs the physician about the effectiveness of therapy, and identifies those patients requiring more intensive therapy, or investigation for new risk factors. Such patients can be investigated for new genetic factors affecting atherosclerosis using quantitative traits including unexplained plaque and unexplained progression.

New therapies for atherosclerosis can be investigated by measuring progression of plaque volume, and treatments to reduce vulnerable plaque can be evaluated by measuring surface plaque roughness. These tools promise to significantly advance the development of new therapies that are anti-atherosclerotic, but which do not affect other intermediate targets such as lipids or blood pressure.
Reference List


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