3D Ultrasound Measurement of Change in Carotid Plaque Volume
A Tool for Rapid Evaluation of New Therapies

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Background and Purpose—New therapies are being developed that are antiatherosclerotic but that lack intermediate end points, such as changes in plasma lipids, which can be measured to test efficacy. To study such treatments, it will be necessary to directly measure changes in atherosclerosis. The study was designed to determine sample sizes needed to detect effects of treatment using 3D ultrasound (US) measurement of carotid plaque.

Methods—In 38 patients with carotid stenosis >60%, age ± SD 69.42 ± 7.87 years, 15 female, randomly assigned in a double-blind fashion to 80 mg atorvastatin daily (n = 17) versus placebo (n = 21), we measured 3D plaque volume at baseline and after 3 months by disc segmentation of voxels representing carotid artery plaque, after 3D reconstruction of parallel transverse duplex US scans into volumetric 3D data sets.

Results—There were no significant differences in baseline risk factors. The rate of progression was 16.81 ± 74.10 mm³ in patients taking placebo versus regression of −90.25 ± 85.12 mm³ in patients taking atorvastatin (P < 0.0001)

Conclusions—3D plaque volume measurement can show large effects of therapy on atherosclerosis in 3 months in sample sizes of ~20 patients per group. Sample sizes of 22 per group would be sufficient to show an effect size of 25% that of atorvastatin in 6 months. This technology promises to be very useful in evaluation of new therapies. (Stroke. 2005; 35:1904-1909.)

Key Words: atherosclerosis ■ carotid artery plaque ■ ultrasonography

With the completion of the human genome project, it is anticipated that many new therapeutic targets will be identified, and new therapies will be developed. One example is apolipoprotein A1 (ApoA1) Milano,1–3 which was found to be protective against vascular events in carriers of this trait, and which led to a therapy that regressed coronary atherosclerosis in a matter of weeks.4 Another potential example of such novel therapy is the inhibitors of acyl-coenzyme A:cholesterol acyltransferase, which are powerfully antiatherosclerotic in animal models but have little effect on plasma lipids in human subjects.5–9

Without a way of measuring efficacy, it would not be possible to develop such drugs because the duration and cost of studies based on vascular end points such as myocardial infarction or stroke would be excessive. Therefore, it will be necessary to use intermediate end points based on progression of atherosclerosis for dose-finding studies and for early proof of principle studies to demonstrate efficacy and thereby make it feasible to go on to larger studies based on end points.

Intravascular ultrasound (US) measurement of coronary plaque volume has been used to show efficacy of antiatherosclerotic therapies,5,10 but this approach is invasive, potentially risky, and very expensive. Noninvasive approaches including measurement of carotid intima-media thickness (IMT) and coronary calcification have been used, but these approaches have relatively high cost because of large sample sizes and long durations of therapy, as well as other drawbacks relating to their biology.11

We have shown that carotid plaque area measured by US is a strong predictor of cardiovascular outcomes.12 Here we report the results of the first study using this methodology for measuring effects of treatment. The purpose was to determine what sample size would be required to show effects of therapy on progression of carotid plaque volume. We studied 80 mg atorvastatin daily versus placebo for 3 months; this high dose of atorvastatin was used to maximize the likelihood of a measurable effect.

Methods

Study Population
We enrolled 53 patients with asymptomatic carotid stenosis >60% as defined by carotid Doppler flow velocities (validated against 200
angiograms in which stenosis was measured in the North American Symptomatic Carotid Endarterectomy Trial), who were participating in a long-term follow-up study to investigate imaging methods that may identify high risk for cardiovascular events. According to Canadian consensus guidelines, endarterectomy is not routinely offered to patients with asymptomatic stenosis because in actual practice, the risk of endarterectomy is higher than the 3% risk in the clinical trials on which benefit is predicated. This was discussed with the patients, and all who agreed to this approach and volunteered for the study were randomized. Patients who were taking lipid-lowering medication underwent a 6-week washout before randomization. Patients with a previous history of angina or myocardial infarction were excluded for safety reasons, but because carotid stenosis is associated with a high risk of cardiac events, we did not wish to expose patients to a long duration of placebo therapy. All subjects gave consent to a protocol approved by the University of Western Ontario standing board of human research ethics and were randomized to placebo versus 80 mg atorvastatin daily for a duration of treatment of 3 months. Subjects were allocated in a double-blind fashion to identical placebo or 80-mg atorvastatin tablets (Lipitor) provided in a randomized sequence by Pfizer Canada Inc.

**Imaging**

Carotid US scans were performed at the Imaging Research Laboratories, Robarts Research Institute, using a Philips/ATL HDI 5000 US machine (Philips/ATL). An L12–5 probe with a central frequency of 8.5 MHz was used in the composite imaging (SonoCT) mode. The probe was attached to a motorized linear mover driven by a Life Imaging Systems L3Di 3D US acquisition system (Life Imaging Systems). The acquisition system consisted of a Pentium III computer with a frame grabber digitizing 2D frames at 30 Hz from the US machine as the probe was moved along the neck at a uniform speed of 3 mm/s without cardiac triggering. 3D volumes were constructed using the acquired frames and displayed using multiaxial reformattng. Imaging was conducted at baseline and 3 months later. Subjects were imaged while recumbent on a gurney with their upper torso inclined ~15°. Both carotids were scanned multiple times (usually 4 to 5) over a scan distance of ~4 cm along the carotid arteries, with the bifurcation located as closely as possible to the center of the volume. We reported previously that using phantoms with known plaque volume ranging from 37.4 to 604.1 mm3, we obtained a mean accuracy in plaque volume measurement of 3.1%; the SD in plaque volume measurement was 4.0% ± 0.9%; the SD in plaque volume measurement depended on the volume of the plaque, and was 4.0 ± 1.0% and 5.1 ± 1.4% for intraobserver and interobserver measurements, respectively. In addition to studies with test phantoms, we investigated the observer variability in the measurement of plaque volumes with 3D US images of 40 individuals with carotid plaques ranging from 37.4 to 604.1 mm3. Our results demonstrated that the intraobserver and interobserver measurement reliability were 94% and 93.2%, respectively. The coefficient of variation (SD divided by the mean) of plaque volume measurement decreased with plaque volume from 27.1% to 2.2% over the range of plaque volumes measured. The variability of measurements from repeat 3D US scans was not different from that of measurements from single scans (P = 0.867). Ainsworth et al 3D Ultrasound Measurement of Plaque Volume

**Results**

From an initial cohort of 53 patients, baseline and 3-month plaque volume measurements were obtained in 38 cases; these measurements form the basis of this report. Reasons for exclusion were that 13 did not have a second US measurement (1 died before the second US, 2 underwent carotid endarterectomy, 3 had a transient ischemic attack or stroke, and 7 did not return for the second measurement). In addition,
images from 2 patients were not used because of poor image quality prohibiting the plaque measurements.

As shown by the baseline characteristics in Table 1, subjects were middle-aged or elderly vascular patients with the expected risk factors on treatment. There were no significant differences in risk factors nor in treatments other than the study medication (such as angiotensin-converting enzyme inhibitors, antiplatelet agents, or previous treatment with statins) between the 2 treatment groups. Baseline plaque volume (mean±SD) was 722.0±473.72 mm$^3$ for the placebo group and 689.53±409.99 mm$^3$ for the atorvastatin group ($P=0.83$). 3-month plaque volumes were 738.81±494.66 mm$^3$ on placebo and 599.29±355.19 mm$^3$ on atorvastatin.

### Table 1. Baseline Characteristics of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>SD</th>
<th>Atorvastatin</th>
<th>SD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>70.14</td>
<td>9.41</td>
<td>68.06</td>
<td>8.60</td>
<td>0.49</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>4.40</td>
<td>0.61</td>
<td>4.35</td>
<td>0.89</td>
<td>0.85</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.53</td>
<td>0.55</td>
<td>1.32</td>
<td>0.65</td>
<td>0.29</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>1.22</td>
<td>0.36</td>
<td>1.47</td>
<td>0.45</td>
<td>0.08</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>2.51</td>
<td>0.72</td>
<td>2.29</td>
<td>0.78</td>
<td>0.38</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>146.48</td>
<td>22.44</td>
<td>147.35</td>
<td>18.75</td>
<td>0.90</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>73.29</td>
<td>15.26</td>
<td>72.53</td>
<td>13.31</td>
<td>0.87</td>
</tr>
<tr>
<td>Sex</td>
<td>15 male, 6 female</td>
<td>0.20</td>
<td>9 male, 8 female</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>13 no, 8 yes</td>
<td></td>
<td>13 no, 4 yes</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>17 no, 4 yes</td>
<td></td>
<td>15 no, 2 yes</td>
<td>0.44</td>
<td></td>
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</tbody>
</table>
Figure 2 shows an example of plaque regression in a subject taking atorvastatin. Over 3 months, plaque volume increased on placebo by 16.81±74.10 mm³, whereas on atorvastatin, there was significant regression of plaque by 90.25±85.12 mm³ (P<0.0001; Figure 3). The operator variability was found to be 53 mm³.

Analysis of covariance gave essentially the same results; the estimated marginal means were: placebo progression 17.31±17.20 mm³ (95% CI, 17.6 to 52.23 mm³) and atorvastatin 90.85±19.12 mm³ (95% CI, 160.39 to 55.93 mm³; Figure 3; P<0.0001). (The SEs convert to SDs of 78.77, slightly smaller than the pooled variance of 80 used for the sample size estimates below.) Observed power was 0.98. There was no difference between the treatment groups with respect to plaque characteristics (P=0.46).

### Sample Size Estimates
Table 2 shows sample size estimates for treatments with effect sizes compared with that of atorvastatin. Assuming that progression on placebo and regression on atorvastatin continue in a linear fashion, with equal variances (an SD of 80 mm³, ie, slightly more than the average variance of the 2 groups), a treatment 25% as effective as atorvastatin would require 86 patients per group treated over 3 months, or 22 per group treated over 6 months to give a power of 90% to show a 2-tailed difference significant at the 0.05 level. Sample sizes using the results from the analysis of covariance were somewhat smaller.

The assumption of linear progression is consistent with our previous published experience with plaque area,21 which bears a strong linear relationship to plaque volume (R=0.93).21

### Discussion
We found that 3D US measurement of plaque progression demonstrated statistically significant effects of a highly efficacious therapy in a small sample over 3 months. This methodology promises to be extremely useful in evaluation of new therapies. A major advantage of 3D plaque measurement relates to the way that plaques grow. Plaque progresses along the vessel (in the axis of flow) 2.4× faster than it thickens22 and also grows circumferentially. Therefore, methods that capture longitudinal and circumferential growth and regression of plaque are inherently more sensitive to change in plaque over time and with therapy than methods limited to measurement of change in thickness.
Power & Effect Size & Sample Size & Duration of Treatment

<table>
<thead>
<tr>
<th>Power</th>
<th>Effect Size (% of atorvastatin)</th>
<th>Regression in mm³</th>
<th>Sample Size Per Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>10%</td>
<td>-9</td>
<td>203</td>
</tr>
<tr>
<td>0.9</td>
<td>25%</td>
<td>-23</td>
<td>86</td>
</tr>
<tr>
<td>0.9</td>
<td>50%</td>
<td>-45</td>
<td>36</td>
</tr>
<tr>
<td>0.9</td>
<td>75%</td>
<td>-68</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>To show treatment effect in 6 months, placebo progression 33.62±80 mm³/6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>10%</td>
<td>-18</td>
<td>51</td>
</tr>
<tr>
<td>0.9</td>
<td>25%</td>
<td>-45</td>
<td>22</td>
</tr>
<tr>
<td>0.9</td>
<td>50%</td>
<td>-90</td>
<td>9</td>
</tr>
<tr>
<td>0.9</td>
<td>75%</td>
<td>-135</td>
<td>6</td>
</tr>
</tbody>
</table>

**TABLE 2.** Range of Sample Sizes Required for Various Effect Sizes and Duration of Treatment

By comparison, measurement of IMT requires much larger sample sizes for much longer times. Bots et al. reported that a treatment with an effect size of 30% would require 468 patients followed for 2 years to give a power of 80% to show an effect significant at the 0.05 level. A similar sample size (366 per group over 2 years) was calculated for the study to evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E (SECURE) trial comparing the effects of ramipril and vitamin E on progression of IMT. Newer methods of IMT measurement using automated edge detection may reduce sample sizes by reducing variability of measurement to as low as 68 per group followed for 1 year to give a 90% power of showing a 30% treatment effect (personal communication, Dr Jacques Barth, May 2004).

However, there remain important differences in biology between IMT and plaque measurement. These have been discussed recently. IMT represents mainly hypertensive medial hypertrophy, and only 15% to 17% of IMT is explained by traditional coronary risk factors; in contrast, 52% of plaque is explained by these factors, thus, treatments expected to affect atherosclerotic plaque should affect plaque volume more than IMT.

Measurement of coronary calcium as a means to study efficacy of antiatherosclerotic therapy has been reviewed recently. The sample sizes required in the past have usually been ≈200 per group over a year; however, recently, some studies have shown differences in effects of treatment with smaller samples and durations of therapy. Again, the issue of biological differences among noninvasive phenotypes may be important; calcification may reflect different biological processes and therefore respond to different therapies than measurement of plaque.

Measurement of coronary plaque volume by intravascular ultrasound has also shown slowing of plaque progression with high-dose atorvastatin compared with pravastatin at a lower dose, but the sample size was 250 per group treated for 18 months. Recently a recombinant ApoA1 Milano/phospholipid complex was shown to regress coronary plaque volume in ≈27 patients per group in 5 weeks. Those results were very similar to ours; however, the much higher cost of IVUS (~60-fold higher), combined with the risk of coronary angiography, makes carotid plaque volume measurement a much preferable approach to studying effects of therapy for atherosclerosis.

Interference with images by calcification, a concern commonly raised in relation to US measurements, was not a significant problem because several images were obtained at each visit using compound imaging with Sono-CT, which permitted images to be obtained that were not materially affected by the calcification. Calcification was not a reason for exclusion from the study nor the measurements.

We have shown previously that total carotid plaque area (the sum of cross-sectional areas of all plaques in the carotid arteries, measured in longitudinal views of each plaque) is a strong predictor of stroke, death, and myocardial infarction. Patients in the top quartile of baseline plaque area have, after adjustment for age, sex, blood pressure, cholesterol, pack years of smoking, homocysteine, diabetes, and treatment of lipids and blood pressure, a relative risk of 3.4 for stroke, death, and myocardial infarction compared with patients in the lowest quartile of baseline plaque area. Patients with progression (~45% of cases) had twice the risk of those with regression (35% of cases) or stable plaque (20% of cases). Plaque volume and plaque area are closely related (the sum of cross-sectional areas of all plaques in the carotid arteries, measured in longitudinal views of each plaque) is a strong predictor of stroke, death, and myocardial infarction. Therefore, it is expected that plaque volume will also be a strong predictor of outcomes; long-term studies will be needed to confirm that.

**Conclusions**

Measurement of 3D plaque volume represents a powerful tool for measuring effects of new therapies on atherosclerosis in small numbers of patients treated for only 3 to 6 months. This technology promises to be very useful in investigation of new antiatherosclerotic therapies.
Acknowledgments

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References

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