Ultrasound and Lipoproteins as Predictors of Lipid-Rich, Rupture-Prone Plaques in the Carotid Artery

Marie-Louise M. Grønholdt

Abstract—The aim of this review is to summarize present knowledge of the ultrasonic detection and determinants of carotid atherosclerosis with lipid-rich cores and to review the evidence that these measures of plaque type may predict cerebral events. With the use of high-resolution ultrasound B-mode imaging, carotid plaques evaluated as only weakly reflecting the ultrasound beam (echolucent) have been associated with a higher risk of neurological events than are plaques reflecting the ultrasound signal strongly (echorich). Histologically, these echolucent plaques have a higher content of lipid and hemorrhage than do echorich plaques, which contain more calcification and fibrous tissue. Findings in the coronary arteries indicate that a lipid-rich plaque with a thin, fibrous cap is more vulnerable, is more prone to rupture, and cause symptoms compared with fibrous plaques. A search for determinants in the blood for these vulnerable plaques suggests that low density lipoprotein (LDL) cholesterol is the best lipid predictor for the extent of atherosclerosis, whereas triglyceride-rich lipoproteins in particular seem to predict an echoluent plaque. Lowering of LDL cholesterol and triglyceride-rich lipoproteins in plasma is associated with reduced progression of coronary atherosclerosis and coronary events. LDL cholesterol reduction is also associated with a reduced stroke rate. These improvements in the prognosis are thought to be the result of a reduction in the lipid content of the plaques, making them more stable and resistant to rupture rather than an actual reduction in plaque volume and degree of stenosis. In conclusion, it appears that ultrasound B-mode imaging as well as lipoproteins presumably may predict dangerous and rupture-prone, lipid-rich plaques in the carotid arteries, thereby being potential diagnostic tools in the prevention of neurological events. (Arterioscler Thromb Vasc Biol. 1999;19:2-13.)

Key Words: atherosclerosis • carotid artery disease • ultrasound • lipoproteins • histology

Atherosclerosis due to accumulation of atherogenic lipoproteins in the carotid arterial wall may lead to cerebrovascular events, causing physical and mental disability and, in one third of these cases, even death. To prevent the possibly fatal course of atherosclerosis, it seems important to understand why this otherwise-benign disease suddenly develops into a life-threatening condition. The questions raised in this article are therefore the following: Which risk factor(s) in the blood determines the development of a vulnerable, unstable plaque? How do we identify these plaques, and what do they contain histologically? (Figure 1).

Ultrasound in Predicting Occurrence of Neurological Symptoms

Stroke is the third leading cause of death in Europe and the United States, requiring enormous resources to be spent on rehabilitation each year. Stroke is defined as a nontransient, acute neurological injury resulting from disruption of blood flow to cerebral tissue. The disruption of blood flow is thought to be a result of embolization arising from degenerative breakdown or thrombotic occlusion of complex plaques in the extracranial vessels,1–4 areas that are readily accessible to ultrasound imaging. The spectrum of clinical presentation is probably related to the size of the embolus, its composition, its propensity to disaggregate, and the state of the collateral circulation. Most strokes occur without warning. Only 5% to 15% of patients experience a transient ischemic attack (TIA) as a premonitory symptom of stroke.5 Because the primary purpose of noninvasive screening is to identify potentially treatable lesions in patients at increased risk of stroke and to treat these patients medically or surgically, it is important to consider all factors that may lead to a cerebrovascular event.

The European Carotid Surgery Trial6 and North American Symptomatic Carotid Endarterectomy Trial7 have shown the benefit of carotid endarterectomy in symptomatic patients with a high-grade stenosis (>70%). Despite the relatively higher risk associated with high-grade stenoses, evaluated in absolute numbers most patients experiencing neurological symptoms have stenoses of <50% in the ipsilateral carotid artery, according to a study by Langsfield et al8 (from 0% to 49% stenosis, 24 new symptoms versus 15 with 50% to 99% stenosis). As a key point in the natural history of carotid plaque development, another multicenter trial, the Asymptomatic Carotid Atherosclerosis Study (ACAS9), has shown...
Recent studies support the notion that not only the degree of stenosis but also the morphology of the carotid artery plaque, as evaluated with ultrasound B-mode imaging, and surface characteristics like ulceration may be of pathogenetic importance (Table 1).\textsuperscript{1,8,16-22} Concerning surface characteristics, ulceration is believed to be important because it causes exposure of the thrombogenic layers of the plaque, with the possibility of subsequent thrombus adhering to the plaque, leading to embolus formation and resultant neurological symptoms. Unfortunately, there is no standardized definition of ulceration. Ulceration can be classified from ultrasonic and angiographic findings or from macroscopic or microscopic pathology of the endarterectomy specimen. Ultrasonographically, an ulceration is an irregularity or break in the echolucent surface of the plaque. Moore et al\textsuperscript{23} defined ulcer size on angiograms as the multiplication of length and width of a

**Figure 1.** Predisposing factors and sequelae of unstable atherosclerotic plaques.

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Study Group</th>
<th>N (&lt;)/70% Stenosis</th>
<th>Design</th>
<th>Classification of Plaque</th>
<th>Symptoms</th>
<th>Analysis</th>
<th>Predictors</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson J.M.\textsuperscript{10}</td>
<td>1985</td>
<td>a + NLS</td>
<td>297</td>
<td>Both</td>
<td>Prospective</td>
<td>Soft, dense, calcified</td>
<td>TIA, stroke</td>
<td>Univariate</td>
<td>Echolucent (soft)</td>
</tr>
<tr>
<td>O’Holleran L.W.\textsuperscript{11}</td>
<td>1987</td>
<td>a + NLS</td>
<td>293</td>
<td>Both</td>
<td>Prospective</td>
<td>Soft, dense, calcified</td>
<td>TIA, stroke</td>
<td>Univariate</td>
<td>Echolucent (soft)</td>
</tr>
<tr>
<td>Matalanis\textsuperscript{1}</td>
<td>1988</td>
<td>a + s</td>
<td>381</td>
<td>Both</td>
<td>Cross-sectional</td>
<td>Gray-Weale\textsuperscript{23} (4 types)</td>
<td>AF, TIA, stroke</td>
<td>Univariate</td>
<td>Echolucent, heterogeneous</td>
</tr>
<tr>
<td>Leahy A.L.\textsuperscript{12}</td>
<td>1988</td>
<td>a + s</td>
<td>108</td>
<td>Both</td>
<td>Cross-sectional</td>
<td>Homo- or heterogeneous</td>
<td>AF, TIA, stroke</td>
<td>Univariate</td>
<td>Heterogeneous</td>
</tr>
<tr>
<td>Sterpetti A.V.\textsuperscript{13}</td>
<td>1988</td>
<td>a + s</td>
<td>214</td>
<td>Both</td>
<td>Prospective</td>
<td>Homo- or heterogeneous</td>
<td>TIA, stroke</td>
<td>Univariate</td>
<td>Heterogeneous</td>
</tr>
<tr>
<td>Steffen C.M.\textsuperscript{14}</td>
<td>1989</td>
<td>a + s</td>
<td>226</td>
<td>Both</td>
<td>Cross-sectional</td>
<td>Gray-Weale\textsuperscript{23} (4 types)</td>
<td>AF, TIA, stroke</td>
<td>Univariate</td>
<td>Echolucent</td>
</tr>
<tr>
<td>Langfield M\textsuperscript{1}</td>
<td>1989</td>
<td>a + s</td>
<td>419</td>
<td>Both</td>
<td>Prospective</td>
<td>Gray-Weale\textsuperscript{23} (4 types)</td>
<td>AF, TIA, stroke</td>
<td>Univariate</td>
<td>Echolucent, heterogeneous</td>
</tr>
<tr>
<td>Giannoni M.F.\textsuperscript{15}</td>
<td>1991</td>
<td>a? + s</td>
<td>75</td>
<td>&lt;45%</td>
<td>Prospective</td>
<td>Homo- or heterogeneous, regular, ulcerated</td>
<td>TIA, stroke</td>
<td>Univariate</td>
<td>Heterogeneous, ulceration</td>
</tr>
<tr>
<td>Polak J.F.\textsuperscript{16}</td>
<td>1993</td>
<td>a + s</td>
<td>5201</td>
<td>Both</td>
<td>Cross-sectional</td>
<td>Modified ECPSG\textsuperscript{36}</td>
<td>Stroke</td>
<td>Univariate</td>
<td>Isoechoic, echolucinct, ulceration</td>
</tr>
<tr>
<td>Bock R.W.\textsuperscript{17}</td>
<td>1993</td>
<td>a</td>
<td>242</td>
<td>Both</td>
<td>Prospective</td>
<td>Echolucent, echolucinct</td>
<td>AF, TIA, stroke</td>
<td>Univariate</td>
<td>Echolucent</td>
</tr>
<tr>
<td>Geroulakos G\textsuperscript{18}</td>
<td>1993</td>
<td>a + s</td>
<td>121</td>
<td>&gt;70%</td>
<td>Cross-sectional</td>
<td>Modified Gray-Weale\textsuperscript{23} (5 types)</td>
<td>AF, TIA, stroke</td>
<td>Univariate</td>
<td>Echolucent</td>
</tr>
<tr>
<td>Belcaro G\textsuperscript{19}</td>
<td>1993</td>
<td>a</td>
<td>300</td>
<td>&lt;60%</td>
<td>Prospective</td>
<td>Homo- or heterogeneous, complex</td>
<td>AF, TIA, stroke</td>
<td>Univariate</td>
<td>Complex, echolucinct, heterogeneous</td>
</tr>
<tr>
<td>Holdsworth R.J.\textsuperscript{20}</td>
<td>1995</td>
<td>a + s</td>
<td>4258</td>
<td>Both</td>
<td>Cross-sectional</td>
<td>Gray-Weale\textsuperscript{23} (4 types)</td>
<td>AF</td>
<td>Univariate</td>
<td>Echolucent, heterogeneous</td>
</tr>
<tr>
<td>Iannuzzi A\textsuperscript{21}</td>
<td>1995</td>
<td>s</td>
<td>536</td>
<td>Both</td>
<td>Cross-sectional</td>
<td>Modified ECPSG\textsuperscript{36} plaque motion</td>
<td>TIA</td>
<td>Univariate</td>
<td>Echolucent, plaque motion</td>
</tr>
<tr>
<td>Cave E.M.\textsuperscript{22}</td>
<td>1995</td>
<td>a + s</td>
<td>116</td>
<td>Both</td>
<td>Cross-sectional</td>
<td>Gray-Weale\textsuperscript{23} (4 types)</td>
<td>AF, TIA</td>
<td>Univariate</td>
<td>Echolucent</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Indicates asymptomatic; s, symptomatic patients in sample; NLS, nonlateralizing symptoms (dizziness, headache, diplopia); AF, amaurosis fugax; ECPSG, European Carotid Plaque Study Group; and IMT, carotid intima-media thickness. Comparisons were done within patient groups in all studies. A P is given for the different predictors of symptoms.
4 Predictors of Rupture-Prone Carotid Plaques

<table>
<thead>
<tr>
<th>Risk</th>
<th>Johnson\textsuperscript{10}</th>
<th>Gray-Weale\textsuperscript{23}</th>
<th>Reilly\textsuperscript{24}</th>
<th>ECPs\textsuperscript{35}</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk</td>
<td>Soft</td>
<td>Echolucent (thin, echorich cap)</td>
<td>Heterogeneous (mixed-level echoes)</td>
<td>Echolucent</td>
</tr>
<tr>
<td></td>
<td>Dominantly echolucent (small, echorich area)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dense</td>
<td>Dominantly echorich (&lt;25% echolucent)</td>
<td></td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low risk</td>
<td>Calcified</td>
<td>Echorich</td>
<td>Homogeneous (medium- or high-level echoes)</td>
<td>Echorich</td>
</tr>
</tbody>
</table>

TABLE 2. Classification of Plaque Morphology in Relation to Risk of Neurological Symptoms

crater (in millimeters), and these authors divided ulceration into 3 groups according to size. Macroscopically, Comerata et al\textsuperscript{26} defined ulcers according to crater width and a depth of 1 mm or more, whereas O’Donnell et al\textsuperscript{27} defined them as a definite surface irregularity with a punched-out characteristic. Seen microscopically, ulceration according to the definitions varies from loss of surface endothelium to deep, undermining depressions in the plaque.\textsuperscript{28,29} The correlation of histology to ulceration investigated with the use of ultrasound\textsuperscript{26,28-33} and angiography\textsuperscript{26,27} is, however, poor. The ability to detect ulceration is affected by the degree of stenosis. With the use of B-mode ultrasound, the sensitivity for identification of ulceration was 77% in plaques with 50% stenosis or less but only 41% in plaques of >50% stenosis (\(P=0.03\)).\textsuperscript{26} The sensitivity for arteriography was similar: 77% and 48%, respectively.\textsuperscript{26} Differing time intervals from the occurrence of symptoms to surgery may also lead to conflicting results, since re-endothelialization was reported by Lusby et al\textsuperscript{34} when this interval was >3 weeks. It may be that ulcers heal after neurological events and therefore cannot be found on ultrasound or angiography when performed some time after the occurrence of symptoms. Reviewing ulcers on 593 angiograms of medically treated patients, Eliasziw et al\textsuperscript{35} found a 2-year risk of nonfatal stroke or vascular death in 30% of the medical group with ulcers as opposed to 17% in the group without ulcers (\(P=0.005\)). These findings indicate that the presence of ulcers may be of prognostic relevance, but if one considers the fact that most patients undergoing surgery have severe lesions in which ulcers are not easily seen preoperatively, this parameter may be difficult to use as an indication for surgery.

Angiography merely yields information on the degree and location of the stenosis, whereas high-resolution B-mode imaging allows characterization of carotid plaques (Table 2) by echogenicity, defined as reflectance of the ultrasound signal. Echogenicity can be classified according to the criteria of Johnson et al\textsuperscript{10} from 1985 (calcified, dense, and soft) or alternatively, the criteria of Gray-Weale et al\textsuperscript{23} from 1988 describing 4 plaque types, from dominantly echolucent with a thin, echogenic cap to dominantly echogenic with small areas of echolucency, through 2 types of mixed echogenicity. Reilly et al\textsuperscript{24} introduced characterization of plaque structure into homogeneous, having uniform high- or medium-level echoes, and heterogeneous, having high-, medium-, and low-level echoes and containing areas with echogenicity similar to that of blood. Other groups use the criteria of the European Carotid Plaque Study Group\textsuperscript{36} from 1994: echorich, intermediate, and echolucent, combined with surface and structural characteristics.\textsuperscript{36,37} With the use of either of these sets of criteria or the modified Gray-Weale criteria (whereby a fifth plaque type includes those impossible to classify according to calcification or acoustic shadowing), the interobserver agreement has been found to be good (\(k=0.79\) and 0.61, respectively).\textsuperscript{18,37} To our knowledge, results of intraobserver studies on plaque morphology have not been published but are regarded as better than the results from the interobserver studies. Given the slow formation of atherosclerotic deposition, day-to-day variation seems negligible.

Most studies relating ultrasonic plaque morphology to clinical outcome agree that echolucent and heterogeneous as well as ulcerated plaques carry a higher risk of neurological symptoms compared with echorich and homogeneous plaques (Table 1).\textsuperscript{8,10,11,13,15,17,19} Langsfield et al\textsuperscript{11} studied 419 patients with asymptomatic plaques for 15 to 22 months and found the echolucent or heterogeneous plaques to be at increased risk of becoming symptomatic compared with dense, echorich plaques (\(P<0.02\)). These findings were reproduced by Belcaro et al.\textsuperscript{19} Following up on a group of both asymptomatic and symptomatic patients, Sterpetti et al\textsuperscript{13} found new neurological events occurring in 19 of 71 carotid arteries (27%) with heterogeneous plaques, whereas new events were only seen in 6 carotid arteries of 167 (4%) with homogeneous plaques (\(P<0.001\)). In prolongation of the work by Johnson et al,\textsuperscript{36} the study by O’Holleran et al\textsuperscript{11} followed up 293 patients for an average of 46 months. Over a 4-year period, all 42 patients with a soft lesion of >75% stenosis became symptomatic while only 60% of those with dense plaques became symptomatic (\(P<0.05\)). Confirming these results, Bock et al\textsuperscript{17} showed that echolucent plaques were associated with a 5.7% incidence of TIA and stroke, compared with only 2.4% for echorich plaques (\(P<0.0001\)). In a cross-sectional study Geroulakos et al\textsuperscript{18} found echolucent plaques to be associated with a higher incidence of brain infarcts on CT scans (\(P<0.02\)). As opposed to positive associations of plaque character to events found by the above-listed studies, Holdsworth et al\textsuperscript{30} in a cross-sectional study of 4258 patients only found amaurosis fugax to be associated with echolucent and heterogeneous plaques, whereas the degree of stenosis overall seemed more predictive of events (\(P<0.00001\)). They concluded that plaque morphology and degree of carotid stenosis are mutually dependent factors, whereas morphology does not add to the
sensitivity of stenosis in predicting the presence of symptoms. Fitzgerald and O’Farrell39 found heterogeneous structure ($P<0.01$) and irregular surface ($P<0.01$) to be associated with the development of subsequent events. Opposed to this, the degree of stenosis and size of a low-echo “pool” within the lesion were not associated with events. However, results are difficult to interpret, as they include, for example, myocardial infarction (MI) in the event group.

The main problem in the majority of these studies is that stroke and TIA were not separated as clinical end points. This seems an important goal for future trials, because a stroke is a condition to be prevented, being the most severe, disabling, and even life-threatening neurological event. Furthermore, these studies are mostly based on symptomatic clinical populations (Table 1), whereas it would be more important to evaluate only asymptomatic patients prospectively in a large sample to gain evidence for the significance of plaque composition. The results of the multicenter studies ACSRS (Asymptomatic Carotid Stenosis and Risk of Stroke Study) and ACST (Asymptomatic Carotid Surgery Trial) are therefore much awaited.40

Summarily, it appears that patients with an echolucent and heterogeneous plaque evaluated by ultrasound B-mode have a higher risk of developing neurological events than do patients with homogeneous, echorich plaques.

**Ultrasound in Predicting Histological Plaque Content**
Table 3 lists the results of studies investigating the histological content of carotid plaques. The echolucent plaques were associated with a high content of lipid and hemorrhage, whereas echorich plaques contained more calcification (causing acoustic shadowing) and fibrous tissue (collagen rich). Ultrasound could not reliably distinguish between lipid and hemorrhage in the plaque, and this is why some studies have combined these 2 constituents in the term soft tissue.36,46

Heterogeneous plaques were found to contain more intraplaque hemorrhage24,27,41 and calcification,36,37 categorizing them as complex, mature plaques. Homogeneous plaques, in contrast, consisted predominantly of dense fibrous tissue.24,33

To characterize B-mode images of plaques more objectively, digital image processing or videodensitometric analysis has been introduced.47–49 Using this method, El-Barghouty et al50 found the content of soft tissue (lipid and hemorrhage) in the plaque to be associated with a low gray-scale median value of the plaque. Conversely, a high fibrous tissue content was associated with a high gray-scale value.

Although all of the findings listed seem consistent, there are many different opinions in the literature: eg, differences exist in the subjective evaluation of B-mode images, techniques of pathological tissue characterization (examined either microscopically or macroscopically), and definitions of specific histological features. This lack of standardization also includes histological sampling errors when only semiquantitative or nonquantitative data were collected and different amounts or parts of the plaque were investigated. In a review by Reilly51 results of the studies using sonographic imaging to determine carotid plaque histology were summarized to yield an accuracy for ultrasonography of 81% (443 of 549), a sensitivity of 90% (287 of 319), and a specificity of 68% (156 of 230). However, in contrast to earlier findings from nonquantitative and semiquantitative studies, recent quantitative studies agree that intraplaque hemorrhage occupies only a very small area of the plaque (≈1%).36,37,45,51–54 Bassiouny et al52 identified hemorrhage in 68% of plaques, comprising 2.5% of the plaque area on average. In the study by Leen et al53 hemorrhage occupied <1% in 65% of the cases and was present in 68% of plaques, whereas Grønholdt et al54 found hemorrhage occupying 0.2% (0% to 4%) of the plaque area on average. Therefore, ultrasound may not reliably identify this small constituent in a plaque, making use of the term soft tissue reasonable.

In summary, correlation of subjective ultrasonic evaluation with histological findings showed that echoluent plaques were associated with a higher content of lipid and hemor-

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**Table 3. Appearance on Ultrasound of Different Histological Constituents of Carotid Artery Plaques**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Design</th>
<th>N</th>
<th>Echolucent Reflectance</th>
<th>Echorich Reflectance</th>
<th>Heterogeneous Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reilly L.M.24</td>
<td>1983</td>
<td>Nonquantitative</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>O’Donnell T.F.27</td>
<td>1984</td>
<td>Nonquantitative</td>
<td>54</td>
<td>Lipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratiff D.A.30</td>
<td>1985</td>
<td>Semiquantitative</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bluth E.I.41</td>
<td>1986</td>
<td>Nonquantitative</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gray-Weale A.C.23</td>
<td>1988</td>
<td>Nonquantitative</td>
<td>220</td>
<td>Hemorrhage or ulceration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spagnoli L.G.43</td>
<td>1989</td>
<td>Quantitative</td>
<td>43</td>
<td>Hemorrhage, lipid*</td>
<td>Fibrous tissue</td>
<td></td>
</tr>
<tr>
<td>Widder B44</td>
<td>1990</td>
<td>Semiquantitative</td>
<td>169</td>
<td>Hemorrhage, lipid*</td>
<td>Fibrous tissue</td>
<td></td>
</tr>
<tr>
<td>Feeley T.M.46</td>
<td>1991</td>
<td>Quantitative</td>
<td>52</td>
<td>Hemorrhage, lipid†</td>
<td>Fibrous tissue</td>
<td></td>
</tr>
<tr>
<td>ECPSG</td>
<td>1995</td>
<td>Quantitative</td>
<td>270</td>
<td>Hemorrhage, lipid†</td>
<td>Fibrous tissue, calcification</td>
<td></td>
</tr>
<tr>
<td>Granholdt M.L.M.37</td>
<td>1997</td>
<td>Quantitative</td>
<td>78</td>
<td>Lipid</td>
<td>Fibrous tissue, calcification</td>
<td></td>
</tr>
</tbody>
</table>

* Atheromatous debris.
† Soft tissue (lipid and hemorrhage combined). Nonquantitative indicates data only on the presence or absence of histological constituents are given; semiquantitative, < or > 50% of plaque area is occupied by a given histological constituent; quantitative, quantitative amount of the histological constituent is given in a percentage of plaque area/volume.

All study designs were cross-sectional.
rhage, whereas echorich plaques contained more calcification and fibrous tissue (Table 3).

### Histological Plaque Constituents Associated With Neurological Symptoms

Controversy also exists on plaque constituents and their prognostic value for developing neurological symptoms (Table 4). Unfortunately, studies concerning plaque constituents as prognostic indicators have used different definitions of symptoms as well as different time intervals from neurological event to surgery, if documented at all. Plaque remodeling is presumed to occur after an event, and this process could have a great influence on the interpretation of results when the time interval before surgery, normally 1 to 3 months, is taken into account. The essence of early articles published on this topic revealed that the finding of intraplaque hemorrhage was associated with a higher rate of symptoms. All but one study did not use quantitative histological methods and apparently did not use “blinded” assessment of the data, whereas more recent blinded studies indicate that this association does not exist. However, problems of interpretation of these studies exist, especially where differences in the definition of hemorrhage are concerned. Moreover, intraplaque hemorrhage seems to be more common in plaques causing high-grade stenosis. Because most centers operate only on asymptomatic patients in the presence of high-grade stenosis, the asymptomatic group in a study often includes more high-grade lesions than does the symptomatic group, and this factor could affect the results. Lusby et al discovered that the age of the hemorrhage was correlated with time elapsed since the neurological event. They described the stages as (1) operative; (2) acute (<1 week), with little inflammatory cell infiltrate; (3) recent (1 to 6 weeks old), characterized by hemosiderin-containing macrophages; and finally (4) remote (>6 weeks), characterized by amorphous material with cholesterol crystals but without an inflammatory reaction. Erroneously, the lattermost stage has also been recognized as hemorrhage by other authors, resulting in the reporting of a high content of intraplaque hemorrhage, leading to incorrect results and

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Design</th>
<th>Classification</th>
<th>N</th>
<th>Predictors</th>
<th>Symptoms</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Lusby R.J.</td>
<td>1982</td>
<td>Nonquantitative</td>
<td>Hemorrhage at 4 age stages</td>
<td>69</td>
<td>Acute, recent hemorrhage</td>
<td>AF, TIA, stroke, nonfocal symptoms</td>
<td>Not given</td>
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<tr>
<td>Persson A.V.</td>
<td>1983</td>
<td>Nonquantitative</td>
<td>Hemorrhage, ulceration</td>
<td>54</td>
<td>Macroscopic hemorrhage</td>
<td>AF, TIA, stroke, nonfocal symptoms</td>
<td>Not given</td>
</tr>
<tr>
<td>Imparato A.M.</td>
<td>1983</td>
<td>Semiquantitative</td>
<td>Hemorrhage, thrombus, ulceration</td>
<td>280</td>
<td>Macroscopic hemorrhage</td>
<td>AF, TIA, stroke</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Reilly L.M.</td>
<td>1983</td>
<td>Nonquantitative</td>
<td>Hemorrhage, lipid, cholesterol, loose stroma, fibrin, ulceration</td>
<td>45</td>
<td>Hemorrhage, ulceration</td>
<td>TIA, stroke</td>
<td>Not given</td>
</tr>
<tr>
<td>Ammar A.D.</td>
<td>1984</td>
<td>Nonquantitative</td>
<td>Hemorrhage at 4 age stages</td>
<td>85</td>
<td>None significant</td>
<td>AF, TIA, stroke</td>
<td>None significant</td>
</tr>
<tr>
<td>Ricotta J.J.</td>
<td>1986</td>
<td>Nonquantitative</td>
<td>Hemorrhage, ulceration, thrombus</td>
<td>84</td>
<td>None significant</td>
<td>TIA, stroke</td>
<td>None significant</td>
</tr>
<tr>
<td>Fryer J.A.</td>
<td>1987</td>
<td>Semiquantitative</td>
<td>Hemorrhage, recent or old, ulceration, neovascularity, inflammation</td>
<td>91</td>
<td>Recent hemorrhage &gt;50% of plaque area</td>
<td>Not defined</td>
<td>Not given</td>
</tr>
<tr>
<td>Lennihan L</td>
<td>1987</td>
<td>Semiquantitative</td>
<td>Hematoma</td>
<td>200</td>
<td>None, but skewed patient selection</td>
<td>AF, TIA, stroke</td>
<td>None significant</td>
</tr>
<tr>
<td>Feeley T.M.</td>
<td>1991</td>
<td>Quantitative</td>
<td>Cholesterol, fibrous, thrombus, hemorrhage, calcification, collagen, necrosis</td>
<td>52</td>
<td>Amorphous material with cholesterol</td>
<td>TIA, stroke</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Avril G</td>
<td>1991</td>
<td>Quantitative</td>
<td>Soft (atherosclerotic debris, hemorrhage) hard (collagen, calcification)</td>
<td>169</td>
<td>Hemorrhage, atherosclerotic debris, macroscopic ulceration</td>
<td>TIA, stroke</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>van Damme H</td>
<td>1992</td>
<td>Nonquantitative</td>
<td>Simple, fibrous, complex hemorrhage, ulceration, thrombus</td>
<td>125</td>
<td>Fresh thrombus</td>
<td>AF, TIA, stroke, nonfocal symptoms</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Seeger J.M.</td>
<td>1995</td>
<td>Quantitative</td>
<td>Lipid, collagen, calcification</td>
<td>74</td>
<td>Lipid-rich plaques</td>
<td>TIA, stroke</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carr S</td>
<td>1996</td>
<td>Semiquantitative</td>
<td>Fibrous, necrotic, calcification, hemorrhage, thinning, rupture, foam cell infiltrate of fibrous cap</td>
<td>44</td>
<td>Thinning, rupture, foam cell infiltrate of fibrous cap</td>
<td>AF, TIA, stroke</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Hatsukami T.S.</td>
<td>1997</td>
<td>Quantitative</td>
<td>Hemorrhage, calcification, fibrous, lipid, necrotic core</td>
<td>43</td>
<td>None significant (few patients)</td>
<td>AF, TIA, stroke</td>
<td>None significant</td>
</tr>
</tbody>
</table>

All studies were designed cross-sectionally. Quantitative indicates amount of histological constituent given in a percentage of plaque area/volume; nonquantitative, only data on presence/absence of histological constituents given; semiquantitative, < or > 50% of plaque area occupied by a given histological constituent. In all studies histological characteristics were related to previous neurological events: AF, amaurosis fugax. A P value is given for the different predictors of symptoms.
conclusions. In contrast, newer studies suggest that the atheromatous material should not be recognized as intraplaque hemorrhage but rather as lipid or necrosis in the plaque.36,37,45,51 Furthermore, 4 studies did not confirm any correlation between age of the plaque hemorrhage and symptoms.24,45,52,59

Thrombus was found in many studies, though not always present in plaques from patients with recent symptoms.63,65,66 This constituent is thought to be associated with the theory of plaque disruption, allowing the thrombogenic lipid core to communicate with the arterial lumen. Its presence may be intermittent, reflecting the dynamic process of rupture, thrombus formation, healing, and remodeling of the plaque.2–4

Recent work with quantified data, especially concerning the relation of atheromatous material (lipid) in the plaque to neurological symptoms, has been reported by Avril et al,60 Seeger et al,61 Feeley et al,62 Widder et al,63 and Hatsukami et al64 (Table 4). Only Hatsukami et al64 did not find any correlation between symptoms and the measured quantitative pathologic constituents. Feeley et al62 showed a significant correlation between plaques containing amorphous material mixed with cholesterol and neurological symptoms ($P = 0.017$). Interestingly, the amount of fibrous tissue found in asymptomatic patients was higher than in patients experiencing increasing numbers of ischemic events.45

Also associated with progression of atherosclerotic disease and neurological symptoms are other plaque features like foam cell infiltration in the fibrous cap; thinning or rupture of the fibrous cap; soft, lipid-rich cores beneath this cap; cholesterol crystals; and necrosis.55,52,53,59,63 Carr et al65 recently found patients with symptomatic plaques ($n = 19$) to have more frequent plaque rupture, fibrous cap thinning, and fibrous cap foam cell infiltration when compared with asymptomatic ($n = 25$) plaques ($P < 0.006$). Bearing in mind that this is a nonquantitative study with relatively few patients, no differences existed for constituents like plaque hemorrhage, presence of a necrotic core, luminal thrombus, smooth muscle cell infiltration, eccentric shape, or plaque type (fibrous, necrotic, or calcified). Leen et al66 found that an eosinophilic amorphous material mixed with cholesterol was the predominant nonfibrous component in symptomatic plaques. Roof rupture (break in the fibrous cap of a nonfibrous plaque) was seen in 66% of these plaques, but this study lacks statistical information.

The above-mentioned findings support the plaque rupture theories mainly based on pathological findings in the coronary arteries.2–4 Theories may not be far from those based on findings in the carotid arteries, because atherogenesis of coronary and cerebrovascular vessels is closely related.67,68 Rupture-prone coronary plaques in particular are thought to consist of a thin, fibrous cap covering an atheromatous, lipid-rich core (cholesterol and its esters).2–4 This plaque may be triggered to rupture by intrinsic stresses (ie, the continued accumulation of lipoproteins from plasma with or without uptake in macrophage foam cells) or extrinsic stresses to rupture. A plaque rupture may lead to intraplaque hemorrhage, formation of superimposed thrombosis, embolism, and subsequent neurological symptoms. Leen et al61 concluded that cholesterol deposition precedes intraplaque hemorrhage rather than the reverse, indicating that intraplaque hemorrhage is a sign of previous plaque rupture rather than a pathogenetic factor itself. A growing lipid-rich core due to lipoprotein accumulation itself makes the plaque vulnerable to rupture, which explains the findings of Grønholdt et al,7 wherein the presence and amount of hemorrhage in the plaque were positively associated with lipid and negatively associated with fibrous tissue content of the plaque.

An alternative pathogenesis to plaque rupture/intraplaque hemorrhage is bleeding and/or transudation into plaques from thin-walled new vessels originating from the vasa vasorum and frequently found at the plaque base.69,70 This bleeding could theoretically increase intraplaque pressure, with resultant cap rupture from the inside.71 However, it is difficult to imagine this phenomenon’s happening against a much higher luminal pressure and with the small amount of hemorrhage found in carotid plaques.52,53 Leen et al72 moreover found that most blood vessels located at the base of plaques were unrelated to hemorrhage in the plaque.

Intrinsic stresses also include ongoing inflammation with degradation of the extracellular matrix, thereby weakening the fibrous cap. The cells involved in inflammation are activated macrophages and/or mast cells that by either phagocytosis or secretion of proteolytic enzymes degrade collagen, thereby thinning and destabilizing the fibrous cap. Smooth muscle cells, on the contrary, are thought to stabilize the plaque by producing collagen.75,76

The exact pathogenesis of calcification in a plaque is still unknown. The amount of calcification increases in plaques with age and the overall plaque burden, but not with the degree of stenosis in coronary arteries.77–79 It is interesting that calcium seems to stabilize the plaque against disruption and thrombosis, as this element is found less frequently in culprit lesions responsible for unstable angina77 and MI.79 This explains the findings of Grønholdt et al,7 wherein the amount of calcification in carotid plaques was found to increase with elapsed time since symptoms occurred. On the contrary, in a multicenter study, a higher content of soft tissue (lipid and hemorrhage) was seen in plaques from patients with recent symptoms, whereas no difference was found in the amount of calcification.56 Regarding the carotid arteries, great controversies exist on the influence of calcification on the risk of neurological events. Some authors believe that calcification of a plaque decreases the risk of subsequent events,10 whereas others19 believe calcification leads to increased risk. Some investigators18 ignore plaques with acoustic shadowing, and no evidence exists that shadowing is actually caused by calcification. Not even the few quantitative histological studies36,37,43 concerning evaluation of calcification are able to prove that the detected calcification is actually producing an echogenic reflectance or acoustic shadowing on ultrasound.

Extrinsic stresses on a plaque are, eg, hemodynamic forces (ie, shear stress, a sudden increase in blood flow, blood pressure, and pulse rate) acting on the weak shoulders of the cap. The carotid plaque is typically situated in the bifurcation of the artery, where a mismatch in flow impedance, abnormal wall tension, and turbulent flow predispose to further atherogenesis and eventually to plaque rupture at this location.
According to the equation by Bernoulli, a flow-restricting stenosis causes a decrease in pressure but a proportional increase in velocity in the stenotic segment. The resulting difference in pressure across the lesion together with the high proximal pressure produces an unroofing force downstream that can cause acute rupture of the plaque, embolization of its contents, or progression to thrombus formation or thrombotic occlusion. Superimposed thrombosis can also build on an intact plaque if hyperthrombogenicity exists because of platelet activation, hypercoagulability, and/or impaired fibrinolysis.29

In summary, the most dangerous, rupture-prone plaques causing neurological events are thought to consist of an atheromatous, lipid-rich core covered by a thin, fibrous cap.

Predictive Value of Plasma Lipoproteins
Plaque vulnerability is assumed to increase with accumulation of lipoproteins derived from the vessel lumen, either stored extracellularly and thereby increasing the lipid-rich core, or phagocyted by macrophages, typically situated in the shoulders of the fibrous cap.80,81 Therefore, the plasma concentration of lipoproteins is of importance in the development of atherosclerosis.82–84 Whereas the independent association of high levels of LDL cholesterol and low levels of HDL cholesterol to coronary atherosclerosis and disease has long been known,85 it is still debated whether they also play a similar role in cerebrovascular atherosclerosis and infarctions. The different pathogenesis underlying the 2 diseases probably contributes to this controversy: whereas overlying thrombosis is almost always the reason for MI’s becoming occlusion, Superimposed thrombosis can also build on an intact plaque if hyperthrombogenicity exists because of platelet activation, hypercoagulability, and/or impaired fibrinolysis.80

In summary, the most dangerous, rupture-prone plaques causing neurological events are thought to consist of an atheromatous, lipid-rich core covered by a thin, fibrous cap.

Plasma Lipoproteins and Carotid Intima-Media Thickness
Since the review,87,88 were published, a number of studies have used ultrasound B-mode to measure the wall thickness of the extracranial part of the carotid arteries (intima-media thickness, or IMT) as an indicator of atherosclerotic diseases.89,90 There is evidence that an increase in IMT is linked to different risk factors for atherosclerosis, such as age, systolic blood pressure, a history of coronary heart disease, diabetes mellitus, levels of LDL cholesterol, and smoking habits.91,92 and patients with familial hypercholesterolemia have also been shown to have increased IMT.97,98 IMT has in addition been recognized as a useful measure in evaluating regression and progression of atherosclerosis in clinical trials. Unfortunately, however, the carotid IMT methodology varies from study to study according to the number of measurements in the different arterial segments. The interobserver and intraobserver reliability (coefficients of variation) of performing repeated measurements of IMT has been tested by Salonen and Salonen94 and found to be 10.5% and 8.3%, respectively.

In 1 case-control study, IMT was correlated positively with plasma cholesterol (r=0.59), LDL cholesterol (r=0.60), and plasma triglycerides (r=0.27).95 In another study in women, premenopausal values of triglycerides and postmenopausal values of LDL cholesterol were independently positively related to average IMT.99 In a third study, multiple regression analysis showed that age directly and HDL cholesterol indirectly were related to IMT (P<0.05).100 Furthermore, in a Japanese population study, IMT was related directly to age, systolic blood pressure, smoking, and total cholesterol both in men and women and indirectly to HDL cholesterol in men only.101 IMT was positively correlated with lipoprotein(a) [Lp(a)] and triglycerides levels but negatively with HDL cholesterol in a prospective study of 59 hypercholesterolemic men without symptomatic cerebrovascular disease.102 Baldassare et al103 reported that IMT in the common carotid artery is higher in hypercholesterolemic patients with plasma Lp(a) levels >30 mg/dL than in those with lower levels (P<0.01). IMT was correlated also directly and independently with plasma levels of Lp(a).103

In summary, recent studies found ischemic stroke correlated positively with LDL cholesterol and negatively with HDL cholesterol levels in plasma.

Influence of Plasma Cholesterol Lowering on Stroke Risk
The lipid composition influences the consistency of a plaque, such that liquid cholesterol esters soften whereas crystalline cholesterol stiffens the atheromatous "gruel."104 Lipid-
TABLE 5. Studies on Plasma Lipoproteins as Predictors of Carotid Plaque Characteristics Evaluated With B-Mode Ultrasound

<table>
<thead>
<tr>
<th>Ultrasound Measures of Atherosclerosis</th>
<th>First Author Year</th>
<th>Design</th>
<th>N</th>
<th>Plasma Lipoprotein Predictors Measured at Hours</th>
<th>Peak Triglycerides Found P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative IMT</td>
<td>Ryu J.E.,125</td>
<td>1992</td>
<td>Cross-sectional</td>
<td>48</td>
<td>TG Po 1 through 8 Yes</td>
</tr>
<tr>
<td></td>
<td>Sharett A.R. (ARIC Study),126</td>
<td>1995</td>
<td>Case-control</td>
<td>602</td>
<td>TG Po 3½, 8  No</td>
</tr>
<tr>
<td>Progression of IMT</td>
<td>Hodis H.N. (MARS Study),127</td>
<td>1997</td>
<td>Prospective</td>
<td>188</td>
<td>IDL TG Only fasting values  No</td>
</tr>
<tr>
<td>Subjectively evaluated echogenicity</td>
<td>Grønholdt M.L.M.,128</td>
<td>1996</td>
<td>Cross-sectional</td>
<td>85</td>
<td>TG Fa+Po, VLDL TG Fa+Po 1 through 4 No</td>
</tr>
<tr>
<td>Computer-estimated echogenicity</td>
<td>Granholm M.L.M.,129</td>
<td>1998</td>
<td>Cross-sectional</td>
<td>137</td>
<td>TG Fa+Po, IDL TG Fa+Po Po, VLDL TG Fa+Po 1 through 4 No</td>
</tr>
</tbody>
</table>

TG indicates triglycerides; Po, postprandial values; and Fa, fasting values.

lowering therapy (reducing plasma cholesterol to less than \( \approx 150 \text{ mL/dL} \)) is expected to influence the relative amounts of these 2 lipid components by depleting the cholesterol esters and matrix-degenerating macrophages and resulting in a stiffer and more stable plaque.\(^{73,106}\) but not necessarily by decreasing the volume of the plaque. This may be why an effect of lipid-lowering therapy on a lower incidence of MI\(^{107,108}\) and stroke\(^{109-111}\) can be seen without a major change in plaque size or volume (ie, degree of stenosis).\(^{104,106,112}\) Lowering of plasma and LDL cholesterol levels has been shown to lead to reduced progression or even regression of atherosclerotic disease in coronary,\(^{113}\) femoral,\(^{114}\) and carotid arteries (IMT)\(^{115-118}\) and to reduced incidence of MI,\(^{114}\) cerebrovascular events,\(^{109-111}\) and even death.\(^{111}\) There are several reasons why lipid-lowering therapy has not shown a pronounced benefit to stroke patients until recently. First, the number of stroke incidences in the studies designed for ischemic heart disease (middle-aged men at low risk for stroke) was small, and consequently the power to detect an effect on stroke was limited. Second, the distinction between hemorrhagic and ischemic stroke was not made, which could confute the results. Third, cholesterol seems to be a less potent risk factor for ischemic stroke than for ischemic heart disease,\(^{92}\) and therefore large reductions in cholesterol or higher numbers of patient-years of observations would be required to demonstrate benefit.\(^{98}\)

Triglycerides and Stroke Risk

Controversies still exist on the relation of triglycerides to cerebrovascular disease. While some studies did not find any association between fasting levels of triglycerides and stroke,\(^{91,109,120}\) others found a positive association to total stroke\(^{121}\) or ischemic stroke.\(^{91,122}\) Like LDL, triglyceride-rich lipoproteins can transfer from plasma into the arterial intima,\(^{85}\) where such particles appear to be retained selectively.\(^{85,123}\) In contrast to LDL, which requires previous oxidation, triglyceride-rich lipoproteins can be taken up directly by macrophages to produce lipid-rich foam cells.\(^{125}\) According to Zilversmit\(^{83}\) accumulation of triglyceride-rich dietary particles in some individuals, due to reduced clearance, results in a prolonged exposure of the endothelial surface, thereby promoting the formation of atherosclerosis. Accumulating evidence suggest that postprandial triglyceride-rich lipoproteins and their remnants as well as reduced levels of HDL cholesterol are independent predictors of carotid IMT,\(^{125-127}\) carotid plaque echolucency\(^{128,129}\) (Table 5), and coronary artery disease.\(^{67,130-133}\) Three intervention trials have found a beneficial effect associated with reduction of triglyceride-rich lipoprotein in plasma, leading to a reduction in the progression of coronary atherosclerosis\(^{134}\) and coronary events,\(^{135,136}\) whereas no beneficial influence on carotid atherosclerosis and cerebral events has been demonstrated. In conclusion therefore, more evidence on the role of triglycerides in cerebrovascular disease and on the risk of stroke is needed.

Conclusion and Perspectives

Both echolucency on ultrasound B-mode imaging and triglyceride-rich lipoprotein levels in plasma appear to predict a lipid-rich plaque in the carotid artery. These plaques may be particularly rupture prone, predicting neurological events. Studies on carotid and coronary endarterectomy specimens indicate the existence of a dynamic process of rupture, thrombus formation, healing, and remodeling of the plaque. A plaque from a symptomatic patient may not show any signs of plaque rupture if the plaque has healed or evolved since the debut of symptoms. Examination of carotid and coronary specimens may give clues to the mechanisms of plaque disruption but cannot entirely explain the above-mentioned dynamic process.\(^{105,137-142}\) The shoulders of a plaque are often heavily infiltrated with activated-macrophage foam cells, indicating ongoing inflammation. Future studies on the inflammatory cell infiltrate in plaques may give more information on the possible role of the foam cell, smooth muscle cells, and other components in plaque rupture.\(^{141-145}\) The

Figure 2. Relationships between ultrasound, histologic, and clinical characteristics of unstable atheromatous plaques.
subjective evaluation of plaque morphology on B-mode ultrasound should be complemented with or substituted by objective evaluation such as videodensitometric analysis. This method is a commercially available, relatively inexpensive, and investigator-independent solution. More studies on the natural history of carotid artery plaques are needed to predict more reliably which plaque types or features are the most dangerous (Figure 2). Earlier identification by noninvasive imaging techniques, screening for inflammatory markers, or measuring plasma lipoprotein levels in the fasting and postprandial state in plasma may allow us to select asymptomatic patients for endarterectomy before they experience a disabling or fatal stroke.

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References


42. Deleted in proof.


Predictors of Rupture-Prone Carotid Plaques


106. Deletred in proof.


116. Deleted in proof.


22. 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.


Ultrasound and Lipoproteins as Predictors of Lipid-Rich, Rupture-Prone Plaques in the Carotid Artery
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