Detection of Carotid Artery Stenosis by In Vivo Duplex Ultrasound Correlation With Planimetric Measurements of the Corresponding Postmortem Specimens

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Background and Purpose—The correct detection and quantification of carotid artery disease are of decisive impact on patient prognosis and adequate treatment. In this study, we evaluated the ability of ultrasonography to detect and to grade carotid artery stenosis through a comparison of the in vivo ultrasound findings with the planimetric analysis of the corresponding postmortem specimens.

Methods—Shortly before their death, 59 critically ill neurological patients (mean age, 70 years) were prospectively examined by extracranial and intracranial Doppler sonography and color-coded duplex ultrasound. Carotid stenosis was classified by hemodynamic and morphological ultrasound criteria. Carotid specimens were removed in toto during autopsy. Under standardized conditions, specimens were redistended, sectioned, and histologically processed. Computed planimetric measurements of the arteries were carried out and compared with the ultrasound findings. Correlation of the ultrasound and postmortem planimetric findings was available in 93 carotid bifurcations.

Results—Through both techniques, 46 carotid arteries were found to be normal. Steno-occlusive carotid lesions ranged from 8.5% to 100% lumen reduction. Overall, \( r = 0.96 \) and adjusted \( R^2 = 0.90 \). For the steno-occlusive carotid lesions, \( r = 0.91 \).

Conclusions—Extracranial and intracranial Doppler and color-coded duplex ultrasound permits reliable detection and quantification of carotid artery stenoses and occlusions even under difficult examination conditions in critically ill patients. (Stroke. 2002;33:2402-2407.)

Key Words: carotid arteries ▪ diagnosis ▪ pathology ▪ ultrasonography

The results of 2 recently published, prospective, multicenter trials on the benefit of carotid endarterectomy, the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and European Carotid Surgery Trial (ECST), showed conclusively the efficacy of carotid endarterectomy in patients with symptomatic high-grade internal carotid artery stenosis.\(^1\,^2\) In these trials, catheter angiography was used to determine carotid artery stenosis, although this invasive technique carries a considerable intrinsic 0.5% to 4% risk of a transient or permanent neurological deficit.\(^3\,^4\)

Carotid ultrasound has been used worldwide as a noninvasive diagnostic test for evaluating extracranial carotid artery disease. Besides its noninvasiveness, ultrasound is cost-effective and easy to perform (bedside technique). In the past, numerous articles confirmed the reliability of carotid ultrasonography in carotid artery disease.\(^5\,^9\) particularly with respect to color-coded duplex ultrasound combining the techniques of high-resolution gray-scale B-mode pictures, color flow imaging, and frequency analysis of the Doppler spectrum. This battery of ultrasound information allows carotid artery stenosis to be quantified. In most previous articles, the ability of carotid ultrasound to predict carotid artery stenosis was validated by angiography, the presumed "gold standard." It is well known, however, that angiography itself is limited in its precision because of the restricted number of imaging planes (mostly 2) and because the frequent clinical practice of eyeballing the images leads to investigator dependency similar to ultrasound.

In this study, we evaluated the ability of extracranial and intracranial ultrasound to detect and to grade carotid artery stenosis by means of the true gold standard, the anatomic specimens of the affected arteries themselves. We compared...
arteries were assessed by transcranial color-coded duplex sonography (8-MHz probe, Multidop X, DWL). The intracranial artery stenosis measurement was possible on 93 carotid arteries. These arteries came from 59 patients, 37 men and 22 women whose mean age was 69.2±13.4 years (range, 37 to 95 years). The median interval between the patients’ ultrasound examinations and their deaths was 3 days (mean, 10.1 days). These patients’ reasons for admission to hospital were ischemic stroke (n = 31), intracranial hemorrhage (n = 25), brain tumor (n = 1), and a deep unclear coma of unknown origin (n = 1). One patient was found to suffer from colon cancer with hepatic metastasis. The presence of increased intracranial pressure was estimated from the initial (and follow-up if available) CT scans.

Patients and Methods

Patients

One hundred eighteen critically ill patients from the Neurological Department of University Medical School of Debrecen (Hungary) were prospectively examined by Doppler and B-mode sonography of the extracranial and intracranial brain-supplying arteries. Eighty-two patients died within a few days or weeks. Of the 82, carotid specimens could be obtained from 67 (118 carotid specimen). In the remaining patients, the carotids were severely damaged or lost during autopsy. Another 25 specimens were damaged during macroscopic workup or histologic processing, so a reliable planimetric measurement was possible on 93 carotid arteries. These arteries came from 59 patients, 37 men and 22 women whose mean age was 69.2±13.4 years (range, 37 to 95 years). The median interval between the patients’ ultrasound examinations and their deaths was 3 days (mean, 10.1 days). These patients’ reasons for admission to hospital were ischemic stroke (n = 31), intracranial hemorrhage (n = 25), brain tumor (n = 1), and a deep unclear coma of unknown origin (n = 1). One patient was found to suffer from colon cancer with hepatic metastasis. The presence of increased intracranial pressure was estimated from the initial (and follow-up if available) CT scans.

Ultrasound

In all patients, the neck arteries were investigated by color duplex ultrasound (7.5-MHz linear transducer, SONOS 2000, Hewlett-Packard), and the periorbital arteries were examined by continuous-wave Doppler (8-MHz probe, Multidop X, DWL). The intracranial arteries were assessed by transcranial color-coded duplex sonography with a dual-frequency, 2.0/2.5-MHz, 90° sector imaging transducer (HP SONOS 2000, Hewlett-Packard) or with a pulsed-wave, 2-MHz probe (Multidop X, DWL). The extracranial duplex examination included longitudinal and axial sections of the common carotid artery (CCA) and internal carotid artery (ICA) and was recorded on videotape. The angle-adjusted peak systolic and end-diastolic flow velocities in the CCA, in the jet of the stenosis, and in the most distal part of the ICA obtainable by duplex imaging were recorded. The degree of stenosis was measured by lumen diameter reduction in the longitudinal projection and by area reduction in the axial projection visible on the B-mode images. The peak systolic velocity of ≥120 cm/s was the threshold for the diagnosis of a stenosis graded ≥50%. Beyond this threshold, higher-degree stenoses were graded in categories of 5%, taking into account (1) the peak systolic velocity in the jet of the stenosis, (2) the peak systolic velocity in the most distal poststenotic ICA, (3) intrastenotic and poststenotic turbulence in the ICA (spectral broadening), (4) direction of the ophthalmic artery flow, (5) the presence of collateral flow via communicating arteries of the circle of Willis, (6) and the absolute asymmetry in flow velocity and pulsatility measured in the CCA and middle cerebral artery.10,11 In addition, continuous-wave Doppler measurements were carried out at the stenotic site. Subfocal ICA stenosis with a variable signal (eg, reduced peak systolic velocity without turbulence or venouslike flow) was defined as 97.5% stenosis. In the case of the indirect, ie, hemodynamic, criteria (fourth or fifth criteria described above), the stenosis was classified as ≥80%. Occlusion was diagnosed in the complete absence of detectable flow in and above the stenosis in conjunction with the above indirect hemodynamic criteria.12 If a contralateral stenotic or occlusive ICA lesion of ≥80% lumen narrowing was present in conjunction with collateral cross flow via the anterior communicating artery, intrastenotic peak systolic flow velocity of the ipsilateral carotid stenosis was correlated with area reduction and lumen diameter reduction visible on B mode. If these B-mode results for lumen narrowing and hemodynamic findings (ie, peak systolic flow velocity) of the ipsilateral carotid stenosis were inconsistent, local area reduction and lumen diameter reduction measured by B-mode were preferred for determining carotid stenosis to avoid overestimation of the ipsilateral carotid stenosis.

In low-grade ICA stenoses of <50% lumen narrowing, the intrastenotic area reduction measured by B mode was given preference over Doppler ultrasound parameters for comparison with the postmortem findings. However, if plaque calcification did not permit clear-cut axial visualization of the vessel walls, the lumen diameter reduction in the longitudinal projection measured by B mode was used for comparison with the pathoanatomic findings.

Stenoses with >50% lumen reduction were classified according to the above criteria. All ultrasound studies were performed by the same investigator (G.S.-A.).

Macropathological Examination of the Carotid Specimen

At autopsy, the CCA, ICA, and external carotid artery (ECA) were excised en bloc. The time between death and autopsy ranged from 1 to 110 hours (mean, 23.8 hours; median, 14 hours). ECA branches were ligated, and the ends of the 3 arterial segments (ie, CCA, ICA, and ECA) were tied to plastic or glass tubes for the injection of the tissue-embedded medium (Cryomatrix, Shandon Inc). This medium is fluid on room temperature but solid at ~20°C. Cryomatrix was pressed manually into the plastic tube at the end of the CCA against an intraluminal pressure of 100 mm Hg. Then, the arterial tree was clamped at the ends with the lumen still under pressure. After the restituted carotid specimens were frozen at −21°C, they were cut into serial 3-mm cross slices. Accordingly, slices distal and proximal to the flow divider were labeled +3, +6, etc, and −3, −6, etc, respectively. Photographs with a 1-mm scale were taken from each slice. Technical details of the procedures for preparing and filling the arteries have been given previously.13

Histological Examination and Planimetry

The 3-mm slices containing the carotid stenosis were selected and slowly defrosted. A dental silicon rubber (President Micro System Jet Bite, Coltene AG) was put into the lumen to prevent shrinkage. The slices were placed for fixation into a 4:1 mixture of 100% ethanol and 40% formaldehyde for 24 to 48 hours. After dehydration, the silicon rubber was carefully removed, and the specimens were embedded in paraffin. Transversal sections 7 to 8 μm thick were cut by microtome and stained with hematoxylin and eosin, dimethyl methylene blue, periodic acid–Schiff stain, and Verhoeff’s elastica combined with picrosirius red.14

Neurolucida (Micro Bright Field, Colchester) software,15 a Leitz Labor Lux S microscope (Leitz, Wetzlar) equipped with an 10×/0.25 numerical aperture objective (Leitz, Wetzlar), and associated instrumentation were used to calculate the original lumen vessel area and remaining lumen area. After system calibration, graphic overlays were made along the luminal surface of the endothelial and the internal elastic membrane according to the microscopic image. Afterward, these bit map format images were analyzed with IMAN1.4 software (KFK). The area of these graphic overlays was calculated. The area bordered by the endothelium (ie, remaining lumen area) was subtracted from the area bordered by the internal elastic membrane (ie, original lumen area), resulting in a value quantifying the ICA or CCA area reduction at the site of the stenosis. This measurement of area reduction was determined to be the gold standard and was compared with the ultrasound findings. Planimetric analysis was carried out by an investigator who was not aware of the ultrasound results.

Statistical Analysis

The degree of carotid stenosis of the in vivo sonographic and postmortem planimetric measurements and the mean differences were determined in percent. Linear regression equation was used to compare carotid stenosis determined by either technique. The correlation coefficients r and $R^2$ were calculated. The corresponding values obtained by ultrasound were plotted against those obtained by pathological investigation. In addition, a Bland-Altman plot was...
applied to illustrate the agreement between anatomic and sono-
graphic measurements. 16

This investigation is part of a more extended research project
comparing ultrasound findings of the brain-supplying arteries with
the corresponding pathological postmortem findings. The study was
performed according to national laws, and patients were studied in
compliance with a protocol previously approved by the local ethics
committee of the University Medical School of Debrecen.

Results

From the above comparison between the carotid ultrasound
examination and corresponding postmortem findings, 46
carotid arteries were found to be normal. In 44 vessels, a
steno-occlusive carotid lesion ranging from 22% to 100%
lumen reduction was discovered both pathoanatomically and
sonographically (Figure 1). In 1 patient, a 70% ICA stenosis
was not diagnosed by ultrasound because unfavorable exam-
ination conditions hampered a sufficient ultrasound
investigation.

The slice (vessel piece) with the most severe steno-
occlusive carotid lesion was 3 to 9 mm below the flow divider
in 5 cases, at the flow divider in 8 cases, and 3 to 15 mm
above the flow divider (ie, ICA) in 33 cases.

In 14 carotid stenoses, a satisfactory B-mode measurement
of the area reduction was not possible because of ultrasonic
shadowing or a difficult-to-delineate vessel wall; in these
cases, the lumen diameter reduction in the longitudinal
projection was used for comparison with anatomy. Nine ICA
occlusions were found by pathological analysis. Seven had
also been detected by ultrasound. In the 2 remaining cases, a
pseudoocclusion had been diagnosed sonographically as a
result of the detection of poststenotic flow. The ICA could
have been occluded in the time interval between ultrasound
and death. One CCA occlusion and an ICA occlusion of the
same vessel were found by both techniques. Sonographic
findings and their corresponding pathoanatomical findings in
stenoses ≥50% identified by both techniques are given in the
Table. In 5 cases (cases 14, 17, and 19 through 21), the
intrastenotic peak systolic velocity was <120 cm/s despite an
anatomical area reduction of >50%. In 1 of these cases (case
14), the stenosis was found in the distal CCA 6 mm below the
flow divider; in the remaining 4 cases, carotid stenosis was
located above the flow divider in the ICA.

Figure 2a and 2b shows the scatterplot, Bland-Altman plot,
and regression equation for both measurements. Overall,
r=0.96, and the adjusted \( R^2=0.90 \); for the 46 steno-occlusive
CCAs and ICAs detected on histopathology only, \( r=0.91 \).

An elevated intracranial pressure resulting from space-
occupying infarction or bleeding was found by CT in 34

![Figure 1. Case of a 70-year-old man with acute hemiparesis of the left side and an ICA stenosis on the right (sonographic measure-
ment, 75% stenosis; planimetric analysis, 83.3% stenosis). Top left, Spectral waveform analysis showed intrastenotic peak velocities of
269/95 cm/s. Top right, Significant asymmetry of the spectral waveform analysis in the CCAs. Velocity and pulsatility are decreased in
the right CCA (bottom). Bottom left, Unfixed 3-mm slice with most severe steno-occlusive lesion 3 mm above the flow divider. ICA left,
ECA right. Scale bar=10 mm. Bottom right, Corresponding histological section (Verhoeff's elastica staining, 13-fold magnification)
showing high-grade ICA stenosis.](http://stroke.ahajournals.org/Content/2001/01/01/STROKEAHA.101.199915.008079AC.large.jpg)
patients; despite this, spectral waveform analysis of the blood flow in the brain-supplying arteries was diagnostic in all cases.

Discussion

There is ongoing discussion as to whether Doppler ultrasound permits sufficient detection and reliable quantification of carotid artery stenosis.\(^5,17–21\) This has become a decisive question in patients with steno-occlusive carotid lesions since the results of NASCET and ECST have revealed that there is a definite threshold qualifying for surgical intervention, ie, \(\geq 70\%\) and \(\geq 82\%\) angiographic diameter reductions, respectively. We investigated the ability of carotid ultrasound to both detect and grade carotid artery stenoses. Unlike previous studies, we did not assess preselected patients with known or probable carotid artery disease screened for endarterectomy but used unselected, critically ill neurological patients.

Alexandrov et al\(^5\) have already pointed out that bidimensional angiographic measurements of carotid stenosis (eg, NASCET and ECST methods) differ significantly from the actual anatomical stenosis because these methods do not consider the area reduction of the residual, often asymmetrical, vessel lumen. In contrast, we used planimetry in this study to evaluate artery luminal area reduction as the gold standard instead of angiographic 1-plane measurements, which are only surrogates of the real anatomy. The histological examination allows identification of both the original and residual arterial lumen. Eccentric lumens pose no problems in estimating the degree of narrowing at necropsy but may be a source of error during angiography.

In accordance with previous studies,\(^6,10\) we could confirm that the sole acquisition of the intrastenotic peak systolic velocity and/or calculation of velocity ratios between the ICA and CCA, as proposed by other investigators,\(^7,9,19,22\) do not provide an adequate sonographic approach for reliable determination of the degree of carotid artery stenosis. All the above-mentioned accessible extracranial and intracranial ultrasound parameters have to be taken into account.\(^10\) For instance, the peak velocities in \(\geq 80\%\) ICA stenosis in conjunction with cross flow to the contralateral side via an anterior communicating artery require different interpretation than the same findings without anterior cross flow.

The Table shows that the synopsis of direct and indirect ultrasound findings evaluated by extracranial and intracranial sonography permits reliable quantification of high-grade carotid stenosis. For the quantification of moderate- and low-grade stenoses, however, the B-mode measurement of the area reduction at the stenotic site or, if not available for

<table>
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<th>Case</th>
<th>Peak Systolic/End Diastolic Velocity (Intrastenotic), cm/s</th>
<th>Spectral Broadening*</th>
<th>Intracranial Collaterals†</th>
<th>Pathological Ophthalmic Flow‡</th>
<th>Asymmetry in the CCA§</th>
<th>Degree of Stenosis (Sonographic), %</th>
<th>Difference Between Ultrasound and Anatomy, %</th>
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*0= no, 1 = slight, 2 = severe.
†0 = no, 1 = yes, 9 = no window.
‡0 = no, 1 = yes.
§Stenosis measured by B-mode ultrasound (area reduction).
||Stenosis measured by B-mode ultrasound (diameter reduction).
technical reasons, the measurement of the lumen diameter reduction provided a good match of in vivo and postmortem findings (Figure 2).

De Bray and Glatt\textsuperscript{10} indicated that the identification and measurement of tight stenoses by area ratio measurement is less accurate, and hemodynamic criteria are preferred. This is widely accepted and is valid for most patients. In 5 of our patients, however, we found a peak systolic velocity of $<120$ cm/s although an anatomic area reduction of $>50\%$ was present (the Table). This fact underlines the above-mentioned sonographer’s rule to consider as many available Doppler and B-mode findings as possible when determining the degree of carotid stenosis. The hemodynamic findings in distal CCA stenoses require an evaluation different from ICA stenosis (see case 14 in the Table).

In previous pathoanatomic studies, ultrasound had been validated through carotid plaques removed during carotid endarterectomy.\textsuperscript{5–7} These specimens, however, may be incomplete in terms of the full extension of the plaque, are frequently damaged by surgery, and because of shrinkage and tissue collapse, would not provide the true in vivo situation distal to, proximal to, or at the site of stenosis. In contrast to shortcomings with endarterectomy specimens, we assessed the complete redistended carotid specimens and evaluated systematically the entire length of the stenotic and nonstenotic arterial segment by performing serial slices. Furthermore, we avoided the collapse of the lumen by filling it with silicon rubber before histological processing.

Several recent studies had focused on the analysis of moderate to severe carotid artery stenosis.\textsuperscript{5–8} The diagnostic value of modern ultrasonography to determine low-grade stenosis and plaques has been investigated less extensively. In the present study, however, we assessed diseased carotid arteries with lumen narrowings ranging from $8.5\%$ to $100\%$. As shown in Figure 2, B-mode ultrasound, combined with Doppler ultrasound, allows reliable measurement of all degrees of carotid artery lesions. Moreover, with 1 exception, all nonstenotic vessels were correctly identified by ultrasound.

In accordance with a previous pathoanatomic comparative study,\textsuperscript{6} we found $r=0.9$ for the $\geq50\%$ stenoses and overall $r=0.96$ for the agreement of anatomic and sonographic measurements as long as a combination of several criteria was used. Other investigators had similar results although they used either hemodynamic criteria or morphological measurements alone. In the study by Ranke et al,\textsuperscript{22} exclusive use of the mean velocity ratio (ie, the intrastenotic mean flow velocity divided by the distally recorded mean blood flow velocity) revealed the closest correlation with angiography ($R^2=0.93$). Griebing et al\textsuperscript{8} measured the cross-sectional area reduction and longitudinal diameter reduction by color flow Doppler and power Doppler, leading to an excellent correlation between power Doppler and angiography in high-grade stenosis with $r=0.93$.

In our study, the ultrasound examination was not carried out under optimal conditions because most patients had to be examined at bedside in the intensive care unit. The uncomfortable position of the sonographer, jugular vein catheters, deep breathing, unextended neck, and agitation of the patient limited the quality of the ultrasound investigation in some cases. Unlike previous investigators,\textsuperscript{23} we included all ultrasound examinations in the analysis, even those with poor quality, on an “intention-to-diagnose” basis. Furthermore, at the time of this study, there was no approval for ultrasound contrast agent. We could not use ultrasound contrast agents for a more accurate differentiation of total ICA occlusion from pseudoocclusion or for the investigation of intracranial pathways.\textsuperscript{24,25}

In summary, using an unique in vivo and postmortem approach, we could show that color-coded duplex ultrasound permits a reliable detection and quantification of carotid artery steno-occlusive disease even under difficult examination conditions as long as the investigator considers the entire spectrum of the available extracranial and intracranial ultrasound parameters.

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References
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