Accuracy of In Vivo Carotid B-Mode Ultrasound Compared With Pathological Analysis

Intima-Media Thickening, Lumen Diameter, and Cross-Sectional Area

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Background and Purpose—This study aimed to determine the correlation of in vivo ultrasound measurements of intima-media thickening (IMT), lumen diameter, and cross-sectional area of the common carotid artery (CCA) with corresponding measurements obtained by gross pathology and histology.

Methods—Sixty-six moribund neurological patients (mean age 71 years) underwent B-mode ultrasound of the CCA a few days before death. During autopsy, carotid specimens were removed in toto. Carotid arteries were ligated and cannulated for injection of a hydrophilic embedding material under standardized conditions. The carotid bifurcation was frozen and cut manually in 3-mm cross slices. Digital image analysis was carried out to determine the diameter and the cross-sectional area of the CCA. IMT was assessed by light microscope. Ultrasonic and planimetric data were compared.

Results—Mean measurements of lumen diameter and cross-sectional area were 7.13 ± 1.27 mm and 0.496 ± 0.167 cm², respectively, by ultrasound, and 7.81 ± 1.45 mm and 0.516 ± 0.194 cm², respectively, by planimetric analysis of the unfixed redistended carotid arteries ($R^2=0.389$ and 0.497). The mean IMT was 1.005 ± 0.267 mm by ultrasound and 0.67 ± 0.141 mm histologically, resulting in a mean difference of −31%.

Conclusions—Transcutaneous B-mode ultrasound provides a reliable approach for in vivo measurements of the cross-sectional area and, less exactly, of the lumen diameter of the CCA. Compared with histological results, in vivo ultrasound measurements of the IMT are systematically larger. (Stroke. 2001;32:1520-1524.)

Key Words: carotid arteries ▪ pathology ▪ ultrasonography

Atherosclerotic changes at the carotid bifurcation are a well-known cause of cerebrovascular disease ranging from thromboembolic transient ischemic attacks due to small emboli of fatty debris and platelet aggregates to completed strokes due to carotid thrombosis and secondary embolism.1 B-mode ultrasound allows for direct visualization of both the vessel wall and the lumen and, subsequently, for detection of early atherosclerosis, indicated by intima-media thickening (IMT). Increases in carotid IMT are directly associated with an increased risk of cardiovascular disease.2-3 In previous experimental studies, the accuracy of carotid B-mode ultrasound to measure IMT had been validated in cadavers by in vitro and in situ measurements but not in living patients.4-7

Unlike angiography, B-mode ultrasound permits the measurement of the cross-sectional area of the common carotid artery (CCA) as well. To the best of our knowledge, there is no report on the accuracy of carotid B-mode ultrasound comparing in vivo measurements with the corresponding postmortem results.

Our purpose was to correlate in vivo ultrasound measurements of IMT, lumen diameter, and cross-sectional area of the CCA with the corresponding measurements obtained by subsequent gross pathology and histology.

Subjects and Methods

Patients

During a 13-month-period, 118 critically ill patients were prospectively investigated by extracranial and intracranial ultrasound. Eighty-two of them died. For 66 patients, a comparison between ultrasound findings and postmortem results was possible. There were 37 men and 29 women (mean age 71.0±13.2 years, range 37 to 95 years). Patients were admitted to the hospital for ischemic stroke...
vessel circumference constant. On Doppler mode, the angle between the external carotid artery (ECA) and internal carotid artery (ICA) could be determined at the flow divider by means of both the superimposed vertical line (representing the ultrasound beam) and the adjustable arrow for angle correction (representing the direction of the Doppler sample volume). In this way, the localization of the IMT measurement site on the CCA circumference was defined by the angle between ECA and ICA at the flow divider and by the distance from the tip of the flow divider (3 cm) (Figure 1). These landmarks enabled us to reconstruct the position of the in vivo IMT measurement on the postmortem tissue slices. Subsequently, IMT measurements were performed in the same way 0.9 cm below the tip of the flow divider. All measurements were performed at the end of a heart cycle and were carried out by the same investigator. Data were recorded onto videotape for later offline analysis.

Gross Pathological Evaluation and Planimetric Analysis of Vessel Diameter and Cross-Sectional Area

The different steps of the gross pathological evaluation are illustrated in Figure 3; technical details of the procedure for preparing and filling the arteries have been described previously.8 The time between death and autopsy ranged from 2 to 72 hours (mean 25.5 hours, median 15.5 hours).

The frozen section labeled 30 (ie, 30 mm below the tip of the flow divider) was digitized by using a Hitachi HV-C20 (Hitachi Ltd) charge couple device (CCD) color camera equipped with a zoom lens (1:1.8, 12.5 to 75 mm) connected to a PC (Figure 4). Total area, maximum diameter, and minimum diameter of the vessel lumen were determined with image analyzer software (IMAN 1.4, KFKI). With each individual sample, a calibration bar was also digitized to determine the magnification of the system and to convert the pixel values into millimeters or square centimeters. The settings of the camera were kept constant throughout the study. The minimum diameter was used for the comparison with ultrasound. The results from ultrasound investigations were unknown to the investigator performing the planimetric analysis.

Histological Preparations and Determination of IMT

After the above-mentioned digitization, the samples were slowly defrosted, and a dental silicon rubber (President Micro System Jet Bite, Coltene AG) was filled into the lumen to prevent shrinkage of the lumen. By means of the photos of the slice 3 mm (+3) above the flow divider (providing the angle between ECA and ICA just above the bifurcation) and 30 and 9 mm below the tip of the flow divider (−30 and −9, respectively) (providing investigation sites), it was possible to reconstruct at which site of the vessel circumference the IMT was determined as the distance between the leading edges of the inner and outer echoes of the double-line pattern of the far artery wall (Figure 2); 3 measurements along a 2- to 3-mm portion of the vessel were performed and were averaged. Wall thickening over 2 mm was called a plaque. If there was a protruding plaque at this site, this particular IMT measurement was excluded from further evaluation. Afterward, the transducer was rotated by 90° exactly at the site of IMT measurement, and the probe was slowly moved up just above the bifurcation, with the vertical course of the vessel followed precisely; when moving the transducer downstream, the sonographer kept the angle between the probe and ECA and ICA at the flow divider just above the bifurcation and by the distance from the tip of the flow divider (3 cm) (Figure 1). These landmarks enabled us to reconstruct the position of the in vivo IMT measurement on the postmortem tissue slices. Subsequently, IMT measurements were performed in the same way 0.9 cm below the tip of the flow divider. All measurements were performed at the end of a heart cycle and were carried out by the same investigator. Data were recorded onto videotape for later offline analysis.

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Figure 1. Schematic representation of carotid artery ultrasound measurements.

Figure 2. Left, B-mode image of an arterial segment 9 mm below the flow divider. The mean distance between the echogenic lines was 1.08 mm. Right, Histological section (Verhoeff elastic staining, original magnification ×125) at the corresponding location. The mean value of IMT was 0.70 mm. The intima and media are within the black arrows. Note the adventitia marked with black ink (small black arrow).

(n=33), intracranial hemorrhage (n=28), brain tumor (n=1), and other reasons (n=4).

The causes of death were determined by experienced pathologists during autopsy. Patients died from cardiopulmonary arrest (n=8), raised intracranial pressure (n=5), pneumonia (n=6), sepsis (n=3), or pulmonary edema (n=1). For 1 patient, the cause of death remained unknown even after autopsy. The mean time interval between ultrasound and death was 10.7 days (median 3 days).

Ultrasound

The ultrasound equipment used was an HP SONOS 2000 system (Hewlett-Packard) with a 7.5-MHz linear transducer. Axial resolution was 0.5 mm, and lateral resolution was 0.6 mm at the focal point at a depth of 3 cm. The 2D image displayed 256 gray levels. Gain (55%) and compression (95%) were held constant throughout the study. The largest magnification was used for IMT measurements. The measurements of lumen diameter and IMT were performed in a longitudinal B-mode projection; cross-sectional area was measured in axial projection while the patient was in a supine position. Details are given in Figure 1. IMT was determined as the distance between the leading edges of the inner and outer echoes of the double-line pattern of the far artery wall (Figure 2); 3 measurements along a 2- to 3-mm portion of the vessel were performed and were averaged. Wall thickening over 2 mm was called a plaque. If there was a protruding plaque at this site, this particular IMT measurement was excluded from further evaluation. Afterward, the transducer was rotated by 90° exactly at the site of IMT measurement, and the probe was slowly moved up just above the bifurcation, with the vertical course of the vessel followed precisely; when moving the transducer downstream, the sonographer kept the angle between the probe and
Excision of the carotid artery including CCA, ECA and ICA en bloc at autopsy. Ligating of the ECA branches.

Filling of the carotid specimen with a fluid tissue embedding material under standardized conditions.

Freezing of the resected specimen at -20°C in a box containing embedding material and two parallel straws for later orientation.

Cutting in 3mm axial slices. Labelling of the slices according to their distance to the flow divider (e.g. slice located 30 mm below the flow divider = -30 mm) and photographing each slice.

Digitalisation and planimetric analysis of slice -30 referring to the lumen diameter and cross-sectional area.

**Figure 3.** Flow-chart illustrating the gross pathological workup of the carotid specimen.

IMT had been measured sonographically. In this site, the adventitia was marked with black ink for later orientation. Afterward, the slices were placed for fixation in perforated plastic boxes in a 4:1 mixture of 100% ethanol and 40% formaldehyde for 24 to 48 hours. After dehydration, the silicon rubber was carefully removed before paraffin embedding. Transverse sections (7 to 8 mm) were cut by microtome and were stained with hematoxylin/eosin, dimethylmethylen blue, periodic acid-Schiff stain, and Verhoeff’s elastic tissue stain combined with picrosirius red.9,10 The IMT was measured at those sites at which black ink could be identified in the adventitia. IMT measurements were performed by light microscope with the use of an object micrometer slide (Leitz) with an accuracy of 0.01 mm. Three measurements along a 2-mm portion of the vessel at a distance of ~1 mm were averaged (Figure 2). Sections with severely damaged intima and/or media at the site of the measurement were excluded. The results from ultrasound investigations were unknown to the investigator performing the light microscopic assessment.

**Statistical Analysis**

For the evaluation of lumen diameter and cross-sectional area of the in vivo sonographic and the postmortem planimetric measurements, the mean values and mean differences were determined in millimeters and square centimeters, respectively. Linear regression equations were performed to compare IMT, diameter, and cross-sectional area measured by either technique. The $R^2$ value was calculated. The values obtained by ultrasound were plotted against those obtained by pathological investigation. Furthermore, Bland-Altman plots were applied to illustrate the agreement between anatomic and sonographic measurements.11

The present study was performed according to national laws, and the patients were studied in compliance with a protocol that had previously been approved by the local ethics committee of our University Medical School.

**Results**

**Comparison of Ultrasound and Gross Pathological Analysis**

Corresponding measurements were available for vessel diameters in 123 arteries and for cross-sectional areas in 92 arteries.

The mean ultrasound and postmortem measurements for vessel diameter and cross-sectional area are compared in Table 1. Correlation of sonographic and pathological measurements resulted in $R^2$ values of 0.389 and 0.497 for diameter and cross-sectional area, respectively. Figure 5 shows the scatterplots, the Bland-Altman plots, and regression equations for both parameters.

**Comparison of Ultrasound and Histological IMT Measurements**

IMT measurements were comparable in 52 arteries at 72 sites; of the 72 sites, 36 measurements had been placed 9 mm ($\pm 9$) and 36 measurements had been placed at 30 mm ($\pm 30$) below the flow divider. Values obtained by ultrasound always turned out to be smaller than those obtained histologically (Table 2), indicating a systematic discrepancy.

The regression equation was as follows: IMT measured histologically (mm) = 0.3586 × IMT measured on ultrasound (mm) + 0.3095. Despite this discrepancy in size, the $R^2$ was 0.463.

**Table 1. Comparison of CCA Measurements Determined by Ultrasound and by Pathology**

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<th>Lumen Diameter, mm</th>
<th>Cross-Sectional Area, cm²</th>
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<tbody>
<tr>
<td>Pathology</td>
<td>Mean ± SD</td>
<td>7.81 ± 1.45</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>3.96–12.53</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Mean ± SD</td>
<td>7.13 ± 1.27</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>4.07–10.9</td>
</tr>
<tr>
<td>Linear regression</td>
<td>$R^2$</td>
<td>0.389</td>
</tr>
<tr>
<td>Pathology</td>
<td>&gt;Ultrasound, n (%)</td>
<td>92 (75)</td>
</tr>
<tr>
<td></td>
<td>&lt;Ultrasound, n (%)</td>
<td>31 (25)</td>
</tr>
<tr>
<td>% Difference*</td>
<td>Mean ± SD</td>
<td>17 ± 12</td>
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*% Difference = [(pathology–ultrasound)/Ultrasound] × 100.
Discussion

The physical basis of the sonographic vessel wall reflexes consisting of 2 parallel echogenic lines separated by an echolucent zone is the difference in acoustic impedance between tissues of different composition. The interfaces between lumen, intima, media, and adventitia lead to the characteristic pattern of reflectivity. Furthermore, the double line pattern is influenced by the axial resolution of the ultrasound device and by the display settings, such as gain and compression. Therefore, measurements comparing anatomic and sonographic findings for each histological layer (intima, media, and adventitia) alone do not show a sufficient agreement. Despite this, previous studies have suggested that the characteristic double reflex of the carotid artery wall can be precisely measured with a 7- to 8-MHz transducer at the far wall of the vessel and correlates well with anatomic intima plus media measurements. Similar to previous investigators, we performed sonographic IMT measurements only in the CCA at the far arterial wall because the reproducibility of in vivo IMT measurements was worse in studies including measurements in the ICA and bulb than in studies limited to the CCA. Gamble et al, using a mechanical 10-MHz probe and different gain settings, measured the thickness of the intima, media, and adventitia by both ultrasound and histology. They found the best correlation with total wall thickness but not with the intima-media complex. A systematic reduction of the IMT, presumably due to shrinkage artifacts during histological preparation, can be observed in the present study. The fixation in formalin, dehydration in ethanol, and embedding in paraffin may contribute to a tissue shrinkage of 30% to 40%. A few recent studies on the validation of ultrasound-determined IMT carried out in vitro and/or in situ had reported a much lower shrinkage of only 2.5% to 14.6%. However, in the study by Zarins et al involving 10 human superficial femoral arteries, the vessel wall decreased by 36% because of postmortem shrinkage, which is in accordance with the present study, establishing a mean difference of −31% (−0.34 mm) between ultrasound and histological findings.

The mean CCA diameter of 7.13 mm measured by ultrasound in the present study is in agreement with the findings in previous ultrasound studies: Marosi and Ehringer established the average diameter of the CCA 1.5 cm below the bifurcation in 53 healthy young adults and found an average diameter of 6.7 mm. Boutouyrie et al measured the CCA diameter and the luminal cross-sectional area 2 cm

<table>
<thead>
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<th>Table 2. Comparison of IMT Measurements</th>
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<tr>
<td>IMT</td>
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<tr>
<td>-----</td>
</tr>
<tr>
<td>Mean±SD</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

*Difference (mm) = histology − ultrasound.
†Difference (%) = [(histology − ultrasound)/ultrasound] × 100.
beneath the bifurcation by means of a pulsed ultrasound echo-tracking system in 3 groups of hypertensive patients. With increasing age, they found average CCA diameters of 6.97, 7.07, and 7.64 mm, respectively. Their sonographic measurements for average luminal cross-sectional areas were 0.39, 0.40, and 0.47 cm², respectively, which is only slightly different from our results (mean 0.496 cm²).

In the present study, values for lumen diameter and cross-sectional area obtained by ultrasound were generally smaller than those obtained by pathology. We are aware of the fact that the postmortem loss of vessel wall elasticity and the removal of the surrounding stabilizing connective tissue may have caused a minor deviation of the specimens during filling with embedding material. The ultrasound measurements had been performed at the end of a heart cycle, whereas postmortem filling of the arteries had been performed under a constant pressure of 100 mm Hg. Previous MRI and ultrasound studies have shown that an increase in both relative diameter and area measurements had been performed at the end of a heart cycle, whereas postmortem filling of the arteries had been performed under a constant pressure of 100 mm Hg. Previous MRI and ultrasound studies have shown that an increase in both relative diameter and area during systole reflects the distensibility of the artery. However, Reneman et al. found a significantly larger relative diameter increase in subjects aged 20 to 30 years compared with subjects aged 50 to 60 years (9.6% versus 5.6%). The mean age of our study population was 71 years, suggesting an already reduced CCA distensibility. Distensibility may have accounted for some of the discrepancies found in the present study.

We found a better correlation of ultrasound and gross pathology for cross-sectional area (R²=0.497) than for lumen diameter (R²=0.389). This might be due to the slightly eccentric shape of the vessel lumen not being correctly represented by the limited number of projections obtained during longitudinal B-mode ultrasound. By contrast, axial sonography allows for measurement of the “real” contour of the whole vessel lumen, considering even small irregularities of the vessel wall. The clinical implication of this finding is that the reliable assessment of the vessel lumen with a potential narrowing requires the use of a multiplane imaging technique, such as ultrasound. In general, all R² values were only moderate in the present study. However, the Bland-Altman plots showed that nearly all provided differences were within mean ± 2 SD (limits of agreement) for cross-sectional area and IMT. The poorest agreement was found for lumen diameter; this finding is probably explained by the eccentric shape of the vessel lumen, as mentioned above.

In summary, transcutaneous B-mode ultrasound in vivo measurements of the CCA with a 7.5-MHz linear transducer showed good agreement with corresponding measurements obtained from standardized macroscopic postmortem findings for the determination of the cross-sectional area. Ultrasound measurements of the IMT were systematically larger than histological results; this discrepancy was presumably due to tissue fixation and processing. The agreement between both methods was poorest for lumen diameter.

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References

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