Ultrasound-Determined Intima-Media Thickness and Atherosclerosis
Direct and Indirect Validation
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Abstract
To evaluate ultrasonographically determined intima-media thickness as a measure of early atherosclerosis, three studies were performed. Ultrasound measurements of intima-media thickness in the carotid artery were directly validated by comparing the same thickness measured by light microscopy. The values were closely correlated \((r=0.82, P<0.001)\). Intima-media thickness determined by light microscopy was consistently smaller than that determined by ultrasound, probably due to shrinkage during histological preparation. As an indirect validation, mean intima-media thickness was calculated in three large groups of patients with no plaque \((n=224)\), one plaque \((n=105)\), and one circumferential or two or more plaques \((n=54)\) in the carotid bifurcation. Intima-media thickness increased significantly with increasing plaque score, indicating that diffuse intima-media thickening is more pronounced with more severe atherosclerosis. The intima-media thickness also increased with increasing multifactorial cardiovascular risk, reflecting a positive relation between signs of early atherosclerosis and the burden of known risk factors for the disease. Our studies support earlier findings that have found that ultrasonographically determined intima-media thickness is a valid way to study early atherosclerosis. \(\text{Arterioscler Thromb.} \ 1994;14:261-264.\)

Key Words
• intima-media thickness • ultrasound investigation • atherosclerosis • light microscopy • plaque • risk factors

Ultrasound methods to quantify the size of atherosclerotic lesions and arterial wall thickness have become frequently used in studies of the progression and regression of atherosclerosis. Early stages of the disease are studied by measuring intima-media thickness (IMT), mostly at different levels of the carotid artery.\(^1\)\(^-\)\(^5\) These measurements are dependent on high-resolution B-mode ultrasonography, a method that presents the arterial wall as a double-line pattern. Measurements are done on the far wall, where the distance between the leading edges of the inner thin and outer thick echoes have been shown to correlate with IMT.\(^1\)\(^-\)\(^3\)\(^,\)\(^6\)\(^-\)\(^8\)\(^,\)\(^10\)\(^-\)\(^11\) Different studies have shown associations between increased IMT and smoking,\(^7\)\(^-\)\(^11\) hypercholesterolemia,\(^4\)\(^-\)\(^5\)\(^,\)\(^8\)\(^,\)\(^10\)\(^-\)\(^11\) hypertension,\(^7\)\(^-\)\(^11\) and increasing age.\(^1\)\(^-\)\(^3\)\(^,\)\(^7\)\(^-\)\(^11\)

Some investigators question the whole concept of measuring IMT with ultrasound. The appearance of a double-line pattern has been suggested as being an artifact.\(^12\)\(^,\)\(^13\) This criticism is based on the observation of artificial "ghost" echoes when ultrasound investigation is performed on materials such as Plexiglas or plastic foil. The ultrasound appearance of the transition between these media and fluid reveals a double-line pattern similar to the pattern on which IMT measures are performed in vivo. An in vitro study on human cadaver aortas, however, shows no correlation between ultrasound-determined IMT and the same distance measured with stereomicroscopy.\(^12\) In another study\(^6\) on specimens obtained from autopsies, the origin of the echoes in the double-line pattern were determined. The results from that study contradict the suggestion that the double-line pattern in the ultrasound appearance of the arterial wall is an artifact.

In the present study, the usefulness of ultrasound-determined IMT in studies of atherosclerosis was demonstrated in three ways. Measurements of IMT with ultrasound were directly validated by comparing measurements of the same thickness as determined by light microscopy. The relevance of the ultrasonographic measurements was indirectly validated in two ways, first by calculating IMT in groups of patients with different occurrences of plaques within the same vessel, and second, by comparing IMT with the estimated risk of coronary events assessed according to the Framingham study.\(^14\)

Methods
The ultrasound equipment used was an Acuson 128 Computed Tomography System (Acuson, Mountain View, Calif) with a 7.0-MHz linear transducer. Longitudinal images of the common carotid artery were recorded on videotape for off-line analysis in a computerized image-analyzing system. The IMT was calculated as the mean distance between the leading edges of the inner and outer echoes of the double-line pattern of the far artery wall along a 1-cm portion of the vessel. The technique and equipment used have been described previously.\(^15\)

Ultrasound and Light Microscopy
In the first part of this study, 19 common carotid arteries with bifurcations were obtained from the Department of Pathology. Ultrasound investigations were performed with the arteries placed in a bath of Dulbecco's buffer solution. The double-line pattern was easily found in all 19 specimens. The arteries were then fixed with formaldehyde for histological preparations. No artificial pressure was used during ultrasound investigation and histological preparations. The staining
method used was elastin-van Gieson’s. Each vessel was cut transversely into 20 slices that were then investigated by light microscopy with equipment that allowed distance measurements with a precision of 0.01 mm. Measurements were made at two sites on each slice; thus IMT was calculated as the mean of 40 measurements on each artery. For both methods the measurements were performed 1 cm proximal to the carotid bulb. The results from ultrasound investigations were unknown to the technician performing the light microscopy measurements.

IMT and Plaque Occurrence

In the second part of this study, results from ultrasound investigations of 385 men and women of different ages were used. The subjects had no clinical symptoms of atherosclerotic lesions in the examined vessel area and no history of transient ischemic attack, reversible ischemic neurological deficit, or stroke. They underwent ultrasound investigation of a predefined “window” extending 3 cm proximal and 1 cm distal to the bifurcation flow divider of the right carotid artery. The plaque occurrence within this window was determined qualitatively according to a three-part scale in which 0 represented an artery without any plaques; 1, a vessel with one plaque; and 2, the occurrence of one circumferential or two or more plaques. Plaques were defined as discernable focal thickenings of the arterial wall confirmed on high-quality recordings. Longitudinal images for IMT measurements in the common carotid artery were captured in end diastole by electrocardiographic R-triggering. Mean IMT was calculated for each of the three groups of different plaque occurrence.

IMT and Estimated Risk for Coronary Event

In the third part of the study the risk for coronary events was estimated for 344 individuals on the basis of each individual’s level of risk factors for cardiovascular disease. Assessment was made according to the Framingham study,14 considering age, sex, smoking habits, glucose tolerance, systolic blood pressure, and total and high-density lipoprotein cholesterol. In the Framingham study, the presence or absence of left ventricular hypertrophy is also considered. As data on this matter were not available, all subjects were considered not to have left ventricular hypertrophy. In the Framingham study, the presence or absence of left ventricular hypertrophy is also considered. As data on this matter were not available, all subjects were considered not to have left ventricular hypertrophy. The figures on the estimated risk for a coronary event consequently do not reflect a true risk estimate in percent, but the figures can be used to rank the subjects according to estimated risk. The subjects were ranked according to increasing estimated risk and divided into tertiles with approximately the same number of subjects in each group.

Statistics

Results are presented as mean±SD. Differences between groups of unpaired data were analyzed with a Kruskal-Wallis test (three groups). Groups of paired data were compared with a Mann-Whitney U test. Standard procedures were applied for calculation of correlation coefficients. The level of significance was .05.

Results

Ultrasound and Light Microscopy

IMTs measured ultrasonographically and microscopically were closely correlated (r=.82, P<.001). IMTs determined by light microscopy were consistently smaller than those determined by ultrasonography (mean, 0.14 mm smaller, P<.001) (Fig 1).

IMT and Plaque Occurrence

To analyze whether signs of late atherosclerotic lesions (plaques) were associated with thicker intima-media complexes, the mean IMT was calculated in three groups of patients with signs of increasing severity of atherosclerosis. IMT increased significantly (P<.001) with increasing plaque occurrence, indicating that IMT is higher in more severe atherosclerosis (Fig 2).
The intima-media complex was significantly thicker in groups with higher estimated multifactorial cardiovascular risk ($P<0.001$), indicating that IMT reflects a vascular process related to the burden of known cardiovascular risk factors (Fig 3).

**Discussion**

In the first part of the study, the difference in mean values between the two techniques (0.14 mm) represented a difference of 14.6%. However, the fact that all soft tissues shrink during histological preparations is well known, most commonly, at least a 10% shrinkage is found. The difference between ultrasound- and light microscopy-determined IMT in our study is thus on the order of what would be expected.

The ultrasound method used normally has a variability of approximately 10% in assessing IMT. When comparing ultrasound to other methods, it is important to include a range of values relevant to those found in patient groups in which the method is to be applied. A wide range in absolute values of the measured distance is also a prerequisite to demonstrate a significant correlation, since a small range usually results in a poor correlation coefficient. In this in vitro study, ultrasound ranged from 0.68 through 1.44 mm, and correlation was high ($r=0.82, P<0.001$). Another in vitro study on human aortas shows no correlation between IMT measured with ultrasound and the same distance determined by stereomicroscopy. In that study, only five specimens were used, and the absolute values ranged from only 1.18 through 1.43 mm. With such a small range, correlation between the two methods is likely to be poor, and with only five specimens a significant correlation calls for a very high $r$ value. We concluded that the low variability of our ultrasound method makes it suitable for epidemiological studies. Its usefulness in the follow-up of individual subjects has yet to be tried.

Subjects with one circumferential or two or more plaques were found to have a significantly thicker intima-media complex than subjects with one plaque, who in turn had a significantly thicker complex than subjects without any plaques. This supports the hypothesis that not only is atherosclerosis represented by focal thickening of the artery wall but also that diffuse IMT might represent early stages in the development of atherosclerosis. The differences were demonstrated even though none of the subjects had symptoms of atherosclerotic disease. If patients with a history of disease caused by atherosclerotic lesions in the investigated vessel area were added, the differences would probably be even greater. This is supported by the finding of higher degrees of stenoses in symptomatic patients than in asymptomatic subjects.

There were significant differences in age between the three groups of subjects with different plaque scores. The youngest subjects were found in the group without any plaques. Subjects were older in the group with one plaque, and the oldest subjects were found in the group with arteries most affected by atherosclerosis. This is no surprise, since the degree of atherosclerosis is known to increase with age. It can be presumed that signs of early atherosclerosis would be present in vessels in which severe lesions are found. Our findings of thicker intima-media complexes in the common carotid artery in patients with plaques in the bifurcation thus supports the hypothesis that ultrasound-determined thickening of the intima-media complex is a sign of early generalized atherosclerosis.

The estimated multifactorial cardiovascular risk is based on factors that have been proven to be statistically associated with ultrasound-based determination of IMT. The function used is primarily calculated on the relation of a combination of risk factors for future coronary heart events. However, our finding of a higher value of IMT with increasing estimated multifactorial risk for coronary heart disease supports the idea that IMT mirrors early atherosclerotic disease. This interpretation is supported by earlier findings of a relation between IMT and coronary atherosclerosis. Valid measurements of IMT are probably important for studies on the progression and regression of atherosclerosis. Evaluation of plaque size has not been thoroughly studied, and some patients have no plaques in arterial regions appropriate for ultrasonographic investigation. We have previously presented a study on the reproducibility of plaque measurements using ultrasound technology. Reproducibility of IMT determinations was better than for measurements of plaque thickness and area. Considering the results from that study and the present results, we conclude that IMT measured with ultrasound will reflect early atherosclerotic lesions in a valid way and hence give valuable information on the regression and progression of such lesions. So far this is true for studies of groups of patients, but the clinical usefulness of this method in the follow-up of individual subjects has yet to be proven.
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