Comparison of In Vivo Carotid 3.0-T Magnetic Resonance to B-Mode Ultrasound Imaging and Histology in a Porcine Model

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OBJECTIVES We compared in vivo 3.0-T magnetic resonance (MR) images of the carotid artery wall in piglets to intima-media thickness measurements of similar carotid segments by B-mode ultrasound (US) and histology to define the corresponding anatomical tissue characteristics and accuracy of carotid MR images.

BACKGROUND Carotid MR is increasingly used for the assessment of cardiovascular risk and cardiovascular drug efficacy. Therefore, determining the anatomical correlate and accuracy of this modality is of major importance.

METHODS In vivo 3.0-T MR and B-mode US scans of the left and right common carotid arteries were performed in 5 piglets (75 to 80 kg). The T_1-weighted MR images were acquired with a noninterpolated pixel size of 0.25 × 0.25 mm for mean wall area (MWA) and mean wall thickness measurements. The B-mode US measured common carotid intima-media thickness and common carotid diameter. We calculated US MWA using common carotid intima-media thickness and carotid diameter. In histology, the intima and media tissue area was defined as histology MWA.

RESULTS Histology MWA was 4.69 (standard deviation [SD]: 0.95) mm², MR MWA was 4.57 (SD: 0.41) mm², and US MWA was 4.90 (SD: 0.50) mm². The mean difference was 0.12 (SD: 1.11) mm² for MR and −0.21 (SD: 1.01) mm² for US when compared with histology. Bland-Altman analysis showed no systematic biases between MR, US, or histology.

CONCLUSIONS Absolute values for carotid artery histology, MR, and US measurements are in good agreement, indicating that both 3.0-T MR and B-mode US measurements can visualize the intima and media. Accuracy of 3.0-T MR is comparable to B-mode US. The present findings imply that carotid MR might be a novel asset in cardiovascular disease risk stratification and a valuable surrogate marker in cardiovascular prevention trials. (J Am Coll Cardiol Img 2009;2:744–50) © 2009 by the American College of Cardiology Foundation

Vascular magnetic resonance (MR) for in vivo assessment of atherosclerotic burden carries the promise to become an asset for cardiovascular disease risk stratification as well as a valuable surrogate marker for cardiovascular drug efficacy assessment. Advantages of this technique include cross-sectional imaging with high reproducibility and estimation of plaque composition (1,2). In addition to visualizing plaque biology, MR can also be used to image the gradual process of arterial wall thickening from healthy arteries to advanced atherosclerosis as a continuous variable. For all of these situations, however, resolution and accurate detection of the submillimeter arterial wall structures is essential.

To date, only indirect evidence exists of the anatomical correlate between carotid MR and B-mode ultrasound (US). Underhill et al. (3), Crowe et al. (4), and Mani et al. (5) were the first to investigate the relation between carotid MR and US and found MR measurements of the carotid artery wall to be larger than by US. The investigators contributed the latter to the fact that, in addition to intima and media, MR measurements also incorporated the adventitial layer (3–5). In addition, resolution was put forward as a potential confounding factor contributing to the discrepancy between the 2 modalities. Of note, in-plane resolution of the MR at that time was between 0.65 mm and 0.50 mm and electrocardiography (ECG) gating was not applied.

Recently, we developed a 3.0-T MR protocol for noninvasive in vivo carotid wall thickness imaging in humans. With this imaging protocol, we increased the in-plane resolution to 0.25 mm^2 and applied ECG gating. Due to these improvements, we decreased the potential confounding of resolution and non-ECG gating contributing to the discrepancy between US and MR.

The aim of the present study was to evaluate the accuracy of this 3.0-T MR protocol of the carotid artery wall and to settle the issue concerning the exact anatomical substrate within the vessel wall corresponding to the obtained MR signal. To this end, we compared in vivo common carotid 3.0-T MR with histology in a porcine model. Simultaneously, B-mode US images were obtained for cross validation.

METHODS

Animal Species and Study Preparation

Five female piglets (*S. domestica*, weight: 75 to 80 kg) were selected for this study. All animals were purchased from a single local farm and were kept according to the guidelines of the animal care facility. The intervention procedures, imaging procedures, and animal handling were approved of by the Institutional Animal Ethics Committee.

After pre-medication with ketamine 10 mg/kg, midazolam 1 mg/kg, and atropine 0.02 mg/kg, oxygen, air, and isoflurane 1% to 1.5% were administered endotracheally. Sufentanyl 7 μg/kg/h and ketamine 7 mg/kg/h were then administered intravenously.
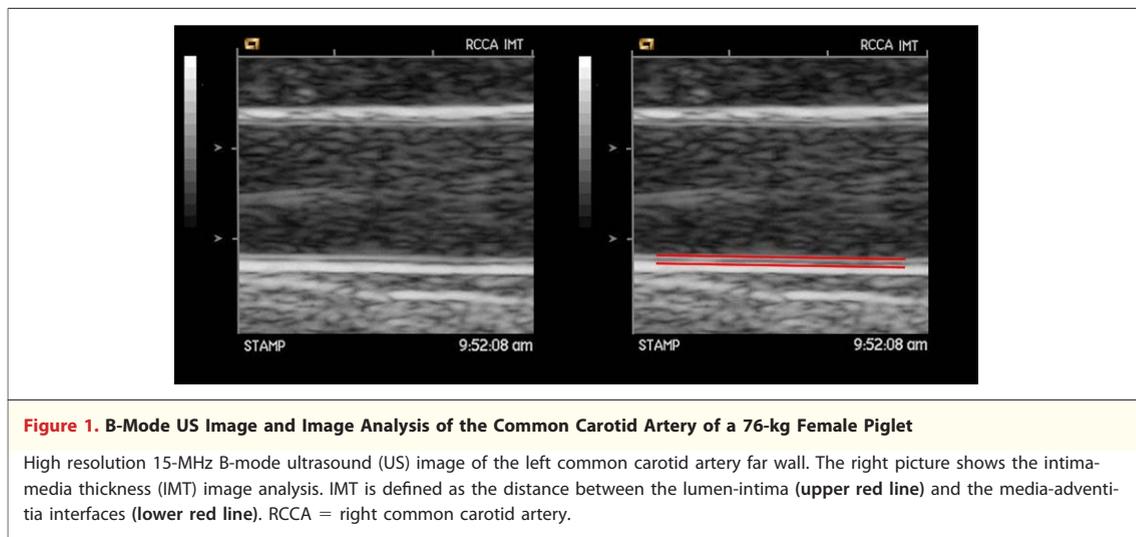
The common carotid arteries were located with US. In all animals, we made left and right paratracheal incisions, approximately 15 cm in length and 5 cm in depth, without exposing the carotid arteries. This enabled MR and US imaging of the carotid arteries from a closer distance, making the distance equivalent to in vivo imaging in humans. Subsequently, a 30-mm synthetic tube was placed over the carotid artery as a landmark. The tube was attached to a paratracheal muscle for fixation.

In vivo carotid IMT measurements. B-mode US intima-media thickness (IMT) measurements were performed using a standardized imaging protocol and a Sequoia 512 scanner equipped with an 8L5 transducer (Acuson-Siemens, Erlangen, Germany). Ultrasound scans of the far wall of both left and right common carotid arteries were performed by placing the transducer in the paratracheal incision under a 45° angle from the anterior posterior direction, right next to the synthetic tube. Subsequently, the sonographer selected the best diastolic image as a DICOM (Digital Imaging and Communications in Medicine) standard still capture (Fig. 1). One image per carotid was made. Standardized equipment and protocols were used for image storage and data management.

In vivo 3.0-T MR. MR imaging scans were obtained on a 3.0-T whole-body scanner (3.0-T Intera, Philips Medical Systems, Best, the Netherlands), using a single-element microcoil (Philips, Hamburg, Germany) with a diameter of 5 cm. Axial T_1-weighted turbo spin echo image stacks were acquired at end-diastole using double inversion recovery preparation (Fig. 2). Sequence parameters were: slice thickness: 3 mm, imaging matrix size: 240, field of view: 60 × 60 mm, noninterpolated pixel size: 0.25 × 0.25 mm, echo time: 9 ms, echotrain length: 8, repetition time: according to the piglets' heart rates. Active fat suppression was applied to improve the definition of the outer wall boundary and avoid chemical shift artifacts. All

Figure 1. B-Mode US Image and Image Analysis of the Common Carotid Artery of a 76-kg Female Piglet

High resolution 15-MHz B-mode ultrasound (US) image of the left common carotid artery far wall. The right picture shows the intima-media thickness (IMT) image analysis. IMT is defined as the distance between the lumen-intima (**upper red line**) and the media-adventitia interfaces (**lower red line**). RCCA = right common carotid artery.

imaging was performed with cardiac gating. To obtain ECG signal, 4 superficial incisions were made on the thorax to enable subcutaneous placement of the ECG leads. The scan time was approximately 30 s per slice, depending on the heart rate.
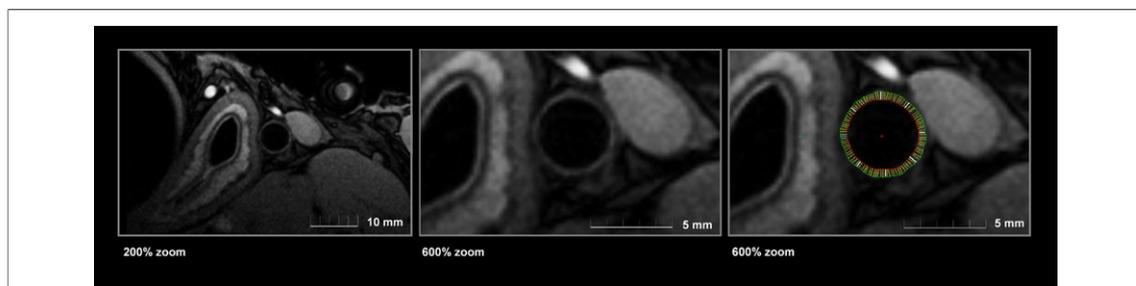
In order to image the same section of the common carotid artery as was imaged by US, the coil was placed in the paratracheal incisions over the synthetic tube. To localize the left and right common carotid artery, axial magnetic resonance angiography images were acquired using a time of flight sequence. These images together with projection images were used for positioning the scan planes perpendicular to the vessel.

Ten slices of the left and right carotid artery were scanned. Each carotid was scanned individually. A total of 20 images were obtained per scan. All images were saved in DICOM format. Standardized equipment and protocols were used for image storage and data management.

Euthanasia, specimen excision, and specimen handling.
Animals were euthanatized after US and MR by an overdose of a potassium infusion, while under deep anesthesia with ketamine and pentobarbital.

The common carotid arteries were exposed in the area of the synthetic tube. The arteries were then marked by placing a small stitch through the outer layer of the artery wall at the level of the upper and lower tip of the tube. The arteries were excised with a 3-cm margin distally and proximally from the upper and lower stitches. After excision, the specimens were put in a Ringer lactate solution.

Subsequently, the carotid arteries were cannulated at both sides with glass tubes and were strung horizontally in a Plexiglas container filled with Ringer lactate solution. The arteries were stretched until the stitches on the artery wall had regained a 30-mm distance, which was the distance they had in vivo. The arteries and glass tubes were attached to a system filled with Ringer lactate solution to

Figure 2. A 3.0-T MR Image and Image Analysis of the Common Carotid Artery of a 76-kg Female Piglet

A cross-sectional image of the left common carotid artery wall. The image analysis is shown on the **right**. The inner and outer borders of the carotid artery are semiautomatically detected with dedicated software (VesselMass, Leiden University Medical Center, Leiden, the Netherlands). The mean wall area has been calculated from the difference between the inner and outer borders. The mean wall thickness is defined as the distance between the inner and outer border (indicated by the **yellow lines**). MR = magnetic resonance.

which a pressure device was attached. The pressure was increased until the artery regained the lumen diameter it had in vivo. Lumen diameter was measured by US. Finally, the Ringer lactate in the system was replaced by 45° C liquid gelatin, and the lumen diameter was kept equal. After the gelatin had cooled off and solidified, both ends of the arteries were tied, detached from the glass tubes, and placed in 4% paraformaldehyde for overnight fixation.

Fixation and Histology

After overnight fixation, cross sections were made perpendicular to the long axis of the artery at 5-mm intervals. The arterial segments were dehydrated with ethanol and xylene, embedded in paraffin at 59°C, sectioned (5 μm), and stained with Elastine von Gieson. The stained slices were photographed with a 2× objective, using an Olympus Soft Imaging Solutions (Münster, Germany) dotSlide imaging system equipped with a BX51 microscope and dotSlide software version 1.2. Images were exported as TIFF (tag image file format) files and had a 3.26 × 3.26-μm resolution.

Ultrasound image analysis. Selected images were analyzed qualitatively and quantitatively offline by a certified image analyzer and validated software (eTrack, Department of Physiology and Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands). One image analyst performed all IMT and lumen diameter measurements. Image analysis was done by identifying the lumen-intima and the media-adventitia boundaries of the carotid arterial far walls (Fig. 1). The method has been described elsewhere (6). Common carotid IMT (CCIMT) was calculated as the average of the mean IMT of the far wall per common carotid artery. Ultrasound mean wall area (US MWA) was calculated using the lumen diameter and CCIMT.

3.0-T MR image analysis. Semiautomated qualitative and quantitative image analyses were performed using semiautomated measurement software (VesselMass, Leiden University Medical Center, Leiden, the Netherlands) (7–9). One image analyst carried out all measurements. The VesselMass software performed automated tracing of the lumen wall boundaries and the outer wall boundaries (Fig. 2). If necessary, the automatically traced boundaries could be manually corrected. The software algorithm for boundary detection is described elsewhere (7–9). Mean wall thickness (MWT) and MWA were measured in all images.

Histology image analyses. Quantitative image analyses were performed using ImageJ software version 1.37 (National Institutes of Health, Bethesda, Maryland). Per carotid artery, 10 slices were used for analysis. Each slice was loaded into ImageJ and converted to 8-bit grayscale. Subsequently, a threshold was applied to create a binary image in which tissue was black and background was white. In this black and white image, the adventitial and periadventitial tissue was removed, and the intima and media remained. The area of the intima and media was then measured (Fig. 3).

Statistical analysis. Continuous variables are expressed as mean ± SD. We calculated the paired difference and the paired mean absolute difference between histology, MR, and US. We calculated the measurement errors of US and MR as follows. Per carotid, we calculated the absolute difference between the imaging modality and histology. Subsequently, we expressed this as a percentage of the histology value of that carotid. We then averaged across samples. Agreement between MR, in vivo US, and histology measurements were assessed with Bland-Altman plots and by describing the mean ± SD of the paired differences among the MR, US, and histology measurements. All statistical analyses were done using SPSS version 14.0 for Windows (SPSS, Inc., Chicago, Illinois).
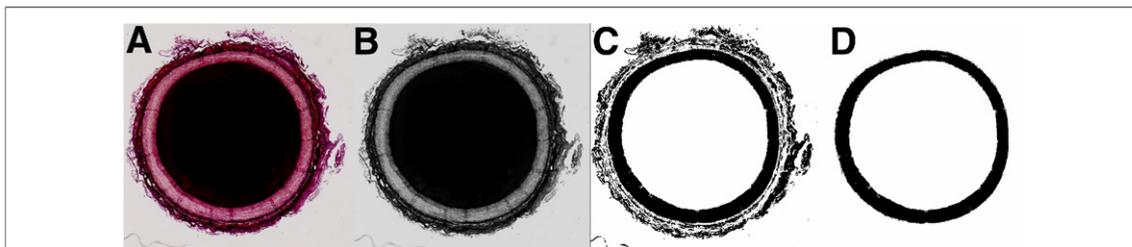

Figure 3. Histology Image and Image Analysis of the Common Carotid Artery of a 76-kg Female Piglet

(A) The Elastine von Gieson stained carotid artery cross section. (B) Conversion of the TIFF image to 8-bit gray scale. (C) Conversion to a binary image. (D) The intima media remains after the adventitia and periadventitial tissue is removed.

RESULTS

Imaging and histology data. Of all 100 MR images, 12 images were inadequate for image analysis. Acquisition time was around 30 s per slice depending on the heart rate. A total of 20 in vivo US images and 100 histology images were made; all were adequate for image analysis.

Mean values (± SD) of histology, MR, and US measurements are listed in Table 1. The mean value (± SD) of the paired difference for histology MWA versus MR MWA was 0.12 (SD: 1.11, p = 0.74) mm^2, histology MWA versus US MWA −0.21 (SD: 1.01, p = 0.53) mm^2, MR MWA versus US MWA −0.33 (SD: 0.72, p = 0.18) mm^2, and MR MWT versus US CCIMT 0.00 (SD: 0.032, p = 0.68) mm. The value (± SD) of the paired mean absolute difference for histology MWA versus MR MWA was 0.87 (SD: 0.65) mm^2, histology MWA versus US MWA was 0.86 (SD: 0.51) mm^2, MR MWA versus US MWA 0.52 (SD: 0.56) mm^2, and MR MWT versus US CCIMT 0.03 (SD: 0.02) mm.

The measurement error for MR MWA was 19% and for US MWA also 19%. The Bland-Altman analysis of histology MWA versus MR MWA and histology MWA versus US MWA are shown in Figure 4. As can be observed, the systematic bias between histology, MR, and US measurements is small and not significant.

DISCUSSION

In the present study, we revealed: 1) a close association between arterial wall dimensions measured by MR or US; and 2) that both MR and US reliably reflected wall dimensions measured by histology. By head-to-head comparison of US, MR, and histol-

ogy, we provide evidence that the IMT, defined as the distance from lumen-intima to media-adventitia tissue boundaries, can be measured using 3.0-T MR. These findings bear relevance for the future application of MR for cardiovascular disease risk stratification and as a surrogate marker for atherosclerosis progression in cardiovascular prevention trials.

Carotid MR versus histology. In this study, carotid artery wall dimensions measured by MR are directly related to the corresponding anatomical tissue. The MR lumen and outer wall boundaries showed a good relation with lumen-intima and media-adventitia interfaces, as measured in histology. Mean values of MR MWA and histology MWA differed by 0.12 mm^2, which is only a 2.6% difference from histology MWA. The fact that the MWA measured by MR is at variance in the order of only tenths of millimeters compared with histology substantiates that T_1-weighted 3.0-T MR images of the common carotid artery wall provide an accurate estimate of the intima-media tissue. From an MR point of view, this is actually a plausible finding as tissue density of the intima and media is higher than adventitial tissue, because adventitial tissue is highly vascularized. As a consequence, T_1 times for intima-media tissue can be expected to be lower than T_1 times in adventitial and periadventitial tissue, resulting in accurate differentiation between these tissues when using a T_1-weighted sequence.

Carotid MR versus US. We cross-correlated our findings by comparing MR and US measurements, for which the anatomical correlate of the US "double line pattern" has been thoroughly investigated. In fact, we observed good concordance between MR and US measurements. We found that MR MWA was only 0.31 mm^2 (6.7%) smaller than US MWA, whereas MR MWT and US CCIMT corresponded even better, with a 0.004 mm (1.2%) difference. The fact that thickness measurements of both modalities were in better agreement than the area measurements pertains to the fact that US MWA is calculated indirectly using both lumen diameter and CCIMT, thereby introducing inaccuracy. The distribution of the MR and US data were also similar, lending further support to the equivalence of MR and US measurements (SD: 0.033 mm for MR MWT and 0.027 mm for US CCIMT).

Accuracy of carotid MR. Concerning the accuracy of imaging the submillimeter carotid artery wall, MR seems to be equally accurate as US. The MR values were on average 0.12 mm^2 smaller than the histol-

Table 1. Mean (± SD) Values of Carotid MR, US, and Histology Measurements

Wall area, mm^2	
Histology MWA	4.69 (0.95)
MR MWA	4.57 (0.41)
US MWA	4.90 (0.50)
Wall thickness, mm	
MR MWT	0.319 (0.033)
US CCIMT	0.323 (0.027
Lumen diameter, mm	
MR	4.27 (0.41)
US	4.52 (0.42)

CCIMT = common carotid intima-media thickness; MR = magnetic resonance; MWA = mean wall area; MWT = mean wall thickness; SD = standard deviation; US = ultrasound.

ogy values, and US values were 0.21 mm^2 larger than the histology values. Also SDs of the paired differences were quite similar for MR and US with regards to the histology (1.11 mm^2 and 1.01 mm^2, respectively). The measurement error for MR was 19%, which is similar to the measurement error for US. The fact that MR and US measurements exhibit substantial agreement in this study is instigated by the use of a 3.0-T MR sequence that images at a very high in-plane resolution of 0.25×0.25 mm. This enables more accurate detection of vessel wall boundaries.

Carotid US versus histology. The US measurements in this study were also similar to the histology values. The US MWA values were 4.90 mm^2, and the histology MWA values were 4.69 mm^2, which is only a 4.5% difference. The anatomical correlate of the US double line pattern has been a matter of debate. Most studies have concluded that the US interfaces at the far wall represent the lumen-intima and the media-adventitia tissue boundaries, respectively. Pignoli et al. (10) was the first to describe this relationship in ex vivo human aorta specimens, and their observations were reproduced in ex vivo experiments by others (11–13). Schulte-Altedorneburg et al. (14) determined the correlation of in vivo common carotid US measurements in 66 moribund neurological patients with postmortem histology measurements, and also reported good correlation. Therefore, the IMT has been widely accepted as the histological correlate to the US measurements. Our findings are in line with these previous results.

Study limitations. A limitation of our study pertains to the fact that ex vivo carotid artery handling and fixation induces artifacts. Specimen fixation induced a shrinkage artifact of 5%. We considered this acceptable. Shrinkage artifacts are a limitation inherent to this type of study, and the magnitude ranges from 5% to 33% in literature (10–14). Another limitation was the fact that 12 of the 100 MR images were not adequate for image analysis. The reason being that the signal-to-noise ratios were poor in these images, mainly due to inadequate coil positioning. Carotid artery imaging in an in vivo porcine model was substantially more challenging than human imaging. Coil positioning was intricate due to the animal's anatomy; the carotid arteries were located much deeper than in the human situation. This made it necessary to make incisions to enable subcutaneous placement of the coil, so the distance of the coil to the artery would be comparable to the human situation.
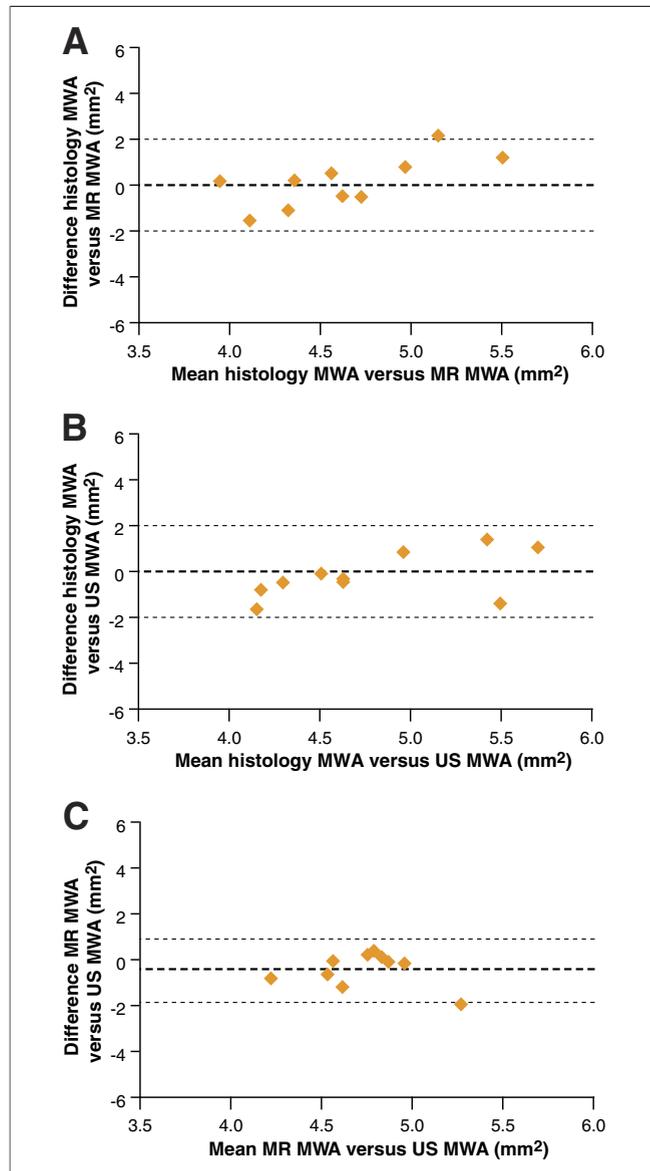

Figure 4. Bland-Altman Plots

Bland-Altman plots of **(A)** histology mean wall area (MWA) versus magnetic resonance (MR) MWA, **(B)** histology MWA versus US MWA, and **(C)** MR MWA versus US MWA. No minimal systematic biases can be observed between the different MWA measurements. The **bold dotted lines** represent the mean differences and the **dotted lines** the 2-SD distance from the mean. SD = standard deviation; other abbreviations as in Figures 1 and 2.

CLINICAL IMPLICATIONS

Imaging the artery wall is an important tool for the assessment of atherosclerosis progression and regression, and has been broadly implemented for cardiovascular disease risk stratification and in cardiovascular prevention studies as a surrogate marker assessing benefit or harm of novel therapeutic interventions. This study shows that 3.0-T MR is

capable of accurate quantification of the dimensions of carotid intima and media and, in fact, is equivalent to histology measurements, which are considered the gold standard.

In addition, the high concordance between 3.0-T MR and B-mode US supports that 3.0-T MR is capable of accurate quantification of carotid intima and media thickness similar to B-mode US, a valid surrogate end point for cardiovascular disease. Moreover, MR allows for circumferential visualization of the vessel wall, thereby increasing reproducibility of the measurements (15). The latter translates into significantly smaller sample sizes and reduction of cost.

Last but not least, ongoing developments in MR are expected to provide additional information on composition of the arterial wall and atherosclerotic plaques, which will assist in identifying individuals at increased risk of developing cardiovascular events and more accurate detection of cardiovascular drug efficacy in terms of plaque stability.

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Key Words: atherosclerosis ■ intima-media thickness ■ carotid artery ■ magnetic resonance ■ ultrasound.