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Accurate Quantification of Atherosclerotic Plaque Volume by 3D Vascular Ultrasound using the Volumetric Linear Array Method

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ABSTRACT

INTRODUCTION: Direct quantification of atherosclerotic plaque volume by three-dimensional vascular ultrasound (3DVUS) is more reproducible than 2DUS-based three-dimensional (2D/3D) techniques that generate pseudo-3D volumes from summed 2D plaque areas; however, its accuracy has not been reported. We aimed to determine 3DVUS accuracy for plaque volume measurement with special emphasis on small plaques (a hallmark of early atherosclerosis).

METHODS: The *in vitro* study consisted of nine phantoms of different volumes (small and medium-large) embedded at variable distances from the surface (superficial vs. >5cm-depth) and comparison of 3DVUS data generated using a novel volumetric-linear array method with the real phantom volumes. The *in vivo* study was undertaken in a rabbit model of atherosclerosis in which 3DVUS and 2D/3D volume measurements were correlated against gold-standard histological measurements.

RESULTS: In the *in vitro* setting, there was a strong correlation between 3DVUS measures and real phantom volume both for small (3.0 to 64.5mm^3 size) and medium-large (91.1 to 965.5mm^3 size) phantoms embedded superficially, with intraclass correlation coefficients (ICC) of 0.99 and 0.98, respectively; conversely, when phantoms were placed at >5cm, the correlation was only moderate (ICC=0.67). In the *in vivo* setting there was strong correlation between 3DVUS-measured plaque volumes and the histological gold-standard (ICC=0.99 [4.02 to 92.5mm^3 size]). Conversely, the correlation between 2D/3D values and the histological gold standard (sum of plaque areas) was weaker (ICC=0.87 [49 to 520mm^2 size]), with large dispersion of the differences between measurements in Bland-Altman plots (mean error, 79.2mm^2).

CONCLUSIONS: 3DVUS using the volumetric-linear array method accurately measures plaque volumes, including those of small plaques. Measurements are more accurate for superficial arterial territories than for deep territories.

Key words (6): Three-dimensional, Vascular Ultrasound, Atherosclerotic Plaque, Plaque Volume, Accuracy, Gold-standard.

Abbreviations:

2D US= two-dimensional ultrasound

2D/3D= 2D-based three-dimensional ultrasound

3DVUS= three-dimensional vascular ultrasound

CT= computed tomography

CV= cardiovascular

ICC= intrasclass correlation coefficient

IFD= inter-frame distance

IMT= intima-media thickness

LoA= limit of agreement

ROI= Region of interest

INTRODUCTION:

Identification of atherosclerotic lesions is increasingly used to improve cardiovascular (CV) risk stratification in asymptomatic populations (1,2). Due to its accessibility and the absence of radiation, vascular ultrasound (US) is widely used for early detection of atherosclerosis. For many years, risk was estimated from measurements of intima-media thickness (IMT) by two-dimensional (2D) US; however, the validity of this approach for individual CV risk stratification has been questioned (2). 2D US plaque measurements such as plaque thickness or area are more accurate predictors of CV events than IMT (3,4), but this method is operator-dependent and shows high variability (5). Recent evidence suggests that quantification of 2D-based plaque burden, rather than area or thickness, is a better predictor of CV events (6,7), prompting the development of new three-dimensional vascular US (3DVUS) methods able to quantify plaque burden with more reproducibility and accuracy.

The most widely-explored "Pseudo-3D" vascular US method for clinical applications is the manual "Freehand 2D/3D-like sweep" method for carotid plaques, in which a conventional 2D probe is manually moved along the patient's neck. Using this 2D/3D-like method, our group recently showed that the identification and quantification of carotid atherosclerotic plaques results in an improvement in risk stratification over traditional equations and coronary calcium score in asymptomatic high-risk individuals (8). However, this 2D/3D-like freehand method is technically challenging because manual sweep speed is not constant and results are not highly reproducible, limiting its use to highly-specialized laboratories (9). In addition, its accuracy for quantifying plaque burden has not been established. To allow estimates of plaque volume and improve reproducibility, the "mechanical sweep" approach emerged, in which an external device is used to move the 2D probe with constant velocity. However, this method is cumbersome and has not been taken into clinical practice. To circumvent these technical limitations, a new 3DVUS methodology has been developed, based on commercially available advanced volumetric-linear array probes (9,10). This modality uses an internally steered array that performs a single-mechanical sweep to acquire high-resolution 3D volume images from a fixed position. 3DVUS

increases the reproducibility of volume quantification for large plaques in the carotid territory (9-11); however, the accuracy of this technique has not been validated *in vivo* and data are lacking on its utility for measuring plaques of different sizes and in different vascular territories. The importance of evaluating early plaques in multiple territories is highlighted by the baseline findings of the PESA (Progression of Early Subclinical Atherosclerosis) study, which reveal a high incidence of plaques in the femoral territory in a low risk population (12).

In this study we present the first validation of the accuracy of 3DVUS for real plaque volume quantification *in vitro* and *in vivo*, with special emphasis on early (small) atherosclerotic plaques and on the influence of the depth of the territory explored.

METHODS

1. *In vitro* phantom study

1.1. Study design

a) Influence of plaque size on volume quantification

Calibration standards or "phantoms" that mimic tissue are widely used to establish the accuracy and reliability of diagnostic ultrasound techniques (13). We designed nine small-volume phantoms, below the 69mm^3 threshold for accurate 3D measurement established for previous methods (14,15), to establish whether 3DVUS can accurately determine small plaque volumes in a controlled laboratory setting. Seven additional phantoms mimicking medium-large plaques (≥70mm^3) were analyzed to establish whether plaque size influences volume quantification accuracy. This phantom set is the largest used to date to validate 3DVUS (9). Phantoms consisted of a rigid polyurethane foam “plaque” placed on the wall of a fake artery: a silicon tube with a 6mm inner diameter and a wall thickness of 0.89mm, similar to the dimensions of a carotid or femoral artery (16) *(Figure 1A)*. The real volumes of polyurethane plaques were determined by weighing them on a high-precision scale and dividing by the polymer density (1.22mg/mm^3;

Sikaflex-1A®, Sika-Industry, Baar, Switzerland). The phantoms were embedded superficially in 2cm-deep agarose blocks to mimic the tissue surrounding arteries (17).

b) Influence of vessel depth on plaque volume quantification

To measure the effect of vessel depth on 3D measurement, three new phantoms were prepared and embedded in an agarose block at a depth of 3cm, a common depth for carotid and femoral arteries. We used small-plaque phantoms in this experiment in order to test the more challenging measurement of small volumes. After imaging and analyzing by 3DVUS more agarose was added to increase the depth to 5cm, mimicking deeper arteries such as the iliac arteries or abdominal aorta, and the phantoms were imaged and analyzed again.

1.2. *In vitro* 3DVUS methodology

The 3DVUS protocol was performed with a Philips iU22 ultrasound system equipped with the VL13-5 3D volume-linear array transducer (Philips Health Care, Andover, MA, USA), which houses an internal mechanically steered array that provides quantifiable 3D volume data. Maintaining a fixed position, the transducer conducts a fan-like continuous automated sweep with a constant angular velocity. The transducer operates over a wide frequency range (13 to 5MHz), and the inter-frame distance (IFD) is controlled by adjusting the angular sweep length from 10 to 30 degrees to obtain inter-frame distances between 0.1 mm and 0.3 mm. The length of vessel scanned thus depends on the defined angular length. The phantom acquisition protocol consisted of a 30 degree sweep. A 30 degree acquisition protocol was selected in order to determine the accuracy achieved with the lowest resolution (the largest IFD) of the novel volume-linear array probe. The probe was centered on an axial view of the phantom. Depth, focus, and gain were adjusted, and automatic scan was initiated. The 3D volumetric image was reviewed and accepted only if the phantom was correctly centered on the *x*, *y* and *z* axes *(Figure 1B)*.

The acquired 3DVUS images were analyzed using the Vascular Plaque Quantification (VPQ) feature of QLAB 10.2 (Philips Health Care, Andover, MA, USA). VPQ fits three 3D meshes, one for the outer-wall of the vessel, another for the inner-wall and a final one for the plaque boundary.

The outer-wall boundary at the level of the plaque and plaque boundary meshes are then used to calculate plaque volume as the three-dimensional space between them. This plaque boundary contour has the advantage that it can be fitted to calculate the plaque volume alone, excluding healthy portions of the surrounding vessel wall (Figure 1C). The 3D dataset is displayed as multiple consecutive transverse slices. To analyze atherosclerotic plaques, a semiautomatic delineation tool was developed based on a gray-scale threshold procedure. The outer and inner vessel borders are manually adjusted using a moldable ellipsoid ROI, and the plaque outline is then automatically traced and smoothness and sensitivity of the gray-scale are adjusted to fit the plaque boundary *(Figure 1C).* After delineating the begin frame, end frame and key frames, the program automatically extrapolates contours throughout the vessel image. Readers review and correct the automatically propagated contours and boundaries, and can manually delineate the plaque outline if the automated tool is affected by artifacts, plaque features, or a poor acoustic window. For phantom analysis, begin and end frames were set at the phantom edges and one key frame was set at the point of maximum phantom thickness.

2. *In vivo* validation by histology

2.1. Study design

The accuracy of 3DVUS for plaque volume quantification in peripheral atherosclerotic plaques was tested in the New Zealand White rabbit model, in which atherosclerosis is induced with the angioplasty-balloon aortic endothelial denudation method followed by a cholesterol-rich (0.2%) diet for 30 weeks (18,19). The adult rabbit abdominal aorta is similar to human carotids in size (≈6mm) and depth (≈2-4cm). Moreover, atherosclerotic plaques generated in this rabbit model resemble human early atherosclerotic plaques in size and composition (19). The distance from the origin of the left renal artery and the right renal artery were used as internal reference points for matching the *in vivo* US imaging studies with histology. Rabbit abdominal aortas were scanned at week 30 by the 2D/3D-like freehand and 3DVUS methods, with focus on the inter-renal segments, to detect atherosclerotic plaques at this level and quantify plaque volume. After

completion of the imaging studies, aortas were removed, fixed in 10% buffered-formalin, and processed for histological analysis. Inter-renal aortic segments were cut into cross-sectional slices every 0.2 mm with a section thickness of 4μm, corresponding to the IFD on US plaque images. Sections were stained with hematoxylin and eosin, and slides were digitalized. All animal studies were performed under the approval of the Institutional Animal Research Committee and carried out in compliance with the Guide for the Care and Use of Laboratory Animals.

2.2. *In vivo* methodologies for 2D/3D-like and 3DVUS ultrasound

Rabbits were sedated with ketamine (10 mg/kg IM) and xylazine (2.5 mg/kg IM) and positioned supine on the exploration table with the abdomen shaved. For the 2D/3D-like method, a Lio9-15 2D high-resolution linear array transducer (Philips Health Care, Andover, MA, USA) was used to scan the abdominal aorta in cross-section; the transducer was moved manually from the proximal to the distal segment of the vessel, including the origin of the left and right renal arteries, in about 10 seconds at a constant scan sweep velocity. The acquired images were read with QLAB software. Consecutive plaque areas expressed in mm^2 from each frame were analyzed and summed in the 2D freehand sweep for plaque burden estimation.

For 3DVUS, the acquisition protocol consisted of a 30 degree sweep, corresponding to a scan length of approximately 6cm, with the probe centered on an axial view of the abdominal aorta at the origin of the left renal artery (*x* axis) and parallel to the long-axis of the aorta (*y* axis). The sweep thus included the origin of the left and right renal arteries. Focus, depth, and gain were optimized, and the 3D sweep was performed automatically in about 2 seconds. The 3D volumetric image was accepted only if the vessel was correctly centered on the 3 dimensional axes and was free of any movement artifact *(Figure 2A)*. The acquired 3DVUS images were read according to the procedure described for the *in vitro* experiment, setting the begin and end frames at the histologically matched aortic segment edges and with one key frame set at the point of maximum plaque thickness *(Figure 2B)*.

2.3. Histological measurements of plaque volume

Planimetric analysis of histological images was performed using NDP viewer software (NDP.view2 Hamamatsu Photonics, UK). In brief, we traced the inner media boundary (yellow), the outer or adventitia-media boundary (red), and the plaque border (green) in all images, obtaining internal, intermediate, and external areas, respectively (*Figure 2C*). We calculated the following parameters: lumen area was the internal area, plaque area was the difference between intermediate and lumen areas, and wall area was the difference between external and lumen areas, similar to the slice-by-slice 3DVUS analysis. Datasets of contiguous slices were obtained for each plaque specimen, and plaque volume was calculated as the sum of plaque areas multiplied by the inter-slice distance.

3. Statistics

For the *in vitro* study, ultrasound phantoms were scanned and analyzed by three sonographers experienced in vascular US and blinded to the real volumes. Interobserver reproducibility was calculated for plaque volume measurement to estimate the effect of variability on the accuracy of plaque volume estimation. Moreover, systematic reading errors were minimized by taking the mean of the 3 readers' measurements, and variation was estimated from the standard deviation (SD) of the calculated mean. We conducted a feasibility analysis to determine the ability of the semiautomated analysis tool to detect early atherosclerotic plaques and the need for manual editing of the plaque contours. Accuracy, agreement and possible observer-induced bias in the 3DVUS methodology were evaluated by Pearson's correlation coefficient (r); the mean of the relative changes ± 1SD, calculated as the mean of the percentage differences between observed volumes relative to real volumes ({observed volume-real volume}/real volume x 100); intraclass correlation coefficient (ICC); and Bland-Altman plots.

For the *in vivo* histological study, one cardiologist experienced in US imaging of rabbits and blinded to the histological measurements scanned and made the offline QLAB reading for the 2D/3D-like and 3DVUS images. Comparisons were made between the summed plaque areas

measured from histological and 2D/3D-like data, as well as between the plaque volume measurements from histological and 3DVUS data. Accuracy, agreement, and bias were evaluated by r correlation, ICC, and Bland-Altman plots. For ICC, good agreement was defined as >0.70 and excellent agreement as >0.90. For Bland-Altman plots, the limits of agreement (LoA) lines were calculated and plotted, representing the mean±2SD of the inter-measurement difference. Statistical analyses were conducted using Stata12 (StataCorp LP, Texas, USA).

RESULTS

1. *In vitro* phantom validation study: accuracy of 3D volume quantification

a) Influence of plaque size on volume quantification

The semiautomatic plaque analysis tool successfully discriminated all phantom plaque outlines. The mean real volume of small phantom plaques was 22±19.7mm^3 (range 3.03-64.5mm^3) and the 3DVUS measured volume was 24±18.7mm^3 (range 3.9-60.8mm^3). For medium-large phantom plaques, mean real volume was 351.6±306mm^3 (range 91.1-965.5mm^3) and the 3DVUS volume was 398.3±323.4mm^3 (range 121.3-1037.2mm^3). Accuracy of volume quantification was excellent for small phantom plaques (ICC=0.99; r=0.992 p<0.001) and medium-large phantoms (ICC=0.98; r=0.999 p<0.001) *(Figure S1 A and B)*. Bland-Altman plots confirmed this good agreement. 3DVUS slightly overestimated the volumes of larger plaques (Figure 3B). The mean measurement error was 1.3mm^3 (LoA -5.9-8.4mm^3) for small plaques (mean of the relative changes 19±12%) and 46.7mm^3 (LoA 87.8-5.6mm^3) for medium-large plaques (mean of the relative changes 14±10%).

There was good reproducibility of plaque volume measurement: ICC = 0.998 (IC95% 0.994-0.999) for observers 1 and 2, ICC = 0.996 (IC95% 0.982-0.992) for observers 1 and 3, and ICC = 0.997 (IC95% 0.986-0.999) for observers 2 and 3.

b) Influence of vessel depth on small plaque volume quantification

The mean real volume of the set of phantom plaques examined in the vessel depth experiment was 26.9±25.3mm^3 (range 6.5-55.2mm^3). 3DVUS-measured volume for superficially embedded phantoms was 27.9±26.8mm^3 (range 6.4-57.9mm^3), showing excellent accuracy (ICC=0.99; r=0.997 p<0.001) *(Figure S1C)*. A Bland-Altman plot confirmed this good agreement *(Figure 3C)*. In contrast, the 3DVUS-measured volume for deeply embedded phantoms was 47.8±41.9mm^3 (range 11.8-93.8mm^3), which deviated significantly from the real volume (ICC = 0.67) *(Figure S1D)*. A Bland-Altman plot showed large dispersion of the differences between observations, with mean = 20.828mm^3 and LoA -12.659 to 54.316mm^3 *(Figure 3D)*.

2. *In vivo* validation by histology

The *in vivo* US studies used to define plaques induced at the inter-renal aortic segment detected nine atherosclerotic plaques, all of which were validated by histology. The 3D semiautomatic plaque analysis tool detected all plaques. For most plaques, the semiautomatic plaque outline required slight manual editing for perfect alignment with the plaque boundaries. The main cause of semiautomatic tool failure was the presence of low-echogenic plaques typical of early atherosclerotic disease. The mean histological volume of the nine plaques was 34.2±29.04mm^3 (range 3.9 to 96 mm^3), and the mean 3DVUS-measured plaque volume was 33.4±27.99mm^3 (range 4.02 to 92.5mm^3). As judged by comparison to the histological volumes, the accuracy of plaque volume quantification by 3DVUS was excellent (ICC=0.99; r=0.998 p<0.001), with Bland-Altman plots showing very small dispersion of the differences, without systematic bias (*Figure 4 A and C*).

When evaluated by the 2D/3D-like method, the mean sum of plaque areas was 203.2±158.1mm^2 (rage 49 to 520mm^2), while in the histological analysis, the mean sum of plaque areas was 124±95.5mm^2 (rage 19.1 to 316mm^2). This results in a weaker correlation with the sum calculated by histological analysis (ICC =0.86), with Bland-Altman plots showing large dispersion of the differences (mean error of 79.212 mm^2 and LoA -55.883 to 214.307mm^2), together with a

systematic increase in the overestimation of plaque area with increasing plaque size *(Figure 4 B and D).*

DISCUSSION:

Accurate quantification of atherosclerotic burden requires a reliable method for measuring plaque volume. In the present study, we show that 3DVUS using a novel volume-linear array probe accurately quantifies plaque volume regardless of plaque size. Notably, 3DVUS precisely quantified small plaques, a hallmark of early atherosclerosis, when risk stratification is of greatest value. However, the accuracy of 3DVUS for plaque volume quantification is limited for deep vessels, suggesting that this technique is better suited for superficial vessels (i.e. carotids and femorals).

Previous studies have evaluated the accuracy of 3DVUS for plaque volume quantification using *in vit*ro phantoms resembling large plaques (9,11,20). Our study is the first to comprehensively evaluate the *in vitro* accuracy of this novel method across a range of phantom volumes, including those resembling small plaques. Although estimated and real plaque volumes correlate closely, the Bland-Altman volume graphs show a slight overestimation for larger phantom plaques with the 3DVUS method. Despite this small systematic error, there is no proportional bias, and all differences fall in a narrow window within the limits of agreement, supporting the overall good performance of 3DVUS for plaque volume estimation. Phantoms were analyzed with the semiautomatic tool, which fits contours to the plaque boundary using a gray-scale threshold algorithm. Although phantoms are calibration imagines with well-defined contours, the semiautomatic fit is imperfect, and minute errors can lead to slight overestimation of plaque volume. These errors can be detected by experienced sonographers, and overestimation could thus be reduced by editing the contours manually. In the in vivo study, we used semiautomatic analysis with manual corrections when needed, and Bland-Altman plots showed no estimation errors and volume measurement proved to be highly accurate. The effect of the error due to semiautomatic fitting is very small and unlikely to be clinically significant; moreover, automatic analysis

improves the reproducibility of volume measurements. Another way to improve semiautomatic tool performance would be to use a smaller interframe distance in 3DVUS acquisition (20 or 10 degree sweep), thereby increasing plaque image resolution.

The present study is also the first *in vivo* validation of this method, showing that 3DVUS-quantified plaque volume correlates closely with plaque volume assessed by the histological gold standard. Moreover, 3DVUS performed better than the 2D/3D-like freehand method, a pseudo-3D method used to indirectly estimate plaque volume (6,8). A key factor for accurate 3D volume acquisition by 3DVUS is the sweep motion along the third dimension (*z* axis) of the image, which determines the IFD and thus the image resolution. So far, three approaches have been developed for acquiring 3D images of vessels *(Figure 5)*. The 2D/3D-like freehand method resulted in a variable IFD, and the resolution of the old "mechanical sweep" methods (from 0.5 to 5mm IFD) remains limited (21). Moreover, these methods are cumbersome and difficult to standardize and implement in clinical practice across centers. In the 3DVUS method, an internally steered linear array makes a single mechanical sweep to acquire high-resolution 3D volume images (0.1 to 0.3mm IFD) from a fixed position, simplifying image acquisition and improving resolution. The reproducibility of volume measurements with these three techniques has been determined as a surrogate of their accuracy in previous studies (22). Although constant sweep velocity improves measurement reproducibility (9), accurate determination of plaque volume is also determined by the IFD (21). Indeterminate or widely separated IFD likely underlie the poor axial resolution of small plaques (< 70 mm^3) reported for the 2D/3D-like freehand method and mechanically steered external 2D probes, limiting their use in early atherosclerosis (14,15,20). Despite its limitations, we used the 2D/3D-like freehand method for comparison with 3DVUS because it is the most widely used pseudo-3D US method for estimating plaque burden (6,8). In the present report, the lower accuracy of the 2D/3D-like freehand method compared with the histological gold standard is thus consistent with the previous studies of reproducibility. In contrast, the 3DVUS method overcomes these limitations both *in vitro* and *in vivo*, and results in a good correlation with the histological gold-standard.

The accuracy of a biomarker such as plaque volume is an important determinant of the reliability of new clinical strategies. A precise knowledge of biomarker accuracy is important for ensuring generalizability of thresholds and cut-off values between centers, achieving precise evaluation of disease progression/regression, and ensuring reliable follow-up data. Our results establish 3DVUS as the first 3D method that accurately estimates plaque burden, particularly in relation to small plaques. This method is therefore especially suited to the evaluation of subclinical disease. 3DVUS is currently being used in the PESA trial to study the determinants of plaque burden presence and progression in the carotid and femoral arteries of more than 4000 apparently healthy middle-aged individuals (23). Our data also show that the depth of the territory being explored has an impact on the accuracy of 3DVUS for plaque volume quantification. Interestingly, the most frequently affected vascular territory in the baseline PESA study was the femoral, followed by the carotids. Both these territories are superficial and thus not affected by the limitations of deep vessels described here. In contrast, our results question the reliability of the 3DVUS method for the evaluation of large arterial territories below a depth of 5cm. The 3D volume-linear array probe has a broad operating frequency in 2D mode that in principle should permit scanning of superficial and deep structures; however, the 3D image is constructed from a series of 2D fan-like images, and the fan-form of the sweeping US beam means that IFD increases slightly with increasing depth *(Figure S2)*. This effect has been reported to degrade resolution in the elevational plane (y axis) (24) and is a plausible source of the observed error in small-plaque quantification at higher penetration depths in our experiment. The fact that most early atherosclerotic plaques in the PESA cohort were found in superficial vascular territories adds value to the use of 3DVUS as a reliable technique for plaque volume quantification, and eventually risk re-stratification, in low-to-mid risk populations.

One limitation of this study is the relatively small number of phantoms designed for the *in vitro* experiment, particularly the 3-phantom experiment assessing the effect of depth. The phantom experiments also did not assess the effect of plaque shape on the accuracy of volume measurement. However, these limitations do not affect the *in vivo* experiment, in which we

evaluated volume measurement accuracy for real plaques with different morphologies and, more importantly, different plaque composition, including varying degrees of calcification. The *in vivo* model also allowed the evaluation of atherosclerotic plaques under physiological conditions of vessel pulsatility, and accuracy was comparable to the *in vitro* model. The use of histology as the gold standard for plaque volume estimation has been linked to fixation and processing problems. Histological assessment can be affected by artifacts and shrinkage, but these in fact depend on the degree of atherosclerosis in the processed arterial segment. Siegel et al (25) first showed that in vessels with mild atherosclerotic narrowing, standard tissue processing results in a non-significant change in absolute measurements of plaque area. More recent studies reported very small changes, of around 5%, when using modern histological techniques. This evidence supports the reliability of our results, and although histological shrinkage cannot be excluded, any effect would similarly influence the precision of the 3DVUS and 2D/3D-like methods.

CONCLUSIONS:

3DVUS with the novel volumetric-linear array method gives highly accurate measures of plaque volume regardless of plaque size. The accuracy is very high for plaques present in superficial territories and lower for those in deeper territories (>5cm). This technique is significantly more accurate than the currently used 2D/3D-like freehand method. 3DVUS has the potential to improve risk stratification based on atherosclerotic plaque burden.

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FIGURE TITLES AND LEGENDS

Figure 1. Small plaque phantom

(A) An ultrasound phantom before embedding in agarose. The polyurethane "plaque" is marked by an arrow. **(B)** 3D automatic sweep acquisition of a small phantom plaque. Panels **1, 2** and **3** show the *X, Y* and *Z* axis views of the phantom. **(C)** Semiautomatic delineation of consecutive axial views of the phantom with VPQ software.

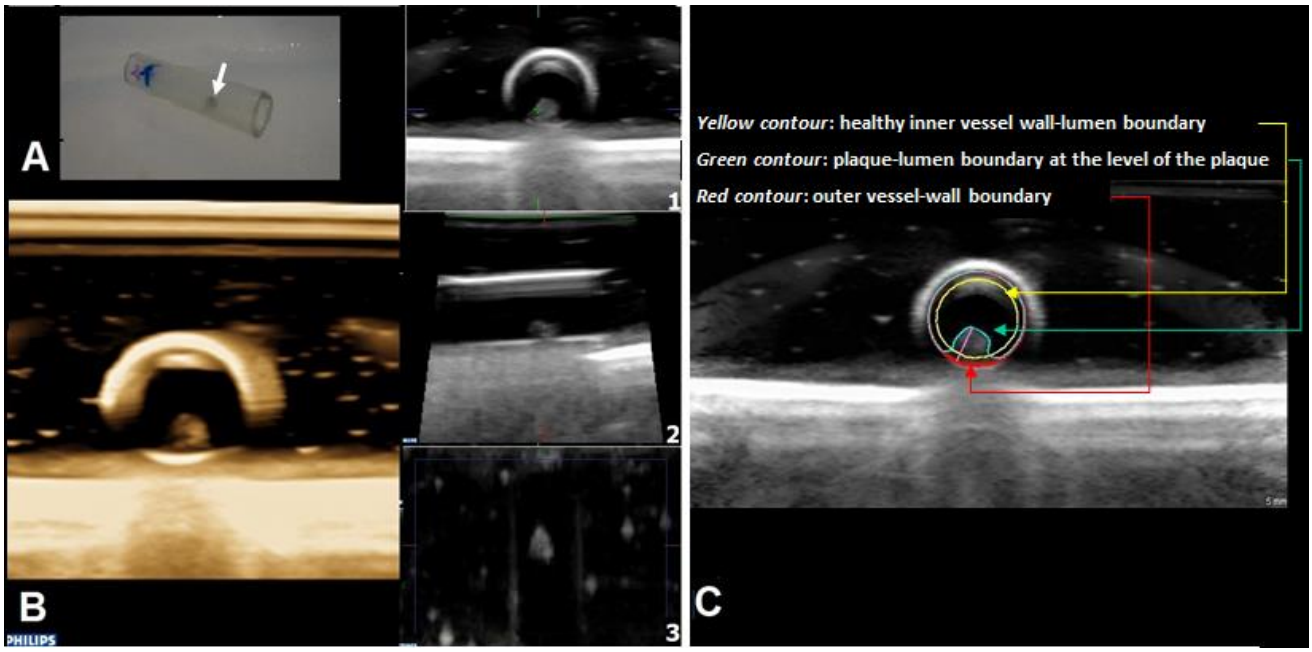


Figure 2. Atherosclerotic plaque quantification on 3DVUS matched with histological findings

(A) 3D automatic sweep acquisition of an atherosclerotic plaque located at the origin of the left renal artery in the abdominal aorta of a New Zealand rabbit. **(B)** Semiautomatic delineation of an axial view of the atherosclerotic plaque with VPQ software and **(C)** a matched histological cross section of the plaque. Red and yellow contours delimitate outer and inner vessel borders. The green contour delimits the plaque border, excluding healthy portions of the surrounding vessel wall or the origin of arterial branches (asterisk).

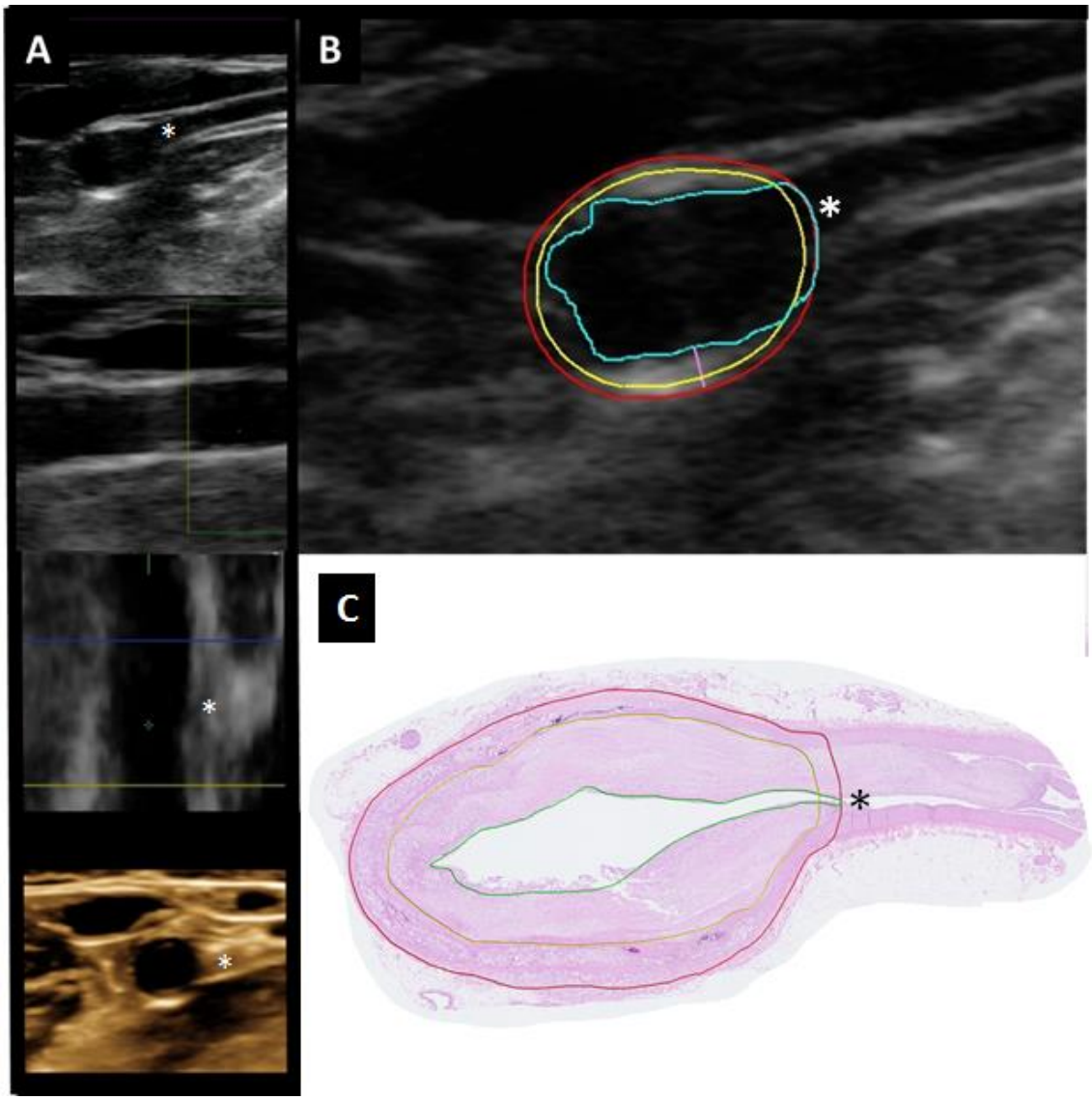


Figure 3. *In vitro* accuracy of volume quantification using 3DVUS

Bland-Altman plots of volume measurement in small and medium-large plaque phantoms **(A and B)**. Bland-Altman plots of volume measurement in small plaque phantoms placed at different depths **(C and D)**. Values are means of readings taken by three independent observers.

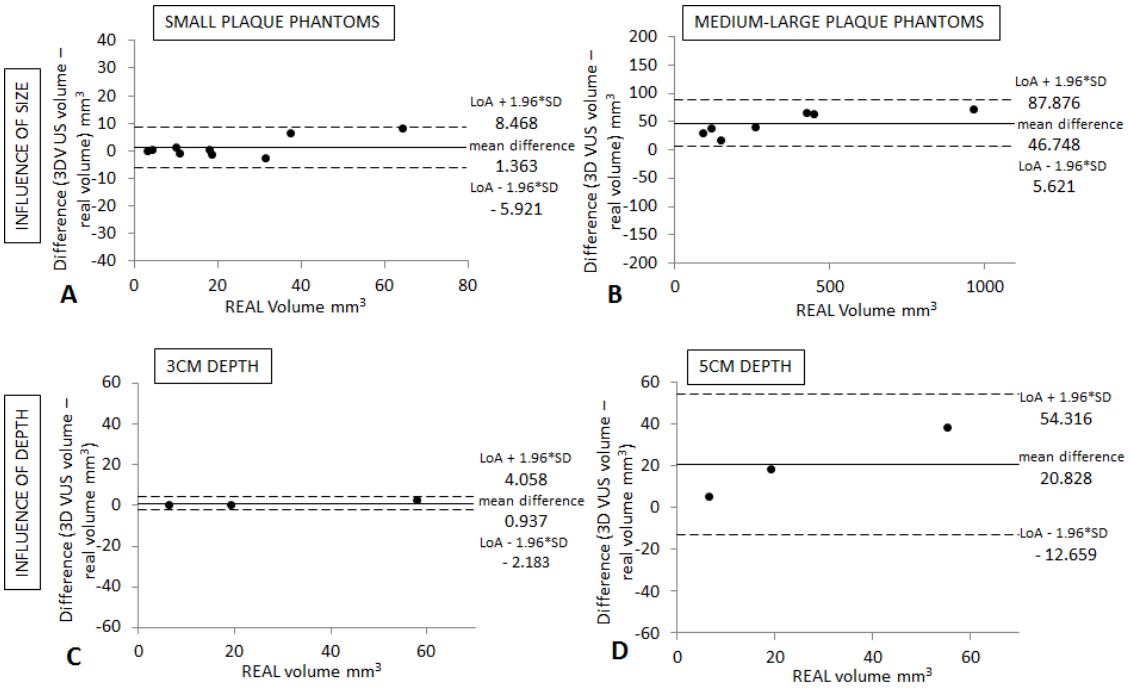


Figure 4. *In vivo* accuracy of 3DVUS and the 2D/3D-like freehand method

Accuracy of 3DVUS volume measurement compared with histology **(A and C)**. Accuracy of volume estimation by 2D/3D-like freehand sum of plaque areas compared with histology **(B and D)**.

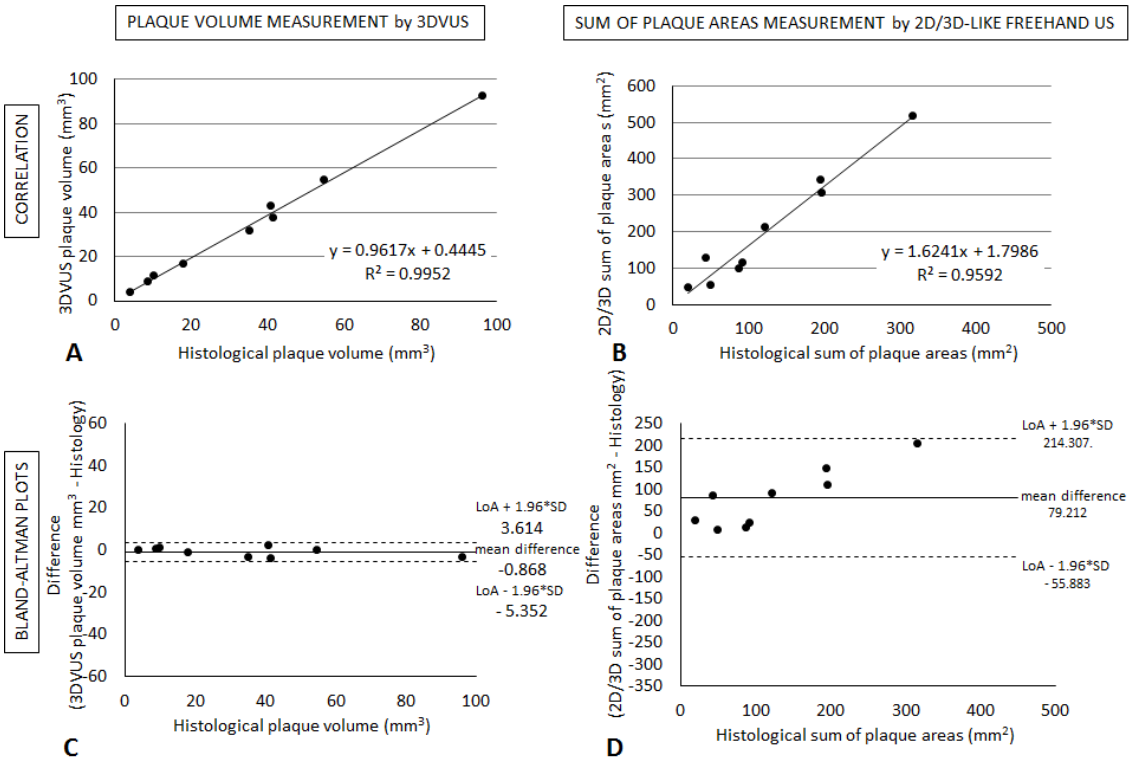
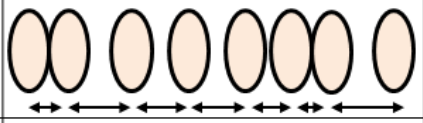
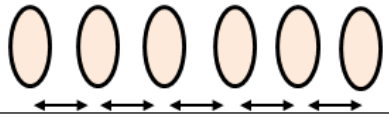
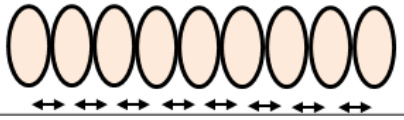


Figure 5. 3D US approaches, their 3D resolution and limitations

		ADQUIRED FRAMES (Scheme)	INTERFRAME DISTANCE (Resolution)	UNITS	LIMITATIONS
MANUAL METHODS	2D/3D-LIKE FREEHAND		Variable & Unknown	Sum of areas (mm^2)	No Volume Poor inter- and intra-observer agreement.
MECHANICAL METHODS	EXTERNALLY STEERED		Constant 0.5 to 5 mm	Volume (mm^3)	Poor accuracy in detecting and quantifying small plaques.
	3DVUS VOLUME-LINEAR ARRAY		Constant 0.1 to 0.3 mm	Volume (mm^3)	For superficial plaques only (<5cm-depth).

↔ Interframe distance O Consecutive axial images of the vessel by US

SUPPLEMENTARY DATA

Figure S1. *In vitro* accuracy of volume quantification using 3DVUS

Correlation of volume measurement in small and medium-large plaque phantoms with the "real" volume (calculated from mass and density). Values are means of the readings by three observers. Vertical error bars represent ± 1 standard deviation. **(A and B)** Correlation of volume measurement for different plaque sizes. **(C and D)** Correlation of volume measurement in small phantoms placed at different depths, showing good results for superficial phantoms but large variation between measurements for deep phantoms.

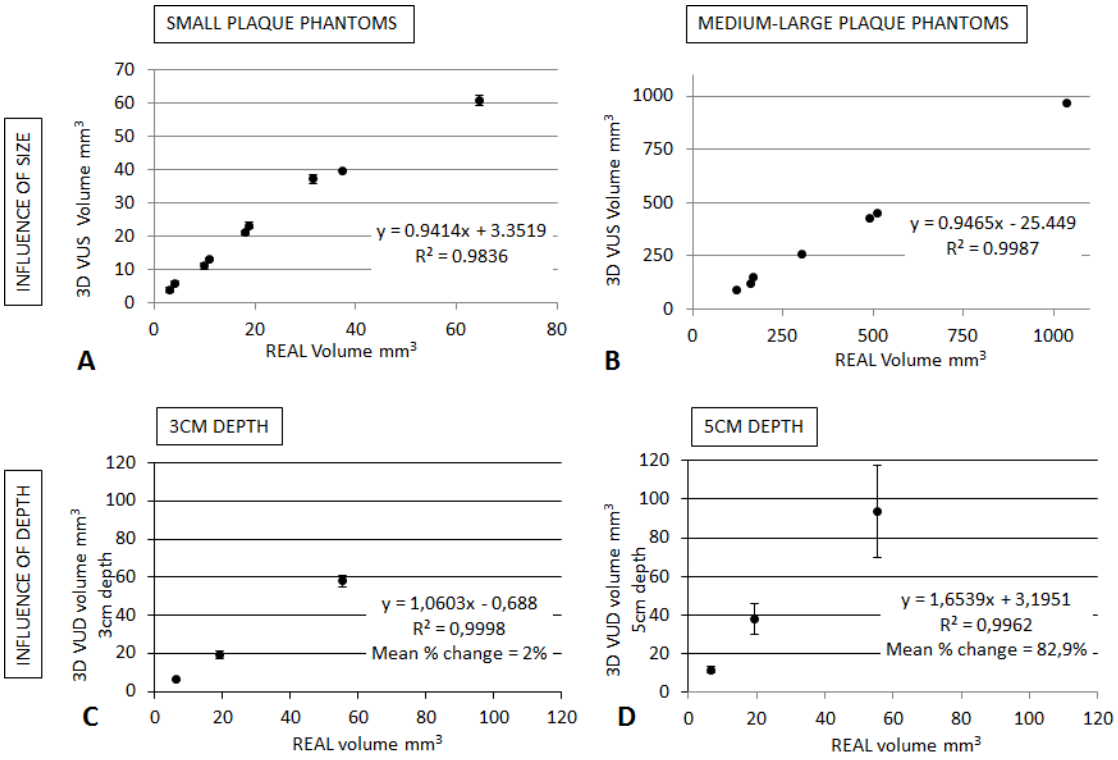


Figure S2. Scheme illustrating the loss of resolution with depth (*y* axis) due to the fan-like movement the 3DVUS volume-linear array probe