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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. >5 cm-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 (3.0–64.5 mm^3 size) and medium-large (91.1–965.5 mm^3 size) phantoms embedded superficially, with intraclass correlation coefficients (ICC) of 0.99 and 0.98, respectively; conversely, when phantoms were placed at >5 cm, 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 [4.02–92.5 mm^3 size]). Conversely, the correlation between 2D/3D

Abbreviations: 2D US, two-dimensional ultrasound; 2D/3D, 2D-based three-dimensional ultrasound; 3DVUS, three-dimensional vascular ultrasound; CT, computed tomography; CV, cardiovascular; ICC, intraclass correlation coefficient; IFD, inter-frame distance; IMT, intima-media thickness; LoA, limit of agreement; ROI, region of interest.

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values and the histological gold standard (sum of plaque areas) was weaker (ICC = 0.87 [49–520 mm^2 size]), with large dispersion of the differences between measurements in Bland-Altman plots (mean error, 79.2 mm^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.

1. 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.

2. Methods

2.1. In vitro phantom study

2.1.1. 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 69 mm^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 ($\geq$70 mm^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 6 mm inner diameter and a wall thickness of 0.89 mm, similar to the dimensions of a carotid or femoral artery [16] (Fig. 1A). The real volumes of polyurethane plaques were determined by weighing them on a high-precision scale and dividing by the polymer density (1.22 mg/mm^3; Sikaflex-1A®, Sika-Industry, Baar, Switzerland). The phantoms were embedded superficially in 2 cm-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 3 cm, 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 5 cm, mimicking deeper arteries such as the iliac arteries or abdominal aorta, and the phantoms were imaged and analyzed again.

2.2. 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 5 MHz), and the inter-frame distance (IFD) is controlled by adjusting the angular sweep length from 10 to 30° 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° sweep. A 30°