

ORIGINAL RESEARCH

New 3-Dimensional Volumetric Ultrasound Method for Accurate Quantification of Atherosclerotic Plaque Volume



Beatriz López-Melgar, MD, PhD,^{a,b,c,*} Virginia Mass, BSc,^{a,*} Paula Nogales, MSc,^a Javier Sánchez-González, PhD,^d Robert Entrekin, MSc,^e Antoine Collet-Billon, MSc,^e Xavier Rossello, MD, PhD,^{a,f} Leticia Fernández-Friera, MD, PhD,^{a,c,g} Antonio Fernández-Ortiz, MD, PhD,^{a,g,h} Javier Sanz, MD,^{a,i} Jacob F. Bentzon, PhD,^{a,j} Héctor Bueno, MD, PhD,^{a,g,k} Borja Ibáñez, MD, PhD,^{a,g,l} Valentín Fuster, MD, PhD^{a,i}

ABSTRACT

BACKGROUND Carotid and femoral plaque burden is a recognized biomarker of cardiovascular disease risk. A new electronic-sweep 3-dimensional (3D)-matrix transducer method can improve the functionality and image quality of vascular ultrasound atherosclerosis imaging.

OBJECTIVES This study aimed to validate this method for plaque volume measurement in early and intermediate-advanced plaques in the carotid and femoral territories.

METHODS Plaque volumes were measured ex vivo in pig carotid and femoral artery specimens by 3-dimensional vascular ultrasound (3DVUS) using a 3D-matrix (electronic-sweep) transducer and its associated 3D plaque quantification software, and were compared with gold-standard histology. To test the clinical feasibility and accuracy of the 3D-matrix transducer, an experiment was conducted in intermediate-high risk individuals with carotid and femoral atherosclerosis. The results were compared with those obtained using the previously validated mechanical-sweep 3D transducer and established 2-dimensional (2D)-based plaque quantification software.

RESULTS In the ex vivo study, the authors assessed 19 atherosclerotic plaques (plaque volume, 0.76 μ L-56.30 μ L), finding strong agreement between measurements with the 3D-matrix transducer and the histological gold-standard (intraclass correlation coefficient [ICC]: 0.992; [95% CI: 0.978-0.997]). In the clinical analysis of 20 patients (mean age 74.6 \pm 4.45 years; 40% men), the authors found 64 (36 carotid and 28 femoral) of 80 scanned territories with atherosclerosis (measured atherosclerotic volume, 10 μ L-859 μ L). There was strong agreement between measurements made from electronic-sweep and mechanical-sweep 3DVUS transducers (ICC: 0.997 [95% CI: 0.995-0.998]). Agreement was also high between plaque volumes estimated by the 2D and 3D plaque quantification software applications (ICC: 0.999 [95% CI: 0.998-0.999]). Analysis time was significantly shorter with the 3D plaque quantification software than with the 2D multislice approach with a mean time reduction of 46%.

CONCLUSIONS 3DVUS using new matrix transducer technology, together with improved 3D plaque quantification software, simplifies the accurate volume measurement of early (small) and intermediate-advanced plaques located in carotid and femoral arteries. (J Am Coll Cardiol Img 2022;15:1124-1135) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

From the ^aCentro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; ^bCardiology Department, Hospital Universitario de La Princesa, IIS-IP La Princesa, Madrid, Spain; ^cHM CIEC MADRID (Centro Integral de Enfermedades Cardiovasculares), Hospital Universitario HM Montepíncipe, HM Hospitales, Madrid, Spain; ^dPhilips Healthcare, Iberia, Madrid, Spain; ^ePhilips Healthcare, Bothell, Washington, USA; ^fCardiology Department, Health Research Institute of the Balearic Islands (IdISBa), Hospital Universitari Son Espases, Palma, Spain; ^gCIBER de Enfermedades Cardiovasculares (CIBERCv), Madrid, Spain; ^hHospital Universitario Clínico San Carlos IdiSSC, Madrid, Spain; ⁱThe Zena and Michael A. Wiener Cardiovascular

Imaging-based biomarkers of subclinical atherosclerosis improve individual cardiovascular risk prediction compared with conventional risk scales based on clinical parameters.^{1,2} The most studied biomarker is the computed tomography-based coronary artery calcium score (CACs); nevertheless, ultrasound is free of ionizing radiation and can detect plaques in large peripheral arteries from very early stages before calcification.³ The European Society of Cardiology guidelines now recommend ultrasound assessment of carotid and/or femoral atherosclerosis burden for cardiovascular risk evaluation,^{4,5} reflecting the ability of atherosclerosis burden measured by 3-dimensional vascular ultrasound (3DVUS) to predict individual cardiovascular risk,⁶ almost matching the prognostic performance of CACS.^{7,8} This good performance is likely because 3DVUS provides a more comprehensive evaluation of overall atherosclerosis burden, avoiding the drawbacks of 2DVUS, together with good reproducibility of plaque measurements.⁹

Several 3-dimensional (3D) approaches have been developed to simplify and standardize 3D image acquisition. The VL13-5 volumetric-linear array probe uses the “mechanical-sweep” method and generates accurate and reproducible measurements of carotid and femoral atherosclerosis burden from early to more advanced disease stages^{9,10} regardless of plaque size.^{11,12} However, the functionality of this approach is limited by the large probe footprint, which hinders examination of angulated surfaces or small fields of view. A new commercially available 3D vascular probe (XL14-3) based on matrix technology performs an “electronic-sweep” that improves image quality for the study of atherosclerosis, and the transducer’s smaller footprint makes it easier to manipulate during the ultrasound exploration. The 3D-matrix probe is supported by dedicated semiautomatic software that allows 3D analysis of the explored arterial segment. A previous report confirmed excellent interscan reproducibility for carotid atherosclerosis assessment¹³; however, the accuracy of the 3D-matrix probe for plaque burden quantification has not been established. More importantly, the new 3D-matrix

transducer has not been tested previously for its ability to detect and quantify early atherosclerosis (plaques smaller than 69 μ L), a cornerstone of primary prevention strategies, that is underestimated by old 3D methods because of technical limitations.^{10,14,15} In this study, we present the first validation of the electronic-sweep 3D-matrix

transducer for accurate plaque volume quantification *ex vivo* in a pig model of atherosclerosis, with a focus on small plaques. In addition, we compared the ability of the 3D-matrix transducer to detect and quantify carotid and femoral plaques in patients with intermediate-advanced atherosclerosis with that of the previously validated mechanical-sweep VL13-5 volumetric-linear array transducer.¹⁰

METHODS

EX VIVO EXPERIMENT FOR GOLD STANDARD HISTOLOGICAL VALIDATION.

The accuracy of the 3D-matrix probe for plaque volume quantification was tested using *ex vivo* carotid and femoral specimens from 13 pigs with liver overexpression of a human gain-of-function mutant (D374Y) of protein convertase/subtilisin kexin type 9 (PCSK9^{D374Y}). When placed on a cholesterol-rich diet, these animals generate atherosclerotic lesions that resemble human atherosclerotic plaques in size and composition.¹⁶ The pig carotid and femoral arteries are of a similar size to their human homologs; however, they are located particularly deep in the adult animal (~5 cm-8 cm for the femoral arteries and >10 cm for the common carotid arteries), limiting ultrasound evaluation *in vivo*. Hence, both carotid and femoral arteries were removed from pigs to create phantoms for use as calibration standards¹⁷ to establish the accuracy and reliability of new 3D probe against gold-standard histological measurements (**Central Illustration A**).

Three arteries were severely damaged during extraction, and thus the final number of *ex vivo* phantoms generated was 49 (23 carotid and 26 femoral phantoms). 3DVUS was performed with a Philips Epiq ultrasound system equipped with the

ABBREVIATIONS AND ACRONYMS

3DVUS = 3-dimensional vascular ultrasound

CACS = coronary artery calcium score

ICC = intraclass correlation coefficient

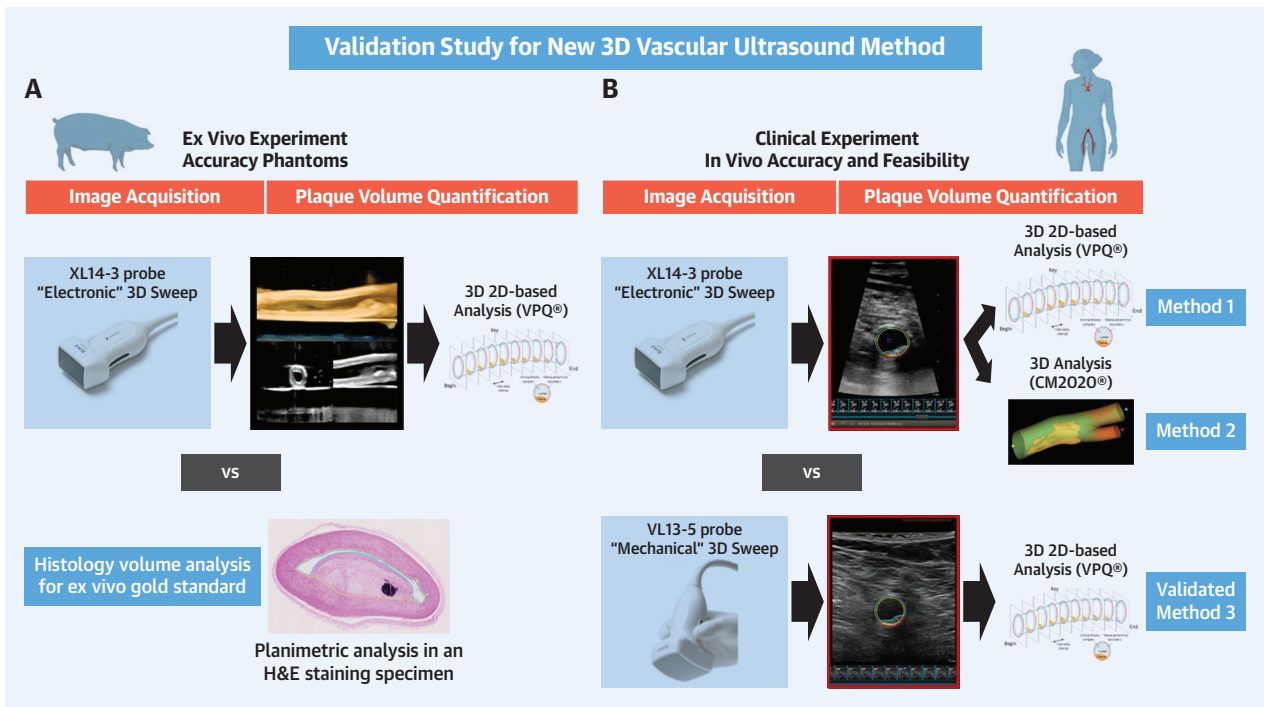
Institute/Marie-Josée and Henry R. Kravis Center for Cardiovascular Health, Mount Sinai School of Medicine, New York, New York, USA; [†]Heart Diseases and Steno Diabetes Center Aarhus, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; [‡]Cardiology Department, Hospital Universitario 12 de Octubre and Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain; and the [§]IIS-Fundación Jiménez Díaz Hospital, Madrid, Spain. *Dr López-Melgar and Mrs. Mass are joint first authors.

Tasneem Naqvi, MD, served as Guest Editor for this paper.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the [Author Center](#).

Manuscript received June 30, 2021; revised manuscript received January 1, 2022, accepted January 6, 2022.

CENTRAL ILLUSTRATION Study Design



López-Melgar B, et al. *J Am Coll Cardiol Img.* 2022;15(6):1124-1135.

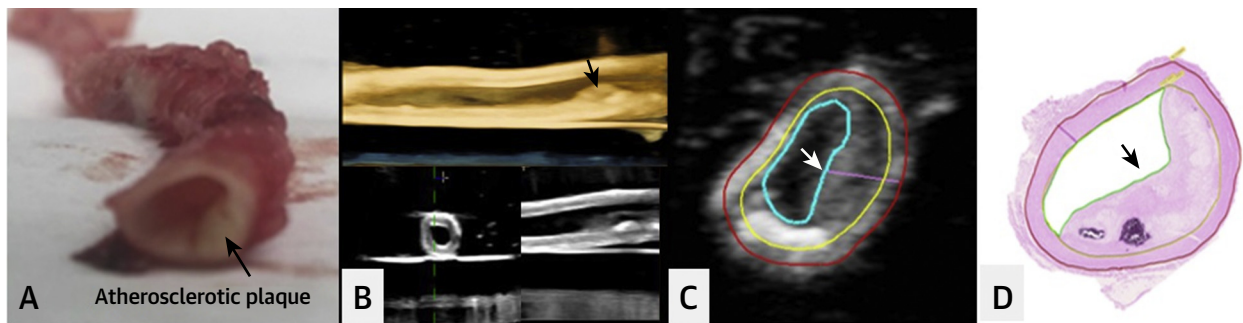
(A) In the ex vivo experiment, plaque volume measurement with the XL14-3 probe and VPQ software was validated against gold standard histological measurements using realistic phantoms prepared from diseased porcine arterial specimens. (B) In the in vivo experiment, the feasibility and accuracy of plaque volume measurement with the XL14-3 probe and VPQ software (method 1) was compared with the previously validated method using the VL13-5 probe and VPQ software (method 3), and the feasibility and accuracy of plaque volume measurement with the new CM2020 software (method 2) was compared with method 1. 2D = 2-dimensional; 3D = 3-dimensional.

XL14-3 3D-matrix array transducer (Philips Healthcare). The XL14-3 transducer performs an “electronic” 3D sweep from a fixed position and provides quantifiable 3D volume data. The phantom acquisition protocol consisted of a 25° sweep because this provided a field of view sufficiently large to visualize all of the plaques embedded in the phantoms. The probe was centered on a longitudinal view of the phantom. Acquired 3D XL14-3 images were analyzed with a modified version (not commercially available) of the previously validated Volume Plaque Quantification (VPQ) software (Philips Healthcare).¹⁰ VPQ enables volume analysis from images obtained with the VL13-5 probe, so it had to be modified to be able to analyze data from the XL14-3 transducer. VPQ displays acquired 3D volumes as multiple consecutive transverse slices (frames) with a fixed interframe distance on which readers trace the outer-wall (red), inner-wall (yellow), and the plaque boundary (green) contours

(Figure 1), which guide the semiautomatic tool to extrapolate contours along the whole vessel image (semiautomatic slice-by-slice analysis of the vessel). Readers can review and manually correct the automatically propagated contours and plaque boundaries. Plaque volume is then calculated as the 3D space between the outer wall and plaque boundaries.

After completion of the imaging studies, carotid and femoral specimens were fixed in 10% buffered-formalin, processed, and cut into cross-sectional slices for histological analysis. Histological measurements of plaque volume were performed with QuPath software by planimetric analysis of histological images. The plaque area for each image was quantified as the difference between the inner media boundary (yellow line) and the plaque border (green line) (Figure 1), similar to the slice-by-slice 3DVUS analysis by VPQ. Datasets of contiguous slices were obtained for each plaque specimen, and plaque volume was

FIGURE 1 Matching of 3DVUS Atherosclerotic Plaque Quantification With Histological Findings



(A) An ex vivo porcine carotid artery (phantom) before embedding in agarose. **(B)** Electronic-sweep 3DVUS acquisition of an agarose-embedded phantom. **(C)** Semiautomatic delineation of consecutive axial views of the phantom with VPQ software. **(D)** Matched histological cross section of the same plaque. **Red lines**, outer-wall boundary; **yellow lines**, inner-wall boundary; **blue/green lines**, residual lumen. **Arrows** mark an atherosclerotic plaque.

calculated as the sum of plaque areas multiplied by the interslice distance. More details on the study design, the technical aspects of the new probe, and the acquisition and analysis of the images are included in the [Supplemental Methods](#).

All animal studies were performed at the Centro Nacional de Investigaciones Cardiovasculares (CNIC) with the approval of the Animal Protection Area of the Comunidad Autónoma de Madrid (Ref. PROEX265/16) and in compliance with the Guide for the Care and Use of Laboratory Animals.

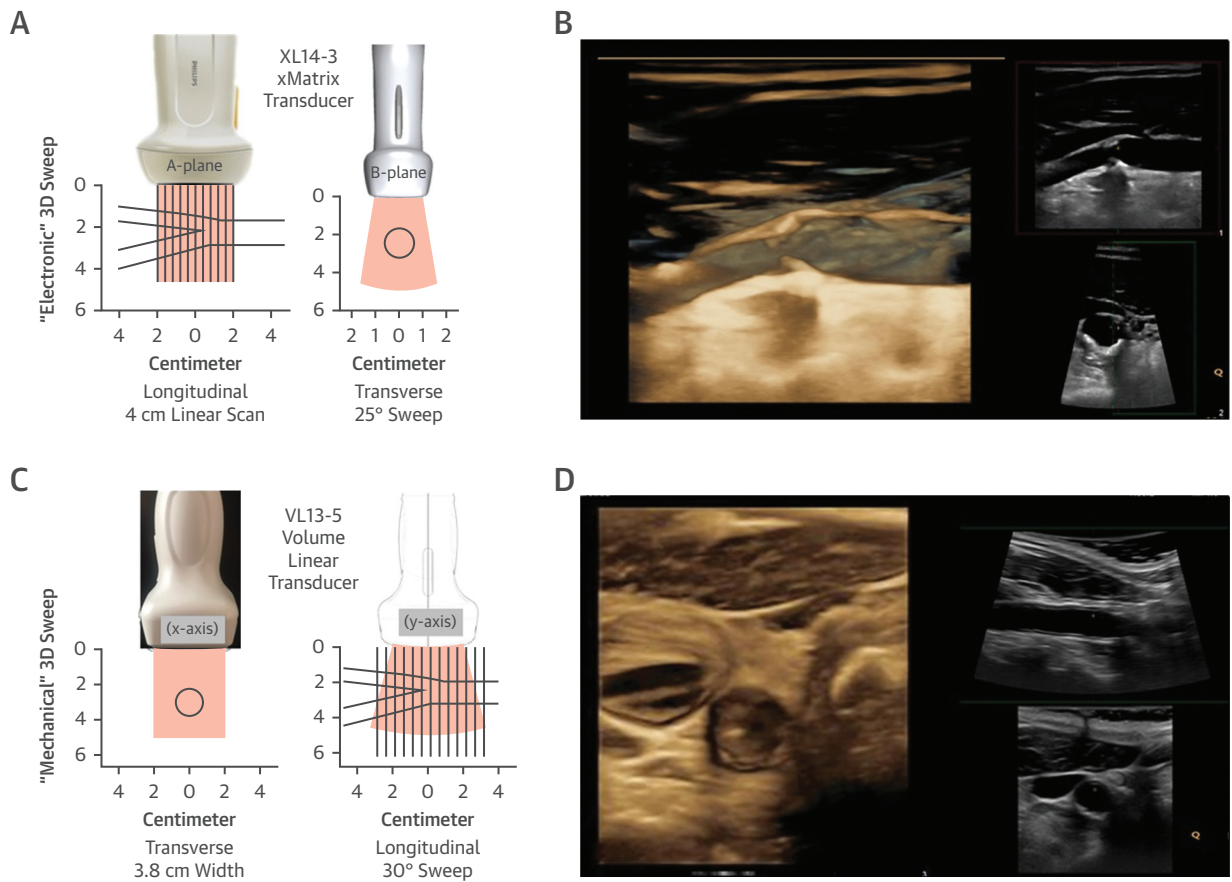
IN VIVO EXPERIMENT TO ASSESS THE CLINICAL FEASIBILITY AND ACCURACY OF THE 3D-MATRIX TRANSDUCER. The feasibility and accuracy of the XL14-3 3D-matrix transducer was tested in a sample of cardiovascular disease-free participants (age 74.6 ± 4.45 years; 40% men) included in the CNIC Athero-Brain: H2H (Head to Heart) study. To avoid sample selection bias, we included all consecutive patients who attended their second H2H study visit until we obtained the estimated sample size of 20 individuals. The date selected for starting recruitment was arbitrary. Participants were scanned in the carotid and femoral territories, first with the electronic-sweep XL14-3 probe, and then with the mechanical-sweep VL13-5 probe. For the XL14-3 3D-matrix transducer, we replicated the acquisition protocol used for ex vivo phantoms. The probe was aligned on a longitudinal view of the carotid artery centered at the carotid bulb and on a longitudinal view of the femoral artery centered at the bifurcation, yielding a scanned volume length of 4 cm in each case. For the VL13-5 mechanical probe, the 3D sweep was performed following previously validated methodology for

patients.⁹ We adjusted the angular sweep length in the longitudinal plane to 30° to obtain a pyramid-shaped 3D volume with a ≈ 6 -cm-long base. However, the border error had to be eliminated to remove the fan-like acquisition, and arteries varied in their depth and tortuosity, so that the usable longitudinal coverage at the arterial level varied between 3 and 5 cm. Acquisition with the VL13-5 probe was also centered on the carotid bulb and femoral bifurcation, but in this case, the central images were oriented in an axial view of the artery following standard methodology. Technical features of carotid plaque burden assessment and images obtained from the same patient are shown in [Figure 2](#).

Images obtained with the XL14-3 3D-matrix transducer were analyzed with VPQ software and according to the procedure described for the ex vivo experiment (method 1) for its comparison against the validated method (VL13-5 and VPQ method 3). The XL14-3 acquisitions were also analyzed with a new unreleased research software that uses a complete 3D approach for plaque quantification, the Carotid Model CM2020 (version 123, Philips Research), identified as method 2, and the results were compared with those obtained by analyzing the same 3DVUS images using the VPQ software for validation as illustrated in [Central Illustration B](#). Plaques were defined according to the Mannheim criteria as focal protrusions into the arterial lumen >0.5 mm, $>50\%$ of the intima-media thickness, or intima-media thickness >1.5 mm.¹⁸ Plaque burden was quantified by measuring the volumes of all atherosclerotic plaques visualized in each acquisition (by territory).

For the feasibility analysis, “difficult arteries” were defined as those with specific features that may

FIGURE 2 Technical Features and Examples of Electronic and Mechanical 3D-Sweep Acquisitions for the Clinical Evaluation of Carotid Atherosclerotic Burden



(A) Technical features of electronic-sweep acquisition with the XL14-3 3D-matrix transducer. **(B)** Standardized 3-dimensional (3D) images obtained with the XL14-3 probe. The acquired 3D volume is centered at the carotid bulb. The XL14-3 probe is designed to obtain the best image resolution when the A-plane of the transducer is aligned with the longitudinal view of the carotid artery. **(C)** Technical features of mechanical-sweep acquisition with the VL13-5 transducer. **(D)** Standardized 3D images obtained with the VL13-5 probe in the same patient as in **B**. The acquired 3D volume is centered at the carotid bulb. Acquisition with the VL13-5 probe is optimally oriented in an axial view of the artery, parallel to the long-axis.

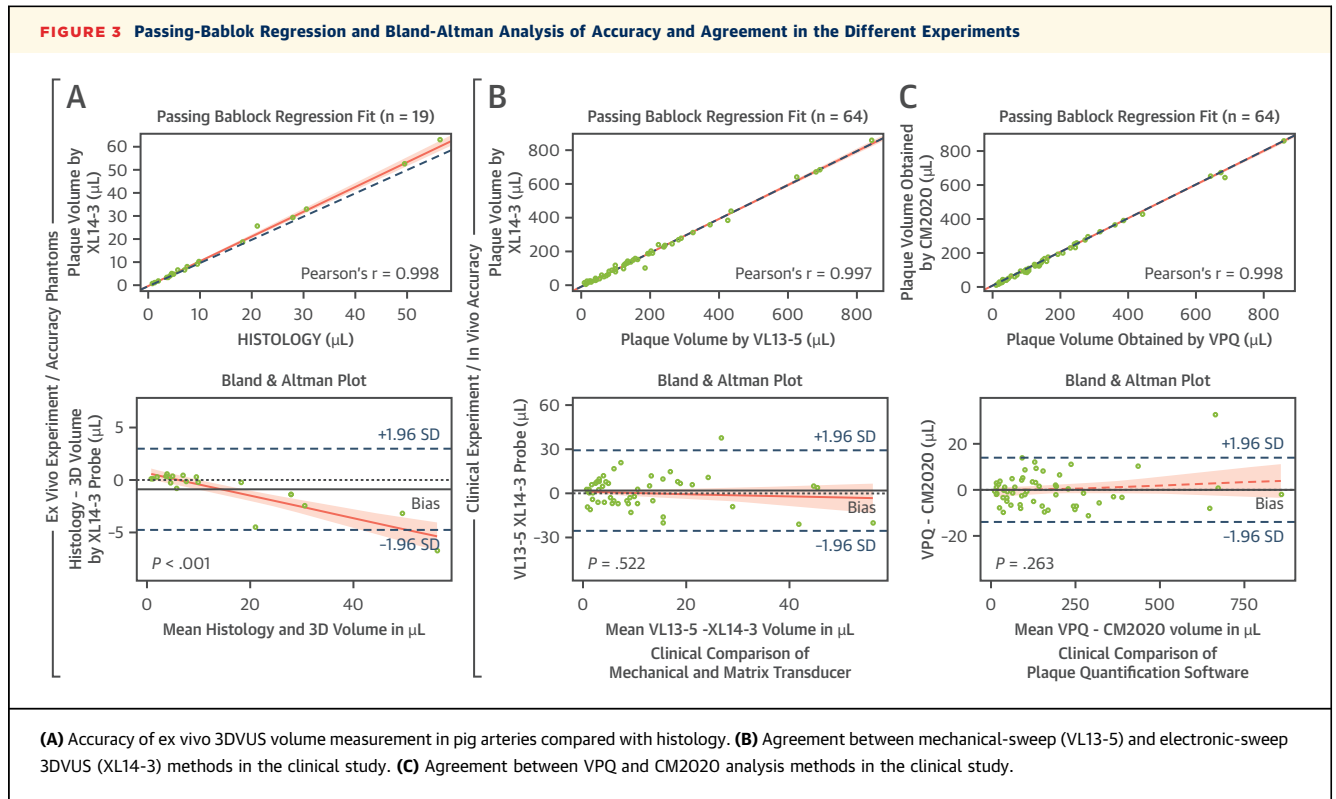
limit plaque acquisition (deep or tortuous vessels that hinder the inclusion of all arterial segments in a single 3D volume). Patients with a “difficult anatomy” were identified as those with a short, narrow, or angulated neck or inguinal area that impeded scanning maneuvers. We also identified 3 specific features that can limit plaque analysis: “low-echodensity” (echogenicity similar to blood), “calcification” (causing significant acoustic shadowing), and “complex morphology” (highly irregular surface and possible surface defects). In addition, efficiency improvement with CM2020 was also assessed by comparing the time required to perform a complete vessel analysis with both applications. All images were analyzed by an expert sonographer specialized in cardiovascular imaging,

who was blind to the histological data (V.M.). The repeat analyses with CM2020 software was separated from the first analysis by at least 1 month. Image analysis with CM2020 is detailed in the [Supplemental Methods](#), and an example of plaque analysis is displayed in [Supplemental Figure 1](#).

The clinical study complied with the principles of the Helsinki declaration and was approved by the Ethical Research Committee of the Instituto de Salud Carlos III (ISCIII 49_2017/HU120 17/027).

STATISTICS AND DATA INTERPRETATION.

Continuous variables are presented as means ± SD. Both the degree of agreement between plaque volumes assessed by different techniques and the



presence of outliers were assessed with the intraclass correlation coefficient (ICC), Passing-Bablok (PB) regression analysis, Bland-Altman plots, and Lin's concordance correlation coefficients (CCC). The intraobserver and interobserver reproducibility of plaque volume analysis using the CM2020 application was assessed in 20 studies of randomly selected plaques using ICC analysis. We used IBM SPSS23, RStudio and Medcalc software to conduct the statistical analysis. Statistical significance was set at $P < 0.05$. A more detailed explanation on statistical analysis and data interpretation is provided in the [Supplemental Methods](#).

RESULTS

EX VIVO VALIDATION BY HISTOLOGY. The ex vivo ultrasound analysis of carotid and femoral artery specimens detected 19 atherosclerotic plaques, all of which were validated by histology. Mean plaque volume by histology was $14.11 \pm 16.23 \mu\text{L}$ (range: $0.76 \mu\text{L}$ - $56.30 \mu\text{L}$) and by 3DVUS was $15.01 \pm 17.98 \mu\text{L}$ (range: $0.63 \mu\text{L}$ - $63.04 \mu\text{L}$). Absolute and relative discordances between imaging and histology are detailed in [Supplemental Table 1](#). The median absolute difference was $0.36 \mu\text{L}$ [IQR: $0.23 \mu\text{L}$ - $1.09 \mu\text{L}$], and

the maximum difference $6.74 \mu\text{L}$. As judged by comparison with histology-determined volumes, the accuracy of plaque volume quantification by 3DVUS was excellent (ICC: 0.992 [95% CI: 0.978 - 0.997]; CCC: 0.991 [95% CI: 0.986 - 0.995]; both with $P < 0.001$). However, PB regression detected a small systematic bias, with 3DVUS slightly overestimating plaque volume, a tendency more marked for larger plaques. These findings were consistent with the results of Bland-Altman analysis ([Figure 3A](#)). ICC, PB, Bland-Altman, and concordance data are detailed in [Tables 1 to 4](#), respectively.

IN VIVO FEASIBILITY AND VALIDATION ANALYSIS IN THE CLINICAL SETTING. All 80 arterial territories from 20 patients (100% of 3DVUS acquisitions) were

TABLE 1 Accuracy Agreement and Consistency of Agreement Between Plaque Volume Measurements in the Different Validation Comparisons

Compared Plaque Volume Measurements	n	Intraclass Correlation Coefficient	
		Absolute Agreement (95% CI)	Consistency of Agreement (95% CI)
XL14-3 3DVUS vs Histology	19	0.992 (0.978 to 0.997)	0.993 (0.983 to 0.997)
XL14-3 3DVUS vs VL13-5 3DVUS	64	0.997 (0.995 to 0.998)	0.997 (0.995 to 0.998)
VPQ QLab vs CM2020 (3DVUS)	64	0.999 (0.998 to 0.999)	0.999 (0.998 to 0.999)

3DVUS = 3-dimensional vascular ultrasound.

TABLE 2 Intercept, Slope, and Residual SD of Passing-Bablok Regression Between Plaque Volume Measurements in the Different Validation Comparisons

Compared Plaque Volume Measurements	n	Passing-Bablok Regression			Test for Linearity, P Value
		Intercept (95% CI)	Slope (95% CI)	Residual SD (95% CI)	
XL14-3 3DVUS vs histology	19	-0.53 (-0.81 to -0.19)	1.08 (1.04 to 1.12)	0.80 (-1.58 to 1.58)	0.66
XL14-3 3DVUS vs VL13-5 3DVUS	64	-2.00 (-2.98 to 0.14)	1.00 (0.98 to 1.02)	10.01 (-19.62 to 19.62)	0.34
VPQ QLab vs CM2020 (3DVUS)	64	0.37 (-1.99 to 2.20)	1.00 (0.98 to 1.02)	6.44 (-12.62 to 12.61)	0.81

3DVUS = 3-dimensional vascular ultrasound.

successfully evaluated, with good image quality defined in most of the XL14-3 and VL13-5 studies. General patient characteristics, including body size, and specific plaque features by patient are shown in [Supplemental Table 2](#). No images were excluded from the analysis caused by technically inadequate image quality. Both transducers coincided in detecting atherosclerosis in 64 of the 80 explored territories (36 carotid and 28 femoral arteries; plaque burden per territory: 10 to 859 μ L).

The VPQ software successfully discriminated all plaques in the images acquired with both transducers. Mean plaque volume with the XL14-3 matrix transducer (method 1) was 158.8 ± 176.9 μ L (range: 10 μ L-859 μ L) and with the VL13-5 mechanical transducer (method 3) was 160.8 ± 175.8 μ L (range: 9 μ L-839 μ L). Plaque volume quantification showed excellent agreement between transducers (ICC: 0.997 [95% CI: 0.995-0.998]; CCC: 0.997 [95% CI: 0.995-0.998]; both with $P < 0.001$). Good agreement was confirmed by PB, Bland-Altman, and Lin's analysis ([Figure 3B](#), [Tables 1 to 4](#)).

In a case-by-case comparison between the XL14-3 and the VL13-5 probes (detailed data not shown), the XL14-3 transducer slightly underestimated total plaque burden compared with the VL13-5 transducer in 2 cases that exceeded the upper limit of agreement on the Bland-Altman plot. On the other hand, the VL13-5 probe slightly underestimated plaque burden in 6 studies (4 carotid and 2 femoral arteries), all featuring difficult arteries and/or difficult anatomies. However, overall plaque volume estimates with the XL14-3 probe maintained close agreement with the VL13-5 acquisitions according to PB and Bland-Altman analysis, with a small mean bias of 2 μ L that was not statistically significant ([Figure 3B](#)).

The performance of the 2 probes with a difficult artery and patient anatomy is illustrated in [Supplemental Figure 2](#), which shows improved image quality and penetration of the XL14-3 transducer.

The same 64 atherosclerotic territories (of the total of 80 arterial studies) were detected with CM2020 software analysis of the XL14-3 acquisitions (method 2). Mean plaque volume with CM2020 was 158.7 ± 176.9 μ L (range: 10.2 μ L-861.5 μ L), and high precision was indicated by close agreement with VPQ assessment of XL14-3 acquisitions (ICC: 0.999 [95% CI: 0.998-0.999]; CCC: 0.997 [95% CI: 0.998-0.999]; both with $P < 0.001$). This excellent agreement was confirmed by PB regression, Bland-Altman plots, and Lin's coefficient ([Figure 3C](#), [Tables 1 to 4](#)). In almost all cases, plaque burden analysis with CM2020 was faster than with VPQ software (394 ± 177 milliseconds vs 735 ± 554 milliseconds, respectively; $P < 0.001$) ([Figure 4](#), [Supplemental Figure 3](#)), with a mean time reduction of $46 \pm 21\%$ for full vessel readings.

Intraobserver and interobserver reproducibility for plaque volume measurement were similarly good, with ICC values of 0.998 [95% CI: 0.996-0.999] for intraobserver agreement and 0.857 [95% CI: 0.669-0.941] for interobserver agreement ([Supplemental Tables 3 and 4](#)).

DISCUSSION

Accurate assessment of cardiovascular disease risk would require precise quantification of individual subclinical atherosclerosis burden. In the present study, we demonstrate that 3DVUS with the novel XL14-3 3D-matrix probe and CM2020 software accurately quantifies plaque volume in a shorter time and shows high precision for the evaluation of early atherosclerotic plaques, a challenging scenario where risk stratification is of greatest value. In addition, electronic-sweep 3DVUS with the XL14-3 transducer accurately quantifies plaque burden in the carotid and femoral arteries, a recommended image-based biomarker for cardiovascular disease risk assessment in clinical guidelines.^{4,5}

Due to poor axial resolution, first-generation 3D volume acquisition methods with external

TABLE 3 Bias and Lower and Upper Limits of Agreement Obtained From Bland-Altman Plots

Compared Plaque Volume Measurements					Bland-Altman Plot		
Method A	Method B	n	Method A Mean ± SD	Method B Mean ± SD	Bias (95% CI)	Lower Limit of Agreement (95% CI)	Upper Limit of Agreement (95% CI)
Histology	XL14-3 3DVUS	19	14.10 ± 16.23	15.01 ± 17.98	-0.90 (-1.85 to 0.04)	-4.78 (-6.42 to -3.12)	2.97 (1.32 to 4.62)
VL13-5 3DVUS	XL14-3 3DVUS	64	160.78 ± 175.76	158.80 ± 176.91	1.98 (-1.52 to 5.49)	-25.54 (-31.62 to -19.46)	29.51 (23.43 to 35.59)
VPQ QLab	CM2020 (3D)	64	144.47 ± 142.57	144.58 ± 140.52	0.22 (-2.49 to 2.25)	-17.40 (-21.93 to -13.70)	17.55 (13.47 to 21.70)

Reference, method A measurements. Comparison method, method B measurements.
 3DVUS = 3-dimensional vascular ultrasound.

mechanical sweep do not accurately detect and quantify early (small) plaques,¹⁵ even for plaque volumes up to 69 μ L.¹⁴ New methods for the assessment of subclinical atherosclerosis cannot be clinically validated without invasive procedures, the exposure of ostensibly healthy individuals to radiation (with computed tomography), or long scan times (with magnetic resonance). Animal models of atherosclerosis provide a helpful alternative, especially those that closely resemble the human disease. Previous studies support the use of artificial phantoms resembling plaques for validation purposes,^{12,15,19} prompting us to design a set of realistic phantoms using diseased porcine arterial specimens. This ex vivo analysis showed that plaque volume quantified by electronic-sweep XL14-3 probe closely matched that measured by gold-standard histology. A key feature of this analysis is that fresh specimens, although evaluated ex vivo, maintain their ultrasound features,²⁰ thus allowing a realistic and detailed assessment of plaques that explores possible quantification biases arising from differences in plaque composition or shape. However, modern histological techniques of fixation and processing have been linked to changes in plaque features and slightly shrinkage-related changes of larger atherosclerotic plaques.²¹ This may explain the small proportional bias in plaque volumes obtained by electronic XL14-3 transducer ex vivo compared with histology. Although histological shrinkage cannot be excluded, the XL14-3 probe and histology estimates of plaque volume showed close agreement.

Advances in 3D probes have not only improved image quality and resolution, but have also simplified and standardized image acquisition, making procedures easier to implement in clinical practice. The XL14-3 probe is compact and has a smaller footprint than high-resolution mechanical-sweep 3D probes. This resulted in a slightly smaller acquired field-of-view (FOV) in electronic acquisitions with the XL14-3 probe than that expected for mechanical

acquisitions with the VL13-5 probe. The overall excellent agreement in volume measurements between the 2 methods with standardized acquisition protocols may reflect the fact that the theoretical \approx 6-cm FOV in the mechanical method translates into an actual variable 3- to 5-cm FOV available for plaque burden analysis, depending on artery depth and tortuosity. This is because the mechanical method uses a fan-like sweep that generates a pyramid-shaped 3D data set, producing a smaller FOV for superficial arteries than for deep arteries, as well as decreased image quality at greater depths and at the lateral image borders (Figure 2). Despite its smaller FOV, the small size of the XL14-3 probe facilitated scanning maneuvers in narrow and angulated areas, allowing more accurate diagnosis of challenging atherosclerotic lesions located in difficult (deep and tortuous) arteries and patient anatomies (short or profound neck or inguinal areas).

We used a standardized protocol centered at the carotid bulb or femoral bifurcation; this is a single-region protocol that acquires a selected region or vessel landmark with the 3D probe. This protocol has been shown to be highly reproducible^{9,13} and to facilitate the monitoring of changes in serial plaque burden evaluations,³ suggesting that 3DVUS methods would be especially appropriate for studies examining plaque progression (mechanistic studies, clinical trials of drug therapies, or lifestyle interventions). More importantly, limiting 3D studies to the evaluation of carotid bulb alone has been shown to reliably predict events,¹⁹ thus simplifying the assessment of atherosclerosis burden for cardiovascular risk estimation. On the other hand, several lines of evidence support the value of imaging the femoral arteries: 1) in young to middle-age individuals, femoral territory is more frequently affected than are the carotid territory or coronary arteries by CACS⁹; 2) femoral atherosclerosis shows a stronger association than carotid plaques with positive CACS, a surrogate marker of cardiovascular

TABLE 4 Concordance Correlation Analysis Between Plaque Volume Measurements Obtained in the Different Validation Comparisons

Compared Plaque Volume Measurements	n	Concordance Correlation Analysis		
		Lin's r_{ccc} (95% CI)	Precision ρ (Pearson's r)	Accuracy C_b (Bias Correction Factor)
XL14-3 3DVUS vs histology	19	0.992 (0.986-0.995)	0.998	0.993
XL14-3 vs VL13-5	64	0.997 (0.995-0.998)	0.997	0.999
VPQ QLab vs CM2020 (3D)	64	0.998 (0.997-0.999)	0.998	1.000

events,²² and femoral burden is strongly associated with the presence of significant coronary artery disease^{23,24}; 3) atherosclerosis progression, also a surrogate marker of events, is more frequently detected in peripheral arteries by ultrasound than in the coronary arteries by CACS in middle-aged individuals³; and 4) more importantly, some prospective outcome-based studies have shown that the presence^{25,26} and the extent²⁷ of both carotid and femoral plaques are associated with clinical cardiovascular events, independently of risk factors. In this regard, our study results will strengthen the case for multiterritorial assessment of plaque burden, because the procedure is equally feasible for the femoral and carotid territories. However, further research is needed to determine the added value of multiterritorial plaque burden assessment by VUS²⁸ and whether the decision to assess both territories or only the carotids should be guided by patient age or disease stage.

The study of atherosclerosis by ultrasound has historically been hindered by limitations derived from the inherent characteristics of ultrasound technique. The most important of these limitations are as follows: 1) the poor detection of low-echogenic plaques and juxtaluminal black areas (JBAs) (those with an echo-density similar to blood and a thin fibrous cap below the resolution of ultrasound); and 2) posterior acoustic shadowing from severe plaque calcification. The methodology validated in this study still has these limitations. To improve the study of low-echogenic plaques, 3D-power Doppler and 3D contrast-enhanced ultrasound technology is currently under development. These future methods will undoubtedly be of value for the detection of JBAs, and more importantly, they will allow exploration of new directions in the assessment of atherosclerosis-based risk markers by ultrasound (ie, the measurement of low-echogenic plaque volume and the combined study of 3D plaque burden and plaque characterization). Nevertheless, notwithstanding the inherent limitations of ultrasound, plaque burden measurement with current ultrasound methods predicts clinical cardiovascular events and has prognostic value. Further studies should seek to

determine the actual effect of the lack of detection of JBAs with current 3DVUS technology for cardiovascular risk assessment. Regarding plaque calcification, in our study cohort, we found no significant limitation in plaque burden assessment caused by posterior acoustic shadowing, indicating that the new 3DVUS method can be used with confidence from early to mid-advanced disease stages. Our results align with those from the HRP study demonstrating the feasibility of carotid 3VDUS in asymptomatic intermediate- to high-risk 65- to 80-year-old individuals.^{7,8} However, previous studies of 3DVUS reported high drop-out rates caused by severe plaque calcification; these rates varied depending on the clinical context, ranging from 23% in patients undergoing revascularization of peripheral artery disease¹² to 33% in patients with recent ischemic stroke.¹¹ These findings suggest that 3DVUS performs worse in very-high risk or symptomatic individuals in whom severe plaque calcification co-occurs with stenotic lesions of complex morphology. Nevertheless, there is currently no consensus recommendation identifying which patients are suitable for plaque burden analysis by 3DVUS, and large prognostic studies will be needed to define the feasibility and prognostic value of 3DVUS in each age stratum and risk category.

The new CM2020 3D analysis software increased analysis speed while maintaining high intraobserver accuracy and reproducibility. This is mainly because CM2020 uses custom-made 3D algorithms that facilitate segmentation and simplify the manual correction of contours, thus significantly reducing analysis time, whereas VPQ is based on slice-by-slice 2D segmentation of the vessel. Also, 3D segmentation produces a 3D bifurcated model of the vessel that allows simultaneous analysis of the proximal segments of the 2 carotid and femoral branches, an approach not possible with VPQ. Altogether, these features make 3D ultrasound a simple, user-friendly, and radiation-free method with true potential to become a population screening tool for bedside cardiovascular risk assessment.

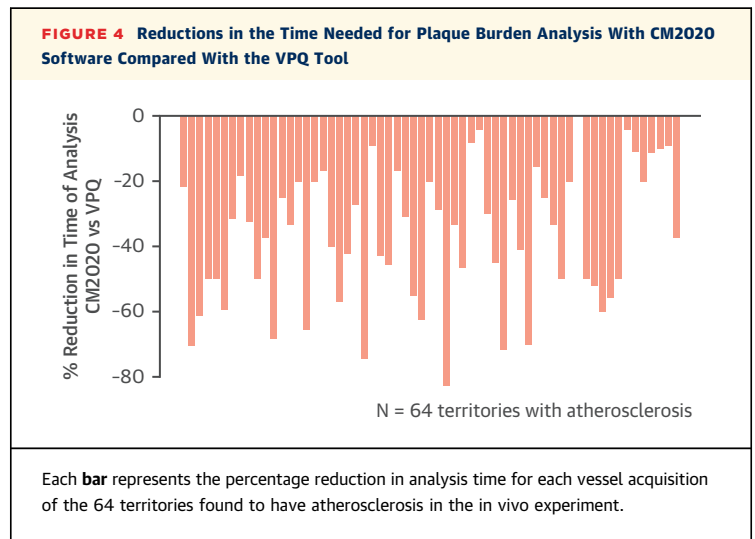
STUDY LIMITATIONS. Our study assessed only the carotid and femoral arteries; however, these are the

territories endorsed in current clinical guidelines for ultrasound assessment of atherosclerosis burden to assist cardiovascular risk estimation. The evaluation of subclinical atherosclerosis burden in deep arteries such as the abdominal aorta has been described as a limitation of previous 3DVUS methods. Given this limitation, we did not test the XL14-3 3D-matrix probe in this territory. This validation study was designed to demonstrate equivalence (noninferiority) of the new probe for plaque volume quantification compared with methods that are currently considered the gold standard in 3DVUS. Further studies would be desirable comparing the feasibility and accuracy of different imaging techniques for the detection and quantification of subclinical atherosclerosis, including the use of 3D probes from other manufacturers, to demonstrate the clinical superiority of the new XL14-3 probe. The improved spatial resolution of the XL14-3 electronic probe compared with the VL13-5 mechanical probe showed an improved feasibility for plaque burden assessment but equivalent for plaque quantification accuracy. These findings may reflect the aforementioned limitation in the study design, along with the use of 2D-based plaque quantitation software for the analyses, as explained in the [Supplemental Methods](#). The complete 3D visualization of atherosclerotic lesions afforded by CM2020 software has the potential to improve plaque quantification and, more clinically relevant, to improve plaque characterization that will further allow the detection of plaque vulnerability features. Although the integration of morphological and burden information for risk assessment is a desirable goal, this would first require a validation study for which we do not yet have the data.

CONCLUSIONS

3DVUS with the XL14-3 3D-matrix transducer gives accurate measures of plaque volume in the carotid and femoral arteries regardless of plaque size. This technique, in combination with the dedicated CM2020 3D plaque quantification software, is significantly simpler and more user-friendly than established approaches and has the potential to improve risk stratification based on atherosclerotic plaque burden.

ACKNOWLEDGMENTS This study is included in a Master Research Agreement between CNIC and



Philips Healthcare. The authors are extremely grateful to the many people who have contributed in so many different ways to setting up and validating this novel technology.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This study was partially funded by grants from the Ministerio de Economía, Industria y Competitividad (MEIC) with cofunding from the European Regional Development Fund (ERDF) (SAF2016-75580-R to Dr Bentzon) and (BES-2016-076633 to Dr Nogales). Research funding was also received from the Instituto de Salud Carlos III Spain (PIE16/00021 to Drs Bueno and Fuster). The CNIC is supported by the Ministerio de Ciencia, Innovación y Universidades (MICINN) and the Pro CNIC Foundation and is a Severo Ochoa Center of Excellence (CEX2020-001041-S). This study forms part of a Master Research Agreement between the CNIC and Philips Healthcare. Drs Sánchez-González, Entekin, and Collet-Billon are employees of Philips Healthcare. All other authors have reported that they have no relationships to disclose related to the contents of this paper.

ADDRESS FOR CORRESPONDENCE: Dr Valentín Fuster, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Mount Sinai Medical Center, Mount Sinai Heart, Icahn School of Medicine at Mount Sinai, Melchor Fernandez Almagro, 3. 28029 Madrid, Spain. E-mail: vfuster@cnic.es. OR Dr Beatriz López-Melgar, Cardiology Department at University Hospital La Princesa, Cardiovascular Disease Prevention Unit, University Hospital HM-Montepíncipe HM-CIEC. Diego de Leon, 62. 28006 Madrid, Spain. E-mail: blmelgar@hotmail.com. Twitter: [@CNIC_CARDIO](#), [@PesaStudy](#), [@Borjaibanez1](#), [@secardiologia](#), [@CIBER_CV](#), [@PhilipsSpain](#), [@Philips](#).

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Atherosclerosis is the leading cause of death and disability worldwide, and the introduction of effective strategies for primary prevention is a health care priority. The new 3DVUS probe and software and the proposed standardized 3DVUS protocol for carotid and femoral plaque burden quantification constitute a feasible and reliable imaging method for screening the general population. Atherosclerosis burden quantification by carotid and femoral 3DVUS has potential as a primary measure of individual cardiovascular risk, complementing scales based on traditional risk factors and improving previous 2DVUS-based atherosclerotic risk markers.

TRANSLATIONAL OUTLOOK: Extending the use of imaging techniques to the general population requires a technique that is simple, reliable, and cost-effective. 3DVUS using new matrix transducer and 3D plaque quantification software is valid for imaging superficial peripheral atherosclerosis burden from early to advanced stages. 3DVUS has the potential to become a key population screening tool for identifying at-risk individuals, targeting preventive therapy, or monitoring the response to treatment. Moreover, this bedside method is simple and radiation-free, encouraging its uptake in clinical practice and large-scale trials.

REFERENCES

- Perk J, De Backer G, Gohlke H, et al. European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur Heart J*. 2012;33:1635-1701.
- Goff DC Jr, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63:2935-2959.
- López-Melgar B, Fernández-Friera L, Oliva B, et al. Short-term progression of multiterritorial subclinical atherosclerosis. *J Am Coll Cardiol*. 2020;75:1617-1627.
- Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J*. 2020;41:111-188.
- Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J*. 2020;41:255-323.
- Wannarong T, Parraga G, Buchanan D, et al. Progression of carotid plaque volume predicts cardiovascular events. *Stroke*. 2013;44:1859-1865.
- Baber U, Mehran R, Sartori S, et al. Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: the BiImage study. *J Am Coll Cardiol*. 2015;65:1065-1074.
- Mortensen MB, Fuster V, Muntendam P, et al. A simple disease-guided approach to personalize ACC/AHA-recommended statin allocation in elderly people: the BiImage Study. *J Am Coll Cardiol*. 2016;68:881-891.
- Lopez-Melgar B, Fernandez-Friera L, Oliva B, et al. Subclinical atherosclerosis burden by 3D ultrasound in mid-life: the PESA study. *J Am Coll Cardiol*. 2017;70:301-313.
- Lopez-Melgar B, Fernandez-Friera L, Sanchez-Gonzalez J, et al. Accurate quantification of atherosclerotic plaque volume by 3D vascular ultrasound using the volumetric linear array method. *Atherosclerosis*. 2016;248:230-237.
- Kalashyan H, Shuaib A, Gibson PH, et al. Single sweep three-dimensional carotid ultrasound: reproducibility in plaque and artery volume measurements. *Atherosclerosis*. 2014;232:397-402.
- Graebe M, Entekin R, Collet-Billon A, Harrison G, Sillesen H. Reproducibility of two 3-D ultrasound carotid plaque quantification methods. *Ultrasound Med Biol*. 2014;40:1641-1649.
- Sandholt BV, Collet-Billon A, Entekin R, Sillesen HH. Inter-scan reproducibility of carotid plaque volume measurements by 3-D ultrasound. *Ultrasound Med Biol*. 2018;44:670-676.
- Fenster A, Blake C, Gyacskov I, Landry A, Spence JD. 3D ultrasound analysis of carotid plaque volume and surface morphology. *Ultrasonics*. 2006;44(Suppl 1):e153-e157.
- Landry A, Fenster A. Theoretical and experimental quantification of carotid plaque volume measurements made by three-dimensional ultrasound using test phantoms. *Med Phys*. 2002;29:2319-2327.
- Al-Mashhadi RH, Sørensen CB, Kragh PM, et al. Familial hypercholesterolemia and atherosclerosis in cloned minipigs created by DNA transposition of a human PCSK9 gain-of-function mutant. *Sci Transl Med*. 2013;5:166ra1.
- Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr*. 2008;21:93-111. quiz 189-90.
- Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim intima-media thickness consensus. *Cerebrovasc Dis*. 2004;18:346-349.
- Johri AM, Chitty DW, Matangi M, et al. Can carotid bulb plaque assessment rule out significant coronary artery disease? A comparison of plaque quantification by two- and three-dimensional ultrasound. *J Am Soc Echocardiogr*. 2013;26:86-95.
- Kole A, Cao Y, Hui J, et al. Comparative quantification of arterial lipid by intravascular photoacoustic-ultrasound imaging and near-infrared spectroscopy-intravascular ultrasound. *J Cardiovasc Transl Res*. 2019;12:211-220.
- Choy JS, Mathieu-Costello O, Kassab GS. The effect of fixation and histological preparation on coronary artery dimensions. *Ann Biomed Eng*. 2005;33:1027-1033.
- Laclaustra M, Casasnovas JA, Fernandez-Ortiz A, et al. Femoral and carotid subclinical atherosclerosis association with risk factors and coronary calcium: the AWHs Study. *J Am Coll Cardiol*. 2016;67:1263-1274.
- Colledanchise KN, Mantella LE, Héту MF, Liblik K, Abunassar JG, Johri AM. Femoral plaque burden by ultrasound is a better indicator of significant coronary artery disease over ankle brachial index. *Int J Cardiovasc Imaging*. 2021;37(10):2965-2973. <https://doi.org/10.1007/s10554-021-02334-9>
- Colledanchise KN, Mantella LE, Bullen M, Héту MF, Abunassar JG, Johri AM. Combined femoral and carotid plaque burden identifies obstructive coronary artery disease in women. *J Am Soc Echocardiogr*. 2020;33:90-100.
- Belcaro G, Nicolaidis AN, Ramaswami G, et al. Carotid and femoral ultrasound morphology screening and cardiovascular events in low risk subjects: a 10-year follow-up study (the CAFES-CAVE study(1)). *Atherosclerosis*. 2001;156:379-387.

26. Davidsson L, Fagerberg B, Bergström G, Schmidt C. Ultrasound-assessed plaque occurrence in the carotid and femoral arteries are independent predictors of cardiovascular events in middle-aged men during 10 years of follow-up. *Atherosclerosis*. 2010;209:469–473.
27. Lamina C, Meisinger C, Heid IM, et al. Association of ankle-brachial index and plaques in the carotid and femoral arteries with cardiovascular

events and total mortality in a population-based study with 13 years of follow-up. *Eur Heart J*. 2006;27:2580–2587.

28. Grubic N, Colledanchise KN, Liblik K, Johri AM. The role of carotid and femoral plaque burden in the diagnosis of coronary artery disease. *Curr Cardiol Rep*. 2020;22(10):121. <https://doi.org/10.1007/s11886-020-01375-1>

KEY WORDS accuracy, atherosclerotic plaque, gold-standard, plaque volume, three-dimensional, vascular ultrasound

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this paper.