New modalities of ultrasound-based intima-media thickness, arterial stiffness and non-coronary vascular calcifications detection to assess cardiovascular risk

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Abstract. – BACKGROUND: Carotid intima-media thickness (c-IMT), arterial stiffness (AS) and vascular calcification (VC) are now considered important new markers of atherosclerosis and have been associated with increased prevalence of cardiovascular events. An accurate, reproducible and easy detection of these parameters could increase the prognostic value of the traditional cardiovascular risk factors in many subjects at low and intermediate risk. Today, c-IMT and AS can be measured by ultrasound, while cardiac computed tomography is the gold standard to quantify coronary VC, although concern about the reproducibility of the former and the safety of the latter have been raised. Nevertheless, a safe and reliable method to quantify non-coronary (i.e., peripheral) VC has not been detected yet.

AIM: To review the most innovative and accurate ultrasound-based modalities of c-IMT and AS detection and to describe a novel Ultra-Sound-Based Carotid, Aortic and Lower limbs Calcification Score (USB-CALCs, simply named CALC), allowing to quantify peripheral calcifications. Finally, to propose a system for cardiovascular risk reclassification derived from the global evaluation of "Quality Intima-Media Thickness", "Quality Arterial Stiffness", and "CALC score" in addition to the Framingham score.

Key Words:
  Vascular Calcification, Intima-Media Thickness, Arterial Stiffness, CALC score.

Introduction

Cardiovascular disease (CVD) is a major health problem in Western Countries, due to its slow and often asymptomatic progression, and the unavoidable impact on morbidity and mortality[1]. In order to prevent cardiovascular events in later life, there is an increasing interest in new markers of atherosclerosis, that may help diagnose CVD at an early stage[2]. Subclinic inflammation, oxidative stress, loss of calcium homeostasis and an impaired "bone-vascular crosstalk" have been recognized as important mechanisms underling the long-lasting natural history of CVD[3,4]. Indeed, arterial stiffness and intima-media thickness can be recognized at the very initial phases of CVD natural history, which slowly evolves toward the formation of atherosclerotic plaques with calcium overload. Vascular calcifications (VC) are often present in the early stages of atherogenesis, of which could be considered an early marker[5]. Recent studies[6] advocated the pathologic deposition of calcium in the vessel wall with the simultaneous reduction of calcium deposition in the bone, to explain the presence of VC, the so called "calcium paradox". This process involves a subclinic deficiency of Vitamin K2, even if several other mechanisms could be involved[7].

Many studies[8] underlined the high prognostic significance of VC. A score based on the quantification of calcifications in tunica intima and media of coronary arteries (coronary artery calcification,

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CAC) detected by computed tomography (CT) has gained popularity[9]. The CAC score is related to the occurrence of new cardiovascular events and CVD mortality[10], and is superior to the Framingham risk score[11], stress echocardiography or perfusion studies in the elderly and in the identification of high-risk asymptomatic patients[12-14]. However, a great limitation of CAC score is that CT imaging follow-up is not suitable for primary prevention, due to the risk related to radiation exposure; moreover, it may have a considerable economic impact on the cost of public health, as it is recently been stated in a report from the European Association of Cardiovascular Imaging and the European American Society of Echography[15].

Thus, many scoring systems, novel markers, and imaging techniques have been proposed to better define individual profile of cardiovascular risk: Ankle-Brachial Index (ABI), high-resolution ultrasound imaging of Intima-Media Thickness (IMT) and plaque, arterial stiffness (AS) and Vascular Calcification (VC) assessment[16]. All of them may have a place in drug development and clinical trial design, but their additional value above and beyond classical risk factors has still to be determined. In this scenario, we reviewed new modalities of detection of carotid IMT and AS, and propose a novel non-coronary calcification risk score to improve the quantification of individual cardiovascular risk.

IMT, AS and VC Measurement "Until Now"

IMT Measurement

IMT is a non-invasive biomarker of early atherosclerosis associated with the risk of subsequent cardiovascular events, independent of all major risk factors[17]. In 1986, Pignoli et al[18] documented for the first time that B-mode imaging represents a useful approach for the measurement of intima-media thickness of human arteries. Comparing measurements obtained by B-mode ultrasound with pathologic findings of gross and microscopic examination, they did not find significant differences. In 2005 a consensus statement by the American Society of Echocardiography provided a protocol for Carotid IMT (cIMT) measurement[19]. It should be measured in both left and right carotid artery, in R-wave gated still frames, acquiring images of the distal 1 cm of the common carotid arteries in three angles. Mean left-side and right-side cIMT should be calculated, and then averaged. cIMT should be measured

1 cm down from common carotid artery bifurcation for few reasons: it is easier to approach compared to other districts, all major clinical papers refer to carotid artery and it is proved that carotid behavior is assumed as general for all other vessels. Today, several vascular ultrasound systems have the possibility to measure cIMT by a semi-automated technique. However, the reproducibility of cIMT is affected by multiple factors, as the reader's experience and ultrasound settings. Inter-reader variability is reduced using semi-automated cIMT measurement, as described by Dogan et al in 2010[20]. They concluded that the number and specific combination of segments, angles and walls interrogated, are associated with differences in reproducibility and precision of progression of cIMT. In their opinion the best protocol is the mean common cIMT protocol, which consists in multiple measurement of cIMT (both the near and the far wall, multiple angles).

More recently, based on results from the Carotid Atherosclerosis Progression Study (CAPS), the incremental predictive value of cIMT in the general population has been questioned[21]. Indeed, although cIMT measured by B-mode ultrasound is associated with future cardiovascular events, when a model using the classical risk factors was compared with a model including cIMT, the classification of individual risk did not result consistently improved. On the contrary, the maximum IMT value along the whole carotid artery or the presence of plaques seem to have the better prognostic value[22].

AS Measurement

Since it occurs long before luminal changes modifications and plaques formation, AS is a more sensitive diagnostic biomarker of atherosclerosis[23]. AS measures vessel walls distension due to pressure wave mechanical push. This wave is generated by the blood flow through the systole and diastole cardiac phases. The primary method to assess AS is Pulse Wave Velocity (PWV), which represents the speed at which a pulse wave moves through an arterial segment. PWV is calculated with the Moens-Korteweg equation[24], which rationalizes the association between the arterial elastic property and PWV:

$$PWV = \sqrt{(E \cdot h)/(2rp)}$$

E: elastic modulus
h: wall thickness
r: internal radius
p: blood density.

As atherosclerosis develops, wall thickening and vessel radius reduction contribute to PWV increase. Arterial stiffness depends on mechanical stretch of arterial wall and on blood pressure at the moment of measurement[25]. Alterations of arterial wall distension and arterial functional changes may anticipate preclinical atherosclerotic lesions (measured by cIMT) at the onset of vascular disease[26]. PWV is the gold standard to assess large arteries stiffness and strongly predicts cardiovascular risk[27]. It is closely associated with age and blood pressure. Hypertension affects PWV independently of age while there is a weak association with diabetes mellitus and no association with the other classic risk factors for atherosclerosis (sex, smoking and lipids)[28]. The application of carotid-femoral PWV has been recently studied in different clinical settings, such as the elderly[29], patients with dyslipidemia[30] or metabolic syndrome[31]. Several reference values for PWV have been reported in the literature and are dependent on the techniques of measurement. The gold standard has been the use of flow meters or catheter-based pressure probes[32]. Pulse wave is detected by pressure and flow waveforms. In case of fiber optic probes, two probes measuring pressure waveforms are placed at a known distance: the temporal shift between the two pressure waveforms is used to calculate PWV in the vessel region between the two probes. Ultrasound flow-meters are placed perivascularly: one ultrasound wave is transmitted with the direction of the flow, another wave is transmitted against the direction of the flow. The difference in transit time between these two ultrasonic waves directly reflects the velocity of blood in the vessel, so PWV can be computed by examining the temporal shift between the two waveforms of two ultrasonic flow-meters placed at a known distance. These two techniques are highly accurate but invasive, so their use is restricted to animal studies or patients undergoing cardiac catheterization.

In the clinical setting, PWV can be measured with applanation tonometry and Doppler ultrasound[33]. In the first case, a tonometer is placed on the skin over carotid and femoral arteries. A pressure waveform is recorded from each location and the temporal shift between the two pressure waveforms is calculated as the transit time. The distance between the two vascular locations can be measured with a tape in two ways: (1) difference between the distance from the sternal notch to the femoral artery and the sternal notch

to the carotid artery; (2) direct distance between carotid and femoral arteries. The distance is then divided by the transit time to calculate PWV. Doppler ultrasound makes use of the same technique of applanation tonometry with ultrasound transducers instead of tonometers. These two method are no invasive, less expensive and without ionizing radiation. However, they show limits, as errors up to 30% for the measurement of PWV, due to inaccuracies in distance measurement. Lack of a standardized method to measure distance between carotid and femoral arteries accounts for variations in PWV in different Centers. Applanation tonometry and Doppler ultrasound provide a global measurement of PWV, but in 2012 it has been proposed a new method to evaluate regional PWV, within the carotid artery, by means of ultrasound[34]. In this study the regional PWV was estimated from the spatial-temporal variation of the wall velocities: authors found values from 4.0 to 5.2 m/s in eight normal subjects, in agreement with the literature. PWV was thus proven operable in the human carotid artery, and is useful to detect vascular disease through mapping the pulse wave and estimating the regional PWV in the carotid artery. In 2010 a large multicenter study used tonometry to measure carotid femoral PWV, and documented a normal PWV as 6 m/sec in healthy young people (less than 30 years old) and PWV up to 10 m/sec in old people (> 70 years old)[35].

It has also been proposed to compute PWV with Magnetic Resonance Imaging (MRI), obtaining time-resolved distension waveforms from multiple data points during cardiac cycle[36]. The major limitation is the lack of commercial software availability; moreover, the variety of MR-based PWV methods make difficult to choose the most suitable approach.

VC Measurement

Today, several studies tried to provide the best method for the quantification of VC in several districts including coronary, and leg arteries, aorta and carotid. They could be divided in quantitative (CT Techniques), considered the gold standard, and semi-quantitative (Plain x-rays, B-mode US, arterial compliance, echocardiography) methods. However, there are substantial limitations to the wide use of CT Techniques, since they are expensive and unsafe to be routinely performed[14]. Moreover, calcifications are often assessed in a single district (e.g. the coronary arteries[8] or the femoral

artery)[37], that might not reflect the overall peripheral calcium load.

The semi-quantitative, and safer, methods, even if easier to be performed and relatively cheaper, generate scores too simple for an effective follow-up; indeed the severity of calcifications is not graduated, and the major part of the scores consider only the presence or the absence of calcifications in the explored artery.

Adragao et al[38] found that in 101 hemodialysis patients a simple vascular calcification score (SVCS) measured by plain X-ray of pelvis and hands predicted cardiovascular risk in haemodialysis patients and was associated with arterial stiffness, measured by pulse wave velocity (PWV) and pulse pressure (PP). Furthermore, higher SVCS, PWV and PP associated with higher mortality[39]. Even if these results provided with simple and inexpensive methods, seem promising, the score was realized exploring the ileo-femoral, radial and digital arteries only, the presence of calcification was not graduated according to severity but based only on their presence or absence, and, finally, the maximum vascular calcification score was relatively low, ranging from 0 to 8.

Another X-ray based score of lumbar aortic calcifications assessed in 617 Framingham Heart Study participants has been published by Kauppila et al[40] Although no correlation with vascular stiffness was provided, aortic calcification severity was graded on a 0-3 scale for each lumbar segment, and the final results were summarized to develop four different composite scores. However, radiation exposure represents a limitation also for this study. Finally, Guerin et al[41] used B-mode ultrasonography (US) to assess the association between carotid, aorta and femoral arteries calcifications and increased arterial stiffness measured by PWV in 120 hemodialysis patients. However, the provided score consisted in only 4 stages and was not based on the severity of calcifications but on the number of involved segments.

The above described calcification scores could be considered as qualitative and only partial descriptions of the presence of calcifications in the arterial districts, rather than a complete and systematic assessment of arterial calcification status. Therefore, there is an undoubted need for new modalities of detection and quantification of VC in aorta and peripheral arteries. The "ideal" method should be non-invasive, repeatable, less expensive and more careful than yet proposed.

## New Technologies: "Quality" IMT (QIMT) and "Quality" Arterial Stiffness (QAS)

Recently, Esaote® developed a new radiofrequency-based technology for a "quality" assessment of IMT and AS, and applied it to the echocolorDoppler MyLab with a 3-9 MHz linear transducer. The accuracy of both QAS and QIMT is superior compared to other currently available conventional technologies. The maximum achievable accuracy, as obtained in the in-vitro experiment, is for diameter 51 μm, for distension 1.4 μm and for IMT 17 μm. The system provides on-line feedback of the measurement by means of quality indicators overlaid on the ultrasound and via the Standard Deviation (SD) over 6 consecutive cardiac cycles.

The QIMT calculation automatically measures the thickness between the intima and the media on the image in real time, using the radiofrequency reception signal. The software of the system supports the Mannheim protocol in the measurement process and in the reporting structure[42]. This system is based on the record of a continuously tracked signal during cardiac cycles. When the standard deviation (SD) of six successive cycles is less than 20 μm the system records QIMT value. Figure 1 shows the details of this modality and shows the result of a single determination. This report include an "Expected QIMT" values table, based on a worldwide database of 40,000 subjects obtained with RF-based techniques which correspond to the patient's age and vascular trending (Figure 2)[43].

The QAS calculation automatically measures the modification of the arterial diameter between the systolic and diastolic phases, e.g. arterial distensibility (Figure 3). The vessel stiffness is calculated starting from this value and from the brachial pressure values. When the SD of arterial distensibility in the last six cycles is less than 30 μm, the system records QAS value. The automatic detection and real time feedback helps to get the best possible measurements. The system provides the local pressure waveform of the common carotid artery, as shown in Figure 4. This waveform is obtained by transforming the last six cycles of the distension curve over time in one pressure curve over time. On the Local Pressure Waveform the following points are indicated:

- Start of Isovolumic Contraction (SIC)
- Aortic Valve Opening (AVO)
- Inflection Point (T1)
- Local Systolic Pressure (PLs)
- Aortic Valve Closure (AVC)

Figure 1. Quality Intima-Media Thickness (QIMT), based on radiofrequency data processing, the best method now available with a resolution lower than 20 µm. The red line represents the adventitia, and the green stripe the intima-media layer, real-time evaluated by the values of 6 cardiac cycles. The QIMT is the value with a standard deviation (DS in the picture) lower than 20 (6 in the picture) during the whole period of measurement. (*Image from personal archive*).
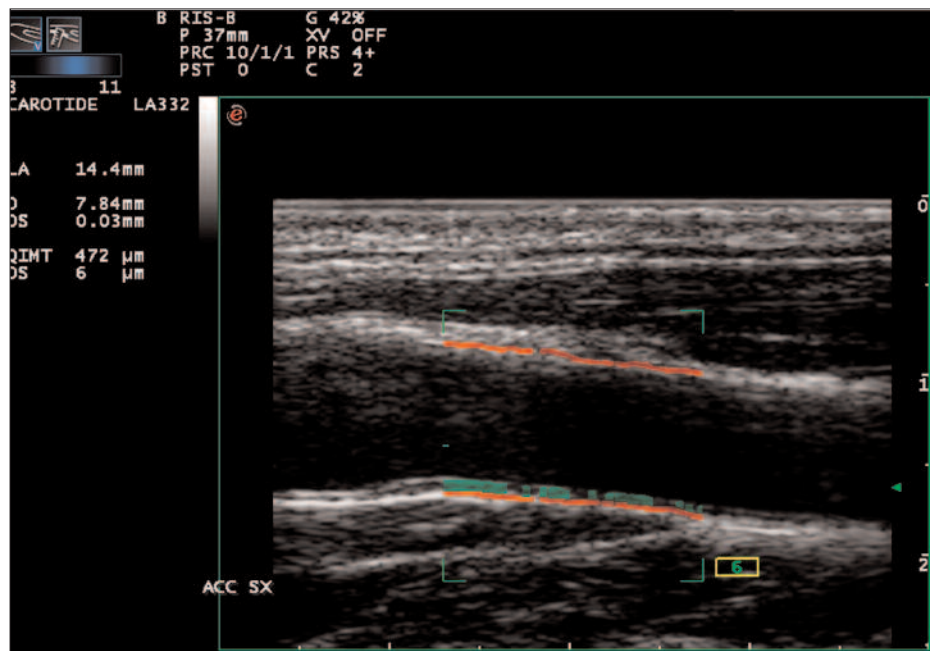

Thus, the pulse wave velocity, which represents arterial stiffness and is expressed in m/s, is automatically detected.

A Novel Modality of VC Assessment: UltraSound-Based Carotid, Aortic and Lower limbs Calcification Score (USB-CALCs)

In our Center, we have recently tested a new ultrasound-based method to evaluate VC in 11 vascular segments, using a linear 4-8 MHz transducer in peripheral arteries (carotid arteries, common femoral arteries, popliteal arteries, posterior tibial arteries, and anterior tibial arteries of both sides), and using a convex 2-5 MHz transducer in subrenal abdominal aorta (Figure 5). Landmarks for the selected 11 vascular segments were the bifurcation of common carotid artery and the bulb of internal carotid artery (segments 1 and 2), the tract of abdominal from the origin of the renal arteries to the bifurcation (segment 3), the common femoral arteries with the bifurcation at the level of the whirlbone (segments 4 and 5), the distal popliteal arteries at the level of the tibial plate (segments 6 and 7), the posterior tibial arteries at the level of the internal malleolus (segments 9 an 10) and, finally, the anterior tibial arteries at the level of the shin (segments 8 and 11). For the determination of CALCs, a single arterial segment of 4 cm in carotids and arteries of the lower limbs, which can be generally captured in single picture regardless the machine used, is selected with the linear transducer; finally, subrenal abdominal aorta is selected using the convex probe. The sequential evaluation of 11 single segments of 4 cm, selected on the basis of the major presence of calcifications according to Authors' experience (Figure 6), and detectable according to well-defined anatomic points, make CALCs determination repeatable among different Centers.
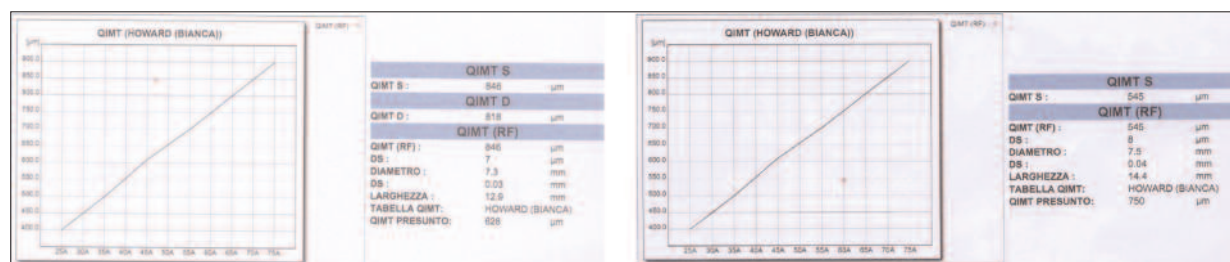

Figure 2. Vascular age": QIMT and age in a "older" 47 years old man (A) and in a "younger" 60 years old man.

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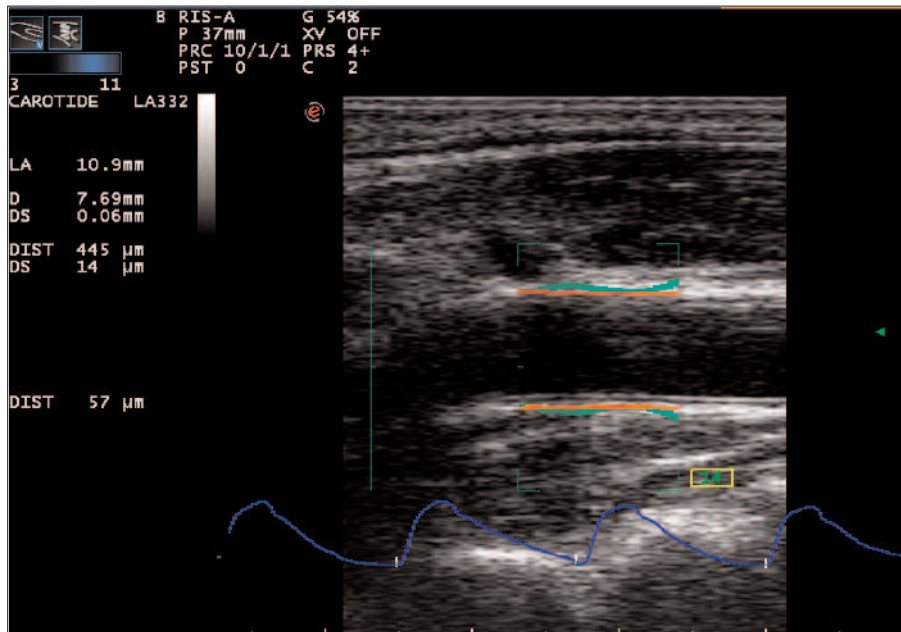
Figure 3. Pressure-dependent vessel wall movement amplification curve (blue wave) of common carotid artery for the determination of Quality Arterial Stiffness (QAS). The average and standard deviation of both the distension and the diastolic diameter of the vessel under measurement are showed on the left side. The red line represents the adventitia, the continue green line the movement of the vessel wall at the moment of image freezing (in this case, at the end of diastolic phase of the last cardiac cycle), the blue curve the vessel wall movement amplification (DIST, 445 μm), the maximum vessel wall distention. Fourteen is the standard deviation (DS in the picture) in μm relative to the previous 6 cardiac cycles and represents the quality indicator (measure allowed only when standard deviation < 30). (*Image from personal archive*).
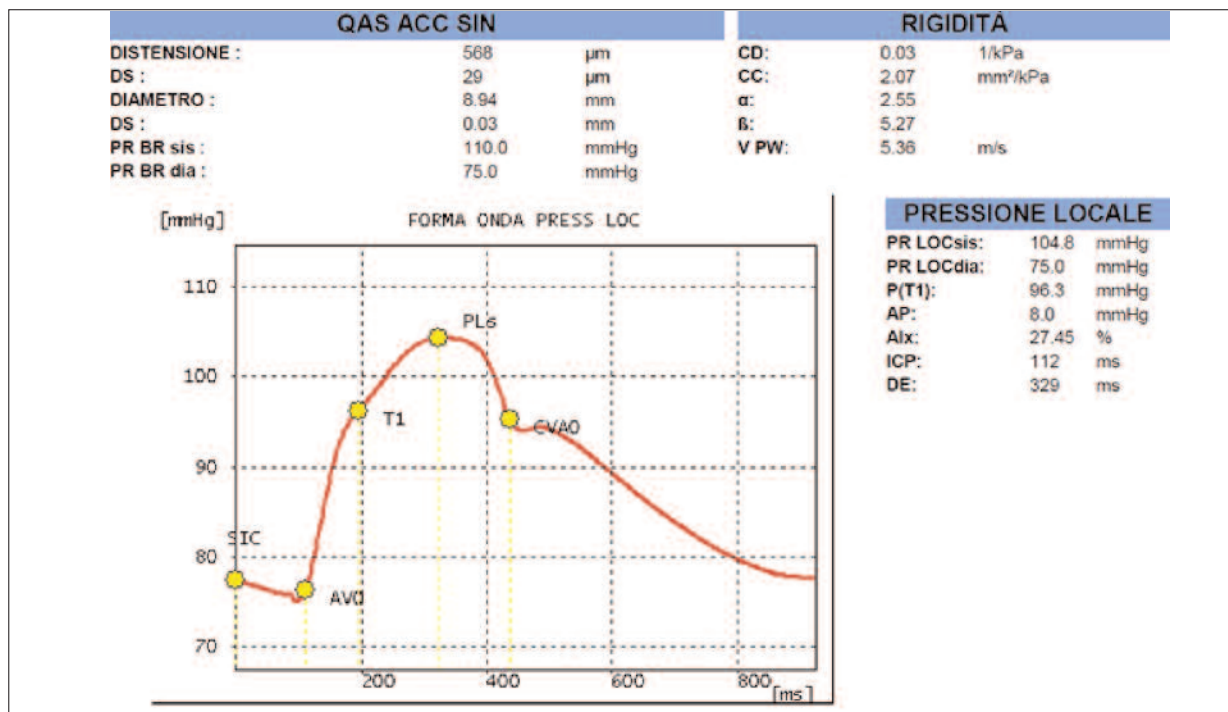
Figure 4. Quality Arterial Stiffness (QAS) report, including Local Pressure Waveform. The waveform is obtained by transforming the last six cycles of the distension curve over time in one pressure curve over time, in order to determinate the Pulse Wave Velocity (V PW, see text for more details). (*Image from personal archive*).
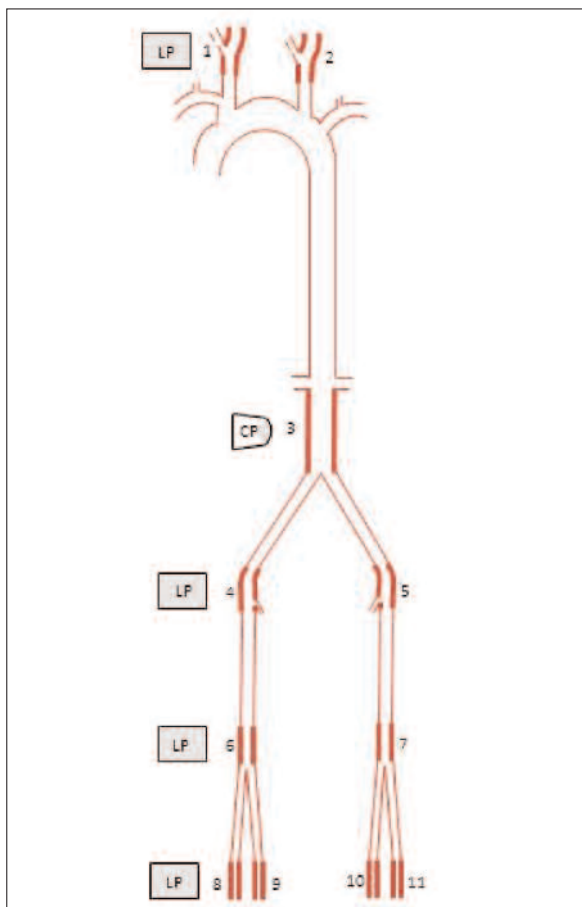
Figure 5. Arterial circulation (*light red line*) and arterial districts (*bold red line*), object of investigation for Carotid, Aortic, Lower limbs Calcification score (CALCs) assessment (*see text for more details*). LP = linear probe; CP = convex probe.

Based on the number and size of VC, a score ranging 1-3 is assigned to each explored vascular segment, evaluated sequentially. One point is given for a single intimal or medial calcification with a thickness less or equal than 1 mm in a longitudinal scan (microcalcification, mC), 2 points for more than 1 mC in the same vascular segment and, finally, the maximum score of 3 points for any calcification thicker than 1 mm (macrocalcification or MC), regardless of the presence of other VC in the same segment (Figure 7). Figure 8 shows a single mC (1 point) in a segment of carotid artery (A) and in the posterior tibial artery too (B); notably, in the latter, no calcification is detectable using the color-flow, and the normal triphasic flow is also present (C). A single MC in the carotid artery (3 points) and a single MC in the abdominal aorta (3 points) are shown in Figure 9.

Therefore, CALC points are assigned distinguishing the severity of VC in many vascular segments. The range of the final PACs score (from 0 to 33) could be wide enough to monitor negative evolution or even improvements of VC, as recently speculated[44].

The accuracy of CALC evaluation is warranted by the optimal correspondence between US-images and optical microscopy of calcific operatory specimens (Figure 10). Moreover, we have detected not only an excellent reproducibility between different expert operators but also between expert operators and advanced trainees. Indeed, as shown in Figure 11, arterial calcification are easily detectable. They appear as well-defined
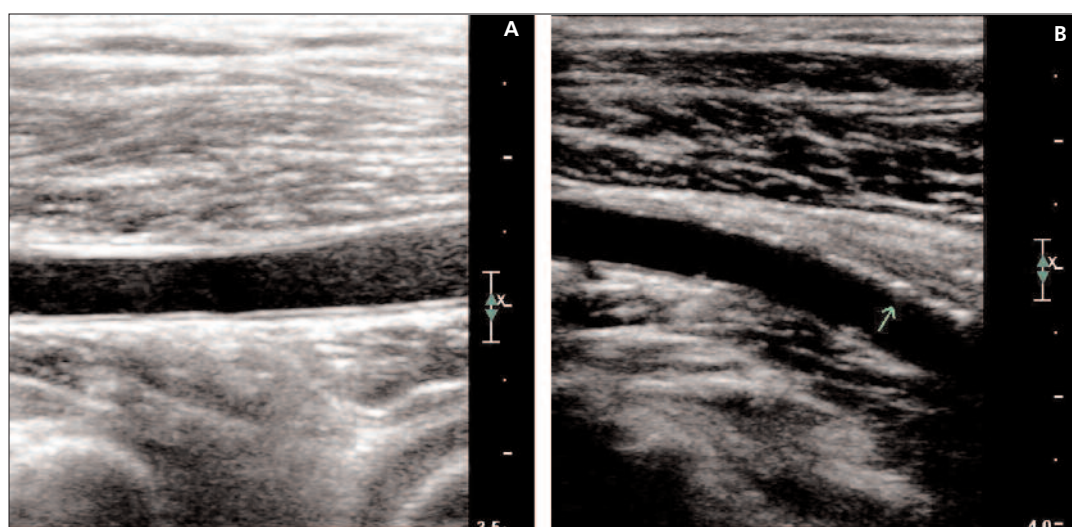
Figure 6. The proximal tract of the popliteal artery is generally spared by vascular calcifications *(A)*; thus, we examine the distal tract of the same artery *(B)*, where vascular calcifications are much more frequent. (Images from personal archive).

Type of Calcification	CALC points
Single Microcalcification (≤ 1 mm)	1
> 1 Microcalcification (≤ 1 mm)	2
Single Macrocalcification (> 1 mm)	3

Figure 7. CALC points were assigned evaluating vascular segments of 4 cm.
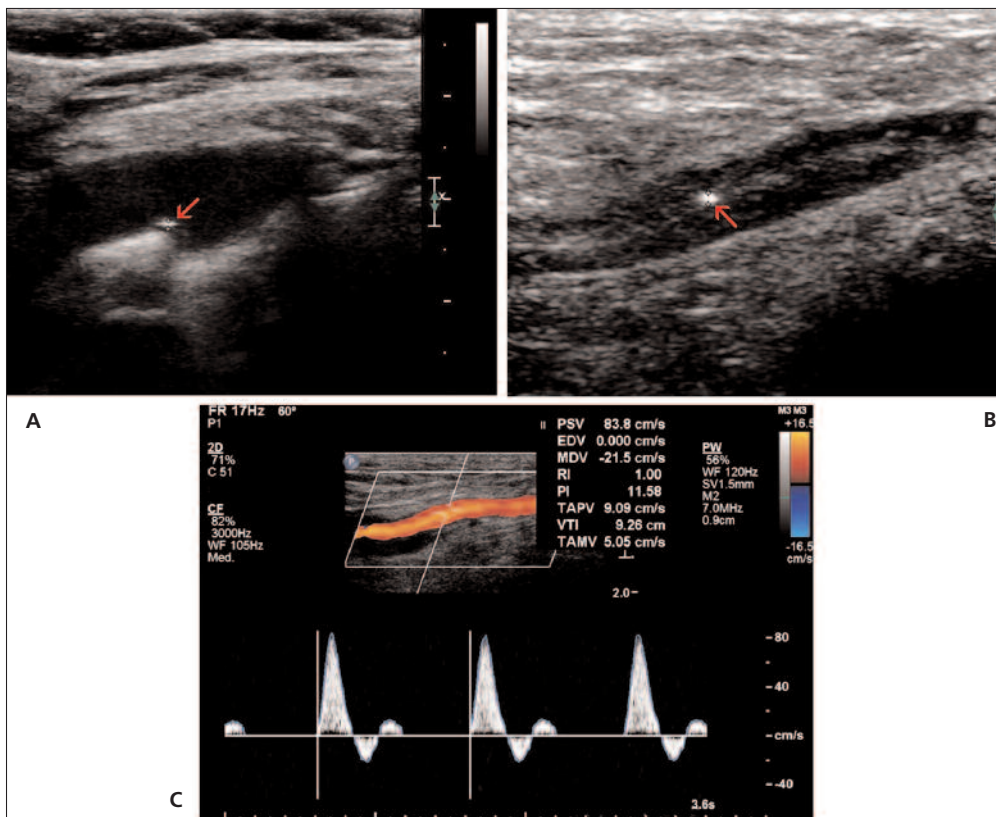
Figure 8. CALC assessment. Single microcalcification (red arrow) in the internal carotid artery (0.79 mm) (**A**, 1 point). Single microcalcification (red arrow) in the posterior tibial artery (0.94 mm). A posterior tibial vein is detectable below the artery (**B**, 1 point); notably, triphasic flow is not modified by the calcification, which is hidden by the color flow *(C)*. (*Images from personal archive*).
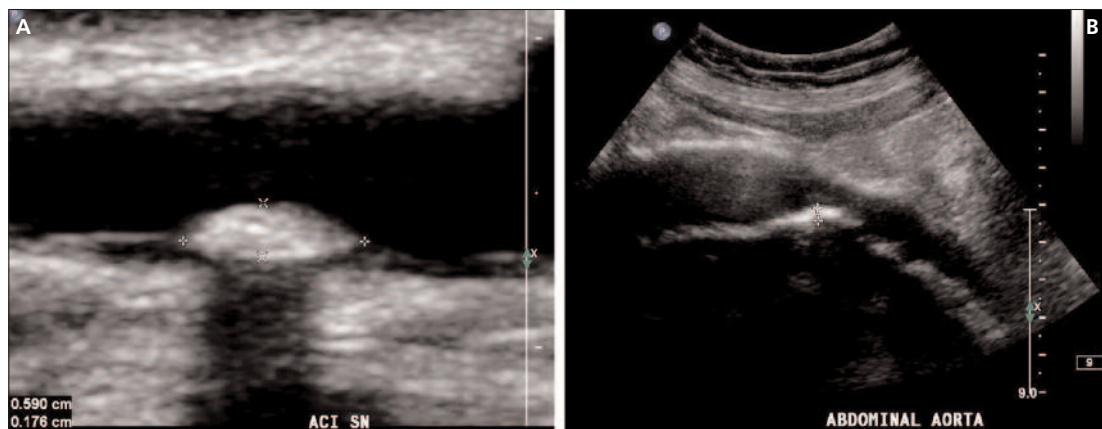
Figure 9. CALC assessment. Single macrocalcification (3 points) in internal carotid artery (*A,* zoomed image) and in abdominal aorta (*B,* 3 points). (*Images from personal archive*).

hyperechoic "white" spot of the intimal and/or medial layer with a posterior acoustic shadow and are isoechoic with the bone.

Future Perspectives

In our opinion, the assessment of QIMT, QAS and CALCs is at present the best available tool to evaluate subclinical organ damage, since represents different expressions of early atherosclerotic process. QIMT and QAS use the best technology to evaluate with great accuracy cIMT and AS, with a real time radiofrequency data processing of six equal waveforms. In this scenario, CALCs is a novel marker of calcific atheroscle-
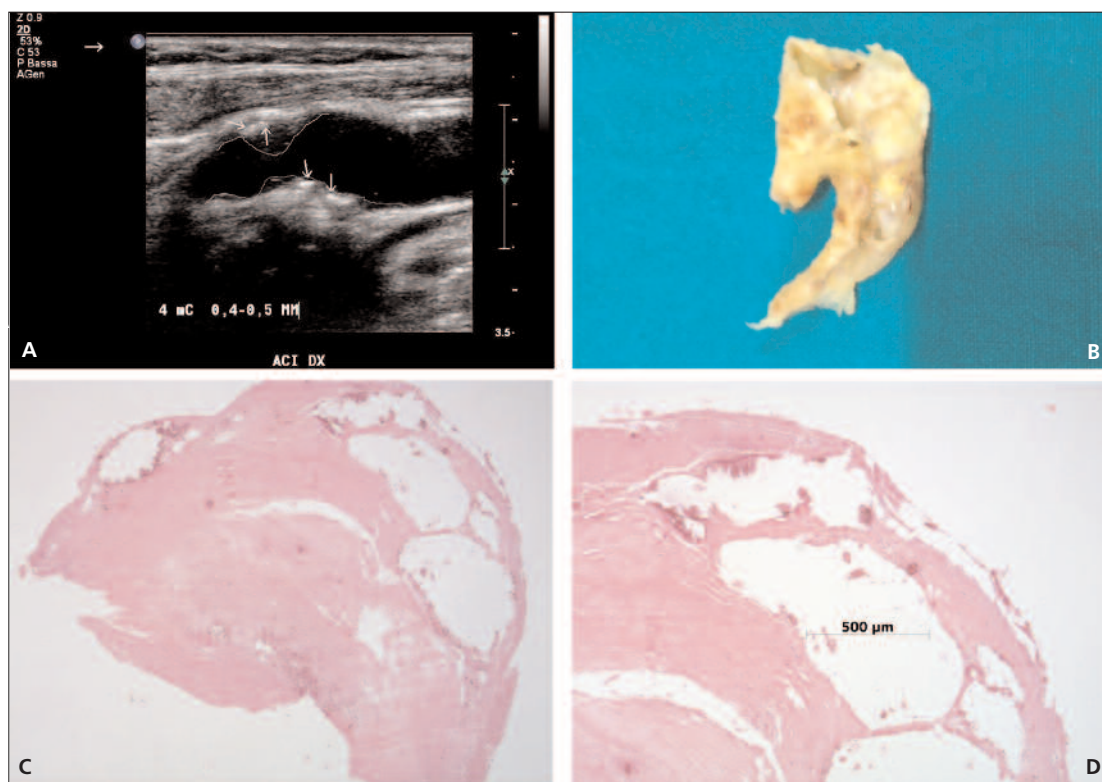
Figure 10. Correspondence between size and number of vascular calcifications in a subject with critical carotid stenosis operated of carotid endarterectomy. Echographic finding *(A)*, macroscopic finding *(B)*, histologic finding *(C)*, zoomed image of C *(D)*. (*Images from personal archive*).
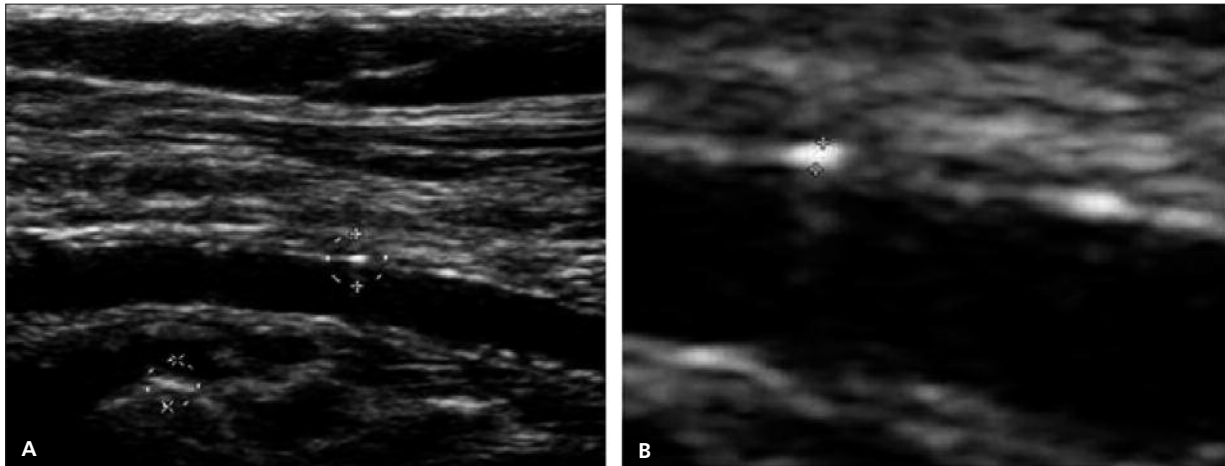
Figure 11. In anteroposterior projection, vascular calcifications appear as "white spots" (dotted line +) isoechoic with tibial plate (dotted line x) using automatic gain setting **(A)**. The zoomed image shows an acoustic shadow under the "white spot" and a submillimetric calcification **(B)**. (*Images from personal archive*).

rosis, useful to assess VC burden in non-coronary circulation. It is based on B-mode ultrasonography, which is safer and relatively cheaper than other currently available imaging techniques (e.g., CT scan). Therefore, it could be useful for risk assessment in several categories of patients, not only asymptomatic individuals with few co-morbidities, but also those with contraindications to more invasive and complex radiologic investigations.

Our unpublished data in subjects at low-intermediate risk, clearly show a strong correlation between QAS, QIMT and CALCs. Moreover, we are evaluating the correlation between CALCs and CACs in patients with thoracic pain. Thus, our ongoing studies are aimed to validate QIMT, QAS and CALCs for reclassification of patients at low-intermediate cardiovascular risk, with important results in terms of cardiovascular disease prevention.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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