Special Article - Vascular Imaging

Vascular Ultrasound and Cardiovascular Risk Assessment

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Abstract

Vascular ultrasound is able to detect endothelial dysfunction, arterial structural remodeling and increased arterial stiffness. These alterations have been shown to be associated with established and emerging cardiovascular risk factors and with incident cardiovascular events. Therefore, vascular ultrasound has been proposed to evaluate the role of different risk factors in the initiation and progression of atherosclerotic process, to study vascular aging and the relationship between arterial stiffness and atherosclerosis, to assess the efficacy of life-style and therapeutic interventions, and to improve the estimation of individual cardiovascular risk. The present paper provides a critical overview of the clinical evidence appraising the association of flow-mediated dilation, carotid and femoral intima-media thickness and plaque presence as well as local arterial stiffness with cardiovascular risk factors and cardiovascular events.

Keywords: Ultrasound; Biomarkers; Carotid artery; Cardiovascular risk; Intima-Media thickness; Arterial stiffness; Flow-Mediated dilation

Abbreviations

CV: Cardiovascular; D: Disease; US: Ultrasound; FMD: Flow-Mediated Dilation; CHS: Cardiovascular Health Study; MESA: Multi-ethnic Study on Atherosclerosis; NRI: Net Reclassification improvement; IMT: Intima-Media Thickness; CCA: Common Carotid Artery; ARIC: Atherosclerosis Risk in Communities Study; ICA: Internal Carotid Artery; 3-D: 3-Dimensional; FA: Femoral Artery; PWV: Pulse-Wave Velocity; CF: Carotid-Femoral

Introduction

Cardiovascular (CV) Disease (D) is the leading cause of death worldwide. In Europe, CVD is responsible for over 4 millions death per year, and causes 42% and 51% of deaths among men and women, respectively, compared with 23% and 19% for all cancers [1]. The medical cost of CVD has continuously increased in the past years, and in 2012 the overall CVD cost in Europe was estimated to be of €196 billion. In the next 20 years this cost is expected to further escalate.

Yet, CVD is largely preventable, and an early detection of individuals at increased risk followed by the implementation of life-style and therapeutic interventions can prevent disability and death, improve quality of life and reduce the global health-care cost. However, the accurate and cost-effective identification of subjects at risk is still a challenge. Various risk scores (Framingham Risk Score, SCORE Charts, and others) have been developed to guide the preventive strategies [2], yet these scores provide estimation of a population-based risk rather than quantification of the individual risk. Furthermore, a substantial part of population belongs to intermediate risk, where it is not clear whether and when an aggressive prevention strategy is beneficial and cost effective.

The use of CV biomarkers in conjunction with risk scores is expected to refine the risk stratification of an individual subject and to guide the preventive/therapeutic strategy. A "biomarker" was defined by the National Institutes of Health as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [3]. Therefore, biomarkers can be used to detect the burden of subclinical disease in order to apply preventive measures, and they should also enable to monitor the response of subclinical disease to preventive interventions. Yet, robust pathophysiologic, epidemiologic and therapeutic evidence is necessary to validate any new biomarker. According to a scientific statement from the American Heart Association, biomarker should differ between subjects with and without outcome, should predict the development of future events over and above established risk markers, and its use should improve clinical outcomes when tested in a randomized clinical trial [4].

Vascular measures might be particularly informative for the assessment of CV risk, as they detect organ damage in different parts of vascular bed, are measurable in a non-invasive way, and reflect both aging process and adverse impact of established CV risk factors, like plasma lipids, smoking, high blood pressure, diabetes, inflammation [5].Nowadays, several vascular biomarkers have been proposed. A position paper from the European Society of Cardiology Working Group on peripheral circulation suggests that the choice of vascular biomarker, or their combination, should depend on the clinical setting and present comorbidities, and may differ for each individual patient [5]. The widespread clinical use of biomarkers depends also on their cost-effectiveness, ease of use, clear methodological consensus and availability of reference values. From this perspective a vascular Ultrasound (US) is an interesting method, as it allows the composite evaluation of vascular structure and function in a non-invasive way at a relatively low cost (Table 1). Vascular ultrasound can be used to assess endothelial function, geometry and stiffness of elastic arteries (like carotid artery) and muscular arteries (like brachial and femoral artery).

Endothelial function

The vascular endothelium is a single layer of cells situated at the interface between the blood and the vascular wall and releasing a wide range of factors that play a central role in the maintenance of vascular

Table 1: Vascular biomarkers obtained by ultrasound.

	Description	Method of measurement	Formulas	Clinical Utility	Recommendation/ Level of evidence for CV prevention	Reference values
FMD	response of BA to reactive hyperemia	B-mode US RF-based US	direct measurement	in intermediate risk subjects NRI=2.4% for CHD [17]	III/B	No
Carotid IMT	thickness of the near/far wall of CCA/bulb/ICA	B-mode US RF-based US	direct measurement	CCA IMT in intermediate risk subjects NRI=3.6% for MI or stroke [34] ICA IMT in general populationNRI=7% for CHD [36]	IIa/A	Yes
Carotid Plaques	number thickness area volume	B-mode US	direct measurement	IMT+plaque in general population NRI=9.9% for CHD [41] IMT+plaque in intermediate risk subjects NRI=21.7%for CHD [41] ICA plaque in general population NRI=7.3% for CVD [42] carotid plaque score in general population NRI=10.2% for CVD [43]	IIa/A	No
Local stiffness	changes in vessel diameter/ area to distending pressure local PWV	B-mode US RF-based wall tracking	distensibility: $\Delta D/(\Delta P*D)$ compliance: $\Delta D/\Delta P$ beta index: $\ln(Ps/Pd)*D / (\Delta D)$ elastic modulus: $(\Delta P*D)/ \Delta D$ Young's modulus: $(\Delta P*D)/ (\Delta D*h)$ Bramwell-Hill equation [71]	--	--	Yes

health, as they regulate vascular tone, vascular growth, inflammation, cell adhesiveness and coagulation [6]. A loss of normal endothelial function is believed a key event in the initiation of atherosclerotic process [7]. Endothelial function can be measured by different techniques, yet the most widely applied technique is brachial artery Flow-Mediated Dilation (FMD). FMD is a nitric oxide-mediated process using high resolution US to monitor the response of arterial diameter to increase in blood flow, which represents a physiologic stimulus for endothelial nitric oxide release [8].

FMD has been shown to be adversely associated with CV risk factors, like hypertension, diabetes, dyslipidemia, smoking, inflammation and abdominal obesity [9-13]. The prognostic value of FMD for future CV events has been also tested. In the elderly population of the Cardiovascular Health Study (CHS) [14] as well as in the population-based cohort of the Multi-ethnic Study of Atherosclerosis (MESA) [15], FMD resulted an independent predictor for CV events, and in a recent meta-analysis of 23 studies including 14 753 subjects, for each 1% increase in FMD the overall CVD risk decreased by 13% in diseased population and by 4% in asymptomatic population [16]. However, the above-mentioned studies also demonstrated that FMD added little to the prognostic accuracy of traditional CV risk factors/scores. In the intermediate risk population of the MESA, the Net Reclassification Improvement (NRI) of FMD for incident coronary heart disease was only 2.4% (Table 1), being lower than that of carotid intima-media thickness (IMT; 10.2%) [17].

FMD has been also used to evaluate the effect of life-style and pharmacologic interventions on endothelial function, and numerous studies have demonstrated that endothelial dysfunction may improve with exercise, weight loss, vitamins, statins or antihypertensive treatment [18-21].

Although the concept of FMD is simple, the correct and reproducible measurement requires significant expertise, and published guidelines suggest a minimum of 100 supervised scans before the independent scanning, and at least 100 scans per year to maintain competency [22]. Additional methodological issues, like the level of inflation pressure, duration of ischemia, localization of inflation and the time point at which the effect of hyperemia on artery is measured, should be standardized and respected by all centers using this method [22-23].

Therefore, at the moment, FMD seems to be above all an interesting research tool that can be employed to study the role of emerging CV risk factors in the development of atherosclerosis and to monitor the effect of interventions and treatment on endothelial function, as the method is non-invasive, does not use pharmacologic stimuli, can be repeated several times and has an adequate reproducibility in expert laboratories [24]. On the other hand, its widespread clinical use for the risk stratification is not currently recommended by guidelines [25-26].

Carotid/Femoral artery IMT and plaques

Arterial wall is histologically constituted of three layers; 1) the inner layer, called tunica intima and composed of a monolayer of endothelial cells supported by internal elastic lamina; 2) the middle layer, called tunica media and consisting of longitudinal smooth muscle cells surrounded by connective tissue with elastic lamellae; 3) the outermost layer, called tunica adventitia and formed by connective tissue with collagen fibers.IMT is a combined measure of tunica intima and tunica media, and may be assessed by high-resolution US in different segments of extracranial carotid tree or in large muscular arteries as the distance between the intima-luminal and the medial-adventitial interfaces of the arterial wall. With aging and the presence of CV risk factor, IMT increases and loses its double-line aspect

Figure 1: A) Thin intima-media complex in adolescent; B) Increased thickness of intima-media complex in elderly healthy subject; C) Diffuse thickening of intima-media complex with structural changes leading to the loss of a 3-layer aspect; D) Irregular and markedly thickened CCA wall - development of the plaque at the posterior wall.

(Figure 1). Plaque, a focal intima-media thickening, appears first in the plaque prone areas [27], i.e. areas with a particular hemodynamic pattern, like arterial branching, that is characterized by a low and oscillating shear stress and a cyclic reversal flow (Figure 2A). It is unclear, whether thickened intima-media complex and the presence of plaques represent two different stages or two different phenotypes of atherosclerotic process.

Numerous large prospective studies, like Kuopio Ischemic Heart Disease study, Atherosclerosis Risk in Communities (ARIC) study, CHS, Carotid Atherosclerosis Progression Study, Rotterdam Study, have demonstrated the association between carotid IMT and incident CVD risk [28-32]. A meta-analysis of 16 studies including 36.984 participants showed an increase in CV events risk of 16% per 0.1 mm difference in baseline common carotid artery (CCA) IMT [33]. However, the studies evaluating the predictive value of carotid IMT over and above established risk scores have been largely negative [17,31], and in a meta-analysis of 14 population-based studies (45.828 participants), the NRI of CCA IMT over Framingham risk score was small, being 0.8% for overall cohort and 3.6% for intermediate risk subjects [34] (Table 1). Still, the meta-analysis was affected by the variability in the IMT methodologies and reporting (number of projections, number of segments, mean or maximal IMT value), and

Figure 2: A) Small fibrotic plaque at carotid bifurcation (atherosclerotic prone area); B) Flat, lipid-rich plaque at the posterior wall of CCA; C) Fibrotic concentric plaque at carotid bulb; D) Irregular fibrotic plaque at carotid bulb and calcified plaque with an irregular surface at the origin of internal carotid artery (acoustic shadowing).

Figure 3: Far-wall CCA IMT is automatically measured within the ROI (green rectangle), and the values of IMT (QIMT) and diameter (D) are displayed beat-to-beat on the screen, together with averaged value (AVG) and standard deviation (SD).
A) Far-wall CCA IMT of healthy middle-aged subject (641μm); B)Far-wall CCA IMT of patient with dyslipidemia and coronary heart disease (1076 μm).

the evaluation was limited only to CCA IMT, while IMT in carotid bifurcation or Internal Carotid Artery (ICA) was not included. It seems that IMT in different carotid segments could be associated with different CV risk factors and events. In the British Regional Heart Study, CCA IMT was strongly associated with risk factors for stroke (above all blood pressure) and with prevalent stroke, whereas bulb IMT and/or plaques were more associated with ischemic heart disease risk factors(smoking, plasma fibrinogen) and prevalent ischemic heart disease [35]. In the MESA, only the mean of maximum IMT in ICA, but not in CCA, has significant NRI (7.0%) for coronary heart disease events [36] (Table 1). The differences between carotid segments can be explained by the fact that the atherosclerotic process tends to develop initially in the areas of arterial branching (Figure 2A), and that increase in CCA IMT may represent not only the atherosclerotic process, but also arterial remodeling reflecting smooth muscle cells hypertrophy and hyperplasia responding to increase in wall tensile stress and pulsatile load [37-38], above all in hypertensive patients.

Carotid plaque (Figure 2 A-D), when compared to IMT, has been shown to have a higher diagnostic accuracy for the prediction of future coronary artery disease events [39], and the presence of carotid plaques predicted CV mortality independently of SCORE stratification [40]. In the ARIC population followed for 15.2 years, adding plaque and carotid IMT (an average of values obtained in 3 segments of both the left and right sides) to traditional risk factors improved coronary heart disease prediction (NRI= 9.9% in the entire population and 21.7% in the intermediate risk groups) [41]. Improvement of CVD risk estimation over Framingham risk score and traditional risk factors was observed for the plaque presence in the ICA (NRI=7.3%) and for the total plaque score (NRI=10.2%) in the Framingham Offspring Study cohort and in Chinese population [43], respectively (Table 1).

Altogether the published data indicate that extensive US examination of extracranial carotid tree is required to fully assess the degree of atherosclerotic burden. Indeed, the American Society of Echocardiography consensus statement specifies that carotid-artery ultrasonography for CVD risk prediction should be based on a thorough scan to detect the presence of plaques, followed by measurement of IMT in the CCA [44], and Mannheim consensus recommends to image each carotid segment from multiple angels as atherosclerotic process is distributed asymmetrically within the vessel wall [45].

In healthy subjects, an average IMT ranges between 400-750 μm (according to age; Figure 1 A and B), IMT progression rate is small - between 1-32μm per year, and the differences between the 25th and 75th percentile are < 1 mm [46-49]. Consequently, a high degree of precision is required for IMT measurement. A higher accuracy and better reproducibility of measurements can be achieved by applications of automated edge-tracking algorithms [50], or by the use of Radiofrequency (RF)-based echo tracking technology allowing automatic measurement of IMT directly from unprocessed RF signal (Figure 3) [51].

The definition of abnormal IMT values is also important, as carotid IMT increases with age and differs between men and women [47-48]. It is generally recommended to consider an abnormal carotid IMT when greater than the 75th percentile for age and sex [5,44]. Several age- and sex-specific reference intervals for CCA IMT as measured by B-mode US [52,53] or by RF-based echo tracking system were published [47].

The definition and quantification of carotid plaque varied in different studies. Mannheim consensus defined plaque as a focal thickening that encroaches into the lumen by 0.5 mm or by 50% of the surrounding IMT, or when IMT is >1.5 mm [45]. Plaque burden has been quantified through plaque number, plaque thickness, plaque area [54] or plaque volume as assessed by 3-D US [55]. Some investigators suggested a characterization of plaque composition, by means of densitometric gray-scale analysis (Figure 4) [56,57], in order to distinguish the less echogenic lipid-rich carotid plaques, which are associated with a higher risk of ischemic stroke, from stable

Figure 5: Distension values (DIST), diameter (D) and distension curves of CCA in: A) Healthy young subject (distension=533 μm); B) Middle-Aged subjects (distension=411 μm); C) Diabetic patient (distension=183 μm).

more echogenic fibrotic plaques [58].

The IMT in muscular Femoral Artery (FA) seems to provide information similar as or complementary to carotid IMT. FA IMT has been shown to be associated with CV risk factors [59,60],extent of coronary atherosclerosis [61] and peripheral artery disease [62], and patients with peripheral artery disease and hypo-echogenic femoral plaques had a 7.24-fold increased CV risk compared with patients with hyper-echogenic plaques after adjustment for possible confounders [63].

Altogether, a detailed US examination of extracranial carotid tree and/or femoral artery, which includes measurement of IMT at different segments, quantification of carotid plaque burden and evaluation of plaque composition, seems to provide clinically useful information on CV risk, above all in intermediate risk subjects (Table 1).

Local arterial stiffness

Arterial stiffness results from a degenerative process affecting mainly the extracellular matrix of elastic arteries under the effect of aging and risk factors. Changes in the mechanical properties of the vessel wall related to arterial stiffening may activate number of mechanisms involved also in the process of atherosclerosis [64]. In fact, arterial stiffening and atherosclerosis could be viewed as two synergic processes that may potentiate each other in the development of vascular changes underlying CVD [65]. Several noninvasive methods are now available to estimate large artery stiffness in the clinical setting, including segmental Carotid-Femoral (CF) Pulse Wave Velocity(PWV) and local arterial stiffness, namely carotid and femoral.

Carotid-femoral PWV, an estimate of aortic stiffness, represents a gold standard method for arterial stiffness assessment [66,67] and is measured directly, as a ratio of distance between two measurement points divided by the time required for the pressure propagation to travel this distance. Number of studies has demonstrated the association between cfPWV and CVD events [68,69], and in a large meta-analysis including 16 studies and 17 635 participants [70], the age-, sex- and risk factors adjusted hazard ratios per 1SD change in logcf-PWV were 1.23, 1.28 and 1.30 for coronary heart disease,

Figure 4: (A) Video-densitometric (gray-scale) analysis of carotid plaques; (B) Histograms of gray levels (256 levels) of echolucent lipid-rich plaque and echogenic fibrotic plaque.

stroke and CVD, respectively. In addition, cf-PWV improved risk prediction in the intermediate risk group by 13%.Carotid-femoral PWV is usually measured by tonometry (Complior, SphygmoCor); however, Doppler US can be also used, assuming that the pulse wave propagation corresponds to the flow wave propagation of spectral Doppler [71,72].

Local arterial stiffness is estimated as systo-diastolic changes in arterial diameter/area over systo-diastolic changes in distending pressure (local pulse pressure). RF-based US technology, thanks to its high frame rate and spatial resolution, is capable to track the movement of the arterial wall throughout the cardiac cycle with a great accuracy [73]. From the real-time distension curves (Figure 5), maximum and minimum arterial diameters are measured, and different indices of arterial stiffness can be calculated, including local PWV (Table 1) [74-76]. Local distending pressure used in these calculations is estimated converting the distension curve to pressure curve by a linear conversion factor and assuming that the difference between mean arterial pressure and diastolic pressure is invariant along the arterial tree [77].

An assessment of local arterial stiffness on a larger scale became feasible only recently, with the introduction of RF-based echo tracking systems, and studies evaluating the association of local stiffness with risk factors and CVD events are still limited. Carotid stiffness has been shown to be associated with blood pressure, plasma lipids, smoking, C-reactive protein, diabetes and physical activity [78-80]. A recent analysis in the Hoorn Study population followed for 7.6 years has demonstrated that both carotid and femoral distensibility indices are independently associated with incident CV events and all-cause mortality [81].Indeed, the hazard ratios per 1 SD for CV events and all cause mortality, respectively, were 1.22 and 1.51 for lower carotid distensibility, and1.39 and 1.27 for lower femoral distensibility; these hazard ratios were comparable with those obtained in the same population for a higher cfPWV (1.56 and 1.13, respectively). The age- and sex-specific percentiles of carotid and femoral stiffness in a healthy population were estimated and the z-scores were established, which enables the comparison of carotid/femoral stiffness values between different patients and helps the interpretation of these measures [82,83].

Therefore, an assessment of local carotid and femoral stiffness by means of US is feasible and seems promising; however, this approach has to be further validated in prospective studies in order to determine its clinical utility in CV risk prediction.

The role of vascular biomarkers in pediatric populations

Assessment of CV risk in pediatric populations gets increasing interest. Although the CV events are rare in children, epidemiologic studies have demonstrated that CV risk factors are already identifiable in childhood and are predictive of adulthood CVD, type 2 diabetes mellitus and hypertension [84]. Moreover, current evidence suggests that childhood and adolescence are particularly vulnerable periods of life to the effects of cardiometabolic risk factors and later development of atherosclerosis, hypertension and diabetes [85]. To slow down the progression of atherosclerosis, and thus to reduce the future burden of CVD, preventive strategies should be developed [86]. A systematic screening of children and adolescents at high cardiometabolic risk (like obese children, children with dyslipidemia and type 1 diabetes,

off-springs of diabetic parents), including also the assessment of preclinical vascular damage, may help to apply targeted early lifestyle interventions and follow their effect.

Several studies have shown that CV risk profile in childhood may influence vascular biomarkers in adulthood. In the Cardiovascular Risk in Young Finns Study, the number of risk factors measured in 12- to 18-year-old adolescents were directly related to CCA IMT measured in young adults at ages 33 through 39 years (P<0.001 for both men and women), and remained significant after adjustment for contemporaneous risk variables [87]. Data from the three large population-based prospective studies (the Bogalusa Heart Study, the Cardiovascular Risk in Young Finns Study and Childhood Determinants of Adult Health Study) have demonstrated that dyslipidemic lipoprotein levels in adolescence (age 12 to 18 years) are associated with increased carotid IMT in adulthood (age 29 to 39 years), and that adolescent dyslipidemic status is more strongly associated with high adult IMT than changes in dyslipidemic status during follow-up [88].

A major current trend that may significantly increase the future burden of CVD is a considerable increase in the prevalence of childhood obesity. Obesity in childhood increases the probability of being obese as adult, represents a risk for adulthood CV morbidity and mortality [89,90], and is associated with wide spectrum of vascular changes. Endothelial dysfunction, the first step towards atherosclerosis, has been described in childhood obesity [91-93]. However, data regarding the impact of obesity on carotid structure and function in children are not conclusive; CCA IMT has been reported as increased or unchanged [89,94-97], whereas arterial stiffness has been found to be increased, unchanged or even decreased [94,98-100].These discrepancies could reflect the fact that morphological and functional characteristics of large arteries in pediatric population are particularly influenced by age, body size, and sexual maturation, as adolescence brings an onset of hormonal changes and growth that are likely to affect the vasculature [101]. During a natural growth and development arterial diameter and distensibility increase [102], and this pattern could be amplified in obese youths, where increased arterial distension compensates for larger luminal diameter and higher blood pressure [103]. Yet, longer-lasting adaptation to obesity-induced increase in hemodynamic load may induce structural changes within the arterial wall leading to its stiffening; the time course of this pathological adaptation is not known. In a population of 14-year old children followed for 5 years, cfPWV at baseline was significantly lower in obese as compared to lean subjects [94]; yet during a follow-up period obese subjects had significantly higher increase in cfPWV than lean ones (25% vs 3%; P<0.0001 vs<0.05, respectively) [104].

CV prevention and risk estimation in pediatric cohorts is challenging, and the interpretation of vascular measures is more demanding than in adults, as an adaptive remodeling of vessel wall in response to physiological development must be considered. A large study including 1051nonobese and nonhypertensive children aged 6 to 18 years, has tried to establish the sex-specific reference charts for CCA IMT and distensibility, and to explore the impact of developmental changes in body size and blood pressure on these measures [101].

Conclusion

Vascular US is capable to detect, during a single examination, the structural and functional changes of elastic and muscular arteries, which increases the probability to detect early organ damage, helps to understand the pathophysiology of vascular impairment, may improve the assessment of individual CV risk and facilitate the decision-making regarding the life-style or therapeutic interventions. Nowadays, vascular US is largely used for studying the impact of established and emerging risk factors and therapeutic interventions on arterial wall, and thus, on initiation, progression and regression of arteriosclerotic and atherosclerotic processes. In the next future, vascular US could become an interesting tool for the assessment of individual CV risk due to its common availability, non-invasiveness, absence of radiation and low cost. There is no evidence that single vascular measures/biomarkers are superior; in contrast the use of different measures could provide complementary information and improve the risk assessment. However, the widespread use of vascular biomarkers in CV risk assessment and CV prevention requires a clear standardization of US methodologies [44,45] and definition of age- and sex-specific reference intervals for different vascular measure in large populations [47,52,53,82,83].

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