What is the role of non-invasive measurements of atherosclerosis in individual cardiovascular risk prediction?

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Abstract
Primary prevention of CVD (cardiovascular disease) is mainly based on the assessment of individual cardiovascular risk factors. However, often, only the most important (conventional) cardiovascular risk factors are determined, and every level of risk factor exposure is associated with a substantial variation in the amount of atherosclerosis. Measuring the effect of risk factor exposure over time directly in the vessel might (partially) overcome these shortcomings. Several non-invasive imaging techniques have the potential to accomplish this, each of these techniques focusing on a different stage of the atherosclerotic process. In this review, we aim to define the current role of various of these non-invasive measurements of atherosclerosis in individual cardiovascular risk prediction, taking into account the most recent insights about validity and reproducibility of these techniques and the results of recent prospective outcome trials. We conclude that, although the clinical application of FMD (flow-mediated dilation) and PWA (pulse wave analysis) in individual cardiovascular risk prediction seems far away, there may be a role for PWV (pulse wave velocity) and IMT (intima-media thickness) measurements in the near future.

Introduction
More than 50 years ago, the Framingham Heart Study [1] identified the traditional cardiovascular risk factors [for example, age, male gender, hypercholesterolaemia, low HDL (high-density lipoprotein)-cholesterol, smoking, hypertension and diabetes mellitus], and many new or emerging risk factors have been identified since. Primary prevention of CVD (cardiovascular disease) is based mainly on the assessment of these individual risk factors, and on the assessment of individual cumulative risk by using a system of graded risk charts. In such a system, individual risk factors are considered as additive in their predictive power and, especially in patients in whom multiple, moderately elevated, risk factors are present, such a graded risk system can be of help in tailoring the intensity of preventive therapy in individual subjects. The Interheart study [2] showed that nine easily measured and potentially modifiable risk factors account for over 90% of the risk of an initial myocardial infarction. However, using a system based on risk factor assessment has its shortcomings in individual cardiovascular risk prediction. Often, only the most important (conventional) cardiovascular risk factors are determined. Furthermore, every level of risk factor exposure is associated with a substantial variation in the amount of atherosclerosis.

Key words: atherosclerosis, cardiovascular risk factor, flow-mediated dilatation (FMD), intima-media thickness (IMT), non-invasive measurement, pulse wave velocity (PWV).

Abbreviations: ACE, angiotensin-converting enzyme; AIx, augmentation index; AT1, angiotensin II type 1; C1, large artery compliance; C2, small artery compliance; CCA, common carotid artery; CT, computer tomography; CVD, cardiovascular disease; CV, coefficient of variation; FMD, flow-mediated dilatation; ICA, internal carotid artery; IMT, intima-media thickness; MAP, mean arterial pressure; MRI, magnetic resonance imaging; PP, pulse pressure; PWA, pulse wave analysis; PWV, pulse wave velocity; SI, stiffness index.

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Atherosclerosis. This could be the result of differences in genetic susceptibility, of interactions with other risk factors and of variation in the duration of exposure to the specific risk factor: prolonged exposure will increase the individual risk of developing a cardiovascular event. However, since there is a huge time-gap between the manifestation of a cardiovascular risk factor on one hand and the development of a clinical event on the other hand, it is complicated to predict individual cardiovascular risk at a given moment. Theoretically, these shortcomings of traditional risk factor screening could (partially) be overcome by measuring the effect of risk factor exposure over time directly in the vessel wall by means of quantifying the amount of subclinical disease. Several non-invasive imaging techniques have the potential to accomplish this, each of these techniques focusing on a different stage of the atherosclerotic/arteriosclerotic process. Early and predominantly functional changes in the vessel wall can be measured by surrogate markers of endothelial function, such as FMD (flow-mediated dilatation). Vascular stiffness, being a dynamic property based on both vascular function and structure, can be quantified by measurement of PWV (pulse wave velocity) or by PWA (pulse wave analysis). Finally, more advanced structural vascular wall changes can be quantified by MRI (magnetic resonance imaging) or cardiac electron-beam CT (computer tomography), with or without the calculation of coronary calcium scores or by measuring IMT (intima-media thickness) using B-mode ultrasound technique (Figure 1). Each of these techniques has the advantage with respect to invasive imaging techniques [such as angiography or IVUS (intravascular ultrasound)] in that they are relatively easy to perform and are more convenient for the patient.

As a result, non-invasive imaging techniques of atherosclerosis (quantified by, for example, FMD and IMT) and arteriosclerosis (quantified by, for example, PWV and PWA) have already been used as a surrogate end point of cardiovascular disease in many observational studies and intervention trials, as reviewed previously [3–5]. However, the role each of these techniques could play in individual cardiovascular risk prediction is less well defined, despite the aforementioned theoretical advantage above a system based on individual risk factor determination.

Therefore, in this review, we try to define the current role of several non-invasive techniques of atherosclerosis measurement in individual cardiovascular risk prediction, taking into account the most recent insights about validity and reproducibility of these techniques and the results of recent prospective outcome trials. A thorough review of all of the methods to assess atherosclerosis non-invasively is beyond the scope of this review. Therefore we have limited the discussion to the potential role of FMD, PWA, PWV and IMT in cardiovascular risk prediction, as these techniques can be performed relatively quickly and easily and at relatively low cost compared with other non-invasive techniques, such as MRI and CT measurements, which have the additional disadvantage of exposing the patient to contrast and/or radiation [6–10], making these techniques less suitable for large-scale individual cardiovascular risk prediction.

FMD

Measurement of brachial artery FMD was first described by Anderson and Mark in 1989 [11]. It began to see a clinical research application in the early 1990s, and nowadays
it is a widely used instrument to determine endothelium-mediated vasodilator function. FMD measures the response of the endothelium to artificially induced hypoxia, which is provoked by inflating an arterial occlusion cuff around the proximal or mid-forearm for several minutes. The (adenosine-mediated) decrease in peripheral resistance distal to the inflated cuff induces reactive hyperaemia in the brachial artery after cuff deflation. This leads to a shear-stress-mediated augmented NO production by endothelial cells. The release of increased amounts of NO, in turn, induces smooth muscle cell relaxation and thereby an increase in vessel diameter. FMD is calculated as the difference between the maximum diameter post-occlusion and the baseline diameter, relative to the baseline diameter, and is expressed as a percentage. In healthy subjects this FMD (%) is highly dependent on the technique used (location and duration of occlusion, and the use of commercially available software to measure vessel diameter continuously), and varies between 0.20 and 19.2 % [12,13].

Previous studies have shown a negative effect of aging and male gender on FMD [14], and various observational studies also demonstrated significant associations of FMD with many clinical and biochemical cardiovascular risk factors, including smoking [15], hypertension [16], elevated plasma levels of total cholesterol [17], decreased plasma HDL-cholesterol levels [18], hyperglycaemia [19] and hyperinsulinaemia [20]. As reviewed by Raitakari and Celermajer [21], many intervention studies, so far, have tested whether an improvement in cardiovascular risk factor profile resulted in an increase in FMD. However, a point of concern about most of these studies is their relatively small sample size, in combination with the moderate reproducibility of the FMD technique. It is not uncommon to find intra-observer CVs (coefficients of variation) > 30 % for FMD measurements [22,23]. Many studies, however, only report the (generally excellent) reproducibility of basal vessel diameter measurement. Corretti et al. [24] estimated that, based on the true reproducibility of the technique, minimal sample sizes of 20–30 patients in a cross-over study and 40–60 patients in a parallel group study design are required to detect an absolute change of 1.5–2 % in FMD. However, taking into account that the mean FMD in healthy subjects using a lower arm cuff occlusion is approx. 5 %, smaller changes might be of clinical significance, requiring much larger sample sizes. The moderate reproducibility of the FMD measurements is not only due to technical aspects of the measurement, but also to biological variability. It has been shown [15,25,26,28] that the intake of caffeine-containing products, alcohol, antioxidants and food (both carbohydrate- and fat-rich meals) could influence FMD. Other factors that have been shown to limit reproducibility are smoking, use of vasoactive medication, sympathetic activation [29], the use of oestrogens and the moment of measurement in the menstrual cycle.

Clinical use of FMD
A direct relationship between brachial artery FMD and coronary endothelial function has been demonstrated [30,31], and several studies have shown that FMD measurements may provide important prognostic information in patients with or at risk of CVD [32–34]. However, some critical notes can be added to these observations. First, it should be emphasized that, to date, no prospective intervention studies have been carried out to demonstrate that an improvement in FMD results in a reduction in CVD (risk). Secondly, several studies report that morphological changes of the brachial artery vessel wall, rather than FMD in the brachial artery, are a better indicator of the extent and severity of coronary artery disease [35,36]. In the Cardiovascular Risk in Young Finns Study [37], it was even shown that these morphological changes themselves are an independent predictor of FMD. Although it is generally assumed that the correlation between FMD and IMT in high-risk populations is the result of the fact that both IMT and FMD are representatives of the same atherosclerotic process, a reciprocal effect of IMT on FMD could not be excluded. Either changes in the composition of the extracellular matrix (passive elastic properties) or the responsiveness of smooth muscle cells to NO (active elastic properties) will limit maximal dilatation, as recently suggested by Witte et al. [38]. This questions the additive value of FMD measurements compared with (or besides) the measurement of morphological vascular wall damage (IMT) in terms of CVD risk prediction, especially in populations where a high prevalence of advanced morphological wall changes can be expected.

The use of FMD as a tool to predict CVD in individuals is also limited by the confounding effect of cardiovascular medication on FMD. Various studies have shown a beneficial effect of statins on endothelial function in healthy subjects [39–41] and subjects with hypercholesterolaemia [42,43]. Similar beneficial effects on endothelial function have also been reported for treatment with ACE (angiotensin-converting enzyme) inhibitors [44] and AT1 (angiotensin II type 1) receptor antagonists [45]. Cardiovascular medication use will therefore render a falsely high FMD and a resultant lower CVD risk.

A final reason for limiting the use of FMD in individual risk prediction is the aforementioned moderate reproducibility of this technique/measurement. Standardization of both patient conditions and the measurement technique should be achieved in order to be able to use FMD as a marker of individual cardiovascular risk. Subjects should not exercise before the measurements, and they should not ingest substances that might affect FMD, such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4–6 h before the study, as reviewed by Corretti et al. [24]. Furthermore, agreement should be reached about the occlusion site and occlusion time...
Table 1 Commercially available devices to measure local, regional/segmental or systemic arterial stiffness

<table>
<thead>
<tr>
<th>Device (company)</th>
<th>Technique</th>
<th>Local, regional or systemic</th>
<th>Outcome measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIUS 02™ (Asulab)</td>
<td>Simultaneous detection of diameter and pressure waveform using the RF-signal of an ultrasound system</td>
<td>Local</td>
<td>Distensibility, elastic modulus and compliance index</td>
</tr>
<tr>
<td>WallTrack system™ (Pie Medical)</td>
<td>Simultaneous detection of diameter and pressure waveform using the RF-signal of an ultrasound system</td>
<td>Local</td>
<td>QCS</td>
</tr>
<tr>
<td>Sphygmocor™ (Atcor Medical)</td>
<td>Pressure wave detection with applanation tonometry</td>
<td>Regional and systemic</td>
<td>PWV, Alx and central arterial PP</td>
</tr>
<tr>
<td>Pulse trace system™ (Micromedica)</td>
<td>Digital volume wave detection with photoplethysmography</td>
<td>Systemic</td>
<td>SI</td>
</tr>
<tr>
<td>Pulse Wave CR2000™ (Hypertension Diagnostic)</td>
<td>Pressure wave detection with a piezoelectric sensor</td>
<td>Regional and systemic</td>
<td>C1 and C2</td>
</tr>
<tr>
<td>PulsePen™ (DiaTecne)</td>
<td>Pressure wave detection with applanation tonometry</td>
<td>Regional and systemic</td>
<td>PWV, Alx and central arterial PP</td>
</tr>
<tr>
<td>PeniScope (Genesis Medical Systems)</td>
<td>Oscillometric pressure wave detection</td>
<td>Regional</td>
<td>PWV</td>
</tr>
<tr>
<td>Complior™ (ArtecMedical)</td>
<td>Pressure wave detection with mechanotransducer</td>
<td>Regional and systemic</td>
<td>PWV, central arterial PP and SI</td>
</tr>
<tr>
<td>AT-form PWV/ABI™ (Colin Co.)</td>
<td>Volume plethysmography</td>
<td>Regional</td>
<td>abPWV</td>
</tr>
</tbody>
</table>

(upper arm occlusion and an occlusion time > 4.5 min yields a significantly higher FMD than forearm occlusion and occlusion times < 4.5 min) [11,13]. A major step in achieving agreement about FMD methodology has recently been set by the European Working Group on Endothelin and Endothelial Factors, by publishing a consensus paper with recommendations about the methodology of FMD measurements [46].

Reproducibility could be enhanced further by commercially available technology, enabling investigators to examine the entire time course of brachial dilatation in response to reactive hyperaemia instead of performing diameter measurements at fixed times only, or enabling investigators to measure vessel diameter automatically by using edge detection [24].

**PWV AND PWA**

Large conduit vessel elasticity depends largely on the elastin/collagen ratio in arterial walls, although endothelial activity, arterial smooth muscle bulk and dynamic properties, such as MAP (mean arterial pressure), systemic vascular resistance and heart rate, also play a role. Any decrease in conduit vessel elasticity has unfavourable cardiovascular consequences. It augments aortic and left ventricular systolic blood pressures (and thereby left ventricular load), whereas, at the same time, it decreases the capacity for myocardial perfusion during diastole [47]. Three types of arterial stiffness can be considered: systemic, regional/segmental and local [48]. Local arterial stiffness can be measured by vessel wall tracking with B-mode ultrasound and subsequent calculation of indices of vascular stiffness [i.e. arterial compliance, arterial distensibility, Peterson’s/Young’s elastic modulus or the β-index/SI (stiffness index)] [49]. Information about regional/segmental and systemic vascular stiffness can be obtained non-invasively by determination of surrogate markers of arterial stiffness, such as PWV, or by analysis of the arterial pressure waveform (PWA). Since most commercially available devices measuring arterial stiffness use one of these latter techniques (Table 1), we have concentrated on the potential use of PWV and PWA for individual cardiovascular risk prediction.

**PWA**

PWA aims to study the form of the arterial pressure or volume wave. Arterial pressure waves are recorded at a peripheral artery using an application tonometer, a mechanotransducer or a piezoelectric sensor, whereas volume waves are usually recorded on the index finger using photoplethysmography (Table 1). The validity of both pressure and volume wave recordings has been criticised, as reviewed extensively by Oliver and Webb [50]. One of the major criticisms of both pressure and volume wave recordings is related to the fact that the waveforms obtained are often calibrated against the (conventionally measured) brachial blood pressure, and subsequently transformed to derive a (central) aortic waveform. This is necessary since peripheral pulse waveform varies markedly from the central pressure waveform due to both ‘general features’, such as wave dispersion, amplification and reflection, and ‘subject-specific characteristics’, such as body height, heart rate, pre-load and blood viscosity. The validity of the use of such a transfer function is a topic of much debate [4,51–56], and the oscillometric cuff method to measure brachial blood pressure for calibration of the radial pressure wave has especially been suggested as a possible weak link.

Once the central arterial waveform is obtained, different components of the waveform can be derived as surrogate markers of arterial stiffness, with a reproducibility that is generally reported to be good [57–61]. These
components include (but are not confined to) the central arterial PP (pulse pressure), AIX (augmentation index), C1 (large artery compliance) and C2 (small artery compliance) for applanation tonometry, and the arterial SI for photoplethysmography. However, since all of these parameters are (in)directly influenced by factors influencing blood pressure, measurement conditions have to be carefully standardized. The international Task Force studying the clinical applications of arterial stiffness [48] advises that subjects refrain from smoking and food for at least 3 h [62–64] and that the subject has a period of rest in a quiet temperature-controlled room before the actual measurements start. When comparisons across groups are desired for PWA-derived parameters, differences in blood pressure and heart rate between the groups should be addressed and (when necessary) statistically controlled for.

**PWV**

PWV is defined as the speed of a pressure wave travelling along an artery of length over a time (Δt) [49], with a stiffer artery giving a higher PWV. Different signals can be used for measurement of PWV, including Doppler and pressure measurements [65]. Most commercial devices currently available use the pressure signal to obtain PWV [66–71] (Table 1). Using this technique, the arterial pulse is recorded using a proximal artery. Usually, PWV is calculated after arterial pressure waves have been recorded at both a proximal and a distal artery by applanation tonometry: the length of the artery segment between both measurement sites is measured with a tape measure, and the wave transit time (Δt) is obtained either by placing a probe on each site and recording the waveforms simultaneously or by recording the waveforms at different sites on separate occasions and using the QRS complex of a simultaneously recorded ECG as a reference frame. Both intra- and inter-observer reproducibility of PWV measurements are reported to be good, regardless of the device used [61,69,72,73]; as for PWA, this could be due to the fact that the technique is relatively simple to learn, even for the inexperienced examiner.

However, as for PWA, there are concerns about the validity of the measurements. First, the determination of the exact foot of the pressure wave (used to calculate Δt) is sometimes difficult to establish, and various algorithms are used for this purpose [74]. Secondly, measurement of the pulse propagation distance is sometimes assumed to be inaccurate, especially in obese subjects and in females with large breasts, where a significant amount of ‘body contour bias’ might be introduced [65], even if measurements are undertaken parallel to the plane of the examination table. In addition, any vessel tortuosity is not accounted for [75]. A third concern about the validity of PWV measurements is the documented confounding influence of distending pressure (represented by MAP) [76] and age on PWV. Finally, the use of the R-wave of a simultaneously recorded ECG as a reference frame to determine pulse propagation time requires that the heart rate is regular and that the ejection duration is relatively constant. However, despite the concerns about validity and the fact that we know of only one study comparing invasively measured PWV with data obtained non-invasively [77], the concept of non-invasively measuring PWV as a surrogate marker of arterial stiffness has been widely accepted. This may be attributed to the fact that validation studies have already been outstripped by numerous outcome studies, as mentioned below.

**Clinical use of PWA and PWV**

PWV waveform is highly dependent on heart rate and blood pressure, which vary not only between subjects, but also within subjects over the course of the day [78], rendering it difficult to obtain valid reference values for, for example, AIX, central arterial PP, SI, C1 and C2. In line with the dependency of PWA parameters on heart rate and blood pressure, several intervention trials have shown that vasoactive medication, such as AT1 receptor antagonists [79], ACE inhibitors [80], β-blockers [81], nitrates [82] and calcium-channel blockers [83], have a profound effect on PWV-derived parameters. Vasoactive medication use will therefore be an important confounder in the prediction of cardiovascular risk by PWA-derived parameters. Unlike when assessing groups, one cannot statistically adjust for the influence of confounders, such as vasoactive medication use, when the aims are to use the PWA-derived parameters for the purpose of individual CVD risk prediction. The value of PWA-derived parameters in individual risk prediction is questioned further by the results of two large prospective studies (CAFE (Conduit Artery Function Evaluation) [84] and ANBP2 (Second Australian National Blood Pressure Study) [85]), which demonstrated recently that the use of central arterial waveforms or central pressures does not add to the prognostic information provided by routine brachial blood pressure measurements. Therefore, at the present moment, PWA-derived parameters and central pressures do not appear to have a place in the routine examination to determine individual risk of CVD.

For PWV, several prospective studies investigating the ability to predict CVD have been carried out. Various studies have demonstrated that PWV is an independent predictor of all-cause mortality in various patient categories, including patients with hypertension [86], end-stage renal failure [87] and diabetes mellitus [88], and in the elderly [89]. Furthermore, PWV has been shown to predict a composite of cardiovascular outcomes above and beyond traditional cardiovascular risk factors in two large prospective population-based studies [90,91]. However, it should be kept in mind that any epidemiological evidence for the additive predictive value of PWV in these studies (besides the traditional risk factors) does not necessarily mean that adding PWV measurements to
the routine examination of individual patients is also of clinical value [92]. The merit of adding PWV depends on the resultant increase in the hazard ratio, and this can vary depending on the population under study. The superior predictive ability of PWV for the development of CVD as found in populations with a low cardiovascular risk has not yet been confirmed in high-risk populations. However, we have demonstrated recently [93] in a cross-sectional study of a high-risk population, consisting of FCH (familial combined hyperlipidaemia) family members, that measuring PWV besides traditional risk factor screening does not increase predictive ability for prevalent CVD. Prospective studies are demanded to confirm or weaken this finding in (other) high-risk populations before conclusions can be drawn about the additive value of PWV alongside traditional risk factor screening in terms of individual cardiovascular risk prediction.

IMT

In 1986, Pignoli et al. [94] showed that arterial wall IMT could be measured non-invasively by B-mode ultrasonography. Most often, IMT is measured in the carotid artery, as this superficial artery is easily accessible, and carotid IMT has been shown to be a good representative of the amount of atherosclerosis throughout the whole body [95–97]. Several different approaches can be used to acquire carotid IMT. Although some studies measure IMT of the CCA (common carotid artery) only, other studies determine IMT of the ICA (internal carotid artery) and/or the carotid bifurcation as well. Although the latter approach takes more time, it also yields more information. A disadvantage of the latter technique, however, is the slightly lower reproducibility of measurements of the bulb and ICA compared with CCA and the more troublesome assessment of these segments, resulting in a higher number of missing values [5,98]. Another source of variation in the IMT measurement technique is the angle of examination. Although some protocols measure IMT in a single optimal B-mode image, others measure IMT in a combination of several (fixed) angles of examination. Even though both approaches have never been compared, the multiple-angle approach has the theoretical advantage of taking into account the irregularity of the atherosclerotic process. Irrespective of the technique used, reproducibility of IMT measurements is usually reported to be good, with intra-observer CVs of < 5 % and inter-observer CVs of < 10 % [5,98,99]. The validity of B-mode ultrasound assessment of IMT is widely accepted, as various studies have shown a close association between ultrasonically and histologically determined IMT [100,101]. In addition, IMT has been associated with a number of important classical cardiovascular risk factors, including increasing age, male gender, smoking, obesity, an impaired glucose tolerance, diabetes, hypertension, alcohol intake and hypercholesterolaemia [102–107], although associations of IMT with several new or emerging risk factors (such as haemostatic factors) are more controversial [108,109].

Clinical use of IMT

IMT is not only associated with individual cardiovascular risk factors (as mentioned above), but also with the development of CVD, as has been shown in a number of large prospective population-based studies [110–112]. Therefore IMT is currently generally accepted as a validated surrogate end point of CVD and has been used in numerous large-scale cardiovascular intervention trials [113–115]. However, despite the important role of IMT in these intervention trials, the value of IMT as a marker of individual cardiovascular risk is as yet less well defined. Very recently, Baldassarre et al. [116] succeeded in deriving ‘best threshold values’ for IMT in a large dyslipidaemic population (n = 1969): an IMT above this threshold value was an independent predictor of cardiovascular events with a hazard ratio of 6.7. However, as for PWV, the optimal threshold value for risk prediction will depend on the population under study and, therefore, threshold values from a broad variety of populations have to be derived. In addition, to be able to use threshold values in clinical practice, it is necessary to highly standardize IMT measurement methods by defining which segment(s) should be measured under which angle of interrogation. If these two requirements can be fulfilled, there seems to be a place for IMT in individual risk prediction in the near future.

CONCLUSIONS

Although the clinical application of FMD and PWA in individual cardiovascular risk prediction still seems far off, there may be a role for PWV and IMT measurements in the near future. However, before the clinical application of PWV and IMT is considered in individual CVD risk prediction, first the additive value of these measurements to predict individual CVD risk above and beyond the measurements of traditional clinical and biochemical parameters has to be demonstrated and appropriate cut-off values have to be derived across various (high) risk populations.

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