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REVIEW

Non-invasive measurements of atherosclerosis (NIMA): current evidence and future perspectives

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ABSTRACT

In clinical practice, cardiovascular (CV) risk stratification is based on the assessment of individual risk factors. Still many cardiovascular deaths occur in individuals who were not at high risk according to the current CV risk stratification models as the Systematic COronary Risk Evaluation chart (SCORE) and Framingham Risk Score. By measuring morphological and/or functional abnormalities in the arterial wall directly, the impact of all CV risk factors together can be determined. In this review, the current status for the use of a panel of non-invasive measurements of atherosclerosis (NIMA) in CV risk prediction in clinical practice is discussed. Some of these NIMA showed predictive value for CV disease, such as intima-media thickness, pulse wave velocity, and ankle-brachial index, both in patients and in healthy and community-based populations. Recommendations have been made to include these NIMA in CV risk stratification in secondary prevention. However, the additional value of NIMA in CV risk stratification in primary prevention settings remains to be determined. Furthermore, the main determinants of NIMA are still unclear. Also the use of different combinations of NIMA should be evaluated, since different NIMA likely reflect different stages and aspects of the atherosclerotic process that leads to CV events. Future prospective studies should focus on repeated measures of NIMA to reveal the main determinants of the different NIMA and evaluate the predictive value of baseline versus repeated measurements.

KEYWORDS

Imaging, atherosclerosis, arterial stiffness, intima-media-thickness, ankle-brachial index

INTRODUCTION

Cardiovascular risk

Cardiovascular disease (CVD) has been a major cause of death for decades now,[1] and it will probably be for years hereafter, although the number of cardiovascular deaths is decreasing.[2] In 2005, 17.5 million cardiovascular deaths were registered, which was 30% of all global deaths[3] and in the Netherlands a comparable trend was observed.[4] Atherosclerosis is the major underlying process, leading to cardiovascular events.[5] Many risk factors have been identified that promote atherosclerosis, including obesity, hypertension, lipid disorders, smoking, and diabetes mellitus.[1] Cardiovascular (CV) risk prediction is mainly based on the assessment of these individual CV risk factors. Often only the most conventional CV risk factors are determined and treated to reduce CV risk. We do not know why some individuals develop early CVD and others do not, despite the presence of risk factors. Many CV deaths occur in patients who were not identified as high-risk patients. Moreover, despite blood pressure control, optimising lipid levels and lifestyle advice, approximately 50% of the patients who died from cardiac arrest were in the intermediate risk category of Framingham Risk Score, as described by Taylor in 2002.[6] Therefore, in the last few years research has focused on new biomarkers of atherosclerosis, including markers of inflammation and oxidative stress. So far, none of the new biomarkers appeared to have additional prognostic power in CV risk prediction beyond the traditional risk scores.[7-9] At every level of traditional risk factor exposure, there is a large inter-individual variation in the amount of atherosclerosis and the development of CVD. This variation is probably due to genetic susceptibility, combinations and interactions between risk factors,

including lifestyle habits, duration of exposure to specific levels of the risk factors, and factors such as biological and laboratory variability. When patients present at the clinic with a CV event, most of the damage has already been done. Therefore, atherosclerosis must be discovered as early as possible in primary prevention settings.

Non-invasive measurements of atherosclerosis (NIMA)

A current concept is that by measuring atherosclerosis directly in the arterial wall, the damage caused by known and unknown risk factors can be determined, which allows us to better predict CV risk for the individual patient. This also provides the opportunity to measure atherosclerosis before clinically overt CVD, as changes in the arterial wall precede the clinical symptoms of CVD. Thus, subclinical disease measurements, representing the final result of risk exposure and genetic susceptibility, may be useful for improving CVD risk stratification, therapeutic strategies and evaluation of risk factor modification.[10]

Several invasive techniques to visualise the arterial system and the extent of atherosclerosis are available, such as intravascular ultrasound and angiography; the latter has been the 'gold standard' imaging technique for the presence of stenosis and/or occlusions in the arterial system in clinical practice for years now. It does not need further explanation that invasive techniques are not suitable as a screening tool in the general population. More recently, less invasive techniques became available, such as computed tomography and magnetic resonance imaging, although sometimes detergents are needed to optimise the pictures, which have to be injected. Moreover, these techniques are not widely available and very expensive at the moment, they cannot be applied to every patient, and expose patients to radiation.

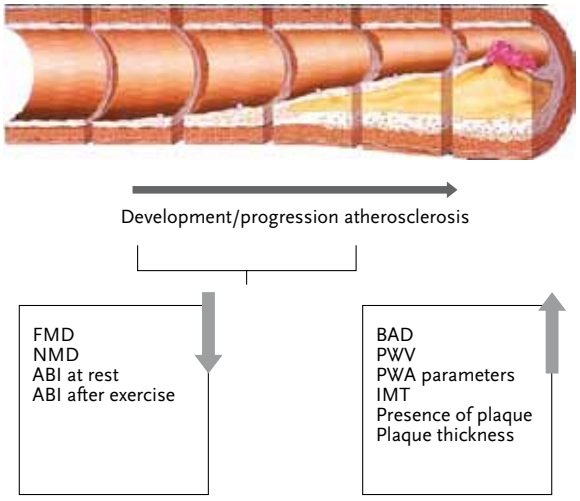
Therefore, many efforts have been made to develop relatively simple and cheap non-invasive measurements of atherosclerosis that can be applied to every individual. A variety of these non-invasive techniques have been developed in the last few years, each measuring different aspects of the atherosclerotic process. In this review, we explore the use of a panel of these non-invasive measurements and derived parameters, as depicted in *figure 1*. The NIMA will be discussed in the sections below based on our own experience, including their current status and evidence for introduction of these NIMA into clinical practice in primary prevention. We will conclude with future perspectives of NIMA in relation to cardiovascular risk prediction.

ENDOTHELIAL (DYS)FUNCTION

Flow-mediated dilation

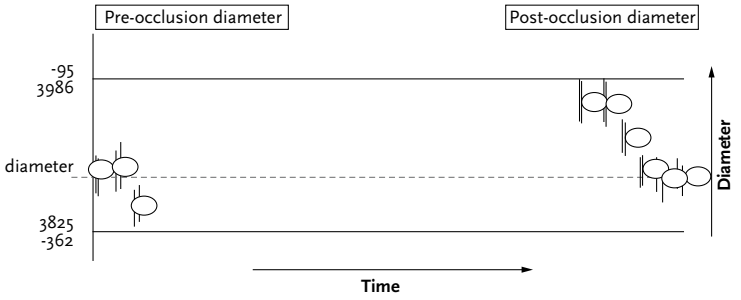
Dysfunction of the endothelium, a monolayer of cells that covers the intima, is one of the first signs of atherosclerosis and is present before structural changes. Endothelial (dys)function can be measured non-invasively by flow-mediated dilation (FMD) with ultrasound at the brachial artery *(figure 2)*.[11] It has been recommended to perform FMD measurements according to the guidelines of the International Brachial Artery Reactivity Task Force.[12] In short: changes in the diameter of the brachial artery are measured at baseline and after releasing a cuff that has occluded the artery for four minutes. This results in an increased blood flow to restore the circulation,

Figure 1. *Cross-section of an artery with progressive atherosclerotic lesions including the different non-invasive measurements of vascular abnormalities and the derived parameters. In the boxes at the bottom, the change in the NIMA parameters with progression of atherosclerosis is depicted*



Non-invasive measurements of atherosclerosis (NIMA)	
1. Endothelial (dys)function Parameters: • Flow-mediated dilation (FMD) • Nitroglycerin-mediated dilation (NMD) • Brachial artery diameter (BAD)	**2. Arterial stiffness** Parameters: • Pulse wave velocity (PWV) • Pulse wave analysis (PWA): augmentation index, central augmented, systolic, and diastolic pressure
3. Thickness of the arterial wall Parameters: • Intima-media thickness (IMT) • Presence of plaque • Plaque thickness	**4. Peripheral flow** Parameters: • Ankle-brachial index (ABI) at rest • Ankle-brachial index after exercise

Figure 2. *On the left, the method of FMD is visualised. On the right the pre- and post-occlusion diameters are depicted as measured with analysing software. The dotted line represents the mean baseline diameter. At baseline, three subsequent measures of the diameter are performed and these are depicted as the dots on the left. After four minutes of occlusion, the cuff is deflated and the diameters are then measured every ten seconds for two minutes; the first six measures are depicted as the dots on the right panel. First there is an increase in diameter after occlusion, and the diameter returns to baseline values in time*



leading to increased sheer stress on the endothelium. A healthy endothelium produces nitric oxide (NO), which causes dilation of the artery to increase the blood flow to the peripheral circulation. When there is endothelial dysfunction, less NO is produced leading to less dilation of the artery. FMD is calculated as the post-occlusion diameter divided by the baseline diameter and is expressed as a percentage. When endothelial function is impaired, a lower FMD is measured.

An important limitation of FMD is its relatively large variability. Numerous factors, such as biological and technical factors, contribute to the variability of FMD as recently summarised by Moens and co-workers.[13] Many efforts have been made to reduce measurement variability, such as the introduction of monitoring software.[14,15] Intra-observer coefficients of variation of 1.8 to 23.0% were previously reported, but when expressed as a percentage, coefficients of variation increased to 28 to 33%.[16] To detect a clinical treatment benefit, a mean improvement of FMD of over 2% is necessary and to account for natural variability even a difference of 4 to 8% is necessary.[17] A power analysis showed us that to detect a difference of 0.5% in the prevalence of CVD, over 14,000 FMD measurements are needed.[18] Furthermore, it is a time-consuming measurement that is relatively uncomfortable for the patient. Finally, there has been an under-reporting of negative studies.[19] Reference values for FMD are lacking and depend on the method used; some report FMD after upper arm occlusion whereas others use forearm occlusion.

Despite the relatively large variability, FMD seems to be a promising technique for cardiovascular risk assessment in selected high-risk patient groups and several papers have reported the usefulness of FMD as a tool in CV risk stratification and prediction,[20-27] although prospective studies are needed to prove this concept. Until recently, prospective data of population-based cohorts were scarce and the reported results were not consistent; FMD was related to CV events in some,[28,29] but not all studies in the general population.[30-32] These inconsistencies might be the result of the reported variability. Previously, we reported that FMD was not related to the traditional CV risk factors or prevalent CVD, neither in a low-risk nor a high-risk population including patients with familial combined hyperlipidaemia (FCH).[18,33] Endothelial dysfunction is a measure of early atherosclerosis and will therefore be present in older populations where multiple risk factors have been present for many years. Very recently, Yeboah and colleagues showed that FMD was a predictor of CVD in a large sample from the general population, although FMD did not improve the prediction of CVD over the Framingham Risk Score. However, adding FMD to risk stratification based on the Framingham Risk Score made many individuals with a normal FMD shift towards a lower risk category. They concluded that FMD might help in CV risk stratification to select those who seemed to be at risk based on Framingham Risk Score, but based on a normal FMD seem to be at lower CV risk.[29] These conclusions have to be regarded with care because of the variability of FMD, as also reported by these authors.

After all these years of research on FMD, there is still no clarity on its possible potential to be a screening tool in CV risk stratification and no uniform results have been reported. Therefore, the use of FMD in clinical practice and especially in primary prevention settings is questionable and the time for FMD to be applied in clinical practice is still far away. Further research should focus on the possible role of FMD in CV risk stratification in younger populations, using standardised methods for FMD in standardised conditions. Improved or other non-invasive measures of endothelial function have to be developed and investigated for their applicability in clinical practice.

Nitroglycerin-mediated dilation

Beside endothelium-dependent vasodilation, the endothelial-independent vasodilation can be determined by administration of nitroglycerin. Nitroglycerin causes relaxation of the smooth muscle cells, which results in dilation of the arteries, and is independent of the function of the endothelium. The maximum diameter after nitroglycerin is divided by the baseline diameter and expressed as a percentage. When the function of the smooth muscle cells is impaired, the nitroglycerin-mediated dilation (NMD) is decreased. The NMD is used to check whether the attenuation of FMD is caused by damage in the endothelium and not a consequence of changes in the smooth muscle cells. Reference values have never been reported and, just as with FMD, depend on the method used. The role of NMD in CV risk stratification is unclear and most likely limited.

Brachial artery diameter

The brachial artery diameter (BAD) is the measure on which FMD is based. The reported measurement variability is much smaller than for FMD.[34] Reference values are lacking, BAD differs between men and women, is dependent on blood pressure, and is influenced by antihypertensive medications. BAD appeared to have predictive value in CV risk assessment in recent publications.[30,35,36] We previously reported that BAD was related to cardiovascular risk factors and other measurements of subclinical atherosclerosis in our population-based sample.[18] The diameter of the brachial artery might be a reflection of systemic vasodilation, as a compensation in reaction to narrowing of the arterial lumen.[37] The BAD might be a potential tool in CV risk stratification when combined with other measurements of atherosclerosis; however, this has to be evaluated prospectively.

ARTERIAL STIFFNESS

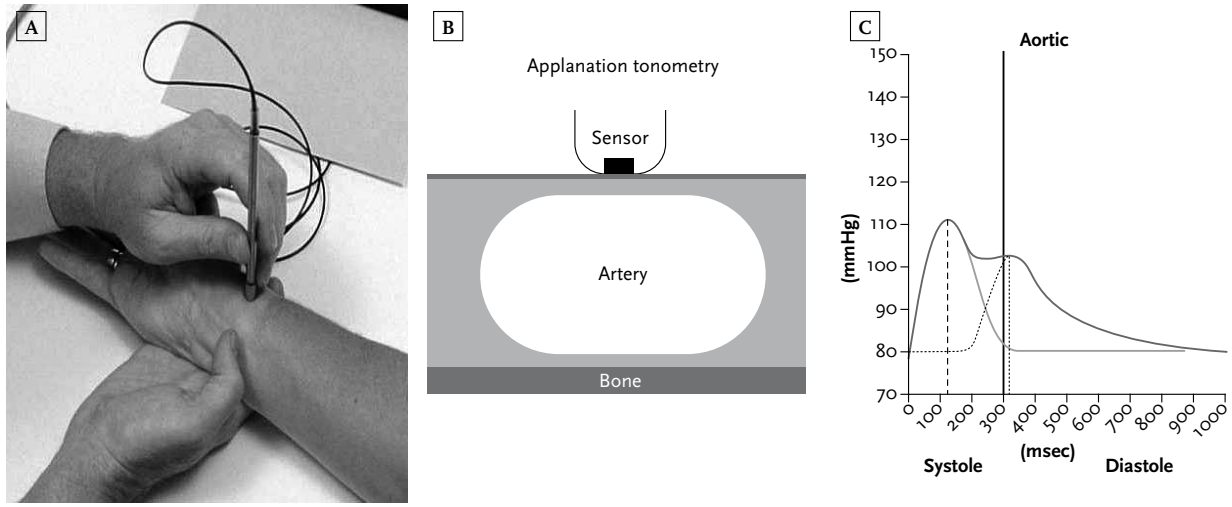
Due to ageing and the progression of atherosclerosis, the arterial wall changes, and besides dysfunction of the endothelium, these changes result in arterial stiffness.[38] Arterial stiffness can be measured non-invasively with pulse wave analysis (PWA) and pulse wave velocity (PWV), using tonometry, as depicted in *figure 3*. The heart ejects a bolus of blood into the arterial system with every heartbeat and this causes a blood pressure wave through the arteries. When the wave arrives at an artery, this causes expansion of the artery. This phenomenon can be observed as the arterial pulse, which can be palpated at the wrist or at the carotid artery. A tonometer is a device that registers the changes in diameter of arteries.

Due to the different composition of central and peripheral arteries, not all arteries stiffen to the same extent. The stiffening of central arteries is greater than the stiffening of the peripheral arteries. The clinical consequences of arterial stiffness are an increased risk of stroke as a result of increased systolic blood pressure, the development of left ventricular hypertrophy as a result of increased cardiac after load, and a decrease in coronary perfusion and heart failure due to the decrease in diastolic blood pressure.

Pulse wave velocity (PWV)

PWV is a measure of wave velocity, which is propagated by contraction of the heart and travels along the arterial tree. To determine PWV, pulse waveforms are recorded at two sites sequentially, and wave transit time can be calculated using the R wave of a simultaneously recorded electrocardiography as a reference frame. PWV measured between the right carotid and the left femoral artery has been described as the gold standard measurement of

Figure 3. *Method of tonometry used to determine pulse wave analysis and velocity; the tonometer is gently pressed against the artery and registers the changes in diameter over time. On the right an example of an obtained waveform, this is composed of a forward wave in the systolic phase and a backward wave in the diastolic phase*



arterial stiffness by a panel of experts.[38] Surface distance between the two recording sites can be measured parallel to the plane of the examination table. The distance between the carotid artery site and the supra sternal notch has to be subtracted from the distance between the supra sternal notch and the femoral artery site. PWV is calculated by dividing the travelled distance by the time. As the arteries become stiffer with ageing and progression of atherosclerosis, PWV increases. To minimise variability and make comparison between studies possible, recommendations for user procedures were provided by Van Bortel *et al.*[39]

Reproducibility of PWV has been extensively studied and measurement variability is rather small.[40-42] Reference values have been provided by several authors;[43,44] in healthy adults the PWV generally ranges from 6 to 11 m/sec.[45] The guidelines for the management of arterial hypertension and the guidelines for cardiovascular screening in the asymptomatic at-risk population included a PWV value >12 m/s as a sign of target organ damage.[46,47]

PWV is an independent predictor of CVD in selected high-risk patient groups, and in the general population[48-50] and could provide additional information in clinical practice for CV risk stratification.[51-54] Very recently, two studies even reported an improvement of CV risk stratification by adding PWV in hypertensive patients[55] as well as in apparently healthy adults.[56] We reported that PWV was increased in FCH patients compared with their unaffected relatives. PWV did predict the presence of CVD equally well as a combination of traditional risk factors, but did not have additive value over and above the traditional risk factors in this high-risk population.[57] PWV was associated with the metabolic syndrome and its individual traits,[58] with increasing waist,[59] and with increasing apolipoprotein B (apoB) levels in our population-based cohort.[132]

In conclusion, PWV is a well established measure of arterial stiffness and is a very promising tool to be included in CV risk stratification in clinical practice in secondary and primary prevention. The additive value of PWV over and above traditional CV risk factors remains to be confirmed in other populations, especially in combination with other NIMA.

Pulse wave analysis (PWA)

PWA is commonly measured at the right radial artery. The pressure wave generated by contraction of the left ventricle travels along the arterial tree. The amplification of the pressure wave increases as it travels distally, resulting in a difference between brachial and central blood pressure of approximately 44% in healthy subjects with a mean age of 45 years.[60] This amplification is known as the augmentation index (AIx) and reflects the overall interaction between the arterial tree and the left ventricle.[61] AIx is principally determined by aortic reservoir function and other elastic arteries and to a minor extent by reflected waves.[62] Men have lower AIx values than women[63,64] and AIx plateaus at the age of 60 and therefore can only be considered a measure of vascular age in younger individuals.[65,66] Also central pressure parameters can be derived from the registered radial wave form, such as central augmented pressure, central systolic pressure, and central diastolic pressure. The derived parameters are indirect measures of arterial stiffness, whereas PWV is a direct measure of arterial stiffness. The main problem of these derived parameters is the calculation by means of a transfer function, which has only been validated in selected patients groups.[67,68] Therefore, care must be taken when interpreting the data in other populations. Furthermore, there is doubt about the formula used to calculate the Aix.[69] Since AIx strongly depends on heart rate, AIx corrected for a heart rate of 75 beats per minute is used.[70] Different techniques are used to measure AIx and not all provide central pressure parameters. Reported reproducibility of the different techniques is good.[40,42] Also recently, reference values were reported in different populations.[71,72] As atherosclerosis increases, AIx increases and this increase has been associated with increased CV risk.[73] In FCH patients we could not report a difference in AIx compared with their unaffected relatives, whereas PWV was increased.[57] This discrepancy might be explained by the fact that the age-related changes in PWV and AIx follow different patterns; changes in AIx are more dominant in younger subjects (<50 years) and changes in PWV are more marked in older individuals (>50 years).[65] In a population-based cohort we found that AIx was associated with the metabolic syndrome and its individual traits, although the association was stronger in men than in women.[58] AIx also modestly but significantly increased with increasing apoB levels,[132] but not with increasing waist circumference.[59]

The use of central blood pressure parameters recently regained interest due to the results of the Conduit Artery Functional Endpoint (CAFÉ) study showing that central blood pressure but not peripheral blood pressure was lowered by one of the drugs administered.[74] Since then, many studies have incorporated the central pressure measurements and many results have to be awaited. Very recently the same authors published additional analyses and concluded that the difference in central pressure reported before was mainly the result of the heart rate reduction with β-blockers. This appeared to be the major mechanism accounting for less effective central aortic pressure reduction per unit change in brachial pressure.[75] Also the Cardiovascular Health Study showed that central pressure was more strongly related to (subclinical) atherosclerosis and CV events than brachial blood pressure,[76] which was strengthened by a

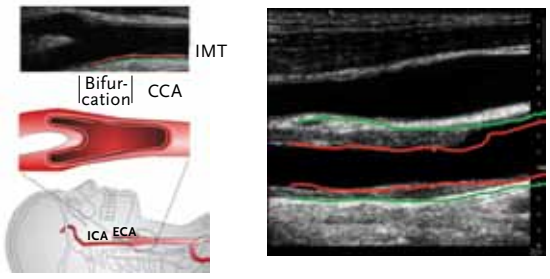
review in 2009.[77] Very recently, a systematic review and meta-analysis provided quite robust evidence that central haemodynamics are independent predictors of CV events and all-cause mortality in different patient groups.[54]
To summarise, evidence is accumulating that central pressures would be more useful in CV risk stratification, although the independent predictive value of central haemodynamics in primary prevention remains to be determined.

THICKNESS OF THE ARTERIAL WALL

Intima-media thickness (IMT)

The arterial wall can be visualised and the thickness of the arterial wall can be measured using ultrasound, as depicted in *figure 4*.
IMT measures structural changes in the arterial wall and is a well-established marker of (subclinical) atherosclerosis. With ageing and progression of atherosclerosis the arterial wall thickens. The increase in IMT is associated with unfavourable levels of cardiovascular risk factors, atherosclerosis elsewhere in the arterial system, and with cardiovascular disease.[78-83] IMT can be measured at different sites of the arterial tree. The most common place to measure IMT is the distal common carotid artery. The presence of a plaque is defined by the Mannheim Intima-media thickness consensus as a focal thickening of the arterial wall of at least 1.5 x the mean IMT.[84]
Numerous studies have reported that IMT can be measured in a reliable and reproducible manner, although different protocols are used in different studies.[85,86] Measurement variability is typically introduced from several resources: ultrasound scanning equipment, sonographers, reading equipment, readers of the scans, scanning protocol, and thickness of the intima-media complex. Since automatic devices were introduced to measure IMT, variability decreased substantially.[87-90] At higher ages, the variability in IMT between subjects is larger.[91] The thickness of the wall also varies during the heart cycle. In the diastolic phase, the IMT is thicker than in the systolic phase. Therefore the measurements have to be performed at the same phase of the heart cycle in every person. Several authors have already extensively discussed the different methods used to measure IMT and international recommendations have been made for standardised IMT measurements.[92] In studies evaluating the effect of drugs in which IMT is the primary endpoint, very small differences have to be detected, demanding a very precise IMT measurement. These studies mostly include IMT measures of the common carotid artery, the bulbus and the internal carotid artery measured from different scanning angles. Other studies use IMT as a screening tool; the measurement then needs to be simple, quick, but reliable and most studies only measure the IMT of the common carotid arteries at the angle that showed the optical thickest IMT.
Reference values were provided by many studies stratified by age.[86,93-96] The guidelines for the management of arterial hypertension and the guidelines for cardiovascular screening in the asymptomatic at-risk population included an IMT value >0.9 mm or the presence of plaque as a sign of target organ damage.[46,47] IMT has shown to predict CVD, in patients as well as in asymptomatic individuals.[80,97-103] In line with these data, we showed that IMT was strongly associated with traditional CV risk factors in both participants from a population based sample,[58,59] and in a high-risk population.[104] The additive value of IMT over and above traditional CV risk factors in CV risk stratification has not been proven yet.[105-107] Only one relatively small study reported that IMT would improve CV risk stratification in a primary prevention setting.[108]
In summary, IMT is a well-established surrogate marker of atherosclerosis and is a very promising tool to be included in CV risk stratification in clinical practice in the near future. The additive value in primary prevention remains to be determined prospectively.

Figure 4. *Measurement of the thickness of the arterial wall using ultrasound at the carotid artery. The most distal 10 mm of the common carotid artery is measured. ECA = external carotid artery, ICA = internal carotid artery, and CCA = common carotid artery. On the left a normal intima-media complex is depicted, on the right an example of increased thickness. The outer layer of the wall is coloured in green and the inner layer of the arterial wall is coloured in red*



The presence of plaque and plaque thickness

The presence of plaque and plaque thickness are measures of advanced atherosclerosis. Not many studies have included these parameters, and those that did used many different methods and definitions. As described in the previous section, the presence of plaque showed predictive value for CVD and is included in some guidelines.[47] Recommendations have been reported on how to define the presence of plaque.[84] We reported in our low-risk population that participants with the metabolic syndrome

had thicker plaques than those without.[58] The number of participants with plaques present increased with increasing apoB levels and increasing waist circumference.[59] The predictive value of plaque thickness in CV risk stratification and the additive value of the presence of plaque in different populations need to be evaluated in prospective studies, taking into account the measuring method.

PERIPHERAL FLOW

Ankle-brachial index at rest

The ankle-brachial index (ABI) at rest measures more advanced stages of atherosclerosis and has been used in clinical practice for years now to determine whether a patient suffers from peripheral arterial disease. The method commonly applied is one measurement of ABI at a single time point and by one single observer based on the publication of Price *et al*, who established the cut-off value of ABI in a very large population-based cohort.[109]

The measurement found its way into clinical practice rather easily. The first publication on the reproducibility of the ABI dates from 1981.[110] The authors recommended performing the measurement more than once and a difference in subsequent measures from 15 mmHg could be regarded as clinically relevant. Reproducibility of the ABI has been studied in selected patient groups and was reliable when performed by trained technicians.[111-115] An ABI at rest below 0.9 is widely considered to be abnormal.[46,47,109] The ABI at rest is a simple, non-invasive and inexpensive test that can be used to identify individuals who are at high risk of developing CVD. Several studies have reported that a low ABI at rest had predictive value for CVD in patients with CVD and in low-risk populations.[116-122] We reported that a decreased ABI at rest was associated with the metabolic syndrome and its individual traits in our population-based cohort.[58] A decreased ABI was also observed with increasing waist circumference[59] and with increasing apoB levels.[132] Further studies need to provide insight into the predictive value of the ABI at rest for CVD in low-risk populations over and above traditional risk factor stratification.

Ankle-brachial index after exercise

In clinical practice the exercise test is performed to confirm that a diminished arterial flow is the cause of a patient's walking disability. A decreased ABI after exercise can also detect atherosclerotic lesions that do not yet cause a drop in blood pressure at rest. When more oxygen is needed during exercise, the obstruction prevents an increase in oxygen supply, which causes a drop of the pressure at the lower limb resulting in a lower ABI after exercise. ABI after exercise might therefore be considered a measure of subclinical atherosclerosis. Peripheral arterial disease is present when the ABI drops by more than 15% after exercise compared with the ABI at rest according to the Dutch guidelines.

Data on the predictive value of the ABI after exercise for CVD are lacking. We reported that individuals with the metabolic syndrome had a decreased ABI after exercise in the general population.[58] ABI after exercise also decreased with increasing waist circumference[59] and with increasing apoB levels.[132] Further studies are warranted to determine the predictive value of ABI after exercise for CVD and its additive value over and beyond traditional CV risk factors, especially in low-risk populations in primary prevention settings.

To summarise: although most of the NIMA described in this review are used for research purposes worldwide, none of these measurements have made their way into clinical practice yet, except for the ankle-brachial index (ABI). Some NIMA, including ABI, IMT, and PWV, have been recommended to be included in cardiovascular risk stratification to determine subclinical organ damage in the guidelines for the management of arterial hypertension, and in the guidelines for CV screening in the asymptomatic at-risk population.[46,47]

PREDICTIVE VALUE OF COMBINATIONS OF NIMA

Each NIMA reflects a different characteristic of the atherosclerotic process, involving functional and/or morphological changes in the vessel wall. It might be better to define the measurements as non-invasive measurements of vascular abnormalities (NIMVA) than NIMA. It is also known that the extent of atherosclerosis differs along the arterial tree. In different populations at risk of CVD, different characteristics of the atherosclerotic process may be present or accelerated. Therefore, simultaneous measurements of different NIMVA could theoretically enhance the power to improve CV risk stratification. Only very few studies have evaluated the predictive value for CVD for different combinations of NIMVA. Very recently, Novo and co-workers reported that IMT in combination with the presence of plaque might provide additional information on CV risk in a primary prevention setting.[108] This was also recently reported in the Atherosclerosis Risk In Communities (ARIC) study by Nambi *et al.*[123] Tu and co-workers reported on the predictive value of the combination of IMT and arterial stiffness, but not all measures of stiffness used in that study showed the same results.[124] In contrast, Muiesan and colleagues found that PWV in combination with echocardiography enhanced CV risk stratification, but adding PWV to IMT did not.[53]

Further studies need to investigate which NIMVA should best be combined to improve CV risk stratification, as well in primary as in secondary prevention.

DETERMINANTS OF NIMVA

The progression rate of atherosclerosis differs among individuals. This might be due to different impact of known and unknown CV risk factors and/or differences in time exposure to these risk factors, and/or genetic predisposition. NIMVA measure the extent of vascular abnormalities reflecting (subclinical) atherosclerosis and the hypothesis is that this amount of atherosclerosis reflects the impact of all different CV risk factors together. Previous studies demonstrated that the predictive power of some individual NIMVA for cardiovascular events is independent of the conventional risk factors as described in the previous sections. Still a large proportion of the variance in NIMVA remained unexplained. In general, the reported percentage explained variance in NIMVA is larger in high-risk populations (i.e. ±50%) than in low-risk populations (i.e. ±30%).[125-131] In line with these data we reported the percentage explained variance in IMT of ±50%, in both FCH patients and in the unaffected relatives.[104] Future studies are needed to identify other main determinants of NIMVA, including exploring potential new CV risk factors. Repeated measurements of NIMVA might help to unravel the impact of ageing, time of exposure to known and unknown CV risk factors, and/or genetic susceptibility.

Practical guide for consideration of use of non-invasive measurement of atherosclerosis in screening for cardiovascular risk:

- Risk factor evaluation in apparently healthy men aged 45-75 years and women aged 55-75 years;
- Risk stratification based on the SCORE risk chart.

✓ Subjects at low risk: lifestyle changes and treatment of modifiable risk factors: no additional screening.

✓ Subjects at moderate/intermediate risk: lifestyle changes and treatment of modifiable risk factors, additional screening by target organ damage measurements (if available); 1 or more positive tests; more aggressive treatment comparable to high-risk patients:
- IMT > 0.9 mm or plaque(s)
- PWV > 12 m/s
- ABI < 0.9

Other measures have been recommended to determine target organ damage of the heart, kidney, eyes and brains; for a detailed description see the 2007 Guidelines for the Management of Arterial Hypertension.[47]

FUTURE PERSPECTIVES

Subclinical disease measurements i.e. NIMVA may be useful for improving CV risk prediction, therapeutic strategies and evaluation of risk factor modification. However, the major pathophysiological determinants of NIMVA are still unknown. Reference values for primary prevention are still lacking for most of the described NIMVA. Furthermore, follow-up data on the panel of NIMVA are not yet available and therefore the relevance of NIMVA in clinical practice for the individual patient is unclear. Measuring changes in a panel of NIMVA values after, for instance, five years of follow-up, in both a low- and a high-risk population, in relation to changes in traditional and new CV risk factors and incidence of CVD, will unravel the major pathophysiological determinants of NIMVA, including ageing, time of risk factor exposure, and genetic risk factors. Also the power of baseline versus repeated NIMVA in CVD risk prediction, over and beyond CV risk factors, can be determined, leading to an evidence-based protocol for NIMVA to improve cardiovascular risk stratification for the individual patient in clinical practice. Furthermore, the combination of NIMVA that will improve CV risk stratification in both low- and high-risk populations in a cost-effective way can be unravelled, allowing earlier and more effective (new) preventive therapy.

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