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Review article

Noninvasive Assessment of Endothelial Function in Clinical Practice

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ABSTRACT

In the fight against cardiovascular diseases, preventive strategies are becoming the focus of attention. One of these strategies proposes to identify individuals who are at a high risk of developing cardiovascular disease. Endothelial dysfunction could improve patient risk stratification and the implementation of preventive strategies. In this review we focus on noninvasive techniques that have recently become available to assess endothelial function: flow-mediated vasodilation as measured by ultrasound of the brachial artery, pulse wave analysis, and finger plethysmography during postischemic hyperemia. We describe the basic principles, the main protocols to perform these techniques, and their clinical value based on the scientific evidence.

Evaluación no invasiva de la función endotelial en la práctica clínica

Palabras clave:
Función endotelial
Vasodilatación mediada por flujo
Velocidad de onda de pulso
Pletismografía digital

RESUMEN

En la lucha contra las enfermedades cardiovasculares, las estrategias preventivas están pasando a ser el principal centro de interés. Una de estas estrategias propone identificar a los individuos con riesgo elevado de sufrir enfermedad cardiovascular. La disfunción endotelial podría facilitar una mejor estratificación del riesgo y la puesta en práctica de estrategias preventivas. En esta revisión nos centramos en las técnicas no invasivas que se han introducido recientemente para evaluar la función endotelial: vasodilatación mediada por flujo medida con ecografía de la arteria humeral, análisis de la onda del pulso y pletismografía digital durante la hiperemia postisquémica. Describimos los principios básicos, los principales protocolos para la aplicación de estas técnicas y su valor clínico basado en la evidencia científica existente.

Abbreviations

CV: cardiovascular
FMD: flow-mediated vasodilation
MRI: magnetic resonance imaging
PAT: peripheral arterial tone
PWV: pulse wave velocity

INTRODUCTION

In the fight against cardiovascular (CV) diseases, preventive strategies are becoming the focus of attention. Comprehensive pharmacological and interventional treatments in recent years have achieved success in certain areas but costs associated with these strategies are high and global results suboptimal. Therefore the fight to achieve meaningful reductions in the incidence of CV events in the general population continues unabated.[1] Two different preventive strategies have been suggested: *a)* to make pharmacological prevention accessible to as many people as possible, ie, the "polypill"[2], and *b)*, to identify those individuals who are at a higher risk of developing CV events within 10 years and thus concentrate preventive efforts on these subjects. The latter is generally preferred at present, given the limited resources available to health systems worldwide and the not uncommon occurrence of side effects associated with the administration of pharmacological agents. Unfortunately, current traditional methods for risk prediction based on conventional risk factors have limitations[3] and there is a need to identify more effective risk stratification algorithms. These should take into account the pathophysiology of the disease, be cost-effective and easy to apply in clinical practice, and provide information over and above that supplied by conventional risk factors and risk scores. Progress in this regard has been made in recent years with the advent of ultrasound techniques for the assessment of carotid intima-media thickness and atheromatous plaque composition, computed tomography (CT) for detection and quantification of coronary

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artery calcification, Doppler measurement of brachial-ankle index pressure, and the determination of aortic pulse wave velocity (PWV),[4] among others. Endothelial dysfunction has also been proposed as a marker of CV risk. This manuscript will focus on the different techniques available for the assessment of endothelial function in clinical practice.

ENDOTHELIAL DYSFUNCTION

The term endothelial dysfunction is widely used to describe any form of abnormal activity of the endothelium. Most commonly, endothelial dysfunction is characterized by impaired nitric oxide (NO) bioavailability due to reduced production of NO by NO synthase (eNOS), increased NO breakdown by reactive oxygen species, or both. Under normal conditions, NO diffuses to the vascular smooth muscle cells and activates guanylate cyclase, which leads to cyclic guanosine monophosphate-mediated vasodilation. Shear stress is a key activator of eNOS under physiological circumstances and this helps adapt organ perfusion to changes in cardiac output. Other signaling molecules such as bradykinin, adenosine, vascular endothelial growth factor (expressed in response to hypoxia), and serotonin (released during platelet aggregation) can also activate eNOS (Fig. 1). [5]

In the early stages of the atherosclerotic process, endothelial function may be partly maintained by the compensatory upregulation of prostacyclin and/or endothelium-derived hyperpolarizing factor(s) (EDHF). Prostacyclin, a product of the cyclooxygenase system, is another endothelium-derived vasodilator that acts independently of NO, but has a limited role in maintaining human vascular tone. The endothelium also mediates the hyperpolarization of vascular smooth muscle cells via an NO-independent pathway, which increases potassium conductance and facilitates the subsequent propagation of depolarization of vascular smooth muscle cells to maintain vasodilator tone. The EDHF involved in this process are only partially understood (such as the cytochrome-derived factors and possibly C-type natriuretic peptide), and may differ between vascular beds.[6] Endothelial dysfunction has been documented in almost every condition associated with atherosclerosis and CV disease[7] and has also been related to the presence of conventional CV risk factors like hypertension, dyslipidemia, diabetes mellitus, age, and obesity.[5,8–13] It has also been described in patients with inflammatory and infectious diseases.[14,15]

As a consequence of endothelial dysfunction, a range of pro-atherosclerotic molecular events occur, including increased lipid permeability and the promotion of oxidative and inflammatory environments within atheromatous plaques that favor plaque rupture and pro-thrombotic events, as seen in the acute coronary syndrome. Endothelial function therefore represents an integrated index of both the overall CV risk factor burden and the sum of vasculoprotective factors in a given individual. Given its role in the atherosclerotic process, it is not surprising that many studies demonstrate a prognostic role for endothelial dysfunction, as measured both in the coronary arteries and the circulation.[16]

ASSESSMENT OF ENDOTHELIAL FUNCTION

Coronary endothelial dysfunction is generally assessed using invasive methods, such as the infusion of acetylcholine (ACh) into the coronary artery, where it has been shown to produce a paradoxical vasoconstriction in those arteries affected by atheroma.[17] ACh promotes the release of NO from vessels with an intact

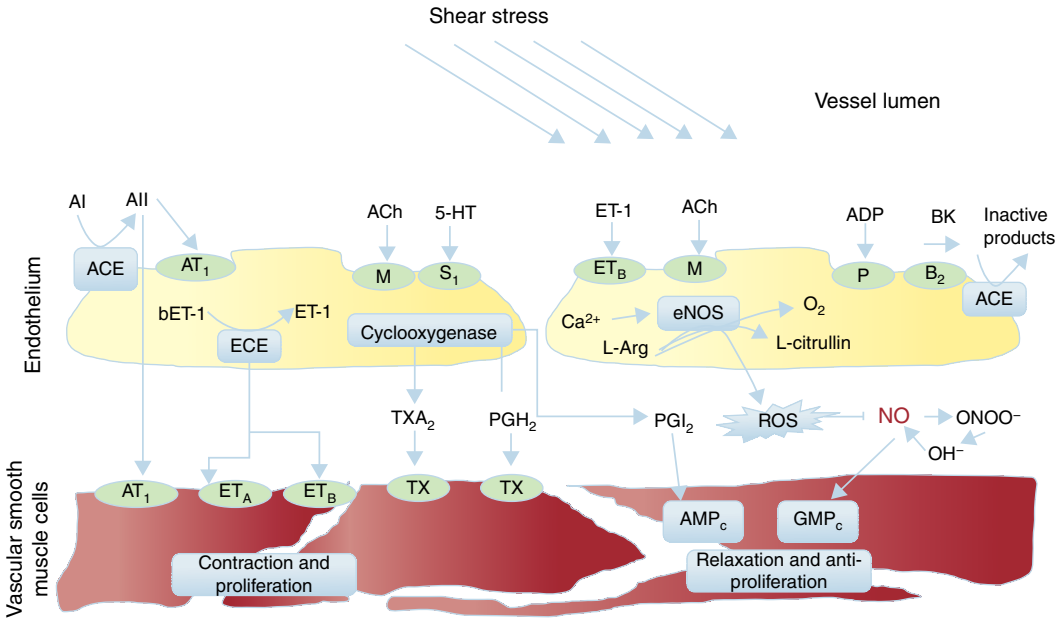

Figure 1. The endothelium. Structure and function. The endothelium is a thin layer of cells that lines the interior surface of blood vessels.[5] It behaves as an autocrine, paracrine, and endocrine gland. Endothelial cells produce several mediators with vasorelaxing, anti-proliferative, antithrombotic, and antiadherent effects, such as nitric oxide, prostacyclin, endothelium-derived hyperpolarizing factor, and C-type natriuretic peptide. The actions of these molecules are balanced by the release of substances with opposing effects, such as endothelin-1, thromboxane A_2, prostaglandin H_2, and superoxide anion. Impairment of endothelium-dependent dilation shifts a net dilator response to a variety of stimuli to a constrictor response that can affect blood flow.[6] 5-HT, serotonin; ACE, angiotensin-converting enzyme; ACh, acetylcholine; ADP, adenosine diphosphate; AI, angiotensin I; AII, angiotensin II; AMP_c, cyclic adenosine monophosphate; AT_1, angiotensin 1 receptor; B_2, bradykinin receptor B2; BK, bradykinin; ECE, endothelin converting enzyme; eNOS, nitric oxide syntetase; ET-1, endothelin-1; ET_A, endothelin A receptors; ET_B, endothelin B receptors; GMP_c, cyclic guanosine monophosphate; L-Arg, L-arginine; M, muscarinic receptor; NO, nitric oxide; OH, hydroxyl radical; ONOO, nitric oxide peroxynitrite; P, purine receptor; PGH_2, prostaglandin H2; PGI_2, prostacyclin, ROS, reactive oxygen species; S_1, serotoninergic receptor; TX, thromboxane receptor; TXA_2, thromboxane. Modified with permission from Flammer et al.[7]

endothelium and this leads to vasodilation. Arteriosclerotic vessels with impaired endothelial function, however, respond with vasoconstriction as a result of a direct vasoconstrictor effect of ACh on the vascular smooth muscle (muscarinic effect) in the absence of NO release. Nitroglycerine is used to test endothelial-independent dilation. Investigators have also used endothelial agonists that include substance P, adenosine, and bradykinin, and the use of these agents has provided important insights into the vascular effects of risk factors. These methods have also allowed the characterization of the vascular effects of pharmacological agents such as angiotensin-converting enzyme inhibitors and statins.[18] The usefulness of this method, however, is limited by its invasive nature and therefore new techniques have been developed in recent years to assess endothelial dysfunction noninvasively. Nonetheless, because endothelial dysfunction is a systemic process that simultaneously affects different vascular territories,[19] it is accepted that noninvasive approaches assessing endothelial function in peripheral vessels are, albeit indirectly, representative of coronary vascular function. Most of the techniques available at present use endothelial-dependent vasomotion as the clinical endpoint for the assessment of endothelial function. Testing involves pharmacological and/or physiological stimulation of endothelial release of NO and other vasoactive compounds, and often a comparison of vascular responses to endothelium-independent dilators such as nitroglycerine. Determination of local NO bioavailability not only reflects its influence on vascular tone, but also the other important functions of this molecule, which include thromboregulation, cell adhesion, and proliferation.[6] The majority of those techniques exhibit a good correlation with the assessment of coronary endothelial function.[20] Currently the main noninvasive techniques to assess endothelial functions are flow-mediated vasodilation (FMD) as measured by ultrasound of the brachial artery, pulse wave analysis, and finger plethysmography during postischemic hyperemia. Other invasive and noninvasive methods for measuring coronary microvascular function have been recently reviewed, such as magnetic resonance imaging (MRI), positron emission tomography scanning, CT scanning, single photon emission CT, Doppler echocardiography, Doppler flow wire, temperature and pressure sensor tripped coronary wire, or thrombolysis in myocardial infarction frame count and myocardial blush score.[19] A completely different approach has also been proposed and this is based on the measurement of plasma levels of endothelium-related analytes, which can reflect -to a certain extent- overall endothelial activation or dysfunction. Circulating soluble fractions of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and von Willebrand factor may be regarded as markers of endothelial function.[20] The concentrations of these peptides raise when the endothelium is activated or damaged and they are predictive of the risk, presence, and severity of vascular disease.[21–23] The assessment of biochemical markers of endothelial dysfunction is beyond the scope of the present review.

ASSESSMENT OF BRACHIAL ARTERY FLOW-MEDIATED VASODILATION

At present, FMD is the most commonly used method for measuring endothelial dysfunction, mainly because of its sensitivity and noninvasive nature. It was used for the first time by Celermajer et al.,[24] after a physiological study by Anderson and Mark.[25] It is based on the endothelial release of NO and other endothelium-derived relaxing factors in response to an increase in shear stress. In this test, this occurs when the forearm blood flow increases during the reactive hyperemia that follows a brief period of transient ischemia in the distal territories. The ischemia is achieved through a pneumatic cuff, placed on the forearm, distal to the ultrasound image site, and inflated to suprasystolic pressure for 5 min (Fig. 2). On deflation of the cuff, the increased flow results in shear stress, which activates endothelial NO synthase to release NO via the L-arginine pathway. The NO diffuses to the smooth muscle cells, causing them to relax, resulting in vasodilation. Finally, FMD is measured as the percentage change in brachial artery diameter (Fig. 3 [26]) from baseline to the maximum increase in diameter (Fig. 4 [27]).[28]

Despite being the most frequently used technique, a closer look at the literature reveals that there are wide variations in mean FMD when different studies in similar populations are compared.[29] At present, the lack of scientific consensus regarding a standardized protocol for measuring FMD precludes the accurate comparison of data between centers. There have been efforts to provide guidelines,[30,31] but a full standardization has not been achieved. Five critical elements of FMD methodology need to be standardized[28]:

- Probe position in relation to cuff: if the cuff is placed proximal to the probe, it is not clear what aspect of endothelial function is being measured. NO-dependent FMD measurement becomes confounded by the presence of additional ischemic (NO-independent) vasodilation.[32] Therefore, if the aim of the study is to assess NO bioavailability, the cuff should be positioned distally to the probe (Fig. 2).
- Shear stimulus (cuff occlusion time): there is general consensus that 5 min is the optimum time to elicit a good reactive hyperemic response and consequent dilation, because a longer period does not guarantee a pure NO-mediated event.[33]
- Image measurement (stereotaxis and automation): it is compulsory to use a stereotactic apparatus to acquire high-quality static images (Fig. 2). To achieve more stable images, a micrometer-adjustable base can be used to reposition the probe to track the artery; alternatively, an anatomical forearm cast helps to preclude pronosupination of the forearm, which is the most important cause of image drifting. Each image should be measured at end diastole, and the maximal dilation should be recorded rather than the dilation at any set time after cuff release; it has been shown that taking a measurement at a set time of 60 s, for example, misses the peak dilation in approximately 70% of subjects.[34] In fact, the moment of maximum dilation is part of the interindividual endothelial response differences,[26] and so far it remains unknown whether is it useful to determine the time to peak diameter. To select the maximal dilation, automatic measurement at each cardiac cycle is desirable, with the additional benefit of avoiding the variability inherent to manual measurements.[35,36]
- Control of environmental factors: this is important for the longitudinal consistency of FMD measurements. The factors which are known to affect FMD measurements include room temperature, time of day, ingestion of fatty foods and caffeine, concurrent inflammation or infection, and stage of menstrual cycle.[28] Although these factors have been shown to influence FMD and current guidelines have issued clear advice regarding certain conditions, recent data demonstrate that this contribution to the variability of FMD is relatively small and should not be considered a limiting factor when the ideal conditions cannot be achieved.[37]
- Finally, quantification of the reactive hyperemic stimulus has been rarely investigated and the variation in the achieved hyperemia may account for substantial FMD variation.[26] Several variables can affect the transduction of shear stress into conduit artery dilation. These are methodological [eg, cuff position, duration of shear stress, and duration of ischemia] and physiological variables [eg, arterial stiffness, flow pattern and blood viscosity] that have not been fully described or accounted

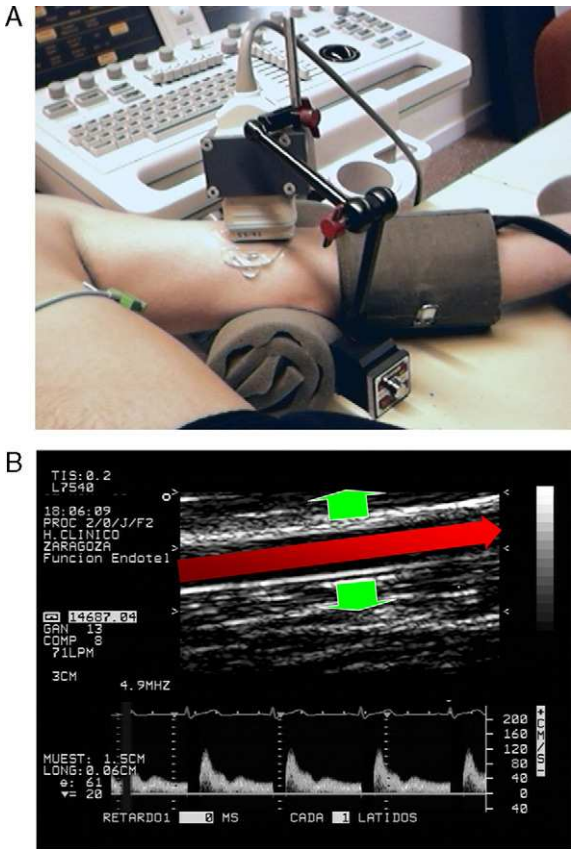

Figure 2. A, Setting for flow-mediated dilation testing. Note the probe position in relation to cuff and the stereotactic apparatus. B, Ultrasound image of braquial artery used to measure both the diameter changes and flow velocity.

for. Shear stress can be calculated by recording the Doppler velocity after cuff release. To avoid shear stress variation, a ratio normalization (FMD/shear) has been proposed.[31] Nonetheless, the usefulness of this normalization is controversial and currently it is not possible to recommend a method for correcting for differences in shear stress.[31]

FMD studies reported in the literature differ regarding their adherence to the key methodological issues outlined above. For accuracy and reproducibility, FMD measurements, like many other imaging modalities, require careful training of the operator, a standardized technique, and well-defined analysis protocols. Recently, it has been shown in follow-up studies and interventional trials that FMD may represent a useful surrogate endpoint. Power curves have been published to assist in the design of both crossover and parallel trials and a nomogram for FMD values is also available for use as a reference for various vessel sizes.[27]

Research on the clinical application of FMD techniques is ongoing and new approaches and measurements are being tested, such as the importance of the diameter reduction of the brachial artery during cuff inflation, which has been suggested to correlate with the presence of risk factors.[38]

Clinical Value of Flow-Mediated Dilation Assessment

Under standardized conditions, brachial artery FMD is a useful measure of NO-dependent endothelial function. In apparently healthy individuals it may serve as a marker for cardiac risk factor exposure and their functional effects.[39–41] Over a 6-year follow-up, FMD has been reported to correlate with the progression of preclinical carotid artery disease, showing a closer relationship with progression than conventional risk factors.[42] In secondary prevention, it provides long term prognostic information both in patients with peripheral vascular disease and acute coronary syndrome as well as short term prognostic information in patients undergoing vascular surgery.[43–45] These are promising studies but the clinical application of FMD is currently limited by the lack of standardized protocols and reference values. Thus FMD testing remains confined to research, where it is a valuable and important tool, mainly for the study of populations rather than individuals. The additive value of brachial ultrasound FMD over and above established traditional clinical tools remains to be proven and ongoing studies are currently addressing this issue.[7]

PULSE WAVE VELOCITY AND PULSE WAVEFORM ANALYSIS

PWV was first described by the Moens-Korteweg equation derived in the 1920s that relates PWV to vessel distensibility, $c0=\sqrt{Eh/2R\rho}$, where c0 is the wave speed, E is the Young modulus in the circumferential direction, h is wall thickness, R is vessel radius, and ρ is the density of fluid. Normal values vary from vessel to vessel and are described in Table 1.[46] Aortic PWV is usually measured between the carotid and femoral artery by synchronically detecting the arrival of the wave at both locations and measuring the distance. This method renders an average velocity value.

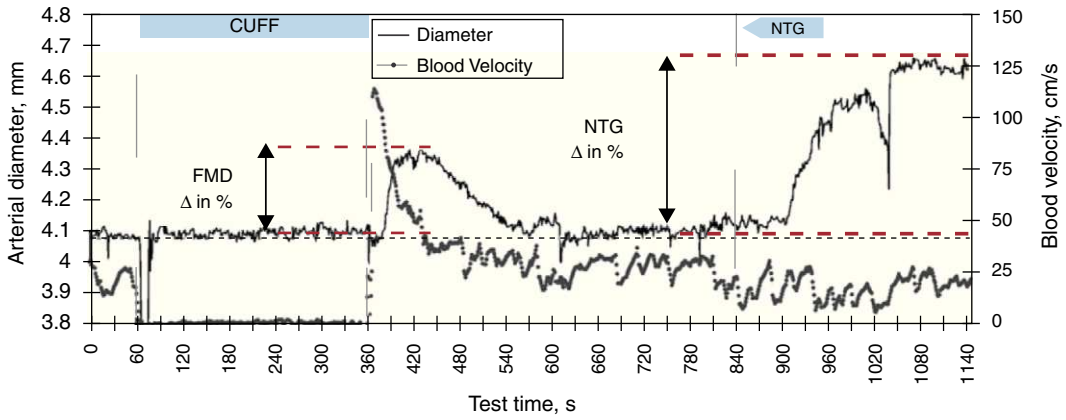

Figure 3. Diameter and blood velocity curves as a function of time in a typical flow-mediated dilation test. The solid black line depicts diameter. The dotted gray line depicts blood velocity. The dotted horizontal line indicates the reference baseline diameter. Distal ischemia period is denoted as CUFF. NTG indicates the instant of nitroglycerin administration. FMD, flow-mediated dilation; NTG, nitroglycerine. Modified with permission from Laclaustra et al.[26]

Another parameter of interest which is related to PWV is the pressure waveform. The pressure waveform in any artery is the result of the summation of the forward transmission of the cardiac pressure impulse and a backward reflection generated by the peripheral vascular system at the interface between large arteries and resistance vessels (arteries and arterioles). The pressure wave travels at such high velocity that it is reflected back to the central arteries during the same ejection cycle and overlaps part of the forward wave. Pressure recorded anywhere in the arterial system is thus the sum of the forward wave and the reflected wave and is dependent on 3 factors: the amplitude and duration of ventricular ejection, the amplitude of the reflected wave, and the PWV.[47]

Using either applanation tonometry or photoplethysmography, pressure vs time data can be acquired in different arteries, such as the radial, femoral or carotid arteries. Several measurements can be obtained using peripheral waveform analysis.[48] By applying a validated integral transfer function, the central aortic waveform can be derived.[49] In particular, central pressures can be inferred and the augmentation index can be calculated as an index of arterial stiffness. Using the electrocardiogram as time reference, readings from arteries at different locations can be compared to measure PWV.

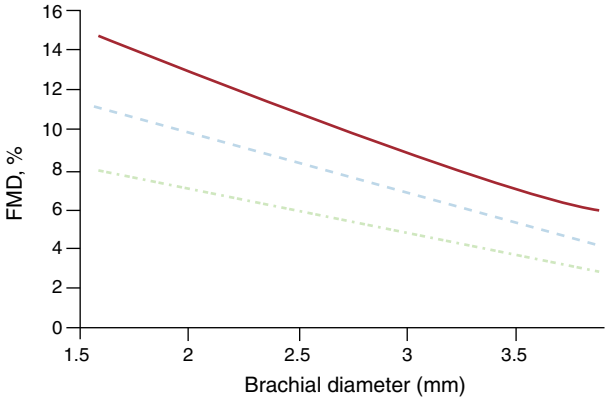

Figure 4. Nomogram for flow-mediated dilation according to the vessel baseline diameter. A negative linear association exists between baseline diameter and flow-mediated dilation. For a specific baseline diameter, flow-mediated dilation can be estimated. The black line shows the 75th percentile, the circular dashed line the 50th percentile, and the rectangular dashed line the 25th percentile of the population. FMD, flow-mediated dilation. Modified with permission from Charakida et al.[27]

Recently, arterial stiffness has been assessed using MRI. Unlike PWV, which is an average measure of overall arterial stiffness, MRI enables the detection of more subtle changes in regional stiffness. In MRI, full 3-dimensional visualization of the vessel is possible, enabling an image-measuring plane to be placed perpendicular to the vessel in a reproducible location. Moreover, velocity data can be acquired simultaneously in 2 aortic locations and the distance between them can be measured precisely.[50]

Radial artery applanation tonometry, as opposed to carotid or femoral artery tonometry, is easier to use in the clinical setting and causes less discomfort to the patient.[51] Tonometry of the radial artery provides an accurate, reproducible, noninvasive assessment of the central pulse pressure waveform. Radial artery applanation tonometry is carried out by placing a handheld tonometer (strain gauge pressure sensor) over the radial artery and applying mild pressure to partially "flatten" the artery (Fig. 5). The radial artery pressure is then transmitted from the vessel to the sensor (strain gauge) and is recorded digitally (Fig. 6).[47]

Recently, a new method of pulse wave analysis using oscillometric data obtained from an arm cuff inflated to suprasystolic pressure has been validated, showing a strong correlation (Pearson's correlation 0.91; $P<.001$) with measurements obtained during cardiac catheterization.[52] This new technique has the advantage of having a high level of automation, which provides better reproducibility.[53]

However, depending on the method, results regarding the reproducibility of PWV are still controversial.[54–56] In general, we could say that when the PWV is recorded by methods like Doppler or tonometry, it is more time-consuming and above all the reproducibility depends on operator skill, whereas when using the

Table 1
Normal Values of Pulse Wave Velocity in the Typical Middle-Aged Adult

Location	Normal values (m/s)
Ascending aorta	4
Abdominal aorta and carotids	5
Brachial artery	7
Iliac arteries	8

Reproduced with permission from Zambanini et al.[46]

new oscillometric techniques (Fig. 7) the procedure is found to be much more simple and reproducible.[57] Nevertheless, strict adherence to standard processes is necessary to ensure that clinically sound data are obtained. Blood pressure should be measured according to the guidelines set forth in the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[58] Given that vasoactive supplements, decongestants, caffeine, nicotine, or exercise can influence vascular reactivity, heart rate, and blood pressure, these should be avoided before blood pressure measurement so as not to invalidate the results.

Clinical Value of Pulse Wave Velocity

Although not the only contributor, endothelial function plays an important role in arterial stiffness. All the factors that reduce distensibility of the vessel (ie, increase "stiffness" of the wall) lead to a faster PWV. Atherosclerotic risk factors cause vascular remodeling, producing arterial "stiffness" within the aorta and other large arteries.[59]

In different studies, PWV correlates with the number of CV risk factors, the fitness level, CV events, and mortality in patient populations with end-stage renal disease, diabetes, or metabolic syndrome, and in healthy elderly adults,[60–63] as well as in those patients with a higher baseline risk, as recently published in a large meta-analysis.[64]

However, due to the lack of bigger studies proving reproducibility, PWV is confined, like FMD, to use as a research tool, applied mainly to study populations rather than individuals.

FINGER PLETHYSMOGRAPHY

In 1937 Alrick Hertzman[65] produced a "photoelectric plethysmograph," which he described as a device that "takes advantage of the fact that the absorption of light by a transilluminated tissue varies with its blood contents." This is a consequence of the Lambert–Beer law, which relates light absorption to optical density. There is still uncertainty about what the photoplethysmographic pulse actually represents at different body sites, but the continuous component is attributed to light absorption by tissue and fixed blood volume, and the pulsatile component is attributed to changes in the blood volume during the cardiac cycle. Photoplethysmography, once calibrated with a blood pressure measurement, can be used on the finger to provide continuous finger blood pressure monitoring (Fig. 8 [66]).

A different technology is also available to measure the same phenomenon. A finger pneumatic plethysmographic cuff has recently been made available, and provides an additional method for a "beat to beat" blood flow volume assessment by recording finger arterial pulsatile volume changes (EndoPAT®) (Fig. 9). With these instruments, blood pulsatile volume changes are used as a surrogate for baseline blood flow. Given that peripheral flow depends on small vessels tone, these procedures are known as peripheral arterial tone (PAT) measurements.

To assess endothelial function, postischemic (hyperaemic) blood flow is compared to baseline blood flow. One finger from each hand is monitored and a pressure cuff is placed on one of the upper arms to produce a transient ischemia, while the other arm serves as a control. After measuring baseline flow, the blood pressure cuff is inflated above systolic pressure during 5 min to induce a postischemic reactive hyperemia. The calculated index (Fig. 10) between the flow in the arm with reactive hyperemia and the control arm represents a measure for endothelial function. Similar flow changes after administering nitroglycerin can be measured to assess the nonendothelium dependent response.[7]

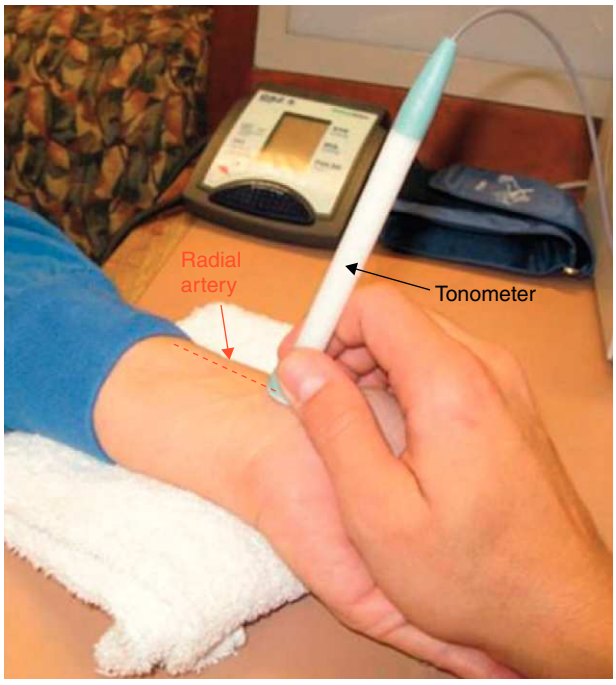

Figure 5. Applanation tonometry is performed by placing a pressure sensor over the radial artery. Modified with permission from Nelson et al.[47]

Clinical Value of EndoPAT®

PAT measurements in patients with chest pain[67] and coronary artery disease[68] seem to correlate well with the assessment of endothelial function performed by both intracoronary infusion of acetylcholine during angiography[20] and FMD brachial artery ultrasound, although the strength of the correlation varies between studies.

The main advantage of EndoPAT® as opposed to the aforementioned techniques is its high reproducibility, mainly because of the simplicity of the procedure itself that leads to very few interoperator variables. A study carried out to compare reproducibility of these techniques indicated that EndoPAT® provides more reproducible results than brachial artery ultrasound assessment of FMD, even when this is performed by a qualified brachial artery ultrasound sonographer.[69] However, the flow increase and subsequent increase of the finger pulse volume during reactive hyperemia is a complex response to ischemia and is only partly dependent on NO.[70] In that sense, another study found that while the reactive hyperemia index is relatively stable for 2 h, even when measured at 30 min intervals, interday reproducibility was low. This might be due to the daily variability of endothelial responses, but also to other factors that may have affected the peripheral vascular responses to ischemia. Once again, it appears as a compulsory requirement to adhere to a strict protocol when performing these assessments.[71]

As with other techniques to assess endothelial dysfunction, EndoPAT® has been correlated with multiple traditional CV risk factors, such as: male sex, body mass index, waist circumference, LDL cholesterol, diabetes, smoking, hypertension, and family history of coronary artery disease.[72] More importantly, a study of

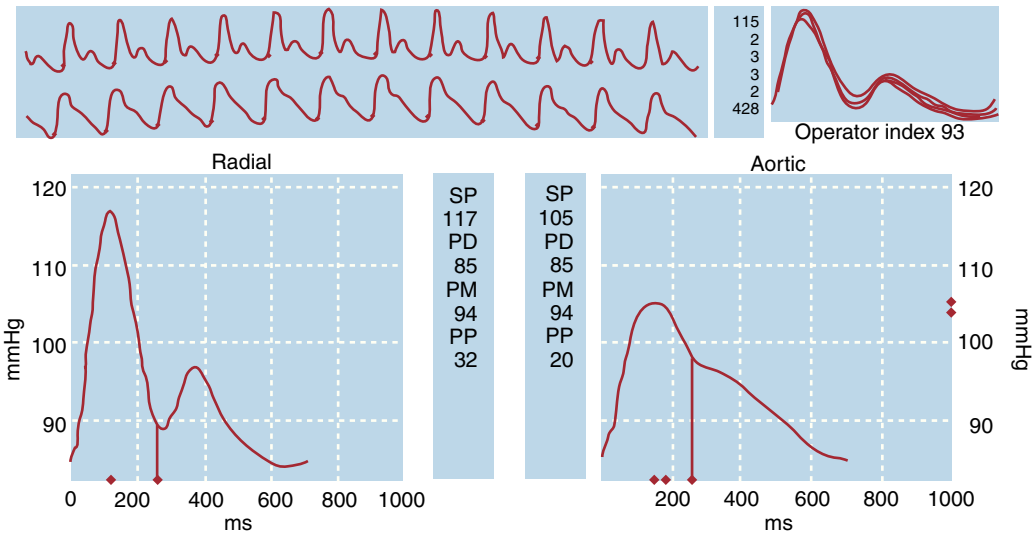

Figure 6. The upper long panel shows the radial pressure waveform above the derived central pressure waveform. The bottom left panel demonstrates a magnified radial arterial waveform. Systolic and diastolic pressures are 117/85 mmHg. The bottom right panel provides a magnified derived central pressure waveform. Central pressure is 105/85 mmHg. Modified with permission from Nelson et al.[47] ms, miliseconds.

270 patients with a follow-up period of 5.8 years demonstrated that this diagnostic procedure adds incremental value to the Framingham risk score.[14] Additionally, EndoPAT® assessment of endothelial dysfunction has been reported to be able to detect improvements after different therapies, including smoking cessation.[73]

USEFULNESS OF NONINVASIVE ENDOTHELIAL ASSESSMENT TECHNIQUES

Given the noninvasive nature of the procedures, these methods can be used in most people and measurements can be repeated to monitor changes in status. These aspects, in addition to the high reproducibility of measurements, mainly due to the high level of automation of at least some of the methods described above, make the techniques useful for clinical practice.

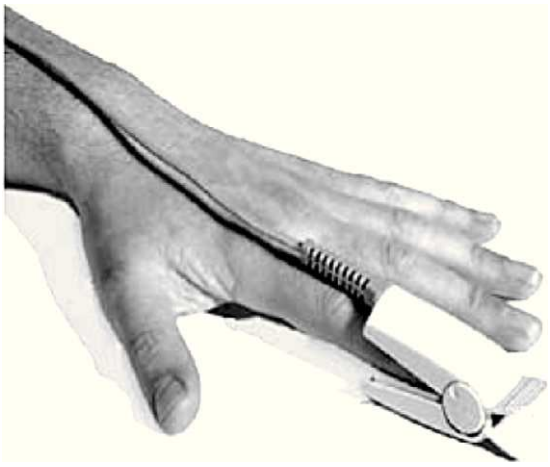

Figure 7. Oscillometric device for assessing pulse wave velocity.

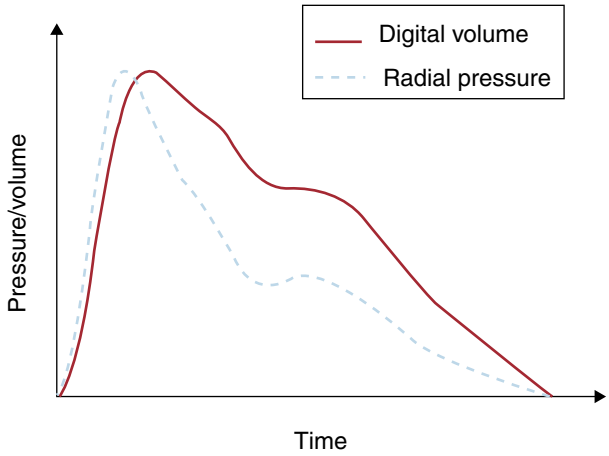

Figure 8. A modern photoplethysmograph incorporating a light-emitting diode and sensor within a finger clip. A typical waveform (solid line) is shown, together with a radial pressure waveform (obtained using a tonometer) in the same individual. Modified with permission from Millaseau et al.[66]

Figure 9. EndoPAT® device.

At present, these techniques are mainly used for research purposes in projects assessing disease mechanisms in humans but they show a remarkable potential as surrogate endpoints and may prove useful in the assessment of pharmacological and non-pharmacological intervention and trials dealing with disease prevention.

Assessment of endothelial function may help in patient risk stratification in primary and secondary prevention, the evaluation of vascular responses to specific therapies, and longitudinal patient monitoring.[16]

PRESENT AND FUTURE DIRECTIONS

Noninvasive techniques have become available which might help in the assessment of endothelial dysfunction in clinical practice. There are however many unresolved issues in relation to the utility of these techniques in everyday clinical practice. From a technical perspective, several issues should be investigated thoroughly before these techniques can be recommended for routine use. Firstly, we need to establish whether they are truly and precisely assessing endothelial function or whether they reflect the combined effect of the many variables—endothelial and nonendothelial dependent—that affect vascular responses. Secondly, it is important to study whether they will truly show a direct correlation with the effects of intervention. As most of these techniques assess endothelial function in an indirect way and are affected by the presence of external agents, protocols should be defined rigorously to minimize the effects of these confounding factors. Efforts should focus on automation to make the results more technician-independent and improve the reproducibility of their measurements.

Conceivably, the use of these techniques in clinical practice could improve patient risk stratification, help in the implementation of preventive strategies, and also improve treatment monitoring. Although several studies have already been performed (Table 2), further research is required in large clinical studies using the appropriate gold standards for the assessment of the role of this methodology in relation to diagnosis and intervention. So far, there is not enough evidence to support the use of endothelial function assessment in clinical practice. Prospective, well designed, clinical studies involving real-life subjects should be performed to clarify whether the endothelial function, as assessed by the techniques described in this manuscript, represent an independent risk factor and/or an independent marker of risk that provides additional information over and above that afforded by the classic CV risk factors. Comparisons among the different tools for risk assessment currently used in clinical practice and the assessment of endothelial function should also be carried out.

Other potential clinical applications include the dynamic monitoring of treatment as, in contrast to conventional CV risk factors, endothelial function is a dynamic event. Hence, these novel techniques should allow a better short-term assessment. Evidence to document that this is the case would require 2 steps. First, it should be established—in long-term observational open-label interventions—whether endothelial function assessment can identify, better than the use of conventional risk factors, successful and ineffective preventive treatments. Second, studies should be performed to demonstrate that intensification of treatment leading to improved endothelial function improves patients' clinical outcomes.

Future areas of interest also include the relationship of endothelial dysfunction to circulating endothelial progenitor cells number and function and their therapeutic modulation, as well as

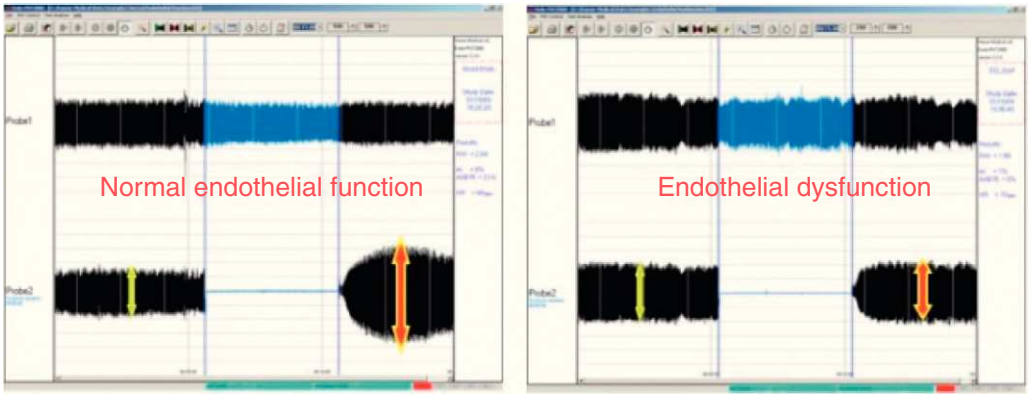

Figure 10. Differences between normal endothelial function and endothelial dysfunction assessed by EndoPAT®.

Table 2
Studies Using Noninvasive Techniques to Assess Endothelial Function

Method of assessment	Number of patients	Population	Type of study	Follow-up	Findings	First author	Year
FMD	500	Asymptomatic subjects	Observational, retrospective	–	Reduced FMD is related to the presence of classic CVRF	Celermajer et al.[39]	1994
FMD	213	Non-smoking subjets	Observational, prospective	6 years	Reduced FMD is related to progresion of preclinical carotid arterial disease	Witte et al.[41]	2009
FMD	199	Patients with peripheral artery disease	Observational, prospective	1.2 years	Reduced FMD independently predicted short and long-term CV events	Gokce et al.[43]	2002-2003
FMD	92	Survivors of ACS without ST-segment elevation	Observational, prospective	2.06 years	Reduced FMD independently predicted CV events	Karatzis et al.[44]	2006
PWV	465	Patients with suspected CAD	Descriptive	–	Aix was an independent risk marker for premature CAD	Weber et al.[59]	2004
PWV	146	Healthy volunteers	Descriptive	–	Reduced Aix was related to higher physical conditioning status (High VO_{2max})	Vaitkevicius et al.[60]	1993
PWV	1045	Hypertensive patients	Observational, prospective	5.7 years	Increasing level of PWV was an independent predictor of primary coronary events	Boutouyrie et al.[61]	2002
PWV	565	Diabetics and non-diabetics	Observational, prospective	10 years	Aortic PWV is a powerful independent predictor of mortality	Cruickshank et al.[62]	2002
PWV	364	Healthy subjects	Descriptive	–	PWV was increased according to the number of MS components present	Kim[63]	2006
PAT	89	Patients with and without CVRF	Descriptive	–	PAT ratio was more impaired in patients with CVRF	Kuvin et al.[67]	2003
PAT	60	Patients with CVRF	Descriptive	–	PAT ratio was related to the extension of CAD	Kuvin et al.[68]	2007
PAT	94	Patients without obstructive artery disease	Descriptive	–	PAT ratio was lower in patients with coronarymicrovascular endotelial function assessed by ACh test	Bonetti et al.[18]	2004
PAT	22	Healthy subjects	Descriptive	–	Repetitive PAT measurements in 30 min, 1 and 2 h have no carry over effects. Interday reproducibility is relatively low	Liu et al.[71]	2009
PAT	1957	General population	Descriptive	–	PAT ratio was related to multiple traditional metabolic and CVRF.	Hamburg et al.[72]	2008
PAT	270	Patients with CVRF	Observational, prospective	7 years	Low PAT ratio was related to higher incidence of CV adverse events	Rubinshtein et al.[14]	2010

Aix, augmentation index; CAD, coronary artery disease; CV, cardiovascular; CVRF, cardiovascular risk factors; FMD, flow-mediated dilation; MS, metabolic syndrome; PAT, peripheral arterial tone; PWV, pulse wave velocity.

genetic factors influencing endothelial function. Until answers to these questions become available, these techniques will remain useful research tools.

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CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Cannon CP, Steinberg BA, Murphy SA, Mega JL, Braunwald E. Meta-analysis of cardiovascular outcomes trials comparing intensive versus moderate statin therapy. J Am Coll Cardiol. 2006;48:438–45.
2. Wald NJ, Law MR. A strategy to reduce cardiovascular disease by more than 80%. BMJ. 2003;326:1419.
3. Law MR, Wald NJ. Risk factor thresholds: their existence under scrutiny. BMJ. 2002;324:1570–6.
4. Simon A, Chironi G, Levenson J. Comparative performance of subclinical atherosclerosis tests in predicting coronary heart disease in asymptomatic individuals. Eur Heart J. 2007;28:2967–71.
5. Taddei S, Virdis A, Mattei P, Arzilli F, Salvetti A. Endothelium-dependent forearm vasodilation is reduced in normotensive subjects with familial history of hypertension. J Cardiovasc Pharmacol. 1992;20 Suppl 12:S193–5.
6. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115:1285–95.

7. Flammer AJ, Lüscher TF. Three decades of endothelium research: from the detection of nitric oxide to the everyday implementation of endothelial function measurements in cardiovascular diseases. Swiss Med Wkly. 2010;140:w13122.
8. Zeiher AM, Schächinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. Circulation. 1995;92:1094–100.
9. Spieker LE, Sudano I, Hürlimann D, Lerch PG, Lang MG, Binggeli C, et al. High-density lipoprotein restores endothelial function in hypercholesterolemic men. Circulation. 2002;105:1399–402.
10. Mäkimattila S, Virkamäki A, Groop PH, Cockcroft J, Utriainen T, Fagerudd J, et al. Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin-dependent diabetes mellitus. Circulation. 1996;94:1276–82.
11. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest. 1996;97:2601–10.
12. Flammer AJ, Vo NTT, Ledergerber B, Hermann F, Gämperli A, Huttner A, et al. Effect of atazanavir versus other protease inhibitor-containing antiretroviral therapy on endothelial function in HIV-infected persons: randomised controlled trial. Heart. 2009;95:385–90.
13. Flammer AJ, Sudano I, Hermann F, Gay S, Forster A, Neidhart M, et al. Angiotensin-converting enzyme inhibition improves vascular function in rheumatoid arthritis. Circulation. 2008;117:2262–9.
14. Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J. 2010;31:1142–8.
15. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med. 1986;315:1046–51.
16. Penny WF, Ben-Yehuda O, Kuroe K, Long J, Bond A, Bhargava V, et al. Improvement of coronary artery endothelial dysfunction with lipid-lowering therapy: heterogeneity of segmental response and correlation with plasma-oxidized low density lipoprotein. J Am Coll Cardiol. 2001;37:766–74.
17. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol. 1995;26:1235–41.
18. Bonetti PO, Pumper GM, Higano ST, Holmes Jr DR, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol. 2004;44:2137–41.
19. Leung DY, Leung M. Non-invasive/invasive imaging: significance and assessment of coronary microvascular dysfunction. Heart. 2011;97:587–95.
20. Van Hoydonck PGA, Schouten EG, Temme EHM. Reproducibility of blood markers of oxidative status and endothelial function in healthy individuals. Clin Chem. 2003;49(6 Pt 1):963–5.
21. De Caterina R, Basta G, Lazzerini G, Dell'Omo G, Petrucci R, Morale M, et al. Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. Arterioscler Thromb Vasc Biol. 1997;17:2646–54.
22. Mocco J, Choudhri TF, Mack WJ, Laufer I, Lee J, Kiss S, et al. Elevation of soluble intercellular adhesion molecule-1 levels in symptomatic and asymptomatic carotid atherosclerosis. Neurosurgery. 2001;48:718–21.
23. Blann AD, Farrell A, Picton A, McCollum CN. Relationship between endothelial cell markers and arterial stenosis in peripheral and carotid artery disease. Thromb Res. 2000;97:209–16.
24. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340:1111–5.
25. Anderson EA, Mark AL. Flow-mediated and reflex changes in large peripheral artery tone in humans. Circulation. 1989;79:93–100.
26. Laclaustra M, Frangi AF, Garcia D, Boisrobert L, Frangi AG, Pascual I. Detailed exploration of the endothelium: parameterization of flow-mediated dilation through principal component analysis. Physiol Meas. 2007;28:301–20.
27. Charakida M, Masi S, Lüscher TF, Kastelein JJP, Deanfield JE. Assessment of atherosclerosis: the role of flow-mediated dilatation. Eur Heart J. 2010;31:2854–61.
28. Al-Qaisi M, Kharbanda RK, Mittal TK, Donald AE. Measurement of endothelial function and its clinical utility for cardiovascular risk. Vasc Health Risk Manag. 2008;4:647–52.
29. Bots ML, Westerink J, Rabelink TJ, De Koning EJP. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. Eur Heart J. 2005;26:363–8.
30. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39:257–65.
31. Thijssen DHJ, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol. 2011;300:H2–12.
32. Doshi SN, Naka KK, Payne N, Jones CJ, Ashton M, Lewis MJ, et al. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. Clin Sci. 2001;101:629–35.
33. Mullen MJ, Kharbanda RK, Cross J, Donald AE, Taylor M, Vallance P, et al. Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia. Circ Res. 2001;88:145–51.
34. Pálinkás A, Tóth E, Venneri L, Rigo F, Csanády M, Picano E. Temporal heterogeneity of endothelium-dependent and -independent dilatation of brachial artery in patients with coronary artery disease. Int J Cardiovasc Imaging. 2002;18:337–42.
35. Frangi AF, Laclaustra M, Lamata P. A registration-based approach to quantify flow-mediated dilation (FMD) of the brachial artery in ultrasound image sequences. IEEE Trans Med Imaging. 2003;22:1458–69.
36. Sonka M, Liang W, Lauer RM. Automated analysis of brachial ultrasound image sequences: early detection of cardiovascular disease via surrogates of endothelial function. IEEE Trans Med Imaging. 2002;21:1271–9.
37. Donald AE, Charakida M, Falaschetti E, Lawlor DA, Halcox JP, Golding J, et al. Determinants of vascular phenotype in a large childhood population: the Avon Longitudinal Study of Parents and Children (ALSPAC). Eur Heart J. 2010;31:1502–10.
38. Gori T, Parker JD, Münzel T. Flow-mediated constriction: further insight into a new measure of vascular function. Eur Heart J. 2011;32:784–7.
39. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol. 1994;24:1468–74.
40. Laclaustra M, Frangi AF, Frangi AG, Casasnovas JA, Cia P. Association of endothelial function and vascular data with LDL-c and HDL-c in a homogeneous population of middle-aged, healthy military men: Evidence for a critical role of optimal lipid levels. Int J Cardiol. 2008;125:376–82.
41. Witte DR, Westerink J, De Koning EJ, Van der Graaf Y, Grobbee DE, Bots ML. Is the association between flow-mediated dilation and cardiovascular risk limited to low-risk populations? J Am Coll Cardiol. 2005;45:1987–93.
42. Halcox JPJ, Donald AE, Ellins E, Witte DR, Shipley MJ, Brunner EJ, et al. Endothelial function predicts progression of carotid intima-media thickness. Circulation. 2009;119:1005–12.
43. Gokce N, Keaney Jr JF, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol. 2003;41:1769–75.
44. Karatzis EN, Ikonomidis I, Vamvakou GD, Papaioannou TG, Protogerou AD, Andreadou I, et al. Long-term prognostic role of flow-mediated dilatation of the brachial artery after acute coronary syndromes without ST elevation. Am J Cardiol. 2006;98:1424–8.
45. Gokce N, Keaney Jr JF, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation. 2002;105:1567–72.
46. Zambanini A, Cunningham SL, Parker KH, Khir AW, McG Thom SA, Hughes AD. Wave-energy patterns in carotid, brachial, and radial arteries: a noninvasive approach using wave-intensity analysis. Am J Physiol Heart Circ Physiol. 2005;289:H270–6.
47. Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT, Tajik AJ. Noninvasive measurement of central vascular pressures with arterial tonometry: clinical revival of the pulse pressure waveform? Mayo Clin Proc. 2010;85:460–72.
48. Hayward CS, Kraidly M, Webb CM, Collins P. Assessment of endothelial function using peripheral waveform analysis: a clinical application. J Am Coll Cardiol. 2002;40:521–8.
49. Rietzschel ER, Boeykens E, De Buyzere ML, Duprez DA, Clement DL. A comparison between systolic and diastolic pulse contour analysis in the evaluation of arterial stiffness. Hypertension. 2001;37:E15–22.
50. Cavalcante JL, Lima JAC, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. J Am Coll Cardiol. 2011;57:1511–22.
51. Adji A, Hirata K, O'Rourke MF. Clinical use of indices determined non-invasively from the radial and carotid pressure waveforms. Blood Press Monit. 2006;11:215–21.
52. Horváth IG, Németh A, Lenkey Z, Alessandri N, Tufano F, Kis P, et al. Invasive validation of a new oscillometric device (Arteriograph) for measuring augmentation index, central blood pressure and aortic pulse wave velocity. J Hypertens. 2010;28:2068–75.
53. Wassertheurer S, Kropf J, Weber T, Van der Giet M, Baulmann J, Ammer M, et al. A new oscillometric method for pulse wave analysis: comparison with a common tonometric method. J Hum Hypertens. 2010;24:498–504.
54. Sigrist MK, Chiarelli G, Levin A, Romann A, Weber C. Pulse wave velocity measurements are reproducible in multiple trained observers: a short report. Nephron Clin Pract. 2010;116:c60–4.
55. Van Leeuwen-Segarceanu EM, Tromp WF, Bos W-JW, Vogels OJ, Groothoff JW, Van der Lee JH. Comparison of two instruments measuring carotid-femoral pulse wave velocity: Vicorder versus SphygmoCor. J Hypertens. 2010;28:1687–91.
56. Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens. 1998;16(12 Pt 2):2079–84.
57. Naidu MUR, Reddy BM, Yashmaina S, Patnaik AN, Rani PU. Validity and reproducibility of arterial pulse wave velocity measurement using new device with oscillometric technique: a pilot study. Biomed Eng Online. 2005;4:49.
58. Eddy DM, Adler J, Patterson B, Lucas D, Smith KA, Morris M. Individualized guidelines: the potential for increasing quality and reducing costs. Ann Intern Med. 2011;154:627–34.
59. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, et al. Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation. 2004;109:184–9.

60. Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, et al. Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation. 1993;88(4 Pt 1):1456–62.
61. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. 2002;39: 10–5.
62. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation. 2002; 106:2085–90.
63. Kim YK. Impact of the metabolic syndrome and its components on pulse wave velocity. Korean J Intern Med. 2006;21:109–15.
64. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55:1318–27.
65. Hertzman AB. Observations on the finger volume pulse recorded photo-electrically. Am J Physiol. 1937;119:334–5.
66. Millasseau SC, Ritter JM, Takazawa K, Chowienczyk PJ. Contour analysis of the photoplethysmographic pulse measured at the finger. J Hypertens. 2006; 24:1449–56.
67. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J. 2003;146:168–74.
68. Kuvin JT, Mammen A, Mooney P, Alsheikh-Ali AA, Karas RH. Assessment of peripheral vascular endothelial function in the ambulatory setting. Vasc Med. 2007;12:13–6.
69. Reisner Y, Lusky R, Shay-El Y, Schnall R, Herscovici S. Reproducibility of endothelial function and arterial stiffness assessed using finger peripheral arterial tonometry. Eur Heart J. 2008;Suppl 29:491.
70. Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. J Appl Physiol. 2006;101:545–8.
71. Liu J, Wang J, Jin Y, Roethig HJ, Unverdorben M. Variability of peripheral arterial tonometry in the measurement of endothelial function in healthy men. Clin Cardiol. 2009;32:700–4.
72. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation. 2008;117:2467–74.
73. Komatsu H, Hara H, Nemoto N. Only short-term smoking cessation improves endotelial dysfunction; non-invasive evaluation used new device, Endo-PAT 2000. Eur Heart J. 2007;28 Suppl 1:587.