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Non-invasive assessment of endothelial function — a review of available methods

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ABSTRACT

The key role of the endothelium in vascular-dependent diseases led to an increase in scientific interest in examining the endothelial function as a tool for screening, as well as for monitoring of the disease and its treatment. In the period from 2016 till 2019, a high level of scientific interest in the assessment of endothelial function has been observed, as expressed in the number of published clinical trials between 369 and 477 per year with the total number of subjects between 49,634 and 75,934.

Currently, none of the known methods of assessing vascular endothelial function is widely used in clinical practice. This may be a result of various factors: scientific (lack of standardization in terms of quantitative indicators of endothelial function), formal (lack of official recommendations for endothelial assessment), financial (the best-validated methods and devices are costly, which renders it unsustainable to use them in screening diagnostics) and technological (high susceptibility of many measurement methods to errors). Nevertheless, it can be expected that non-invasive methods for the early detection of endothelial dysfunction in screening programs will gradually gain importance.

Key words: endothelium, endothelial function, endothelial assessment

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Introduction

Endothelial dysfunction precedes the appearance of atherosclerotic lesions and their clinical symptoms. Early pathology detection allows the implementation of adequate preventive treatment [1–6]. The study aimed to review non-invasive methods of endothelial function assessment as an attractive option for early diagnosis and an interesting research area.

Endothelial function assessment — general assumptions

The vast majority of non-invasive methods for endothelial function assessment are based on the phenomenon of reactive hyperaemia. The flow-mediated dilation (FMD) method is based on the ability of endothelium cells to regulate peripheral resistance, while endothelial dysfunction is defined as the impairment of vasodilation due to the secretion of endothelium-derived relaxing factor EDRF [7–10]. The identification of EDRF as nitric oxide (NO) was awarded the Nobel Prize in 1998 [11, 12].

Among the factors modulating the vascular endothelium function, shear stress is the most convenient to apply in clinical practice. The sudden change of shear stress leads to the reactive hyperaemia commonly used in non-invasive tests of the endothelium. The post-occlusion reactive hyperaemia (PORH) is defined as an increase in blood flow following the artery occlusion due to EDRF release [13, 14]. While not all details of the mechanism of reactive hyperaemia have been established so far, a general pattern can be drawn in the form of a cause-and-effect sequence following the artery occlusion (i.e., deflation of the occlusive cuff). The decrease in peripheral resistance due to vasodilation of the arterioles causes an analogous blood flow increase in the conduit arteries. An increase in blood flow causes a proportional increase in shear forces, and this directly induces endothelial cell mechanoreception pathways resulting in the increased secretion of nitric oxide, which, in turn, causes vasodilation of the conduit arteries [15, 16].

Application of endothelial function assessment in clinical studies

The key role of the endothelium in vascular-dependent diseases has led to an increase in scientific interest in examining the endothelial function as a tool for screening, as well as for monitoring of the disease and its treatment [13–16]. Apart from the already existing atherosclerosis, the functioning of the vascular endothelium is influenced by, among others:
— Arterial hypertension,
— Hyperlipidaemia,
— Diabetes mellitus,
— Hyperhomocysteinaemia,
— Heart failure,
— Tobacco smoking,
— Age,
— Inflammatory factors,
— Menopause [1, 2, 17].

Recently, a considerable number of studies applying endothelial function assessment as a research tool have been published. The authors conducted a search covering the period from 1st January 2016 to 31st December 2020 using the MEDLINE database with "endothelial function" as the only keyword. The initial search revealed 61,399 records. Only 1,873 of those articles, classified as clinical trials, were included for further analysis. References of those studies were searched manually for studies in which endothelial function was assessed. The final analysis comprised 1,742 studies in which several subjects who underwent endothelial function assessment was reported. For each eligible study, the number of studied subjects was defined (Tab. 1).

In the period from 2016 till 2019, a high level of scientific interest in the assessment of endothelial function has been observed, as expressed in the number of published clinical trials between 369 and 477 per year with the total number of subjects between 49,634 and 75,934. The sudden decline in the number of published studies and the total number of participants seen in 2020 is likely an effect of the COVID-19 pandemic. Therefore, a further increase in the number of such studies should be assumed once the restrictions are eased. It should be emphasized that the performed analysis was limited using only one keyword in one of the most prestigious databases, which groups only the best scientific journals and the yet impressive total number of 262,218 people who underwent assessment of endothelial function was reported. Thus, the actual number of endothelial function tests carried out in the analysed period might have been much higher.

Methods of endothelial function assessment

Understanding the importance, and thus the growing interest in endothelial pathophysiology has led to the development of several methods for invasive or non-invasive, quantitative evaluation of its function [1–4]. Most of these methods are based on the assumptions: that firstly certain types of stimulation trigger the nitric oxide production leading to local vasodilation, and secondly endothelial dysfunction is a systemic disorder, therefore can be tested in any arterial vessels, most often on easily accessible arteries of the upper limbs [18]. To determine the severity of the endothelial reaction the baseline (reference level) of the assessed parameter should be defined as a reference to its value after the stimulation. Some methods need to employ additional mathematical methods to compensate for the effects of extra-endothelial factors that may interfere with the measurement of the local response [19].

In-vitro methods

The in-vitro methods of endothelial function assessment are based on the analysis of the concentration of the vasoactive factor in the plasma. Direct measurement of NO concentration is not feasible in everyday clinical practice, and the available techniques are burdened with a considerable error. However, it is possible to assess the concentration of other substances functionally related to the endothelium [1, 2, 17]. The most commonly used markers of endothelial function include:

Table 1. Trials applying endothelial function assessment

Year	Number of clinical trials	Number of clinical trials reporting of number of participants	Number of participants
2016	477	434	75,934
2017	432	399	54,673
2018	369	352	50,095
2019	400	374	49,634
2020	195	183	31,882
Total:	1,873	1,742	262,218

— Von Willebrand factor,
— Thrombomodulin,
— Intercellular adhesive molecule (ICAM-1),
— Vascular adhesive molecule (VCAM-1),
— Plasminogen inhibitor type 1 (PAI-1),
— P-selectin and E-selectin,
— Vascular endothelial growth factor (VEGF) [1, 2, 10, 17].

Another in-vitro test showing damage to the vascular endothelium may be a measurement of the number of circulating endothelial cells (CEC) in the blood. The increase of CEC correlates with other endothelial markers, including von Willebrand factor, E-selectin and thrombomodulin [1,2,4-6,10].

Invasive in-vivo methods

Invasive methods of endothelial function assessment are mainly used in scientific research due to the significant risk of complications resulting from the specificity of endovascular procedures [9, 14]. The use of invasive techniques was necessary to establish a reference standard for the validation of completely safe non-invasive methods. Assessment of coronary response to local acetylcholine injection introduced in the 1990s is a widely accepted method. Vasoconstriction caused by endothelial dysfunction is recorded indirectly by measuring the coronary flow with an endovascular Doppler probe or with thermodilution. The measure of the coronary arteries lumen with quantitative angiography is an alternative to the coronary flow assessment [20]. A positron emission tomography (PET) scanner [22] and magnetic resonance imaging (MRI) with phase contrast [21] were also used to quantify the hyperaemic response to the administration of vasoactive agents.

Non-invasive in-vivo methods

Flow-mediated skin fluorescence (FMSF)

One of the non-invasive methods of endothelial function assessment is the measurement of fluorescence [23]. The FMSF is based on the fluorescence measurement of the reduced form of nicotinamide adenine dinucleotide (NADH), emitted by the skin cells in the band of 420 to 480 nm (peak emission in the range 455–465 nm) in response to excitation by UV light in the 300 to 400 nm range (recommended range is 345–355 nm). The test consists of three phases: recording the baseline intensity of the fluorescence (usually 1–2 minutes); occlusion phase (usually 5 minutes); registration of the response (decay of the NADH fluorescence intensity to the baseline, time — up to 15 minutes) [23]. FMSF allows determining the tissues and vascular bed response during ischaemia, while the classic techniques based on FMD are based on a vasomotor reactivity examination [24].

FMD-based methods

The phenomenon of reactive hyperaemia is the methodological foundation for almost all currently used non-invasive techniques for endothelial function assessment. This group of methods is derived from the classic technique based on the use of ultrasound for direct visualization of brachial artery dilation, which is associated with operator-dependent bias. Therefore, intensive research is conducted on the development of semi-automatic or fully automatic techniques, i.e., techniques that do not require the active participation of the operator during the test.

Classic FMD method

The classic FMD method assessing the intensity of reactive hyperaemia consists of three phases: measurement of the diameter of a selected artery (e.g., brachial); stimulation phase; measurement after stimulation. As a stimulation, transient ischaemia caused by tightening the sphygmomanometric cuff (by inflating it to pressure 30 to 50 mmHg higher than the baseline systolic blood pressure), is usually applied [9–12,16, 22, 25]. Only a few publications have reported the use of intra-arterial injection of a vasoactive agent: nitro-glycerine [16], acetylcholine or methacholine [9–11, 26]. The relative change in vessel diameter is rather small, assuming values ranging from a few to several per cent compared to the pre-occlusion measurement [14, 16, 25].

Reactive hyperaemia peripheral arterial tonometry (RH-PAT)

RH-PAT — a modified FMD method is used in the EndoPAT device (Itamar Medical Ltd.) [27–30]. This method uses finger probes with a two-compartment pneumatic cuff. The proximal cuff is pumped up to a pressure close to the diastolic blood pressure of the patient, and its task is to relieve the arteries walls and reduce the volume of venous blood in the area of the second phalanx of the examined finger [27]. The distal cuff, separately connected to the measurement system, is used for the actual measurement by pneumatic plethysmography. The method has been extensively validated in many publications as a diagnostic and predictive tool in terms of vascular risk assessment [27–32].

Thermal method

The thermal method used in VENDYS devices (Endothelix Inc.) is based on recording the temperature of two fingers (e.g., index fingers), with one used as a reference, and the other stimulated with a classic 5-minute occlusion with an automatically pumped cuff [33]. The tightening of the cuff causes a decrease in the occluded limb's skin temperature in comparison to the baseline temperature recorded before cuff inflation. Based on the rate of the temperature decay and with the use of

a mathematical model, a zero-reactivity curve (ZRC) is determined. The third segment of the temperature curve recorded after the restoration of perfusion refers to the ZRC line and normalized to the reference record from the unoccluded (reference) limb. Based on the record from the third phase of measurement, a curve of the hyperaemic response is drawn [33]. The peak temperature, defined as the maximum point of this curve, represents the Vascular Reactivity Index (VRI) [33, 34].

Enclosed-zone FMD (EZ-FMD) method

This method is based on the oscillometric blood pressure measurement, where the cuff inflation and deflation cycle is repeated six times: at the beginning of the measurement (once) to determine the patient's maximum pulse amplitude and five times after a 5-minute of a total occlusion. The EZ-FMD coefficient is calculated from the formula based on the maximum systolic peak in the pre-occlusion phase and the highest peak in the post-occlusion phase [35, 36].

Photoplethysmography method — reactive hyperemia peripheral arterial volume (RH-PAV)

Classic pulse oximetry sensors in the form of finger clips can also be used to test endothelial function. A LED diode operating in the near-infrared region (940 nm) is used for the test. The methodology of calculating the result is analogous to that of the RH-PAT index: the average amplitudes are calculated from two signal intervals recorded: 40 s before occlusion and 40 s after cuff deflation. The results are normalized to the opposite non-occluded limb. The authors of the described technique called it RH-PAV [37].

Impedance plethysmography method

Impedance plethysmography uses changes in the electrical impedance of tissues caused by pulsatile blood flow. For reactive hyperaemia studies, local injection of acetylcholine or methacholine directly into one of the forearm arteries was used [21].

Laser speckle contrast imaging (LSCI) method

LSCI is based on the analysis of speckle pattern images, formed when illuminating the patient's skin surface with laser light. LSCI allows for two-dimensional visualization of peripheral perfusion in the form of a colour map. This method was used to acquire images of perfusion changes occurring under the influence of acetylcholine and in response to classical stimulation with temporary artery occlusion [38, 39].

Laser Doppler flowmetry (LDF) method

LDF is used to assess peripheral microcirculation, including the measurement of reactive hyperaemia. With regard to vascular endothelial studies, the LDF method is used to assess reactive hyperaemia in the vascular bed of cutaneous vessels. Laser flow meters are well suited for recording the early component of the hyperaemic response associated with rapid dilation of resistance-type vessels (skin arterioles) [41, 42].

Pulse velocity wave (PVW) measurement method

This method is based on the measurement of the delay of the peak of the peripheral pulse wave, usually in relation to the QRS complex in a simultaneously recorded ECG — the greater the stiffness of the vessels, the faster the pulse wave propagation is observed [43].

The delay is calculated from measurements taken at two different points of the body, such as the carotid and femoral arteries. This technique can also be used for endothelial studies [44]. Depending on the adopted pulse wave recording points, it is possible not only to study the central arterial stiffness but also the peripheral regardless of the condition of the aorta. The stiffness of the arterial wall depends on the vessel ultrastructure, blood pressure and tone of the vascular muscle layer, all varying with age. The use of pulse wave velocity measurement in endothelial studies is based on the assumption that the administration of vasodilators to the patient allows the extraction of the last of these components. Available studies showed a negative correlation between PWV values and endothelial function [43, 44].

Pulse wave analysis (PWA) method

PWA is based on the mathematical decomposition of the pulse waveform recorded on the peripheral (usually radial) artery. Based on the relation of the timing and the amplitude data several parameters reflecting different aspects of central haemodynamics are determined. Similarly to the PWV technique, the pulse wave analysis may also be supported by pharmacological stimulation to determine the range of vascular endothelium response to the administration of vasoactive agents [44, 45].

Summary

Endothelial dysfunction playing a pivotal role in the pathophysiology of several civilization diseases can be detected at the asymptomatic stage. Therefore, a non-invasive assessment of endothelial function can be applied for early screening in people at increased cardiovascular risk. Moreover, endothelial function assessment can be used as a method for evaluation of treatment effectiveness, as well as for adherence to treatment [46–57]. However, until now these methods have usually been used only for research purposes, and their potential clinical application should be preceded by clinical trials.

Recent studies indicate the endothelium as the main target of SARS-CoV-2, which often causes thrombotic complications [58–60]. Therefore, studies aimed to assess the usefulness of testing the endothelial function also seem advisable in patients with COVID 19.

Currently, none of the known methods of assessing vascular endothelial function is widely used in clinical practice. This may be a result of various factors: scientific (lack of standardization in terms of quantitative indicators of endothelial function), formal (lack of official recommendations for endothelial assessment), financial (the best-validated methods and devices are costly, which renders it unsustainable to use them in screening diagnostics) and technological (high susceptibility of many measurement methods to errors).

Nevertheless, it can be expected that non-invasive methods for the early detection of endothelial dysfunction in screening programs will gradually gain importance.

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