

# NON-INVASIVE ASSESSMENT OF ENDOTHELIAL DYSFUNCTION

**Daniel Lighezan**

## REZUMAT

Endoteliul vascular secretă numeroase substanțe chimice, printre care: oxidul nitric (NO), prostacilinele, factorul hiperpolarizant endotelial, prin intermediul cărora reglează tonusul vascular, proliferarea celulară, răspunsul inflamator, agregarea plachetară și coagularea. Vasele de sânge au capacitatea de a răspunde la stimuli fizici și chimici prezenți în lumen prin autoreglarea tonusului vascular. Multe vase răspund la flux crescut sau stress parietal prin dilatare. Funcția endotelială este evaluată prin vasodilatația dependentă de endotelium. Vasodilatația mediată de flux este o funcție dependentă de endotelium, explorată în principal prin tehnici neinvazive la nivelul arterei brahiale. Această metodă are avantajul că este neinvazivă, sigură și mai rapidă decât metodele invazive, precum și faptul că rezultatele sunt strâns corelate cu funcția endotelială de la nivelul arterelor coronariene. Această metodă neinvazivă de explorare a disfuncției endoteliale depinde mult de operator, necesită cooperare foarte bună a pacientului și are o rezoluție relativ slabă în relație cu dimensiunea arterei. Determinarea funcției endoteliale prin tehnici neinvazive la nivelul arterelor periferice poate fi o deschidere spre explorarea corelată a arterelor coronariene. Disfuncția endotelială la nivelul arterei brahiale poate identifica pacienții cu risc pentru boala coronariană, vasculară cerebrală, sau boala vasculară periferică. Evaluarea funcției endoteliale prin screeningul cu tehnici neinvazive, este importantă pentru identificarea precoce a aterosclerozei la copiii și adulții tineri, oferind astfel oportunitatea maximă de prevenție pentru bolile cardiovasculare.

**Cuvinte cheie:** disfuncție endotelială, vasodilatație mediată de flux

## ABSTRACT

The vascular endothelium secretes numerous factors such as nitric oxide (NO), prostacyclin, and the endothelium derived hyperpolarizing factor, through which regulates vascular tone, cell growth, inflammatory response, coagulation, and thrombocyte adhesion. The blood vessels have the capacity to respond to physical or chemical stimuli present in the lumen through self regulation of vascular tone. Many blood vessels respond to an increase in flow or shear stress by dilating. Endothelial function has been largely assessed as impaired endothelium-dependent vasodilatation. Flow-mediated vasodilatation (FMD) is an endothelium-dependent function, mainly explored by a noninvasive technique, in the brachial artery. This method has the advantage of being non-invasive, safer, and faster than invasive methods, while its results are closely correlated with endothelial function in the coronary arteries. However, it seems to be highly operator dependent, requires excellent patient cooperation, and has relatively poor resolution relative to arterial size. Assessment of endothelial function with invasive and non-invasive techniques in the peripheral circulation is believed to be a window to the coronary arteries. Endothelial dysfunction in the brachial artery will identify patients at risk for developing coronary artery disease, cerebral or peripheral vascular disease. By screening with a non-invasive technique, evaluation of endothelial function is important for the study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention.

**Key Words:** endothelial dysfunction, flow-mediated vasodilatation

## INTRODUCTION

The vascular endothelium secretes numerous factors such as nitric oxide (NO), prostacyclin, and the endothelium derived hyperpolarising factor, through which regulates vascular tone, cell growth, inflammatory response, coagulation, and thrombocyte

adhesion. The blood vessels have the capacity to respond to physical or chemical stimuli present in the lumen through selfregulation of vascular tone. Many blood vessels respond to an increase in flow or shear stress by dilating.

Endothelial function has been largely assessed as impaired endothelium-dependent vasodilation because endothelium-derived nitric oxide, is a major mediator of endothelium-dependent vasodilation, involved in the regulation of other protective properties of endothelium.<sup>1,2</sup>

Common conditions such as dyslipidemia, hypertension, diabetes and smoking are associated with endothelial dysfunction, being in the same time risk factors which promote the development, progression, and complications of atherosclerosis.

---

ASCAR Clinic of Cardiology, Victor Babes University of Medicine and Pharmacy, Timisoara

Correspondence to:  
Daniel Lighezan, ASCAR Clinic of Cardiology, 12 Revolutiei Blvd., Timisoara, Romania, Tel: +40 256 259224.  
Email: dlighezan@hotmail.com

---

Received for publication: Oct. 18, 2005. Revised: Dec. 14, 2005.

Accumulating clinical studies suggest an important pathophysiological role of endothelial dysfunction, as determined by impaired endothelium-dependent vasodilation, by demonstrating a close association of the degree of coronary or peripheral endothelial dysfunction with cardiovascular events.<sup>3</sup>

Endothelial function is altered in patients with cardiovascular risk factors and this alteration is thought to play an important role in the development, progression, and clinical complications of atherosclerosis. That is why the evaluation of endothelial function is useful in identifying subjects at high risk for atherosclerosis. Endothelial dysfunction is also an independent prognostic factor in patients with advanced atherosclerosis.<sup>4</sup>

### **HIGH RESOLUTION ULTRASOUND: ASSESSMENT OF ENDOTHELIUM DEPENDENT FLOW MEDIATED VASODILATATION OF THE BRACHIAL ARTERY**

During the last few years a number of non-invasive techniques have been developed to assess endothelial function in humans. These techniques are easily applied directly into the coronary arteries or in peripheral conduit arteries, and can be used as screening tests for identification of early onset atherosclerosis.<sup>4</sup>

Flow-mediated vasodilation (FMD), is an endothelium-dependent function, mainly explored by a noninvasive technique, in the brachial artery.

The brachial arteries are scanned, with high resolution ultrasound imaging, at rest and during hiperaemia. Hiperaemia can be induced by inflation and deflation of a sphygmomanometer cuff around the forearm, distal to the site scanned later with ultrasound.

Peripheral arteries, including the brachial artery, respond to physical and chemical stimuli by adjusting vascular tone and regulating blood flow.<sup>5</sup>

Increased blood flow in peripheral arteries is leading to increased shear stress stimuli, increased NO production, and vasodilation.<sup>4,5</sup> The vasodilatory response of the brachial artery to increased shear stress is called flow mediated dilation (FMD), and reflects the ability of vascular endothelium to produce NO.<sup>5</sup>

NO is the main mediator for shear induced vasodilatation, there have been identified but other internal molecules such as endothelium derived prostanoids or endothelium derived hyperpolarising factor that may also contribute in various degrees of flow mediated vasodilation.<sup>4</sup>

### **Methodology**

Endothelial function, defined as flow mediated dilatation is defined as the percentage increase in vessel diameter from baseline conditions to maximum vessel diameter, during hiperaemia. Most laboratories estimate FMD as the percentage change of the brachial artery diameter from rest, to the vessel diameter 60 seconds after the ischaemia cuff is released.<sup>4</sup>

### **Subject preparation**

After a 12 hour fasting period, the subject is positioned in a supine position, in a quiet room, with constant temperature, the arm is placed in a comfortable position for assessing the brachial artery, and must remain under constant conditions for at least 10 minutes.

All vasoactive drugs must be discontinued at least 12 hours before the study, and the patient must not ingest substances that might affect FMD such as caffeine, high-fat food and vitamin C nor smoke, for at least six hours before the study.<sup>6</sup>

### **Equipment and image acquisition**

The brachial artery is imaged above the antecubital fossa in the longitudinal plane, using a linear array transducer (with frequency 7–12 MHz) attached to a high quality mainframe ultrasound system. The ultrasound systems must be equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler, an internal electrocardiogram monitor and a high-frequency vascular transducer.

Initially the diameter of the brachial artery is determined at rest, and blood flow is estimated by time averaging the pulsed Doppler velocity signal obtained from a mid artery sample volume. The diameter of the brachial artery can be determined manually with electronic calipers or automatically using edge detection software. To decrease the variability of the measurements, the brachial artery diameter should be determined by the average derived from multiple diameter measurements along a segment of the vessel. During image acquisition, anatomic landmarks such as veins or fascial planes should be noted in order to help maintain the same image of the artery throughout the study.<sup>6</sup>

Usually, ischaemia is produced by inflating a cuff placed at the distal forearm, at a pressure 50 mm Hg greater than the systolic blood pressure, for 5 minutes. Alternatively the ischaemia cuff can be placed at the upper arm instead of the forearm, resulting in a greater hyperaemic flow and higher brachial artery vasodilation after its release. Another possibility in

studying FMD is to use other vessels such as radial, axillary or superficial femoral arteries, but these vessels must have a diameter larger than 5 mm. Although it is still unclear which is the most preferable position to place the ischaemia cuff, it seems that placing it at the upper arm results are less accurate, data acquisition is poor because the image can be distorted by collapse of the brachial artery or a shift in soft tissues.<sup>4,7-9</sup>

The ischaemia cuff is released after five minutes and is leading to an increase in forearm blood flow, resulting in a vasodilation of the brachial artery. The maximum blood flow velocity is detected by analysing mid artery pulsed Doppler signal immediately after or up to 15 seconds after cuff release, while the maximum diameter of the brachial artery is determined approximately 60 seconds after release or 45–60 seconds after the peak hyperaemic flow.<sup>9</sup>

Additional information may be offered by the evaluation of the “area under the curve” of the diameter versus time from ischaemic cuff release. It is important to measure the diameter of the brachial artery at the same period of the cardiac cycle, in order to avoid any effect of arterial compliance on the measurements. Therefore, simultaneous ECG recordings are essential to achieve the most reliable results.

All the determinations are carried out on tape by two independent observers, since the brachial artery is continuously monitored from 30 seconds, before to 120 seconds after ischaemia cuff release.<sup>4</sup>

Often, the comparison of FMD with endothelium dependent dilation in the brachial artery, produced after glyceryl trinitrate administration, helps to elucidate changes in smooth muscle function or arterial compliance, that may affect the observed changes in FMD.<sup>4,6</sup>

Flow-mediated vasodilation is an endothelium-dependent process that reflects the relaxation of a conduit artery when exposed to increased shear stress.

This method has the advantage of being non-invasive, safer, and faster than invasive methods, while its results are closely correlated with endothelial function in the coronary arteries. However, it seems to be highly operator dependent, requires excellent patient cooperation, and has relatively poor resolution relative to arterial size.<sup>4</sup>

The method of FMD is reproducible, with a coefficient of variation for repeated measurements of brachial artery diameter, in a good laboratory, of about 3–4% in short term (two hour interval), as well as in longer term (three week interval) repeated measurements.<sup>10</sup>

### **Normal values**

The absolute mean FMD values vary across population, ranging from -1,9 to 19,2. These differences are due to differences in risk factor profiles of various study populations or are consequences of various aspects of measurement methodology, because the methodology which excludes mainly the reference values of FMD. The technical aspects of measurements, the location and the duration are the most important factors that influence FMD.<sup>11,12</sup>

In various studies published in the literature, the FMD response with an upper arm occlusion showed a higher FMD value, compared with a lower arm occlusion.<sup>13-17</sup> A longer duration of ischemia increased the FMD and the measurement of FMD at the radial artery can lead to a lower absolute FMD value.<sup>18</sup>

The type of equipment, location of the measurement and occlusion pressure had no effect on FMD values. Neither the differences in technical aspects nor the risk factor distribution can completely explain the differences among different FMD studies, that is why it is necessary to implement a standardization of the FMD measurements.

### **CLINICAL IMPLICATIONS**

Recent data suggest that endothelial function is a useful prognostic marker in coronary artery disease patients.<sup>19</sup>

In these patients, it has been reported that endothelial dysfunction is an independent prognostic factor and may predict future events, irrespective of the angiographic severity of the disease.<sup>19,20</sup>

Individuals with endothelial dysfunction require better control of their lipid profile, C reactive protein, serum glucose, blood pressure, and smoking, since it is well known that all these factors affect endothelial function significantly.

Moreover, blunted endothelial function may reflect early atherosclerosis, and should lead to a detailed evaluation (and control) of all the conventional and newer risk factors, such as infection/ inflammation, homocysteine.

Persisting severe endothelial dysfunction in patients with advanced atherosclerosis may require a more aggressive control of risk factors and probably modification of current medication.<sup>21</sup>

### **CONCLUSIONS**

Assessment of endothelial function with invasive and non-invasive techniques in the peripheral

circulation is believed to be a window to the coronary arteries. Ultrasound assessment of brachial artery FMD provides important information about vascular function in health and disease.

Endothelial dysfunction in the brachial artery will identify patients at risk for developing coronary artery disease, cerebral or peripheral vascular disease. By screening it with a non-invasive technique, such as the evaluation of endothelial function, is important for the study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention.

## REFERENCES

1. Moncada S, Higgs A. The L-arginine-NO pathway. *N Engl J Med* 1993;329:2002-12.
2. Widlansky ME, Gokce N, Keaney JF, et al. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 2003;42:1149-60.
3. Landmesser U, Hornig B, Drexler H. Endothelial function: a critical determinant in atherosclerosis? *Circulation* 2004;109(Suppl.1):27-33.
4. Tousoulis D, Antoniadis C, Stefanadis C. Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart* 2005;91:553-8.
5. Anderson EA, Mark AL. Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation* 1989;79:93-100.
6. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *J Am Coll Cardiol* 2002;39:257-65.
7. Tousoulis D, Davies G, Lefroy DC, et al. Variable coronary vasomotor responses to acetylcholine in patients with normal coronary arteriograms: evidence for localised endothelial dysfunction. *Heart* 1996;75:261-6.
8. Tousoulis D, Tentolouris C, Crake T, et al. Basal and flow-mediated nitric oxide production by atheromatous coronary arteries. *J Am Coll Cardiol* 1997;29:1256-62.
9. Anderson AJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995;26:1235-41.
10. Lind L, Hall J, Johansson K. Evaluation of four different methods to measure endothelium-dependent vasodilation in the human peripheral circulation. *Clin Sci* 2002;102:561-7.
11. Bots ML, Westerink F, Rabelink S, et al. 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;26:363-6.
12. Landmesser U, Drexler H. The clinical significance of endothelial dysfunction. *Curr Op Cardiol* 2005;20:547-51.
13. Agewall S, Doughty RN, Bagg W, et al. Comparison of ultrasound assessment of flow-mediated dilatation in flow-mediated vasodilatation: effects of measurement on response the radial and brachial artery with upper and forearm cuff positions. *Clin Physiol* 2001;21:9-14.
14. Berry KL, Skyrme-Jones RA, Meredith IT. Occlusion cuff position is an important determinant of the time course and magnitude of human brachial artery flow-mediated dilation. *Clin Sci* 2000;99:26-7.
15. Doshi SN, Naka KK, Payne N, et al. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci* 2001;101:629-635.
16. Mannion TC, Vita JA, Keaney JF, et al. Non-invasive assessment of brachial artery endothelial vasomotor function: the effect of cuff position on level of discomfort and vasomotor responses. *Vasc Med* 1998;3:263-7.
17. Vogel RA, Corretti MC, Plotnick GD. A comparison of brachial artery flow-mediated vasodilation using upper and lower arm arterial occlusion in subjects with and without coronary risk factors. *Clin Cardiol* 2000;23:571-5.
18. Corretti MC, Plotnick GD, Vogel RA. Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound. *Am J Physiol* 1995;268:1397-404.
19. Tagawa T, Imaizumi T, Endo T, et al. Role of nitric oxide in reactive hyperemia in human forearm vessels. *Circulation* 1994;90:2285-90.
20. Hashimoto M, Miyamoto Y, Matsuda Y et al, New methods to evaluate endothelial function: Non-invasive method of evaluating endothelial function in humans. *J Pharmacol Sci* 2003;93:405-8.
21. Hashimoto M, Eto M, Akishita M, et al. Correlation between flow-mediated vasodilatation of the brachial artery and intima-media thickness in the carotid artery in men. *Arterioscler Thromb Vasc Biol* 1999;19:2795-800.