

RESEARCH ARTICLE

Assessment of Post-Occlusive Reactive Hyperaemia in the Evaluation of Endothelial Function in Patients with Lower Extremity Artery Disease

Cosmin Carasca^{1*}, Annamaria Magdas², Ioan Tilea³, Alexandru Incze²

¹ University of Medicine and Pharmacy of Tirgu Mures, Romania

² Department of Internal Medicine IV, University of Medicine and Pharmacy of Tirgu Mures, Romania

³ Cardiovascular Rehabilitation Clinic, Department of Family Medicine, Institute of Cardiovascular Diseases and Transplantation Tirgu-Mures, Romania, University of Medicine and Pharmacy of Tirgu Mures, Romania

Background: The aim was to assess endothelial function with photoplethysmography (PPG), by post-occlusive reactive hyperaemia (PORH) combined with alprostadil challenge test in patients with peripheral artery disease (PAD). **Methods:** Forty-nine PAD patients stage II-III Fontaine (39 male, 10 female, mean age 68.45±5.86 years) and a control group of 49 healthy individuals (24 male, 25 female; mean age 25.1±3.8 for a young subgroup; 71.0±0.16 years for an elderly subgroup) were included. Ankle-brachial index (ABI) was assessed at baseline, peripheral perfusion (PP) and PORH were assessed at baseline and after the 30 minutes administration of parenteral alprostadil. **Results:** After 3 minutes of arterial occlusion, peripheral perfusion increased from 0.69±0.94 mV/V to 2.27±2.42 mV/V ($p<0.0001$). After alprostadil challenge, peripheral perfusion increased from 0.84±1.24 mV/V to 4.52±3.52 mV/V ($p<0.0001$). In controls PP was 2.4±1.7 mV/V versus 3.8±1.5 mV/V, $p<0.0001$. **Conclusion:** In patients with PAD, an increase in PORH after alprostadil challenge due to the release of nitric oxide (NO), provides information on the endothelial function and could reflect the presence of collaterals. In the healthy control group, the increase in PORH could reflect the integrity of main arterial branch. In PAD patients with an increase in PORH, conservative therapy should be preferred over surgical revascularisation.

Keywords: peripheral perfusion, post-occlusive reactive hyperaemia, alprostadil

Received 31 March 2017 / Accepted 22 June 2017

Background

The prevalence of peripheral arterial disease (PAD) of the lower limb is rising and it is still underdiagnosed [1]. Despite a decrease in ankle-brachial index (ABI), only one-half of the population with occlusive atherosclerotic vascular disease is symptomatic [2]. This compensatory mechanism might be present in case of progressive arterial occlusion, accompanied by the development of collateral blood vessels that by-pass the stenotic lesions [3]. The growth of these small arteries—called arteriogenesis is triggered by the shear stress due to arterial occlusion [4]. So far, to assess collateral circulation, several high-cost, invasive diagnostic methods have been proposed, but their use at the primary care level is still limited. Therefore, there are several studies searching for a reliable, non-invasive method to assess the presence of collateral microcirculation [5, 6, 7, 8]. In this field, the non-invasive vascular reactivity test, called post-occlusive reactive hyperaemia (PORH) gained attention. This procedure is based on the ability of the endothelium to release vasodilator agents like nitric oxide (NO) as a response to proximal arterial occlusion [9]. After occlusion, when the peripheral perfusion (PP) is restored, flow is increasing because of the reduced vascular resistance. An inexpensive method, the photoplethysmography (PPG) has been proposed to determine these blood volume changes [10]. This optical technique allows detecting blood volume

changes during a cardiac cycle, which is recorded by a sensor consisting of a light source and a photo detector, and is represented as perfusion units in mV [7].

According to the International guidelines on management of patients with PAD, administration of a prostaglandin E1 (PGE1) for 7 to 28 days, facilitate ulcer healing in patients with critical limb ischemia (CLI) [11]. Therefore the aim of the study was to assess endothelial function in patients with PAD using PPG, and to evaluate whether post-occlusive reactive hyperaemia combined with alprostadil-challenge test is a suitable method to identify patients who are candidates for conservative therapy.

Methods

A number of 98 subjects were included in our study: 49 subjects (39 male, 10 female) diagnosed with lower extremity artery disease stage II-III Fontaine, and a healthy control group of 49 subjects (24 male, 25 female); the control group was divided in 2 subgroups of young and elderly individuals. In the PAD group the mean age was 68.45±5.86 years. In the young control group, the mean age was 25.1±3.8 years while in the elderly group 71.0±0.16 years. All of them gave written informed consent and the study was approved by the Ethics Committee of the County Clinical Hospital, Tirgu Mures, Romania. Inclusion criteria were: diagnosis of PAD - according to the current

* Correspondence to: Cosmin Carasca
E-mail: cosmincarasca@yahoo.com

guidelines of the European Society of Cardiology (ESC): ABI<0.9, multiple arterial stenotic lesions or total iliofemoral or femoral-popliteal arterial occlusions confirmed on duplex ultrasound. Patients with connective tissue diseases, lymphoedema, and venous thrombosis were excluded from the study. Intermittent claudication was evaluated according to the Edinburgh Claudication Questionnaire [12]. Ankle-brachial index and PORH test were performed at the inclusion. Smoking and caffeine cessation was required for at least two hours before testing. Patients were placed in supine position for at least ten minutes prior to measurements. During examination, room temperature was maintained at 23-24°C. Ankle-brachial index was determined with a 5-10 MHz handheld Doppler device (BiDop ES-100V3 Hadeco®). The PPG measurements were performed with Bidop ES-100V3® hand-held Doppler and transmitted to the Smart-V-Link® software. The sensor was fixed with an adhesive pad on the plantar surface of the affected leg's hallux. A pneumatic cuff was placed proximal to the pad. After baseline peripheral perfusion (PP_b) recording, blood flow was interrupted for a period of three minutes by inflation of the cuff with 20 mmHg above systolic pressure. After cuff deflation at maximum speed, post-occlusive flow was measured for further 4 minutes (1 measurement /minute). Post-occlusive reactive hyperaemia was denoted as the maximum value of the four measurements performed at one minute intervals (PORH_b). After obtaining the PORH_b value, intravenous alprostadil (10µg/kg/min) was administrated for 30 minutes. Peripheral perfusion and post-occlusive reactive hyperemia were again determined after alprostadil challenge test (PP_c, PORH_c). Patients with a lack of increase of peripheral perfusion by at least 50% after administration of alprostadil, were categorized as non-responders (NR) and were referred for surgical revascularization. In the control groups, only baseline measurements were performed (ABI, PP_b, PORH_b), alprostadil challenge test was not performed.

Patients' characteristics were collected as raw data. Numerical data were represented as mean±SD. Means were compared using t-test for continuous variables. Correlations were studied using Pearson's correlation test for data

presenting Gaussian distribution. Statistical analysis was performed with Graph Pad Instat program. Statistical significance was set at $p \leq 0.05$ with confidence interval of 95%.

Results

Among responders ABI was 0.4 ± 0.2 , and in non-responders 0.30 ± 0.22 . In young controls, ABI was 1.27 ± 0.5 , and in elderly controls 0.99 ± 0.16 .

In responders, the peripheral perfusion increased by 1.58 mV/V (from 0,69 mv/V to 2,27 mV/V) after occlusion (Figure 1) and after vasodilator challenge, peripheral perfusion increased by 3.68 mV/V (from 0,84 mV/V to 4,52 mV/V) (Figure 2). In responders, we found no correlation between the ABI and PORH after alprostadil challenge, $p=0.83$ (Figure 3).

In the non-responders group, PP_b versus PORH_b was: 0.6 ± 0.85 mV/V versus 0.67 ± 0.78 ($p=0.09$) and after alprostadil challenge PP_c versus PORH_c was: $0,68 \pm 1.18$ mV/V versus 0.74 ± 1.13 mV/V ($p=0.08$) (Figure 4).

In the young controls group, PP_b versus PORH_b was 3.7 ± 2.25 mV/V versus 5.3 ± 2.8 mV/V ($p < 0.0001$), while in older controls 2.4 ± 1.7 mV/V versus 3.8 ± 1.5 mV/V, $p < 0.0001$.

Discussion

In our study we evaluated endothelial function as a vasodilator response to arterial occlusion with the non-invasive finger PPG in patients with lower extremity artery disease and critical ankle-brachial index. We found that in patients with PAD, despite the low ABI, post-occlusive peripheral perfusion increased significantly and this vasodilator effect was more pronounced after alprostadil challenge. In the young control group, as well as in elderly patients without PAD, despite the preserved values of ABI, we also observed an increase in peripheral perfusion after arterial occlusion. In our experience, these findings could be explained by the fact that in PAD patients the increase in peripheral perfusion after alprostadil could reflect the endothelial function of the collateral circulation that by-pass the stenosis. In patients with critical ABI, proximal arterial obstruction and the increased shear stress that occurs, trigger a series of

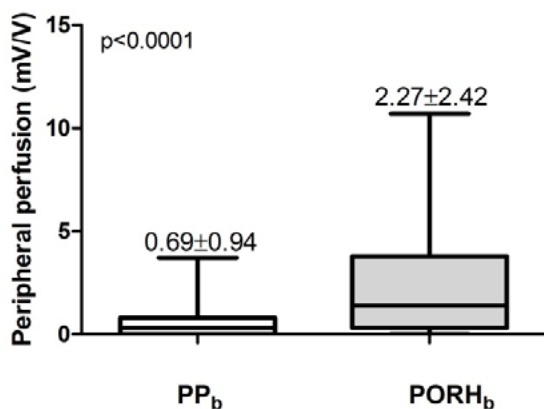


Fig. 1. Paired t-test for evaluating difference in baseline peripheral perfusion and after reactive hyperaemia in responders

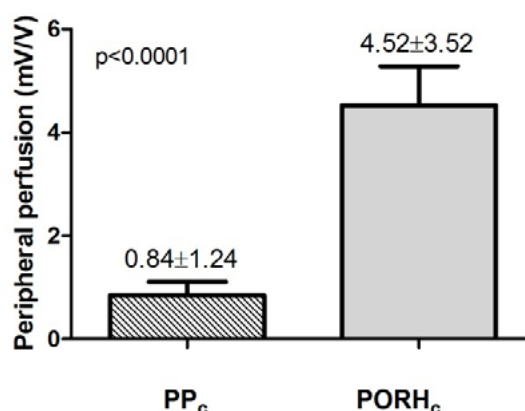


Fig. 2. Difference in peripheral perfusion and PORH after alprostadil challenge test in responders

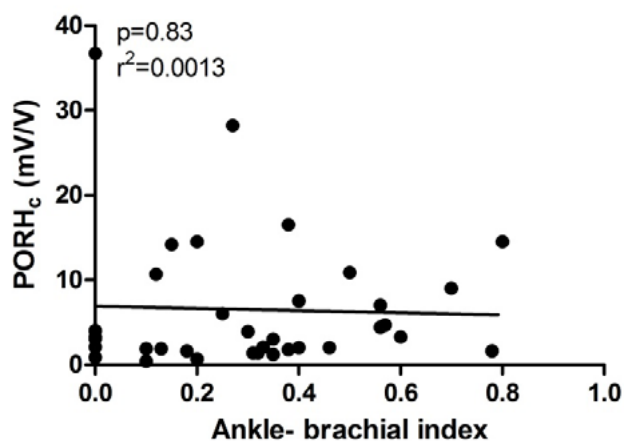


Fig. 3. Pearson's correlation between PORH after alprostadil challenge and ankle-brachial index

inflammatory reaction that results in homing of stem cells and inflammatory cells to the “injured” tissue and the native collaterals are increasing in diameter, ensuring blood supply to the distal zones [13]. This could be the substrate for the observations that this group of patients did not develop gangrene. In our experience in non-responders with critical ABI (<0.5) and the lack of increase in PORH by at least 50% after alprostadil challenge, reflects altered endothelial function of the main arterial branch and maybe the absence of collateral vessels. Thus, for limb salvage this population should benefit of invasive high-cost investigations and surgical revascularisation. The assessment of endothelial function involves expensive equipment with a great financial burden on the healthcare system and unavailable for the primary care system. Therefore, in patients with PAD, in order to triage patients for conservative or revascularization therapy, low-cost, operator-independent screening techniques are required. Post-occlusive reactive hyperaemia assessed by finger PPG showed significant increase in peripheral perfusion due to nitric oxide release after occlusion, which could be due to the presence of collateral microcirculation that by-pass stenotic areas. This effect was potentiated after alprostadil challenge. We found no correlation between ABI and post-occlusive reactive hyperaemia, because while ankle-brachial index is a screening method to identify large vessel disease, the assessment of post-occlusive reactive hyperemia reflects the function of the endothelium and maybe the presence of small collateral circulation. Our experience is that in PAD patients with an increase in PORH after alprostadil challenge, the way towards limb salvage consists in lifestyle modification, smoking cessation, exercise therapy, as well as pharmacotherapy including cilostazol, pentoxifyllin, antiplatelet agents, lipid-lowering agents as well as parenteral administration of PGE1 for 7 to 28 days [14].

Finger PPG is a low-cost, operator-independent optical technique that enables us to detect blood volume changes in response to post-occlusive hyperemia. Although it seems to be a promising method that helps physicians in therapeutic decision making at the primary care level, there is

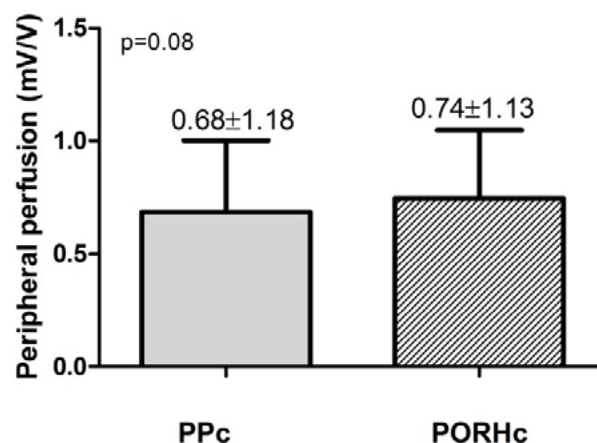


Fig. 4. Paired t-test to evaluate the difference in PP and PORH after alprostadil challenge in non-responders

no consensus available for this technique regarding occlusion time as well as a normal range for PORH.

Conclusions

The fact that in PAD patients with low ankle-brachial index, associated with stenotic lesions, as well as in the healthy control group, PORH increased significantly, allows us to conclude that the post-occlusive reactive hyperemia test reflects the endothelial function. In our opinion, an increase in PORH after alprostadil challenge in PAD patients despite a low ABI could reflect the endothelial function of the collateral vessels, while in healthy subjects the increase in PORH could reflect the endothelial function of the main arterial branch. Although, in PAD patients a comprehensive investigation has to be done, for a screening method at the primary care level, PORH test combined with alprostadil challenge test, could offer an inexpensive screening method in order to help physicians in therapeutic decision making. In our experience, in patients with lower extremity artery disease, and an increase of PORH after vasodilator challenge by at least 50%, conservative therapy with alprostadil, cilostazol, lipid-lowering agents, antiplatelet agents as well as lifestyle modification are able to delay surgical revascularisation. The long-term follow-up of these patients is of major importance, and if conservative therapy fails, surgical approach should be performed.

Conflict of interest

Authors declare no conflict of interest.

References

1. Criqui MH, Aboyans V. Epidemiology of peripheral artery disease. *Circ Res* 2015; 116(9):1509-1526
2. Aronow WS. Peripheral arterial disease in the elderly. *Clin Interv Aging* 2007; 2(4):645-654
3. Hernando FJS, Conejero AM. Peripheral artery disease: pathophysiology, diagnosis and treatment. *Rev Esp Cardiol* 2007; 60(9):969-982
4. Cai W, Schaper W. Mechanism of arteriogenesis. *Acta Biochim Biophys Sin* 2008; 40(8):681-692
5. Fedorovich AA. Non-invasive evaluation of vasomotor and metabolic functions of microvascular endothelium in human skin. *Microvasc Res*

- 2012; 84(1):86-93
6. Hosseini ZS, Zahedi E, Attar HM, Fakhryadeh H, Parsafar MH. Discrimination between different degrees of coronary artery disease using time-domain features of the finger photoplethysmogram in response to reactive hyperemia. *Biomed Signal Proc Contr* 2015;18:282-292
 7. Barwick A, Lanting S, Chuter V. Intra-tester and inter-tester reliability of postocclusive reactive hyperemia measurement at the hallux. *Microvasc Res* 2015; 99:67-71
 8. Kuznetsova T, Szczesny G, Thijs L, Jozeau D, D'hooge J, Staessen JA. Assessment of peripheral vascular function with photoplethysmographic pulse amplitude. *Artery Res* 2011; 5(2):58-64
 9. De Mul FF, Morales F, Smit AJ, Graaff R. A model for post-occlusive reactive hyperemia as measured with laser-Doppler perfusion monitoring. *IEEE Trans Biomed Eng* 2005; 52:184-190
 10. Selvaraj N, Jaryal AK, Santhosh J, Anand S, Deepak KK. Monitoring of reactive hyperemia using photoplethysmographic pulse amplitude and transient time. *J Clin Monit Comput* 2009; 23:315-322
 11. Anderson JL, Halperin JL, Albert N et al. Management of Patients With Peripheral Artery Disease (Compilation of 2005 and 2011 ACCF/AHA Guideline Recommendations) A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *JACC* 2013; 61(4):1555-7150
 12. Leng GC, Fowkes FG. The Edinburgh Claudication Questionnaire: an improved version of the WHO/Rose Questionnaire for use in epidemiological surveys. *J Clin Epidemiol* 1992; 45:1101-1109
 13. Heil M, Schaper W. Influence of mechanical, cellular, and molecular factors on collateral artery growth (arteriogenesis). *Circ Res* 2004; 95:449-458
 14. Tendera M, Aboyans V, Bartelink ML, et al. The Task Force on the diagnosis and treatment of peripheral artery diseases of the European Society of Cardiology (ESC). *Eur Heart J* 2011; 32:2851-2906