ORIGINAL ARTICLE

Endothelial function assessment in atherosclerosis

Comparison of brachial artery flow-mediated vasodilation and peripheral arterial tonometry

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KEY WORDS

atherosclerosis, endothelial function, flow-mediated dilation, nitric oxide, peripheral arterial tonometry

ABSTRACT

INTRODUCTION Endothelial dysfunction, characterized by the loss of nitric oxide bioavailability, is a key element in the pathogenesis of atherosclerosis and an important prognostic factor in cardiovascular diseases. Therefore, the development of reliable, safe, and noninvasive methods of endothelial function assessment is important for their use in cardiovascular risk stratification. Brachial artery flow-mediated dilation (FMD) is widely used in research but technical difficulties and problems with calibration between laboratories limit its clinical use. Reactive hyperemia–peripheral artery tonometry (RH-PAT, EndoPAT) has been developed as a simpler, cheaper, and potentially more reproducible method.

OBJECTIVES We aimed to investigate associations between RH-PAT and FMD in relation to atherosclerotic risk factor profile.

PATIENTS AND METHODS The study involved 80 subjects (52 men, 28 women) aged 43.6 ±14.8 years, with moderate-to-low cardiovascular risk (mean SCORE, 2.2% ±2%), in whom FMD, RH-PAT, and intima–media thickness (IMT) were determined.

RESULTS The reactive hyperemia index (RHI) measured by RH-PAT correlated with FMD ($r = 0.35$, $P < 0.01$). However, no significant correlation was observed between RHI and IMT, SCORE, or the number of classical atherosclerotic risk factors (hypertension, smoking, diabetes, hypercholesterolemia), while FMD was significantly correlated with IMT ($r = –0.53$, $P < 0.001$), risk factors ($r = –0.55$, $P < 0.05$), and SCORE ($r = –0.4$, $P < 0.05$).

CONCLUSIONS Despite its technical requirements, FMD is a more sensitive method than RH-PAT in evaluating the effect of classical atherosclerotic risk factors on vascular endothelial function. Microvasculature response during RH-PAT needs to be further studied, including the assessment of nonendothelial factors that may affect the measurements, before RH-PAT becomes the universal tool for the evaluation of the endothelial cells.

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INTRODUCTION Endothelial dysfunction, characterized by the loss of nitric oxide bioavailability, is the key element in the pathogenesis of atherosclerosis. The significance of endothelial dysfunction has been demonstrated by numerous animal model studies[1] and has been confirmed in clinical studies showing that it precedes clinical and morphological atherosclerotic changes.[2] Numerous studies have described associations between endothelial dysfunction and risk factors for cardiovascular diseases.[3-5] Impaired endothelial function is a feature of aging and is present in patients with high levels of low-density lipoproteins, arterial hypertension, diabetes, acute coronary syndromes, autoimmune diseases, and in smokers.[3-6] Moreover, the number of atherosclerotic risk factors has been linked to the degree of endothelial dysfunction.[7,8]

Considering the above data, endothelial dysfunction is an important prognostic factor in cardiovascular diseases. However, the determination of endothelial function has not yet been routinely included in risk assessment recommended by major cardiovascular guidelines. This has been mostly associated with the difficulties in developing fully reproducible noninvasive methods for the endothelial function assessment. Such techniques are based on the fact that nitric oxide leads to vasodilation, which can be quantified becoming a measure of assessing endothelial function or its disturbances.

Quantitative intravascular ultrasound coronary angiography following intracoronary acetylcholine infusion is an example of an invasive method. It is accurate and sensitive but, at the same time, invasive, expensive, and bears substantial risk of complications, limiting its wider clinical application (imperative for the use in risk stratification). Flow-mediated dilation (FMD) measurement is an example of noninvasive approach, which uses ultrasound measurement of an increase in the brachial artery diastolic diameter in response to blood flow. The measurement requires high-frequency linear transducers, good ultrasound system, and an experienced operator. Therefore, new noninvasive techniques have been developed in an attempt to make endothelial dysfunction assessment more accessible in general medical practice. The reactive hyperemia–peripheral artery tonometry (RH-PAT) is a much simpler and cheaper method and can potentially provide better interobserver reproducibility. A number of questions regarding the mechanism of the response or the role of ischemic metabolites in vasodilation have been raised, challenging the value of RH-PAT. Accordingly, the aim of the present study was to investigate the associations between RH-PAT and FMD as well as between these measures of endothelial function and atherosclerotic risk factors and severity of atherosclerosis.

PATIENTS AND METHODS

Subjects and protocols

The study was conducted in 80 subjects (52 men, 28 women) aged 43.6 ±14.8 years, in whom atherosclerotic risk was assessed. In each patient, detailed, questionnaire-based medical history was taken, with emphasis on atherosclerotic risk factors (hypertension, hypercholesterolemia, obesity, diabetes, and smoking), and a physical examination was performed, including 3 measurements of resting blood pressure as well as anthropometric assessment with the calculation of the body mass index (BMI). Coronary artery disease was defined by clinical history of acute coronary events or positive result of coronary angiography. None of the patients suffered from myocardial infarction. Clinical risk factors were defined as follows: hypercholesterolemia (total plasma cholesterol level >5 mmol/l); diabetes (fasting glucose level ≥7 mmol/l, hemoglobin A_{1c} >6.5%, or current treatment with insulin or oral hypoglycemic agents), hypertension (blood pressure ≥140/90 mmHg or current treatment with antihypertensive agents), and smoking (current or within last 6 months).[9] The exclusion criteria included active cancer, acute and chronic infections (past 3 months), necessity for continued use of vasoactive drugs known to interfere with endothelial function measurements, life expectancy of <3 years, pulmonary hypertension, use of steroids or immunosuppressive agents, renal insufficiency, history of acute coronary syndromes, pregnancy or breastfeeding, and history of alcohol abuse. Ultrasound assessment of atherosclerosis based on the measurement of the carotid artery intima–media thickness (IMT) was performed. Endothelial function was assessed using brachial artery FMD and RH-PAT measurement. Nitroglycerine-mediated dilation (NMD) assessment was used as control for FMD. FMD and NMD were studied at least 30 min after RH-PAT to allow for the recovery between hyperemia stimuli. In preliminary experiments, investigations were also performed on separate days but this was identified as not necessary. Both measurements were performed in a dimmed, temperature-controlled room. Participants were recommended to fast and refrain from smoking for at least 8 h and to refrain from coffee and caffeine/vitamin C-containing drinks, alcohol, fatty foods, or exercise for at least 10 h prior to the study. Moreover, before the study, vasodilating drugs known to affect endothelial function measurements were discontinued for at least 12 h. Peripheral blood from the antecubital vein was taken and fasting blood glucose and lipid profile were measured. The study was approved by the Local Ethics Committee and written informed consent was obtained from all participants.

Brachial artery ultrasound flow-mediated dilation

Endothelial function was assessed by FMD, which is based on the measurement of the brachial artery diameter before and after acute 5-minute-long brachial artery occlusion (using the sphygmomanometer cuff) as described before, using techniques consistent with the guidelines.[10] Measurements were performed with the Toshiba Xario Diagnostic Ultrasound System (Toshiba, United States) and a 8MHz linear transducer. All tests were performed by 2 experienced operators (GW and GO). Before the examination, patients were maintained in a supine position for 15 minutes in a calm and darkened room. Assessments were performed on the right brachial artery, after arm immobilization. Blood pressure cuff was placed around the right arm. The measurement site was identified and marked 3 to 8 cm proximally from the right antecubital fossa and a vascular transducer was fixed at the site. Depth and gain settings were optimized to identify the lumen and vessel wall interface. The measurements were recorded before cuff inflation to at least 200 mmHg (50 mmHg above the systolic blood pressure level, to achieve brachial artery occlusion, for 5 min) and then subsequently 1, 2, and 5 min after cuff deflation. The measurements during diastole were recorded and maximum dilation was usually detected within approximately 60 to 120 s of reactive hyperemia.

NMD was measured to study non-endothelium-dependent vasodilations. Similarly to the measurements of FMD, a vessel diameter was assessed before the use of nitroglycerine (Nitromint, aerosol, 400 µg *s.l.* EGIS Pharma) and 1, 2, and 5 min after sublingual nitroglycerin application.

Studies were recorded on CDs and images were transferred to a computer for measurement aided by the edge detection software, Image Pro Plus, or with Vascular Research Tools 5 (Medical Imaging Applications, LLC, United States). Calculations of the vessel diameter, measured as a distance between the two M-lines, the one close to the transducer and the one distant from the transducer, were performed according to the original Celermajer's methodology.[10] Maximum FMD ($FMD_{max\%}$) and NMD ($NMD_{max\%}$) were defined as: $FMD_{max\%}$ = 100 × (maximum vascular diameter after reactive hyperemia – basal vascular diameter) / basal vascular diameter, while $NMD_{max\%}$ = 100 × (maximum vascular diameter after nitrogycerin – basal vascular diameter) / basal vascular diameter.[11] In a subset of patients (n = 10), the data were acquired by 2 independent observers and the Bland–Altman analysis was performed using GraphPad Prism. The mean difference between the measurements (bias) was 0.02 for interobserver variability for testing with the limits of agreement between 1.8 and 1.77. For interobserver variability for analysis these value was 0.03 with the limits of agreement between 0.68 and 0.62. Corresponding correlation coefficients were r = 0.95 and r = 0.99, and coefficients of repeatability were 1.8 for testing and 0.8 for analysis.

Reactive hyperemia–peripheral artery tonometry The RH-PAT measurement was performed using Endo-PAT 2000 (Itamar Medical Ltd, Caesarea, Israel) in a quiet, dimmed, and temperature-controlled room following the manufacturer's recommendations. The measurements were automatically analyzed to obtain the reactive hyperemia index (RHI) using the EndoPAT™ software, version 3.1.2 (Itamar Medical, Israel). Before the examination, patients remained in a supine position for 15 min. First, PAT sensors were placed on the index fingers of the right and left hands. The right arm was tested, while the left provided information about the whole body homeostasis and served as control. Each measurement consisted of 5 min of basal recording, 5 min of right brachial artery occlusion, and 5 min of reactive hyperemia measurement. Brachial artery occlusion was performed using the sphygmomanometer cuff, which was inflated to at least 60 mmHg over a patient's systolic blood pressure level (minimum, 200 mmHg; maximum, about 300 mmHg). All studies were done strictly according to the manufacturer's instructions and in accordance with the current literature.[12-15]

Carotid artery intima–media thickness The IMT measurement was performed using the same device and by the same investigator. The measurements were taken using the standard method. Briefly, assessments were performed at 12 different points (2 cm below the common carotid arteries bulbs, about every 1 cm, omitting visible coronary plaques), on the right and left common carotid arteries, measuring the distance between the border between the artery lumen and carotid artery intima and second bright M-line (the border between the media and adventitia). The obtained images were digitally recorded, transferred to the computer, and analyzed using the Image Pro Plus software. On the basis of the 12 measurements, the mean and maximum IMT were calculated (IMT_{mean} and IMT_{max}, respectively). Normal IMT was defined as the IMT of less than 0.9 mm.[16]

Statistical analysis The results were shown using descriptive statistical methods, including ranges, means, standard deviations, and percentage distributions. Statistical analyses were performed using Statistica 10.0 PL, (StatSoft, Poland). The Box–Cox transformation was applied to achieve normal distribution of FMD, NMD, IMT, and RHI results. The comparison of normally distributed variables in the groups with and without atherosclerotic risk factors was performed using the t test, while when normal range was lacking, the Mann–Whitney U test was used. The analysis of associations between FMD, NMD, IMT, and RHI and atherosclerotic risk factors was performed using the Spearman's correlation coefficient calculation. For determining interobserver variability of the FMD measurements, Bland–Altman plots were constructed and the coefficient of repeatability was calculated (1.96 times the standard deviation of the difference between the measurements by independent observers). The limits of agreement were defined as the mean difference plus and minus 1.96 times the standard deviation of the differences. The results were statistically significant at a P value of less than 0.05. The power analysis indicated that to detect a simple correlation r (r = 0.4) using a two-sided test, 5% significance level test (α = 0.05) with 80% power (β = 0.2), the required minimal sample size was approximately 47.

RESULTS

Patient characteristics The study group included subjects at the age of 19 to 68 years (52 men and 28 women; mean age, 43.6 ±14.8 years). The most prevalent atherosclerotic risk factors were hypercholesterolemia (n = 53; 66.2%), overweight or obesity (BMI ≥25.0 kg/m^2; n = 51; 63.7%), and hypertension (treated and untreated; n = 41; 51.2%). Six patients suffered from diabetes. The mean SCORE was 2.2% ±2%. Treatments used in these patients

TABLE 1 Clinical characteristics of the subjects (n = 80)

Characteristics		Values
age, y		43.6 ±14.8
sex, male/female, n (%)		52/28 (65/35)
BMI, kg/m^2		28.4 ±6.1
overweight/obesity (BMI ≥25.0 kg/m^2), n (%)		51 (63.7)
coronary artery disease, n (%)		11 (13.8)
CRP, mg/l		3.24 ±3.5
TC, mmol/l		5.3 ±1.0
hypercholesterolemia (TC ≥5.0 mmol/l), n (%)		53 (66.2)
arterial hypertension, n (%)		41 (51.2)
SBP, mmHg		129 ±13.4
DBP, mmHg		79.3 ±9.5
smoking, n (%)		16 (20)
type 2 diabetes, n (%)		6 (7.5)
PAD/stroke/TIA		0 (0)
main medications	aspirin	17 (21.3)
	ACEIs/ARBs	40 (50)
	diuretic	35 (43.7)
	β-blocker	33 (41.2)
	statin	33 (41.2)
	oral hypoglycemic	6 (7.5)
	insulin	0 (0)

Data are presented as number (percentage) or as mean ± SD where appropriate.

Abbreviations: ACEI – angiotensin-converting-enzyme inhibitor, ARB – angiotensin receptor blocker, BMI – body mass index, CRP – C-reactive protein, DBP – diastolic blood pressure, PAD – peripheral artery disease, SBP – systolic blood pressure, SD – standard deviation, TC – total cholesterol, TIA – transient ischemic attack

corresponded to a risk factor profile. Clinical characteristics of the patients are summarized in **TABLE 1**. The mean IMT was 0.67 ±0.13 mm.

Endothelial dysfunction Endothelial function was assessed independently using FMD/NMD and RH-PAT. The mean maximum brachial artery FMD ($FMD_{max\%}$) was 9.4% ±4.7% (0.5%–19.7%), while the mean RHI was 1.8 ±0.4 (1.0–3.1). The mean maximum NMD ($NMD_{max\%}$) was 13.8% ±6% (4.07%–39.5%). The values of FMD_{max} varied significantly more than those of RHI. Importantly, becasue the data for FMD, NMD, or RHI were not normally distributed, we performed the Box–Cox transformation to achieve normal data distribution in subsequent statistical analyses (**FIGURE 1ABC**).

Correlations between the measures of endothelial dysfunction Despite the differences in variability of the studied parameters of endothelial dysfunction, we observed a modest but statistically significant correlation between RHI and FMD ($r = 0.35$, $P = 0.001$; **FIGURE 2A**), while no association was found between RHI and NMD ($r = 0.05$, $P = 0.6$; **FIGURE 2B**).

Correlations between the measures of endothelial function and carotid atherosclerosis The IMT values in the study group were not pathological and ranged between 0.4 and 0.9 mm. As expected, there was a significant negative correlation between the IMT_{mean} with maximum FMD ($FMD_{max\%}$; $r = -0.53$; $P = 0.000001$; **FIGURE 3A**), while no association was found with NMD (**FIGURE 3B**). Surprisingly, there was no association between the IMT_{mean} and RHI in the study group ($r = 0.007$; $P = 0.94$; **FIGURE 3C**).

Quantitative measures of vascular dysfunction and atherosclerotic risk factors In further analyses, we compared the noninvasive methods of endothelial function assessment ($FMD_{max\%}$, RHI), IMT in 2 groups of subjects: patients with at least 1 classical risk factor for atherosclerosis (n = 69) and controls without known classical risk factors for atherosclerosis (n = 11). As expected, $FMD_{max\%}$ was significantly decreased and IMT_{mean} was significantly increased in patients with classical atherosclerotic risk factors when compared with those without analyzed risk factors (**FIGURE 4AC**). The NMD_{max} did not differ between the groups (**FIGURE 4B**). There was no difference in the RHI between the groups (**FIGURE 4D**). Subsequently, we analyzed correlations between the number of atherosclerotic risk factors and measures of endothelial dysfunction or carotid atherosclerosis. The study group consisted of 11 subjects without any atherosclerotic risk factors, 19 with 1 risk factor, 12 with 2, 28 with 3, and 10 with 4 risk factors. An increasing number of the risk factors was associated with lower $FMD_{max\%}$ (analysis of variance [ANOVA]: $P = 0.0007$; Spearman's correlation: $Rs = -0.55$, $P < 0.05$; **FIGURE 5A**), but not NMD (**FIGURE 5B**), and increased IMT (ANOVA; $P < 0.05$; $Rs = 0.78$, $P < 0.05$; **FIGURE 5C**). There was no significant association between the RHI and number of major atherosclerotic risk factors (ANOVA: $P = 0.54$; $Rs = -0.06$, $P > 0.5$; **FIGURE 5D**). These relationships were maintained when the analysis was performed in 66 patients having excluded high-risk patients with coronary artery disease or diabetes. In the subgroup with the average SCORE of 1.7 ±2, a significant correlation between the number of risk factors and FMD was observed ($r = -0.5$, $P < 0.05$), while still no relationship was found with the RHI ($r = 0.12$, $P = 0.3$). Similarly, no correlation between the RHI and IMT ($r = 0.06$, $P = 0.3$) was noted in this subgroup. Finally, in the whole group, there was a significant correlation of the risk calculated by SCORE with FMD ($r = -0.4$, $P < 0.05$) but not with RHI.

Correlations between individual atherosclerotic risk factors and FMD and RH-PAT Certain risk factors might be associated with significantly different results using RH-PAT compared with FMD. Therefore, we next analyzed the effect of individual risk factors on FMD and RH-PAT and found that FMD was associated with several risk factors, predominantly hypertension in a multivariate analysis (**TABLE 2**), while there was a borderline association between RH-PAT and hypercholesterolemia (**TABLE 3**).

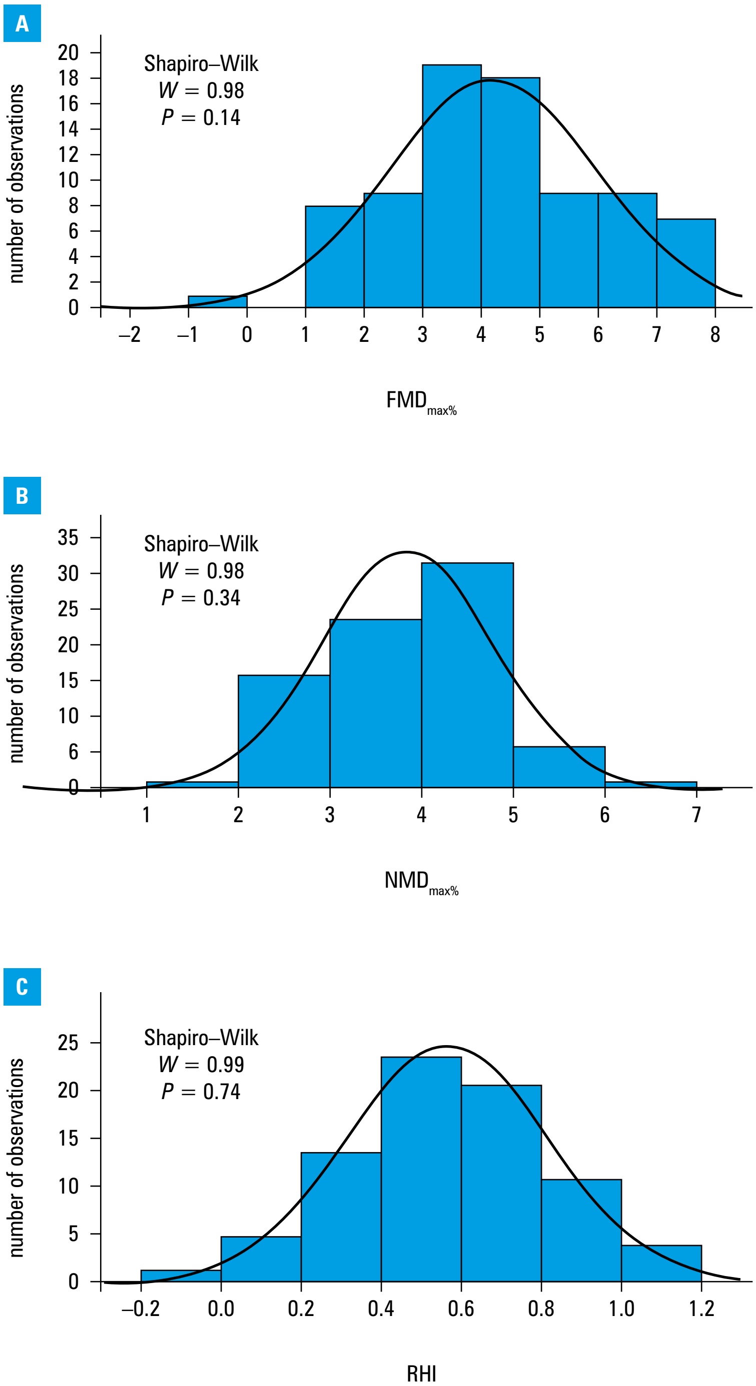

FIGURE 1 Distribution of the measurements of maximum flow-mediated dilation ($FMD_{max\%}$; **A**) or maximum nitroglycerin-mediated dilation ($NMD_{max\%}$; **B**) and reactive hyperemia index (RHI; **C**) in the study group (n = 80) following the Box–Cox transformation

DISCUSSION

Increasing understanding of the significance of endothelial dysfunction in the development of cardiovascular diseases creates an urgent need for the development of a simple, reproducible, and sensitive measure of assessing endothelial function. While brachial artery FMD has served this purpose so far, it is technically challenging, relatively complex, and the results are difficult to compare between clinical laboratories. Therefore, an introduction of a simplified method based on the measurement of reactive hyperemia (RH-PAT; RHI measurement) has been welcomed with enthusiasm, and an increasing number of studies have reported using this technique for endothelial function assessment.[17] The EndoScore scale has been introduced, which categorizes patients into 3 RHI zones reflecting endothelial health. However, at the same time, sensitivity of RH-PAT/RHI for the detection of the effects of numerous conditions on endothelial function has been questioned.[13]

In the present study, we compared classical brachial artery FMD with RHI in the measurement of the effects of the major atherosclerotic risk factors on the endothelial function. Our population was characterized by low carotid IMT and a low-to-moderate SCORE risk. We found that RHI significantly correlated with FMD. However, while FMD significantly correlated with the degree of carotid atherosclerosis measured by IMT or with a risk factor profile, RHI failed to show such correlations. This led us to conclude that while the RHI remains an interesting modality for measuring vascular function, the component of non-nitric oxide-mediated dilation effects may be so strong that it does not allow to detect the relationships with the measures of IMT or with the major atherosclerotic risk factors. Possibly when the differences in the endothelial function are dramatic and substantial, such as the recently reported differences in sex or race, the RHI could be useful.[18] This is also in line with a recent study showing that FMD was weakly associated with the RHI in patients with atherosclerosis, but not among control subjects.[19]

We showed that brachial artery dilation ($FMD_{max\%}$) modestly correlates with the RHI. A similar positive correlation between $FMD_{max\%}$ and RHI was shown by several previous studies.[15,17,20,21] However, other studies did not observe such correlation.[22-24] The discrepancies between the studies have been attributed to population characteristics and methods of FMD measurement. Of note, similarly to our study, reports showing the relationship between RHI and FMD indicated that it was of modest-to-low degree.

Interestingly, when we then compared the relationship between classical major risk factors for atherosclerosis, such as diabetes, hypercholesterolemia, smoking, and hypertension, with FMD or RHI, we did not observe an expected association between a decrease in the RHI and the number of coexisting risk factors, while FMD decreased significantly. These results are in line with those by Bonetti et al.,[17] who reported an association between coronary artery endothelial function (coronary artery response to acetylcholine) and RHI but did not observe an association between RHI and total cholesterol level, blood pressure, diabetes, and smoking. While initial studies demonstrated links to classical cardiovascular risk factors,[21,25,26] a number of recent studies have shown no difference in the RHI despite widely reported and expected endothelial dysfunction in these conditions.[13] These data, in combination with our current report, suggest caution when recommending peripheral arterial tonometry for cardiovascular risk stratification, but do not exclude its usefulness. A low RH signal detected by RH-PAT, has been shown to be associated with a higher

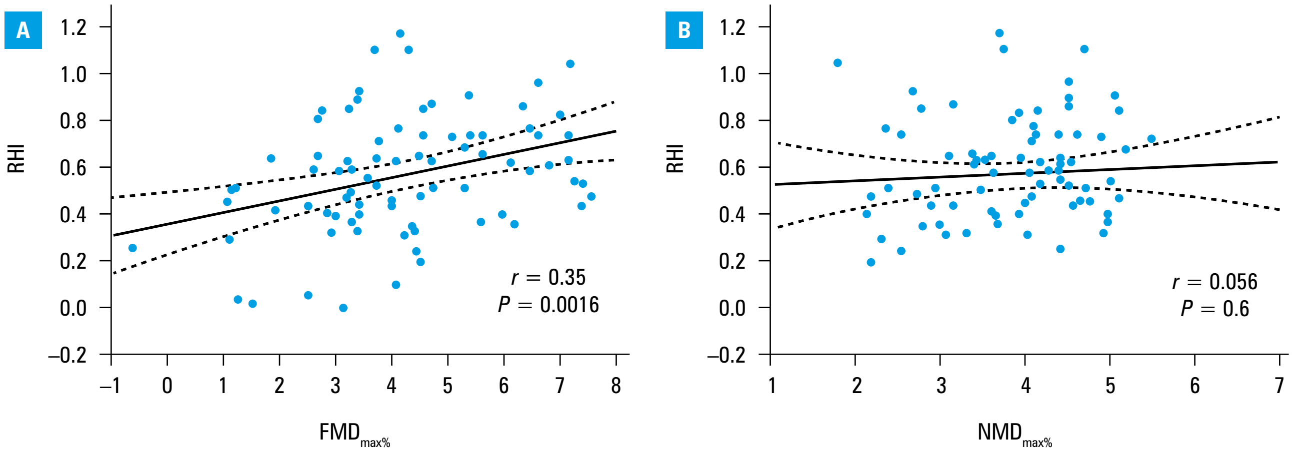

FIGURE 2 Correlations between reactive hyperemia index (RHI) and maximum flow-mediated dilation ($FMD_{max\%}$; **A**) or between RHI and nitroglycerin-mediated dilation ($NMD_{max\%}$; **B**); data are presented after the Box–Cox transformation; Spearman's correlation analysis is presented on the graphs

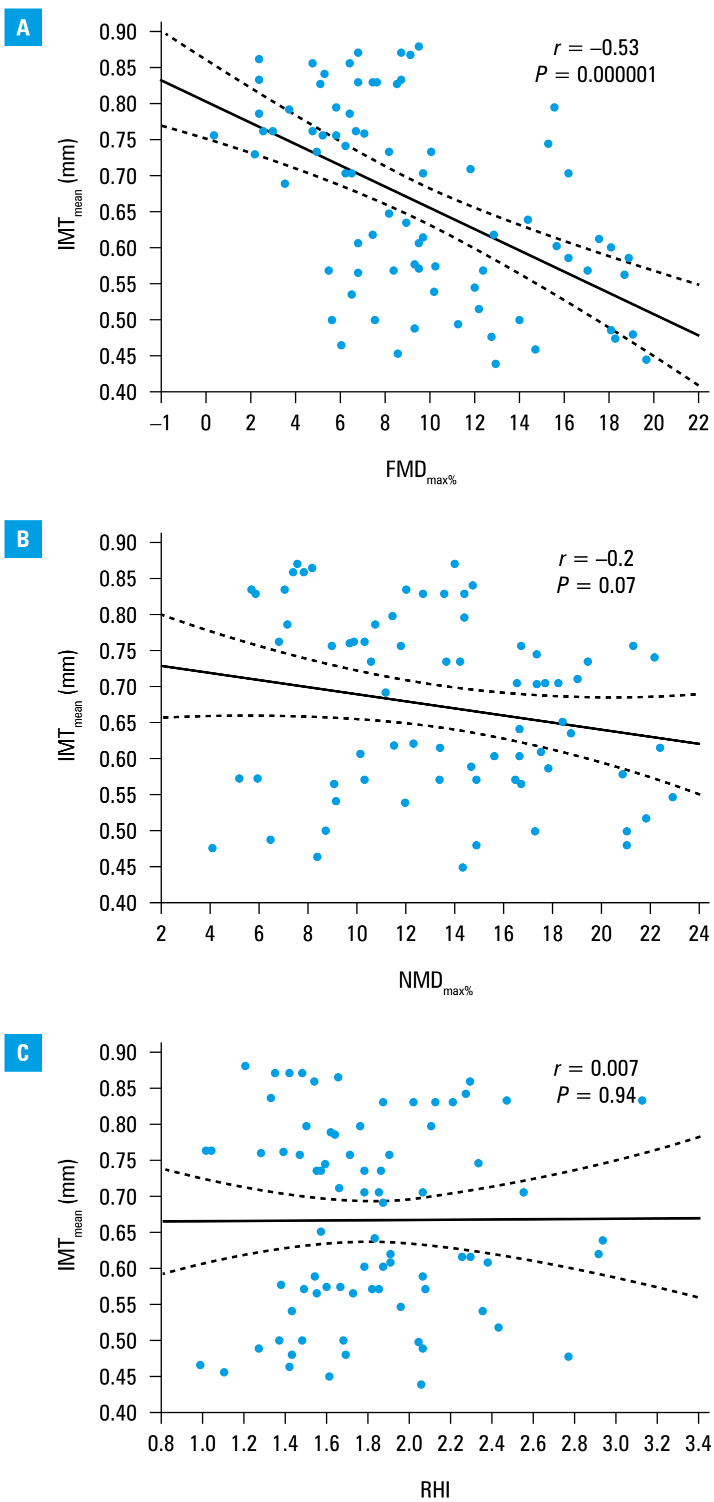

FIGURE 3 Correlations between the mean intima-media thickness (IMT_{mean}) and maximum flow-mediated dilation ($FMD_{max\%}$; **A**), nitroglycerin-mediated dilation ($NMD_{max\%}$; **B**) or reactive hyperemia index (RHI; **C**); data represent the actual values of IMT, FMD, or RHI for better visualization of the correlations; correlation values represent the Spearman's correlation coefficient

rate of cardiac events in a 7-year follow-up of over 200 subjects.[27] However, more and more data indicate that this may be the case only with respect to carefully selected clinical subpopulations such as patients with metabolic syndrome or initially high cardiovascular risk.

One of the most comprehensive reports on RHI has been provided by Hamburg et al.[23] who included over 4000 subjects. They found that while FMD represented endothelial dysfunction related to aging, hypertension, and obesity, the abnormal PAT ratio has been associated with lower systolic blood pressure, increasing total/high-density lipoprotein cholesterol ratio, diabetes, and smoking. Interestingly, the PAT ratio was not associated with FMD.[23] Thus, the brachial and digital measures of vascular function provide distinct information and relate to different clinical conditions.[23] This makes the RHI complementary to FMD but unlikely to substitute it.

There are also several other modalities for the assessment of vascular function, such as the cold pressor test or ultrasonographic detection of vasomotor responses of the left anterior descending coronary artery, which can be used as complementary methods, although RHI will remain a valuable and simple alternative.[9]

Obviously, there may be numerous methodological reasons for the differences in endothelial function assessment utilizing both methods.

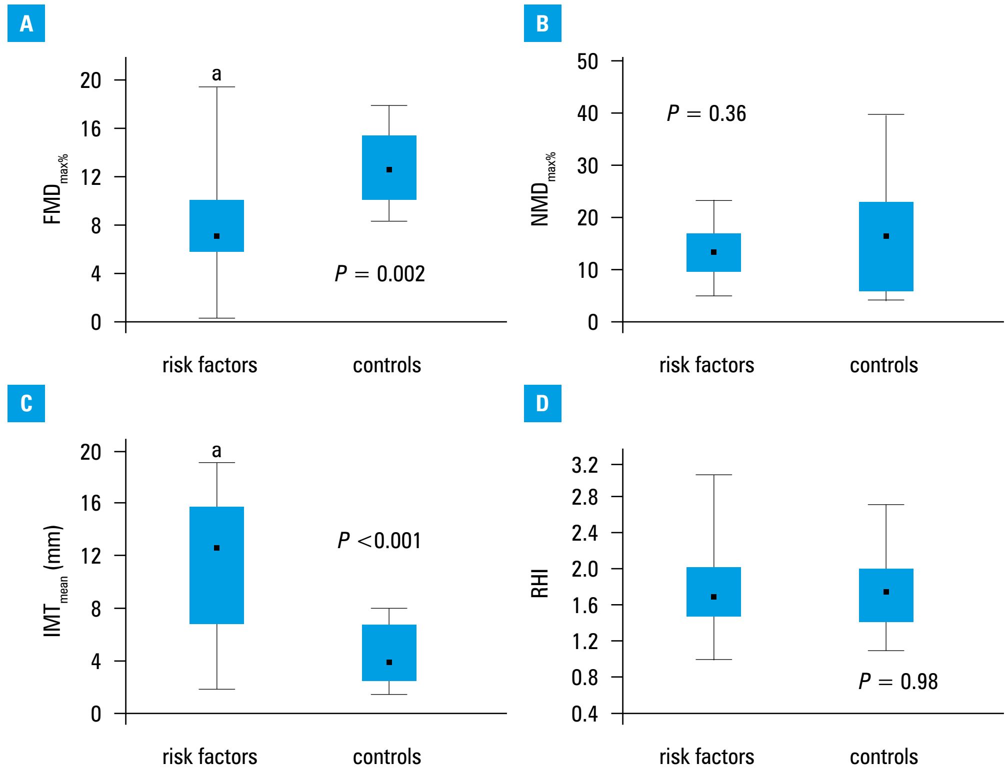

FIGURE 4 Differences in maximum brachial artery flow-mediated dilation ($FMD_{max\%}$; **A**) or nitroglycerin-mediated dilation ($NMD_{max\%}$; **B**) and mean intima–media thickness (IMT_{mean}; **C**) and reactive hyperemia index (RHI; **D**) between subjects with at least 1 atherosclerotic risk factor (n = 69) and controls (n = 11); data represent the actual values of IMT, FMD, or RHI for better visualization of correlations; dots represent median values, boxes represent 25%–75% percentiles, and whiskers represent minimum and maximum values;
a $P < 0.05$ vs. control

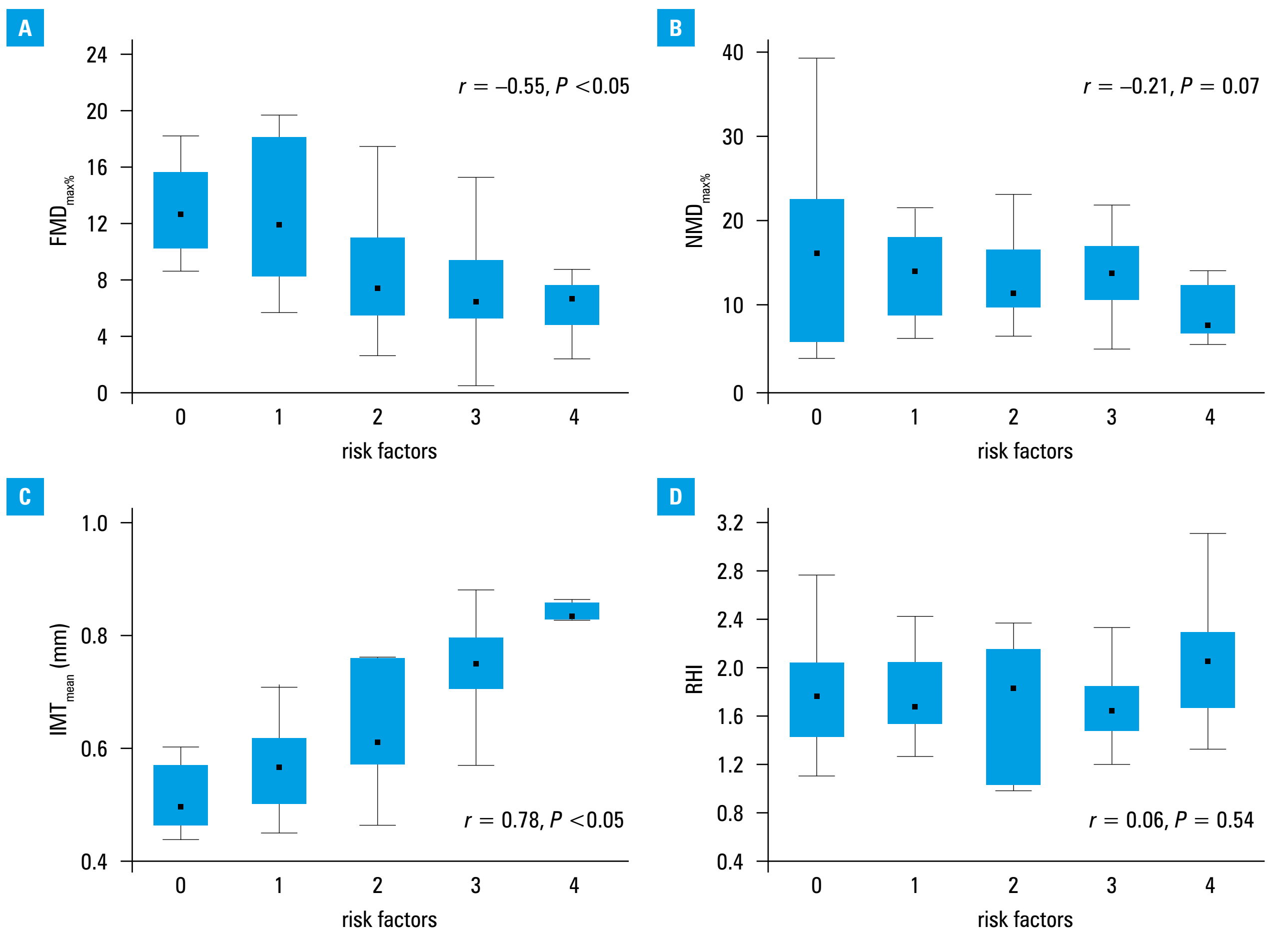

FIGURE 5 Correlations between the number of atherosclerotic risk factors and maximum flow-mediated dilation ($FMD_{max\%}$) ($P < 0.05$; **A**), maximum nitroglycerin-mediated dilation ($NMD_{max\%}$) ($P = 0.07$; **B**), mean intima-media thickness (IMT_{mean}) ($P < 0.05$; **C**), and reactive hyperemia index (RHI) ($P = 0.54$; **D**;) the following risk factors were considered: diabetes, hypercholesterolemia, smoking, and hypertension (see the Methods section); data represent the actual values of IMT, FMD, or RHI for better visualization of correlations; dots represent median values, boxes represent 25%–75% percentiles, and whiskers represent minimum and maximum values; correlation values represent Spearman's correlation coefficient

TABLE 2 Differences in flow-mediated dilation in relation to individual major risk factors of atherosclerosis

			FMD, %			
risk factors	with factor, n	no factor, n	with RF	no RF	*P* (univariate)[a]	*P* (multivariate type III sums of squares)[a]
hypercholesterolemia	53	27	8.9 ±4.9	10.3 ±4.4	0.17	0.6
hypertension	41	39	6.6 ±3.4	12.3 ±4.2	<0.01	<0.01
smoking	16	64	8.2 ±3.6	9.7 ±4.9	0.37	0.6
diabetes	6	74	6.5 ±2.4	9.6 ±4.8	<0.05	0.7
overweight/obesity	51	29	7.8 ±4.1	12.2 ±4.4	<0.05	0.1

Data are presented as mean ± SD.

a statistical analysis was performed on Box–Cox-transformed values

Abbreviations: FMD – flow-mediated dilation, RF – risk factor, others – see TABLE 1

TABLE 3 Differences in reac tive hyperemia index in relation to individual major risk factors of atherosclerosis

			RHI			
risk factors	with factor, n	no factor, n	with RF	no RF	*P* (univariate)[a]	*P* (multivariate type III sums of squares)[a]
hypercholesterolemia	53	27	1.9 ±0.4	1.7 ±0.5	0.055	0.6
hypertension	41	39	1.77 ±0.4	1.83 ±0.4	0.5	<0.01
smoking	16	64	1.9 ±0.4	1.8 ±0.4	0.3	0.6
diabetes	6	74	1.6 ±0.3	1.8 ±0.4	0.4	0.7
overweight/obesity	51	29	1.7 ±0.4	1.8 ±0.4	0.7	0.1

Data are presented as mean ± SD.

a statistical analysis was performed on Box–Cox transformed values

Abbreviations: RHI – reactive hyperemia index, others – see TABLE 1

FMD measures macrovascular response (brachial artery), while RH-PAT measures microvascular response, which may be more variable and may depend on many more factors than just endothelial nitric oxide release.[15]

In the present study, we also assessed the relationship between the severity of carotid atherosclerosis (IMT) and the measures of endothelial dysfunction. As expected, an inverse correlation between FMD and IMT was observed. Similar observations have been reported in numerous populations making combination of these 2 parameters a potent predictor and measure of atherosclerotic risk.[28] However, we did not observe a correlation between RHI and IMT. This is in contrast to a study by Fitch et al.[29] who described such correlation in 54 healthy individuals or to reports of a possible prognostic value of the PAT measurement for IMT progression.[30] Interestingly, most studies published to date were conducted in much younger populations than that in our study (mostly in adolescents).

Some methodological considerations of FMD and IMT assessments include its interobserver and intraobserver measurement variability. In our study, the measurements were always performed by the same 2 experienced operators. Correlation coefficients for these variabilities in FMD measurements performed by experienced investigators were high and in our laboratory equaled to 0.98, which is in line with the values reported elsewhere.[31] Similarly, the Bland–Altman analysis showed high reproducibility of the measurements.

As both RHI and FMD measurements were performed, a concern could be raised that the prior measurement affected the subsequent one. Therefore, nitroglycerin was always applied at the end of the experiment and at least 30 min of rest was allowed between the measurements involving a reactive hyperemia stimulus. This is sufficient because in a recent study, Barton et al.[32] demonstrated that 15 min is a minimum recovery time between a reactive hyperemia stimulus in repeated measurements of brachial FMD.

Finally, the sample size in our study may not be sufficient to fully discriminate the effects of individual risk factors in a multivariate analysis, which should be kept in mind when interpreting the results presented in TABLES 2 and 3.

In summary, our study indicates that FMD, despite its technical requirements and operator-dependence, is a more sensitive method than RH-PAT for assessing the effects of classical atherosclerotic risk factors. It also allows to assess additional parameters such as blood flow, flow-mediated constriction, and baseline brachial artery diameter. It is now important to determine clinical populations in which RH-PAT might better serve risk stratification. Of note, while even in 80 subjects hypertension was associated with reduced FMD, hypercholesterolemia was more related to RH-PAT in a multivariate analysis. It is also possible that RHI/RH-PAT may be more sensitive when repeated measurements in the same patient are necessary, such as in studies on the effects of various treatments and medications on endothelial function. RH-PAT would eliminate the confounding factors associated with FMD. We also need a more detailed physiological response characteristics of microvasculature during RH-PAT study to address the issue of endothelium vs. non-endothelium dependence of recorded responses and understand the effects of sympathetic nervous system activity[21] before we can further consider RH-PAT as universal tool for endothelial health stratification.

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ARTYKUŁ ORYGINALNY

Ocena funkcji śródbłonka naczyniowego w miażdżycy

Porównanie pomiarów rozkurczu pod wpływem zwiekszonegoprzepływu krwi oraz pletyzmograficznego pomiaru wskaznika reaktywnej hiperemii

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SŁOWA KLUCZOWE

funkcja śródbłonka naczyniowego, miażdżyca, obwodowa tonometria tętnicza, rozkurcz pod wpływem zwiększonego przepływu krwi, tlenek azotu

STRESZCZENIE

WPROWADZENIE Dysfunkcja śródbłonka, charakteryzująca się utratą biodostępności tlenku azotu, jest kluczowym elementem w patogenezie miażdżycy i ważnym czynnikiem prognostycznym w schorzeniach sercowo-naczyniowych. Dlatego rozwój wiarygodnych, bezpiecznych i nieinwazyjnych metod pomiaru funkcji śródbłonka naczyniowego jest kluczowy, aby można je było wykorzystywać w stratyfikacji ryzyka sercowo-naczyniowego. Pomiar rozkurczu naczyń pod wpływem zwiększonego przepływu krwi (*flow-mediated dilation* – FMD) jest metodą szeroko stosowaną w badaniach naukowych, jednak trudności techniczne i problemy z kalibracją wartości pomiędzy laboratoriami ograniczają jej zastosowania kliniczne. Obwodowa tonometria tętnicza wykorzystująca zjawisko reaktywnej hiperemii (*reactive hyperemia–peripheral artery tonometry* – RH-PAT, Endo-PAT) została opracowana jako prostsza, tańsza i potencjalnie bardziej powtarzalna metoda.

CELE Celem pracy było zbadanie związków pomiędzy pomiarami FMD a RH-PAT w odniesieniu do profilu czynników ryzyka miażdżycy.

PACJENCI I METODY Do badania włączono 80 pacjentów (52 mężczyzn, 28 kobiet), w wieku 43,6 ±14,8 roku z umiarkowanym i niskim ryzykiem sercowo-naczyniowym (średnia wartość SCORE = 2,2 ±2%), u których badano FMD, RH-PAT oraz grubość błony wewnętrznej i środkowej (*intima–media thickness* – IMT).

WYNIKI Pomiar indeksu reaktywnej hiperemii (*reactive hyperemia index* – RHI) za pomocą RH-PAT znamiennie korelował z pomiarami FMD ($r = 0,35$; $p <0,01$). Nie zaobserwowano jednak znamiennej korelacji między RHI a IMT, SCORE ani liczbą klasycznych czynników ryzyka miażdżycy (nadciśnienie, palenie, cukrzyca, hipercholesterolemia), podczas gdy FMD wykazywało znamienną korelację z IMT ($r = –0,53$; $p <0,001$), czynnikami ryzyka miażdżycy ($r = –0,55$; $p <0,05$) oraz wartością SCORE ($r = –0,4$; $p <0,05$).

WNIOSKI FMD, mimo wymogów technicznych, jest metodą bardziej czułą niż RH-PAT, w odniesieniu do oceny wpływu klasycznych czynników ryzyka miażdżycy na funkcję śródbłonka naczyniowego. Odpowiedź mikronaczyniowa na hiperemię podczas badania za pomocą RH-PAT musi być bardziej szczegółowo zbadana, z określeniem znaczenia czynników niezależnych od śródbłonka, które mogą wpływać na wykonywane pomiary, zanim RH-PAT będzie można uznać za uniwersalne narzędzie oceny stanu komórek śródbłonka.

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