Impaired endothelium-dependent and endothelium-independent systemic vasodilatory reserve in pulmonary hypertension regardless the clinical group: A generalized dysfunction beyond the pulmonary arteries?

Muhittin Demirel, Şeyhmus Külahçıcıoğlu, Hacer Ceren Tokgöz, Özgür Y. Akbal, Aykun Hakgör, Ali Karagöz, Seda Taniveri, Berhan Keskin, Barkın Kılltürşay, Süleyman Çağan Elc, Züleyde Bayram, İbrahim Halil Tanboğa, Nihal Özdemir, Cihangir Kaymaz

Department of Cardiology, University of Health Sciences, Hamidiye Faculty of Medicine, Koşuyolu Yüksek İhtisas Training and Research Hospital; İstanbul-Turkey
1Department of Cardiology, Bingöl State Hospital; Bingöl-Turkey
2Department of Biostatistics, Nişantaşı University; İstanbul-Turkey

ABSTRACT

Objective: Endothelium-dependent (ED) and endothelium-independent (EI) flow-mediated vasodilatation (FMD) have been used as measures of systemic arterial vasodilatory reserve. In this study, we aimed to assess both ED-FMD and EI-FMD in different groups with pulmonary hypertension (PH), and to investigate the relationship of these measures with clinical, echocardiographic, and invasive parameters of diseases severity and targeted treatment status.

Methods: Our study population comprised 41 patients with PH [28 (68.2%) women, age 46.3±19.6 years] including idiopathic pulmonary arterial hypertension, Eisenmenger syndrome, and chronic thromboembolic PH in whom diagnosis were confirmed in accordance with current guidelines and 17 age and sex-matched healthy controls. The brachial artery (BA) was used for assessment of FMD with Duplex ultrasound, and serial changes in diameter were recorded at baseline, 1, and 3 minutes after termination of 2-minute external occlusive compression for ED-FMD, and after sublingual intake of glycerol trinitrate for EI-FMD, respectively.

Results: Compared with controls, overall the PH group showed significantly lower ED-FMD (0.65±0.21 vs. 0.30±0.23 and 0.65±0.18 vs. 0.24±0.21) and EI-FMD (0.67±0.15 vs. 0.37±0.25 and 0.75±0.20 vs. 0.32±0.24) responses at 1st and 3rd min (p<0.001 for all). All these changes in the values of ED-FMD and EI-FMD were comparable among the PH subgroups. Neither ED-FMD nor EI-FMD were correlated with measures of PH severity and targeted therapy (TT) status (p>0.05).

Conclusion: Our results suggest an impaired BA vasodilatory reserve in patients with PH regardless of the clinical subgroup. Although these findings seem to be consistent with systemic dysfunction, acute FMD may not reflect the severity of PH and cannot be used as a potential surrogate for outcome in this setting.

Keywords: chronic pulmonary hypertension, endothelium, pulmonary circulation, right ventricle

Introduction

Pulmonary hypertension (PH) is a progressive and debilitating disease resulting in right ventricular dysfunction, heart failure, and eventually death (1-3). Group 1 PH or pulmonary arterial hypertension (PAH) has been considered as a prototype of hemodynamically pre-capillary PH generalized to other PH subsets, except group 2 PH which is characterized by post-capillary pressure overload owing to left-sided cardiovascular pathologies (1-3). The main pathologic pathway of PAH is narrowing of the pulmonary artery lumen because of vasoconstriction, arterial concentric remodeling, and in situ thrombosis mainly involv-
Eisenmenger syndrome in different groups of PH, and to investigate the relationship of patients with different PH etiologies remains to be determined. Various cardiovascular diseases, their clinical relevance in endothelium-independent (EI) FMD have been used for non-(NO) (5, 14-20). Although endothelium-dependent (ED) and effective mediator of FMD is endothelium-derived nitric oxide with treprostinil and iloprost (5-13). Although pulmonary endothelial system is the main target of pulmonary hypertension treatment, presence of generalized endothelial dysfunction should also be considered.

**HIGHLIGHTS**

- Although flow-mediated vasodilatation has been used as a measure of systemic arterial vasodilatory reserve in cardiovascular diseases, its clinical relevance in patients with pulmonary hypertension remains undetermined.
- Our study demonstrated that brachial artery vasodilatory reserve was significantly compromised in patients with pulmonary hypertension than in healthy subjects.
- Although pulmonary endothelial system is the main target of pulmonary hypertension treatment, presence of generalized endothelial dysfunction should also be considered.

Flowing the distal pulmonary arteries (1-3). Pulmonary vascular endothelium has a primary role in maintaining low pulmonary vascular resistance. Increased vasoconstriction in pulmonary vasculature owing to increased endothelin-1 and thromboxane-A2 levels plays a critical role in pathogenesis of PAH (1-4). However, pulmonary arterial hemodynamics seem to be dependent on those in systemic arterial circulation, and current studies in PAH suggest a systemic disease comprising abnormal endothelial responses to vasodilatory triggers, metabolic dysregulation, and inflammation which might be associated with clinical outcome (5, 6). All of the three pathways which have been proven to be involved in the pathogenesis of PAH are essentially related with endothelial dysfunction (2-5), and currently available specific therapies targeted at these pathways not only provide endothelium-mediated vasodilatory effects and antiproliferative effects on pulmonary vasculature, but also vasodilatory effects on different components of systemic arterial circulation (5-13). However, peripheral vasodilatory effects of drugs proven for PAH therapies seem to be ignored despite the robust evidence for effectiveness of these agents on systemic arterial circulation, such as accelerated healing of Raynaud’s syndrome and skin ulcers via cutaneous vasodilation in scleroderma with bosentan and iloprost, improvement in erectile dysfunction and systemic vasodilation in heart failure with sildenafil, portal vein vasodilation with epoprostenol, and improvement in limb blood flow in peripheral arterial disease with treprostinil and iloprost (5-13).

Most of the blood vessels respond to shear stress by vasodilatation known as flow-mediated dilatation (FMD), and the most effective mediator of FMD is endothelium-derived nitric oxide (NO) (5, 14-20). Although endothelium-dependent (ED) and endothelium-independent (EI) FMD have been used for non-invasive assessment of systemic arterial vasodilatory reserve in various cardiovascular diseases, their clinical relevance in patients with different PH etiologies remains to be determined (5, 14-20).

In this study, we aimed to assess both ED-FMD and EI-FMD in different groups of PH, and to investigate the relationship of these measures with clinical, echocardiographic, and invasive parameters of disease severity.

**Methods**

The study population comprised of 41 patients with PH [age 46.32±19.61 years, 28 (68.3%) women] and 17 healthy controls who underwent assessment of FMD in brachial artery (BA) with duplex ultrasound. The PH population were as follows; idiopathic PAH (n=17), Eisenmenger syndrome (n=17), and chronic thromboembolic PH (CTEPH) (n=7). Healthy volunteers with normal echocardiographic right and left ventricular functions and patients with tricuspid regurgitation velocities <2.8 without supportive PH criteria, clinical history, and were free of a history of recent cardiovascular diseases, alcohol, drug, or tobacco use were included (2).

The study protocol was approved by the Ethics Committee and was performed according to the guidelines of the Declaration of Helsinki. A written informed consent was obtained from all the patients before enrollment.

The World Health Organization functional status of PH group varies from class II to III, and none of them was hospitalized last month before inclusion in the study. The diagnostic criteria of the European Society of Cardiology and the European Respiratory Society 2015 PH guidelines were used for hemodynamic and clinical definitions of PH, and hemodynamic definitions of PH was based on the criteria including the mean pulmonary arterial pressure of (mPAP) ≥25 mm Hg, pulmonary capillary pressure (PCWP) or left ventricle end-diastolic pressure (LVEDP) 15, and pulmonary vascular resistance (PVR) ≥3 measured by heart catheterization (2).

**Definition of Eisenmenger syndrome:** Eisenmenger syndrome is elevated pulmonary vascular resistance driving right-to-left intracardiac or great arterial shunting leading to systemic arterial desaturation (2).

**Definition of CTEPH:** The diagnosis based on findings of PH obtained after at least 3 months of effective anticoagulation, with mismatched perfusion defects on lung scan and specific diagnostic signs for CTEPH seen by multidetector CT angiography or invasive angiography (2).

**Exclusion criteria:** The patients with known coronary artery disease, peripheral arterial disease, cerebrovascular disease, systemic hypertension, diabetes mellitus, those who used drugs that could affect endothelial functions such as angiotensin converting enzyme inhibitor, angiotensin receptor blocker, alpha or beta blockers, nitrate, and alcohol or smoking history were excluded.

In accordance with the guidelines for ultrasound assessment of ED flow-mediated vasodilation of the brachial artery, Duplex assessment of BA for FMD was performed in a quiet and temperature controlled room, and under ECG monitorization (14). The patients and controls were positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery was imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior...
intimal interfaces between the lumen and vessel wall was selected for continuous 2D grayscale imaging. Patients with suboptimal imaging were excluded.

A linear array transducer with a frequency of 10 MHz, attached to a high-quality mainframe ultrasound system (General Electric Medical-Systems, Horten, Norway) was used to acquire images. Serial changes in diameter were recorded at baseline, 1, and 3 minutes after termination of 2-minute external occlusion for ED-FMD, and glyceryl trinitrate (GTN) was used for evaluation of EI-FMD after ED-FMD session. After 15 minutes of rest, the patients were given 400 mcg of GTN sublingually (14). At the 1st and 3rd minute of GTN exposure, arterial radius was recorded as nitrate phase. Percent FMD was described as percentage increasing of arterial radius after basal recording.

$$FMD\% = \frac{[FMD \times 1st \text{-} \text{min}-\text{basal} \text{radius}] \times 100}{basal \text{radius}}.$$ 

### Table 1. Baseline demographic and clinical variables

| Variables          | Patients | Controls | P-value |
|--------------------|----------|----------|---------|
| Age (years)        | 46.32±19.61 | 42.35±15.29 | 0.463   |
| Sex (female)       | 28 (68.3%) | 10 (58.8%) | 0.698   |
| Body mass index (kg/m²) | 24.39±5.39 | 23.95±4.37 | 0.763   |

Continuous variables presented as mean ± standard deviation.

### Table 2. Medications in subgroups of patients with pulmonary hypertension

| Drug type | IPAH (n=17) | Eisenmenger (n=17) | CTEPH (n=7) |
|-----------|-------------|--------------------|-------------|
| Bosentan n (%) | 13 (76) | 12 (70) | -           |
| Tadalafil n (%) | 11 (64) | 11 (64) | -           |
| Sildenafil n (%) | 1 (6) | 1 (6) | -           |
| Iloprost n (%) | 1 (6) | - | -           |
| Treprostinil n (%) | 2 (12) | 1 (6) | -           |
| Riociguat n (%) | - | - | 6 (85)     |
| Diuretic n (%) | 6 (35) | 5 (29) | 5 (71)     |
| Warfarin n (%) | 2 (12) | 2 (12) | 7 (100)    |
| Ambisentan n (%) | 1 (6) | - | -           |

IPAH - idiopathic pulmonary arterial hypertension, CTEPH - chronic thromboembolic pulmonary hypertension

### Table 3. Serial changes in ischemia-flow mediated dilatation (ED-FMD) and nitrate mediated dilatation (EI-FMD) across the PH group

| Groups | Basal | 1st minute | 3rd minute | P-value | Basal | 1st minute | 3rd minute | P-value |
|--------|-------|------------|------------|---------|-------|------------|------------|---------|
| Controls | 3.20 (2.6–3.4) | 3.8 (3.4–4.1) | 3.8 (3.3–4.0) | 0.001 | 3.2 (2.73.4) | 3.8 (3.4–4.2) | 3.9 (3.3–4.1) | 0.001 |
| IPAH | 3.20 (2.9–3.8) | 3.5 (3.2–4.0) | 3.4 (3.1–4.1) | 0.001 | 3.2 (2.9–3.7) | 3.4 (3.2–4) | 3.5 (3.0–4.1) | 0.001 |
| Eisenmenger | 3.2 (3.0–3.9) | 3.4 (3.2–4.1) | 3.4 (3.1–4.0) | 0.001 | 3.2 (2.9–3.8) | 3.5 (3–4) | 3.4 (3.0–4.2) | 0.001 |
| CTEPH | 3.3 (3.0–3.4) | 3.6 (3.3–3.7) | 3.5 (3.3–4.0) | 0.002 | 3.3 (3.0–3.4) | 3.8 (3.2–3.8) | 3.7 (3.2–4.0) | 0.004 |

Continuous results, continuous variables given as median and interquartile range [1st to 3rd].

ED - endothelium dependent, EI - endothelium independent, FMD - flow-mediated vasodilatation, PH - pulmonary hypertension, IPAH - idiopathic pulmonary arterial hypertension, CTEPH - chronic thromboembolic pulmonary hypertension

### Results

The age, sex, and body mass index were comparable between PH and control groups (Table 1). Medications in PH subgroups are given in Table 2.

Control group, IPAH, Eisenmenger, and CTEPH patients showed significant increases in the “ischemia-flow mediated dilatation” at the 1st and 3rd minutes compared with baseline (p=0.001, p=0.001, p=0.001, and p=0.002, respectively) (Table 3). The change from basal to 3rd minute measurements were statistically significant among PH subgroups (p=0.001 and p<0.001).
However, the change between the 1\textsuperscript{st} minute and 3\textsuperscript{rd} minute measurements were comparable among PH subgroups (p>0.05) (Fig. 1, Table 4). Compared with the control group, IPAH group (p=0.001), Eisenmenger group (p=0.001) and CTEPH group (p=0.003) showed significantly lower ischemia change from baseline to 3\textsuperscript{rd} minute measurements, (p<0.001). However, 3\textsuperscript{rd} minute ischemia change was comparable among PH subgroups (p>0.05) (Table 4).

In the control, IPAH, Eisenmenger, and CTEPH groups, “nitrate mediated vascular dilatation” at the 1\textsuperscript{st} and 3\textsuperscript{rd} minutes were statistically different than the basal measures (p=0.001, p=0.001, p=0.001, and p=0.004, respectively). Moreover, changes from the baseline to the 1\textsuperscript{st} and 3\textsuperscript{rd} minutes and from the 1\textsuperscript{st} to
In this study, we demonstrated that brachial artery vasodilatory reserve assessed with ED-FMD (ischemia response) and EI-FMD (nitrate response) was significantly impaired in patients with IPAH, Eisenmenger, and CTEPH than in healthy subjects. There was no difference in ED and EI vasodilatory responses among the etiologic PH subgroups. However, these impairments in systemic arterial vasodilatory reserve were not correlated with the clinical and hemodynamic measures of pulmonary vascular disease severity. No significant difference was observed in ED-FMD (FMD%) between the 1st and 3rd minutes.

The growing evidence from studies performed in patients with IPAH and scleroderma-PAH suggest the presence of a wide spectrum of systemic metabolic and inflammatory alterations and vascular dysfunction (5, 6, 15-40) manifested by Impaired acidosis and modulation of ergoreflex (5, 6, 32-36, 44, 45). An increased ventilatory response to exercise as a result of the ergoreflex overactivation related with reduced muscle strength due to switching from type I toward more fatigable type II fibers, disturbed mitochondrial function and excitation–contraction coupling, increased muscle protein breakdown and decreased capillary density in nailfold on capillaroscopy (5, 6, 29). Reduced capillary density in sublingual vessels (31). Skeletal myopathy characterized by ergoreflex overactivation preceding the occurrence of PAH (5, 6, 29).

**Discussion**

In this study, we demonstrated that brachial artery vasodilatory reserve assessed with ED-FMD (ischemia response) and EI-FMD showed weak correlations to pulmonary vascular resistance, PA pressures, pulmonary vascular and systemic vascular resistances, and pulmonary to systemic vascular resistance ratio (p=0.05). Moreover, the percent changes in ED-FMD and EI-FMD showed weak correlations to pulmonary vascular resistance and pulmonary to systemic vascular resistance ratio with Spearman test (0.312 and 0.239, respectively) (Table 5).

| Variables                                      | ED-FMD (P-value) | EI-FMD (P-value) |
|------------------------------------------------|------------------|------------------|
| Doppler PA systolic pressure (mm Hg)           | 0.04 (0.803)     | 0.14 (0.366)     |
| Doppler PA mean pressure (mm Hg)               | 0.01 (0.362)     | 0.06 (0.735)     |
| Invasive PA systolic pressures (mm Hg)         | 0.07 (0.702)     | 0.03 (0.884)     |
| Invasive PA mean pressures (mm Hg)             | 0.14 (0.475)     | 0.08 (0.685)     |
| PVR (Wood U)                                  | 0.31 (0.127)     | 0.31 (0.127)     |
| SVR (Wood U)                                  | 0.03 (0.955)     | 0.01 (0.967)     |
| PVR/SVR                                        | 0.23 (0.253)     | 0.22 (0.288)     |
| Six-minute walk distance (m)                   | -0.09 (0.544)    | 0.08 (0.597)     |

Continuous variables given as median and interquartile range (1st to 3rd minutes).

ED - endothelium dependent, EI - endothelium independent, FMD - flow-mediated vasodilatation, PH - pulmonary hypertension, IPAH - idiopathic pulmonary arterial hypertension, CTEPH - chronic thromboembolic pulmonary hypertension.
The vascular pathologies of PAH including cell overgrowth, neo-intima formation, fragmentation of the elastic lamina, and vasoconstriction, muscularization, and calcium deposits in the large pulmonary arteries have also been reported in atherosclerotic cardiovascular diseases, aortic aneurysms, diabetic retinopathy, hypertensive nephropathy, and high-grade glioblastoma multiforme (5, 6, 16, 18).

When assessed with multiparametric approach, activation of BRD4 (bromodomain protein 4), a transcriptional regulator protein associated with pulmonary artery muscularization and calcification via activation of RUNX2 (Runt-related transcription factor 2) and cytokines, are considered to have contributions in accelerated coronary artery disease in patients with PAH, and inhibition of abnormal BRD4 activity with apabetalone is proposed as a novel potential target for PAH treatment (5, 22).

Flow-mediated dilatation (FMD) was first described in femoral and brachial arteries and is mediated by potassium channel dependent release of NO produced from the nitroglycerine that leaks into the smooth muscle cells (5, 6, 14-20). ED-FMD (ischemia response) assesses the hyperemia occurring in medium muscular arteries as a response to shear stress following ischemia induced by inflating the blood pressure cuff (5, 6, 14-20). In patients with PAH, studies with duplex ultrasound in BA revealed impaired FMD as a marker for systemic endothelial disfunction (5, 6, 14-20). Hughes et al. (17) reported significant reductions in BA FMD in 2.7% of patients with IPAH and 6.3% of patients with SSC-PAH, and a trend toward a reduced response in family members of the patients with IPAH. Peled et al. (18) documented impaired ED-FMD in patients with PAH, a correlation between the magnitude of the impairment and severity of disease as assessed by clinical and hemodynamic parameters. Wolff et al. (19) evaluated BA FMD in healthy controls and patients with PAH undergoing acute vasoreactivity testing with iloprost, and an impaired ED-FMD correlated with percent decrease in pulmonary vascular resistance with iloprost challenge was noted. However, in contrast to the study by Peled et al. (18), they found that impaired ED-FMD was not correlated with the hemodynamical severity of PAH (19).

Although our analyses confirmed the impairment in systemic vasodilatory reserve as assessed by ED-FMD and EI-FMD in all the patients with PH in accordance with the study by Wolff et al. (19); and in contrast to the study by Peled et al. (18), none of these impairments correlated with the echocardiographic, hemodynamic, and 6MWT measures of PH severity and targeted therapy (TT) status. Although ED-FMD was comparable among PAH subgroups, difference mechanisms might be involved in systemic endothelial dysfunction in these patients. Lower ED-FMD in patients with Eisenmenger is considered to be a compensatory response to chronic hypoxia rather than caused by increased vasoconstrictor mediator levels. Nakamura et al. (20) reported that cardiovascular shunt lesions with increased pulmonary blood flow may be associated with a trend toward decreased cardiac output owing to decreased vascular wall shear stress and endothelial dysfunction. Besides, Ciftel et al. (41) found that compared with the control group, the mean FMD was significantly reduced in the cyanotic PH group. Furthermore, polycythemia and hypoxia are considered to induce endothelial injury (5, 6, 19). Lower ED-FMD in other subgroups might be caused by release of primary and secondary mediators. Interestingly, FMD was also found to be disturbed in patients with CTEPH similar to those in other PH subsets, and this finding suggests the presence of generalized endothelial dysfunction in the systemic circulation. Sirmagul et al. (42) showed that in monocrotaline (MCT) induced PH rats, the plasma NO level was not significantly different in the control and MCT groups; however, the iloprost and sildenafil treatments significantly decreased NO level in plasma. After administration of sublingual nitrate to evaluate EI-FMD, a significantly lower FMD was observed in all PH subgroups than in the controls. All the PH groups showed similar changes in the EI-FMD response.

**Study limitations**

The design of the current study suffered from some limitations. First, the population size may be considered relatively small. However, majority of the previous data for FMD in patients with PAH have also been derived from studies having the same limitation (16-20). Uninterrupted PAH treatments targeted to three specific pathways because of ethical considerations might be another inevitable methodological limitation in the assessment of FMD in these patients; along with intra- and inter-observer variability of FMD method, daily changes according to biological circadian rhythm, and unsatisfactory signal to noise ratio related to artery diameter. The relationship between the measures of FMD and clinical, echocardiographic, and hemodynamic measures of PH severity was based on a cross-sectional analysis. Despite the absence of a relation between the impairment in FMD and these outcome measures at the time of assessments, long-term follow-up of these patients might provide more robust data regarding the prognostic impact of the impaired ED or EI FMD on clinical course of PH. The new guideline stated that it would be better to measure up to 5 minutes; however, the fact that it was done according to the previous guidelines at the time of study may have affected the results. Another limitation of study included taking single measurements at 1 and 3 minutes post deflation instead of the recommended continuous monitoring for the whole 180 seconds to find the peak vessel diameter (43). PAH-specific therapies that the patients received might affect the FMD measurements. In this analysis, all the patients had an intermediate risk in which the annual event rate expectation of 5%-10% according to the ESC/ERS 2015 multiparametric risk algorithm. In addition, the clinical parameters could not be analyzed according to the patient groups owing to the low number of patients in groups. The clinical, hemodynamic, and neurohumoral relationship with Endotel function analysis was not performed because of the small number of patient in the groups.

**Conclusion**

Our results suggest significantly impaired BA vasodilatory reserve as assessed by ED-FMD and EI-FMD in patients with PH regardless of the etiological clinical subgroup and imply a general-
ized vasodilatory dysfunction beyond the pulmonary vasculature. However, acute FMD may not reflect the severity of PH and cannot be used as a potential surrogate for outcome in this setting.

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