Peripheral endothelial dysfunction in patients with pulmonary arterial hypertension

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Received 8 February 2008; accepted 25 June 2008
Available online 3 August 2008

KEYWORDS
Pulmonary hypertension; Endothelial dysfunction; Systemic

Summary
Background: Pulmonary endothelium plays an important role in the mechanism of pulmonary arterial hypertension (PAH). However, there is only a few data regarding the systemic endothelium in this syndrome. This study focused on the systemic endothelial involvement in PAH.

Methods: Endothelial function was evaluated in 54 patients with idiopathic (n = 28), scleroderma-associated (n = 10), chronic thromboembolic (n = 7), or Eisenmenger (n = 9) PAH and 21 controls (13 healthy; eight scleroderma and normal pulmonary pressure). All underwent clinical evaluation, pulmonary assessment, echocardiography, and pulmonary cardiac stress test. Endothelial function was evaluated by measuring the forearm blood flow dilatation response to brachial arterial occlusion by a non-invasive plethysmograph, yielding a peripheral arterial tone (PAT) ratio.

Results: The PAT ratio was significantly lower (p < 0.05) than healthy controls in all patients except the Eisenmenger group (control: 2.20 ± 0.25; idiopathic 1.84 ± 0.51; scleroderma 1.66 ± 0.66; thromboembolic 1.89 ± 0.32; Eisenmenger 2.17 ± 0.62). The impaired hyperemic response significantly correlated with disease severity, as measured by NYHA classification (r = −0.210, p = 0.035), pulmonary pressure (r = −0.228, p = 0.035), 6 min walking distance (r = 0.215, p = 0.047), and oxygen desaturation on effort (r = 0.207, p = 0.038). Mean systolic pulmonary pressure among patients was 54–99 mmHg.

Conclusion: A systemic component of endothelial dysfunction might be involved in idiopathic, scleroderma-associated and chronic thromboembolic PAH that is correlated with disease severity.

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0954-6111/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2008.06.014
Introduction

Pulmonary arterial hypertension (PAH) is a rare, incurable disease. However, the histopathologic features of PAH suggest endothelial injury and proliferative processes. Pulmonary endothelial dysfunction is apparently a significant component of the underlying mechanism of PAH. Studies have reported a chronically impaired production of vasoactive mediators, such as nitric oxide and prostacyclin, in patients with PAH, along with prolonged overexpression of vasoconstrictors, such as endothelin 1 and thromboxane A2.

The term "primary pulmonary hypertension" was originally coined to describe a condition of hypertensive vasculopathy of unknown cause that occurs exclusively in the pulmonary vasculature. In the last 20 years, however, researchers have recognized that pulmonary hypertension may be associated with several systemic conditions including connective tissue diseases, portal hypertension, and human immunodeficiency virus infection, in addition to the use of appetite suppressant medications. However, the precise nature and extent of the peripheral (or systemic) vasculature involvement remain unclear.

The aim of this study was to investigate peripheral endothelial function in four types of PAH: idiopathic PAH, scleroderma-associated PAH, Eisenmenger syndrome due to congenital systemic-to-pulmonary shunt, and chronic thromboembolic PAH (CTEPH). Peripheral endothelial function was measured by a non-invasive plethysmograph (EndoPAT 2000), assessing post occlusion brachial artery flow-mediated dilatation (FMD).

Patients and methods

Study participants

The study was prospective and cross sectional. Participants included 54 patients with pulmonary hypertension (28 idiopathic PAH, 10 scleroderma-associated PAH, nine Eisenmenger syndrome, and seven chronic thromboembolic PAH) being treated in our department, and 21 control subjects (13 healthy and eight with scleroderma and normal pulmonary arterial pressure). In no case was the medication changed from 3 months before enrollment to the end of the study period. Patients with diabetes, ischemic heart disease, cerebrovascular event, renal failure, or systemic hypertension were excluded, as were patients taking nitrates, alpha/beta blockers and angiotensin-converting enzyme inhibitors.

Study protocol

All subjects underwent a full medical history, function class grading (NYHA), physical examination, and laboratory assessment that included pulmonary function test, oxygen saturation, carbon monoxide diffusion capacity (DLCO), 6 min walk test (for distance and oxygen saturation), and echocardiography (for systolic pulmonary pressure). Thirty-six patients had undergone cardiac catheterization prior to the study, and data on mean pulmonary pressure, cardiac index and pulmonary vascular resistance (PVR) were obtained from their records.

Endothelial function testing was performed in the morning under stable conditions, after an overnight fast. Subjects were asked to refrain from smoking and from drinking alcohol or caffeinated drinks for 12 h. At testing, subjects were seated on a special comfortable chair with both hands placed at the level of the heart. The EndoPAT 2000 device (Itamar Medical Ltd, Ceasarea, Israel) was used to obtain a beat-to-beat plethysmographic recording of the finger arterial pulse-wave amplitude (PWA). A pneumatic probe was placed on the index finger of each hand to record peripheral arterial tone (PAT). Following a 20 min equilibrium period (20 °C constant temperature), baseline levels were measured for 5 min at rest, and then for 5 min of occlusion of one arm. Occlusion was induced by inflating the cuff on the upper arm to 50 mmHg above systolic pressure and then releasing it to induce reactive (flow-mediated) hyperemia. The other, non-occluded, hand was used for reference, to correct for potential systemic changes. The postobstructive PWA was measured starting 90 s after cuff deflation, for 210 s. Endothelial function was calculated as the ratio between the average postobstruction PWA and the average 3 min baseline PWA, corrected for systemic changes and baseline signal amplitude. The signals were analyzed with a computerized automated algorithm to eliminate intra- and inter-observer variability. Absolute endothelial dysfunction was defined as a PAT ratio of less than 1.67.

This FMD test has been used and compared with other methods in many previous studies. Figure 1 presents examples of the normal and pathological (reactive hyperemia) pictures using the Endo-PAT 2000 device.

This study was approved by the ethics committee of our tertiary center. All subjects signed an informed consent form to participate in the study.

Statistical analysis

Descriptive statistics were calculated for each group. Differences among the patient groups were analyzed by analysis of variance and chi-square test (contingency
tables), and differences between patients and controls were analyzed by Student's t-test. For post hoc analysis, the Scheffe test was used. A p value of <0.05 was considered significant. The Statistical Package for Social Sciences (SPSS Corp., version 11) was used for data handling and analysis.

**Statement of responsibility**

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**Patient characteristics**

The patient characteristics are shown in Table 1. The distributions of age and body mass index were similar in all groups. Comparison of the patients with the control subjects yielded a statistically significant difference in NYHA class, baseline oxygen saturation, and DLCO. Among the various patient groups, the patients with Eisenmenger syndrome had a significantly lower oxygen saturation than the patients with idiopathic or thromboembolic disease ($p = 0.030$ and $p = 0.022$, respectively; Scheffe post hoc test); no significant differences were found for the other parameters. Mean distance on the 6 min walk test was similar in all groups; however oxygen desaturation on effort was significantly worse in the patients with Eisenmenger syndrome and scleroderma.

Cardiac catheterization had been performed in 36 of the 54. No statistically significant difference in pulmonary pressure, measured by right heart catheterization, was noted among the patient groups. Cardiac output and PVR were also similar among the patient groups, although there was a trend toward a higher PVR in the patients with thromboembolic disease. Echocardiographic assessment showed a significantly higher pressure in the Eisenmenger syndrome group than in the idiopathic PAH and scleroderma-PAH groups ($p = 0.009$ and $p = 0.031$, respectively). The difference from the thromboembolic groups was not significant ($p = 0.194$).

**Table 1 Clinical and epidemiologic characteristics of patients with PAH and controls**

<table>
<thead>
<tr>
<th>Background factors</th>
<th>Healthy Controls</th>
<th>Idiopathic PAH</th>
<th>Thromboembolic PAH</th>
<th>Eisenmenger syndrome</th>
<th>Scleroderma-PAH only control</th>
<th>p (pt. groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>28</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Age (y)</td>
<td>46.0 ± 14.1</td>
<td>51.9 ± 13.6</td>
<td>50.0 ± 12.4</td>
<td>42.0 ± 20.1</td>
<td>54.3 ± 14.2</td>
<td>0.743</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 3.6</td>
<td>26.3 ± 6.7</td>
<td>28.7 ± 6.0</td>
<td>23.8 ± 3.7</td>
<td>23.7 ± 4.5</td>
<td>0.287</td>
</tr>
<tr>
<td>NYHA</td>
<td>1.0</td>
<td>1.9 ± 0.7</td>
<td>2.0 ± 0.6</td>
<td>2.0 ± 0.5</td>
<td>2.2 ± 0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>I, n (%)</td>
<td>13 (24%)</td>
<td>7 (14%)</td>
<td>1 (14%)</td>
<td>1 (11%)</td>
<td>1 (10%)</td>
<td>8</td>
</tr>
<tr>
<td>II, n (%)</td>
<td>17 (61%)</td>
<td>5 (72%)</td>
<td>7 (78%)</td>
<td>6 (61%)</td>
<td></td>
<td>0.550</td>
</tr>
<tr>
<td>III–IV, n (%)</td>
<td>4 (14%)</td>
<td>1 (14%)</td>
<td>1 (11%)</td>
<td>3 (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epoprostenol</td>
<td>4 (14%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ilomedin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remodulin</td>
<td>6 (21%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bosentan</td>
<td>3 (11%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select. EB</td>
<td>9 (32%)</td>
<td>1 (14%)</td>
<td>7 (78%)</td>
<td>3 (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viagra</td>
<td>8 (29%)</td>
<td>1 (14%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCB</td>
<td>5 (18%)</td>
<td>2 (33%)</td>
<td>1 (11%)</td>
<td>2 (20%)</td>
<td></td>
<td>2 (25%)</td>
</tr>
</tbody>
</table>

**Clinical findings**

<table>
<thead>
<tr>
<th>Pulse (l/min)</th>
<th>73.5 ± 10.6</th>
<th>82.8 ± 12.0</th>
<th>75.4 ± 13.3</th>
<th>74.9 ± 12.6</th>
<th>81.2 ± 12.6</th>
<th>83.2 ± 7.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL_sat (%)</td>
<td>97.5 ± 1.4</td>
<td>94.3 ± 5.1</td>
<td>97.0 ± 1.5</td>
<td>87.9 ± 10.9</td>
<td>94.2 ± 5.5</td>
<td>0.675</td>
</tr>
<tr>
<td>6MWT (m)</td>
<td>512 ± 35</td>
<td>399 ± 118</td>
<td>388 ± 143</td>
<td>361 ± 66</td>
<td>315 ± 77</td>
<td>0.207</td>
</tr>
<tr>
<td>6MWT sat (%)</td>
<td>98.3 ± 0.8</td>
<td>89.8 ± 9.1</td>
<td>93.7 ± 3.8</td>
<td>72.3 ± 21.7</td>
<td>85.0 ± 12.8</td>
<td>0.003</td>
</tr>
<tr>
<td>DLCO (%)</td>
<td>98.5 ± 3.5</td>
<td>58.1 ± 20.3</td>
<td>67.0 ± 8.8</td>
<td>62.4 ± 21.5</td>
<td>43.0 ± 28.1</td>
<td>0.098</td>
</tr>
<tr>
<td>PPR (mmHg)</td>
<td>23.0 ± 4.2</td>
<td>70.5 ± 21.6</td>
<td>75.9 ± 19.0</td>
<td>99.6 ± 22.9</td>
<td>69.3 ± 20.1</td>
<td>0.006</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>4.3 ± 1.4</td>
<td>2.7 ± 1.1</td>
<td>4.1 ± 1.4</td>
<td>4.4 ± 1.5</td>
<td>5.9 ± 2.3</td>
<td>0.671</td>
</tr>
<tr>
<td>PVR (MPa s/m³)</td>
<td>11.4 ± 5.6</td>
<td>22.9 ± 11.2</td>
<td>18.7 ± 9.7</td>
<td>14.9 ± 5.7</td>
<td>2.8 ± 0.7</td>
<td>0.054</td>
</tr>
<tr>
<td>PAT ratio</td>
<td>2.20 ± 0.25</td>
<td>1.84 ± 0.51</td>
<td>1.89 ± 0.32</td>
<td>2.17 ± 0.62</td>
<td>1.66 ± 0.66</td>
<td>2.03 ± 0.38</td>
</tr>
</tbody>
</table>

* = $p < 0.05$ compare to control; $^5 = p < 0.01$ compare to control.

BMI = body mass index. CCB = calcium channel blocker. BL_sat = baseline oxygen saturation. DLCO = monoxide diffusion in the lung. Select EB = selective endothelin blocker. 6MWT = 6 min walking test. NYHA = New York Heart Association. PPR = pulmonary pressure. CO = cardiac output. PVR <hsp sp = 0.25> = peripheral vascular resistance in pascal seconds per cubic meter (MPa s/m³).
The PAT ratio was significantly lower than control values (2.20 ± 0.25) in the patients with PAH (1.84 ± 0.51, p = 0.023), chronic thromboembolic PAH (1.89 ± 0.32, p = 0.031), and scleroderma-PAH (1.66 ± 0.66, p = 0.014). There was no difference from the healthy controls in either between the Eisenmenger syndrome group (2.17 ± 0.62) or the control scleroderma group (2.03 ± 0.38) (Table 1). The PAT ratio was significantly correlated with disease severity, as measured by NYHA classification (r = −0.210, p = 0.035), systolic pulmonary pressure (r = −0.228, p = 0.035) (Figure 2), distance on the 6 min walk test (r = 0.215, p = 0.047), and oxygen saturation on effort (r = 0.207, p = 0.038). Exclusion of the Eisenmenger syndrome group — the only group with a normal PAT ratio — from the analysis yielded stronger correlations, as follows: NYHA: r = −0.253, p = 0.020; pulmonary pressure: r = −0.375, p = 0.002; 6 min walk distance: r = 0.255, p = 0.033. Furthermore, comparison of the patients with a PAT ratio above and below the cutoff for endothelial dysfunction (1.67) yielded a significantly higher pulmonary pressure (71.9 ± 20.8 vs. 66.7 ± 30.8 mmHg, p = 0.0473) and lower 6 min walk distance (376 ± 112 vs. 395.8 ± 106 m, p = 0.695) for those with endothelial dysfunction (Figure 3). When the Eisenmenger syndrome group was excluded from this analysis, the difference in pulmonary pressure was stronger (71.9 ± 21.3 vs. 57.9 ± 25.9 mmHg, p = 0.034).

Analysis of baseline PAT signal amplitude as a measure of vasodilatory potential (which is inversely related to the baseline signal) showed a significantly lower value in the scleroderma group compared to the other patient groups among whom the results were similar (not shown).

Discussing Cl

In the present study, 54 patients with PAH and 21 control subjects were assessed for endothelial dysfunction by the FMD test using the EndoPAT 2000 system. Our analysis yielded two main findings: (a) peripheral endothelial dysfunction occurs in idiopathic PAH, scleroderma-associated with PAH, and chronic thromboembolic PAH, but not in Eisenmenger syndrome. (b) The impaired hyperemic response significantly correlates with disease severity. These results appear to suggest a systemic component of endothelial dysfunction in these types of PAH. This is the first report demonstrating a quantitative association between systemic endothelial dysfunction and pulmonary arterial pressure in patients with PAH.

Support for systemic vascular involvement in PAH was provided by Hughes et al., who reported a significant reduction in brachial arterial dilatation in patients with idiopathic PAH and their relatives, and in patients with systemic sclerosis with pulmonary hypertension, however they did not demonstrate quantitative association with disease severity. Wolff et al. have described recently impaired peripheral endothelial function in severe IPAH. They did not find quantitative association between endothelial dysfunction and the hemodynamic measures, but they did find a significant association between endothelial dysfunction and the decrease in mean PAP in response to inhalation of aerosolized iloprost. Additionally,
Bull et al. observed a higher rate of circulating endothelial cells in patients with pulmonary hypertension, which was correlated with pulmonary pressure. Affected patients had higher values of CD36, a marker of microvascular origin, and of E-selectin, a marker of endothelial cell activation. The systemic involvement might be related primarily to the disease pathobiology, or mediated by a high activity of circulatory mediators in PAH such as pro-inflammatory cytokines, alterations in metabolic pathways of serotonin, prothrombotic abnormalities, hypoxia and sympathetic muscle nerve overactivity, or alteration in systemic metabolism. Our findings, together with the previous reports of a positive correlation of disease severity (measured by pulmonary pressure, 6 min walk test and oxygen desaturation) with the number of circulating endothelial cells and sympathetic overactivation, may indicate a tight linkage between the pulmonary disease and the peripheral endothelium. However, the exact contribution of each factor to the peripheral endothelial dysfunction needs to be further investigated in longitudinal studies.

The histopathological changes in various forms of PAH are qualitatively similar, but there are quantitative differences in their distribution and prevalence in the different components of the pulmonary vascular bed (arterioles, capillaries or veins). The main histopathological features of pulmonary arteriopathy are medial hypertrophy, intimal thickening, adventitial thickening, and complex lesions. Arteritis may be associated with plexiform lesions, and it is characterized by necrosis of the arterial wall with fibrinoid induration of inflammatory cells. All these findings are typical of the clinical classification groups of idiopathic PAH, familiar PAH, and PAH associated with connective tissue disease, congenital systemic-to-pulmonary shunts, portal hypertension, human immunodeficiency virus, and drugs, but different in CTEPH. Therefore, we expected to find qualitatively similar responses in all our patient groups except in the CTEPH. We speculated that the low baseline oxygenation in the patients with Eisenmenger syndrome was attributable to a compensation mechanism induced by long-standing disease that normalized the baseline signal amplitude and the consequent patient response to acute ischemia challenge on the hyperemic FMD test. Alternatively, it may indicate that in idiopathic, scleroderma-associated and thromboembolic PAH, the peripheral endothelial involvement is not a consequence of the pulmonary pressure itself, or of the hypoxia, but rather is associated with a potential primary or secondary mediator; this is not true of Eisenmenger syndrome, which is due to an anatomic abnormality. However, our findings are in disagreement with the study of Nakamura et al. wherein endothelial dysfunction was reported in patients with long-standing Eisenmenger syndrome and pulmonary hypertension. Additionally, it is interesting that although CTEPH mechanism is different from PAH, it seems that the endothelial function has altered in a similar fashion as it is in the PAH patients. This finding again supports the existence of unknown mediator.

Some authors claim that the pulmonary endothelial dysfunction in PAH leads to the chronic impairment of the production of vasodilators such as nitric oxide and prostacyclin, along with overexpression of vasoconstrictors, such as thromboxane A2 and endothelin-1. Since FMD is mediated by a nitric oxide-dependent mechanism, our findings may support an abnormality in peripheral nitric oxide-dependent vasodilatation in these subgroups of pulmonary hypertension. Accordingly, levels of sublingual glyceryl trinitrate, which induces nitric oxide-dependent vasodilatation, were found to be in the normal range in patients with idiopathic and scleroderma-associated PAH. Our results further emphasize Xu W et al. results who demonstrated inverse association between serum substrate arginine levels and PAH, as well as higher expression of Arginase II and lower NO synthesis in PAH.

Our study has a few limitations. First, the patients were treated with different regimens (Table 1), but ethically, we could not stop the medication prior to the study, nor testing sublingual Nitroglycerin effect on FMD. Nevertheless, the treatment regimen apparently had no effect on the PAT signal (baseline or ratio; data not shown). A longitudinal study is needed to understand the nature of this measure in PAH, its response to treatment, and its role in monitoring disease activity and/or severity. Second, the baseline signal amplitude was reduced in the patients with scleroderma and PAH, but normal in the control patients with scleroderma. Therefore, the use of the PAT signal as a parameter of scleroderma should be considered with caution.

In summary, this study supports the systemic involvement of endothelial dysfunction in idiopathic PAH, chronic thromboembolic PAH and scleroderma-associated PAH, which was correlated with disease severity.

**Conflict of interest statement**

None of the authors have a conflict of interest to declare in relation to this work.

**References**


