Effects of polycythemia on systemic endothelial function in chronic hypoxic lung disease

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Polycythemia occurs in the course of hypoxemic lung disease to maintain oxygen delivery despite the impaired gas exchange. The hematocrit is moderately elevated in 20% of patients with chronic obstructive pulmonary disease (COPD) (8). Because COPD is becoming increasingly prevalent in most countries and is now recognized as a major risk factor for atherosclerosis and cardiovascular complications (6, 29), understanding the effects of polycythemia on vascular function in patients with COPD is crucial.

One consequence of polycythemia is increased whole blood viscosity, which may promote circulatory disturbances including systemic arterial hypertension, thrombotic events, and stroke (18). Convincing data indicate that marked hematocrit elevation is associated with adverse vascular effects, including increased resistance and decreased blood flow to the brain or other organs (18). In patients with severe polycythemia, therapeutic interventions to lower the hematocrit improve cerebral blood flow, limb blood flow, and limb oxygen transport (31, 32). On the opposite side, moderate hematocrit elevation improves exercise tolerance and exercise capacity in athletes, as well as in individuals living in high altitudes (4, 13). The incidence of coronary artery disease also seems lower in hypoxemic polycythemic individuals living in high altitudes and in adults with congenital cyanotic heart disease compared with appropriate normocytic controls (13, 14). Thus the clinical impact of polycythemia in patients with chronic hypoxemia is debated, and the mechanisms by which polycythemia may affect the vasculature are incompletely understood.

Polycythemia is associated with increases in whole blood viscosity and vessel wall shear stress (WSS), which affect endothelial function (19). WSS is the main determinant of basal endothelium-derived nitric oxide (NO) release (24). In normocytic patients, lower hematocrit levels were associated with decreased flow-mediated vasodilation (FMD), in accordance with the shear stress reduction (15). In apparent contradiction to this finding, FMD was impaired in patients with polycythemia vera (21). Endothelial function in systemic arteries as assessed by measuring the vasodilator response to an acetylcholine infusion was severely altered in adults with congenital cyanotic heart disease (22), in keeping with our previous findings in the pulmonary circulation of polycythemic patients with COPD (11). In these studies, however, neither WSS nor FMD was measured, which hinders the interpretation of the results. Thus, whether polycythemia per se causes endothelial dysfunction is still controversial. Another point is that polycythemia occurring in response to chronic hypoxemia is often associated with pathological conditions such as inflammation, smoking, sleep apnea syndrome, and systemic hypertension, which may contribute to alterations in systemic vascular function.
we therefore designed the present study to assess endothelium-dependent vasodilation in 15 polycythemic and 13 non-polycythemic patients with COPD responsible for similar degrees of hypoxia and airway obstruction. Endothelium-dependent vasodilation was evaluated by recording brachial artery diameter variations in response to hypoxia and by using venous occlusion plethysmography to measure the forearm blood flow (FBF) response to brachial artery infusions of endothelium- and non-endothelium-dependent vasodilators. Infusion of the nitric oxide synthase (NOS) inhibitor L-arginine analog N-monomethyl-L-arginine (L-NMMA) was performed to assess the vascular effect of basal NO release. In addition, we examined a larger cohort of patients with COPD recruited prospectively at the Henri Mondor Teaching Hospital (Table 2). The data from 76 of these patients were published previously, but these earlier studies did not assess potential effects of the hemoglobin level on exercise performance (27). We defined normocytocemia as a hemoglobin level between 12 and 15.5 g/dl and moderate polycythemia as a hemoglobin level between 15.5 and 19 g/dl. This study was approved by the institutional review board of the Henri Mondor Teaching Hospital. All patients and controls signed an informed consent document before study inclusion.

**Measurements**

**Blood viscosity.** Blood viscosity ($\eta$) was measured using a calibrated coaxial cylinder Couette viscometer at shear rates of 0.01 s$^{-1}$ to 33 s$^{-1}$, under atmospheric pressure, at the native hematocrit and at 37°C (2). The shear-thinning curve (viscosity decrease against shear rate) was then analyzed using Quemada’s model, and viscosity at 100 s$^{-1}$ was derived (2).

**Wall shear stress.** WSS was calculated as previously described (2) from blood viscosity and brachial artery blood flow computed as $Q = V\pi r^2$, where $V$ is the mean blood velocity measured over three cardiac cycles using pulse-wave Doppler coupled to high-resolution B-mode ultrasound (Acuson Sequoia 512) and $r$ is the radius of the artery at the flow measurement site. WSS was calculated using the Poiseuille formula assuming non-Newtonian fluid and laminar flow (verified by determination of the Reynolds number) as $WSS = 4nQ/(\pi r^4)$, where WSS is wall shear stress, $\eta$ is blood viscosity, $Q$ is local blood flow at rest, and $r$ is the radius of the blood flow measurement site (23). Brachial arterial diameter and blood flow were not indexed to arm size or body surface area.

**Table 2. Characteristics of 93 normocytic and 23 polycythemic patients in the study of inflammation and exercise performance**

<table>
<thead>
<tr>
<th>N</th>
<th>Normocytic</th>
<th>Polycythemic</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>64.4 ± 1.0</td>
<td>61.1 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>F/M</td>
<td>26/67</td>
<td>3/20</td>
<td></td>
</tr>
<tr>
<td>Pack-yr</td>
<td>53.3 ± 3.3</td>
<td>42.6 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>25.8 ± 0.6</td>
<td>27.7 ± 0.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise specified. Cytokines were measured in 75 normocytic and 16 polycythemic patients. IL, interleukin; TNF-$\alpha$, tumor necrosis factor-$\alpha$; MCP-1, monocyte chemotactic protein-1.
FMD. FMD was evaluated as previously described, after 30-min bed rest, in a temperature-controlled room (22°C), with continuous blood pressure monitoring (Finapres 2300, Ohmeda, Madison, NJ) (1, 2). A high-resolution ultrasound Wall Track system (Pie Medical, Maastricht, The Netherlands) with a 7.5-Hz linear probe was used to measure the systolic and diastolic internal diameters of the distal brachial artery. This echo-tracking system, which analyzes radiofrequency signals, has 30-μm precision for diastolic diameter measurements. FMD was measured as the percentage increase in brachial artery diastolic diameter after 5 min of ischemia of the ipsilateral hand induced by inflating a wrist cuff (hyperemia test). The mean of three diastolic diameters was used as the basal diameter (D_B). The maximum diameter (D_M) was the greatest diastolic diameter following cuff deflation; measurements were made at deflation then every 30 s for 5 min. The measurement at deflation was the minimum diameter (D_h, for basal diameter). FMD was calculated as 100 × (D_M − D_h)/D_h.

FBF measurement by venous occlusion plethysmography. FBF was measured at the forearm using strain-gauge venous occlusion plethysmography (1). FMD was usually assessed 1 or 2 days before FBF assessment by venous occlusion plethysmography. A mercury-in-silastic strain gauge placed around the widest portion of the upper third of the forearm was electrically coupled to a plethysmograph (Perivin JSI 0539/H) calibrated to measure normalized changes in volume. For each measurement, venous flow was occluded just proximal to the elbow by rapidly inflating a blood pressure cuff to 40 mmHg. A wrist cuff was inflated to suprasystolic pressures starting 1 min before each measurement to exclude the hand circulation from the blood flow determination. FBF values are reported in milliliters per minute per 100 ml of forearm volume, and each value is the mean of at least three measurements. Systolic blood pressure (BP), diastolic BP, mean arterial BP, and heart rate were monitored continuously (Finapres 2300, Ohmeda). All studies were performed in the morning in a quiet temperature-controlled (22°C) room. While the patient was in the supine position, a catheter (Seldicath, diameter 1.0, Teflon ORX, 3P, Prodimed, Le Plessis Bouchard, France) was inserted after local anesthesia (1% xylocaine) into the brachial artery of the non-dominant arm, which was elevated slightly above the right atrium. To establish resting control FBF values, we administered 0.9% saline for 30 min. Vasoactive agent infusions were then started. Between each series of drug infusions, FBF was allowed to return to the basal value (this required ~20 min). Three drugs were used to explore endothelium-dependent vasodilation: acetylcholine (250–500 nmol/min, Clinalfa, Bubendorf, Switzerland) and isoptin (40 nmol/min). Each concentration of each drug was infused in random order except for isoptin and L-NMMA, which were infused in that order at the end of the procedure.

Plasma cytokine assays. Plasma levels of interleukins (IL)-6, -8, and -1β; monocyte chemotactic protein-1 (MCP-1); and tumor necrosis factor alpha (TNF-α) were determined using ELISAs (R&D Systems, Lille, France).

Statistical Analysis

All data are reported as the means ± SE. Normocytic and polycythemic patients were compared using the unpaired t-test for quantitative variables and the chi-square test for categorical variables. When the median was equal to the mean, the distribution was considered normal. ANOVA for repeated measurements was used to assess changes in FBF induced by drug infusions. When a significant difference was found, we compared individual means using the Scheffé test. Correlations between variables were evaluated using least-squares linear regression techniques (Pearson). Probability (P) values < 0.05 were considered statistically significant.

RESULTS

Study Population

The 15 polycythemic and 13 normocytic patients were similar regarding age, sex ratio, smoking history, blood pressure, and heart rate (Table 1). Lung function tests and arterial \( P_{O_2} \) (\( P_{ACO_2} \)) and \( P_{CO_2} \) (\( P_{ACO_2} \)) were not significantly different between the two groups. Hemoglobin concentrations ranged from 11.8 to 15.3 g/dl in the normocytic group and from 16.0 to 19 g/dl in the polycythemic group. Compared with the polycythemic patients, polycytic patients had higher body mass index (BMI) values, but the percentage of patients with treated systemic hypertension (3 patients in each group) or diabetes (1 patient in each group) was similar in the two groups. The two groups were subjected to a similar regimen of bronchodilator drugs. The two patients with OSA in the normocytic group were treated at the time of the study, compared with only two of the five patients with OSA in the polycythemic group. In accordance with the higher blood hematocrit and hematocrit levels in the polycythemic patients, this group had higher blood viscosity compared with the normocytic group (Table 1). However, the ratio of viscosity over hematocrit was not significantly different between the two groups.

Evaluation of Endothelial Function Using Brachial Arterial Diameter Monitoring

At baseline, blood flow, and calculated WSS were not significantly different between polycytic and normocytic patients (Table 1). The absence of difference in WSS resulted from the larger brachial artery diameter in the polycythemic group, which compensated for the higher blood viscosity. In the overall population, brachial artery diameter correlated positively with hemoglobin levels (\( P < 0.01 \); Fig. 1A). Brachial artery diameter did not correlate with \( P_{O_2} \) or oxygen saturation. In response to the hyperemia test, brachial artery flow increased to similar values in the two groups, but the accompanying increase in brachial artery diameter was greater in the polycythemic group (0.25 ± 0.02 vs. 0.15 ± 0.02 mm, respectively, \( P = 0.01 \); Fig. 1B; or 3.97 ± 0.39 vs. 2.85 ± 0.25%, respectively, \( P < 0.02 \); Fig. 1D). FMD correlated positively with the hemoglobin level (\( P < 0.01 \); Fig. 1C). No relationship was observed between FMD and other variables including age, sex, forced expiratory volume in 1 s (FEV_1), \( P_{ACO_2} \), systolic BP, and BMI.

Pharmacological Evaluation of Forearm Blood Flow Using Venous Occlusion Plethysmography

Baseline FBF values were not significantly different between the two groups. The FBF increase induced by incremental acetylcholine infusion rates was markedly blunted in the polycytic patients compared with the normocytic patients (\( P = 0.03 \), Fig. 2A). The percentage FBF increase after 80 μg/min of acetylcholine correlated negatively with hemoglobin (\( P = 0.02 \); Fig. 2B). No correlation was observed between the percentage FBF increase after acetylcholine and...
BMI, age, sex, FEV$_1$, Pa$_{O_2}$, or systolic BP. In contrast, the FBF increases induced by bradykinin and substance P were not significantly different between the polycythemic and normocytic patients (Fig. 3). Neither were significant differences found for the vasodilator response to the non-endothelium-dependent vasodilators SNP and isoptin (Fig. 4).

We then infused the specific NOS inhibitor L-NMMA to evaluate the biologic effect of basal NO on vascular tone.
Figure 5 shows that increasing l-NMMA infusion rates induced similar vasoconstrictor responses in polycythemic and normocytic patients, as judged by the absolute or percent decrease in FBF.

**Exercise Capacity in Polycythemic and Normocytic Patients**

To investigate whether polycythemia affected the functional status of the patients, we examined a larger cohort of patients with COPD investigated at our institution. We found that polycythemic patients (hemoglobin, 15.5–19 g/dl) did not differ from normocytic patients (hemoglobin, 12–15.5 g/dl) regarding PaO$_2$ or 6-min walking distance (Table 2). However, the 6-min walking distance correlated positively with PaO$_2$ ($P < 0.05$) and hemoglobin concentration ($P < 0.05$) in the overall study cohort. Plasma cytokine levels did not differ between polycythemic and normocytic patients.

**DISCUSSION**

Here, we investigated the effects of moderate polycythemia on endothelial function in patients with COPD, a condition associated with a high risk of cardiovascular disease. We found
the normocythemic patients, and this difference was mainly
significant between polycythemic and normocythemic pa-
tients. Blood viscosity was higher in the polycythemic than in
the normocythemic patients, although it tended to be higher in the polycythemic
patients; ANOVA detected no significant difference between the 2 groups. 

endothelial function plays a critical role in protecting
against platelet activation, thrombosis, and vascular remodeling. Endothelial cells respond to circulating vasoactive compounds and to variations in WSS, the dragging frictional force created by blood flow and blood viscosity (10). One conse-
quence of an acute or chronic WSS increase in large and small arteries is vessel caliber adjustment that restores WSS to its baseline value (10, 24). We first investigated baseline brachial artery flow, blood viscosity, and brachial artery diameter in polycythemic and normocythemic patients to determine base-
line WSS. We found that brachial artery flow did not differ significantly between polycythemic and normocythemic patients, although it tended to be higher in the polycythemic group. Blood viscosity was higher in the polycythemic than in the normocythemic patients, and this difference was mainly
due to a higher hematocrit level, since blood viscosity normal-
ized for hematocrit did not differ significantly between the two groups. Because the polycythemic patients also had a larger baseline brachial artery diameter, their baseline brachial artery WSS did not differ significantly from that in the normocythemic patients. Moreover, the acute WSS increase in response to reactive hyperemia induced a larger brachial artery diameter increase in the polycythemic than in the normocythemic patients. This difference occurred despite the similar relative increase in brachial artery flow generated by hyperemia in the two groups. Of note, FMD correlated positively to the hemoglobin concentration. These results suggest tight regulation of WSS, with the blood viscosity increase due to polycythemia being associated with an arterial diameter increase that main-
tains WSS within the normal range.

Both acute and chronic vessel diameter increases in response to shear stress involve endothelial NO release (5, 24). The similar vasoconstrictor response to L-NMMA, as judged by the FBF decrease, in polycythemic and normocythemic patients suggests that similar amounts of NO were released by the endothelium under baseline conditions in the two groups, in keeping with the similar basal WSS values. These results also suggest structural enlargement as opposed to active vasodilatation as the explanation to the increased vessel diameter in polycythemic patients under baseline conditions. Indeed, a chronic flow increase is associated with structural enlargement of systemic vessels (24). In contrast, the larger brachial artery diameter increase following hyperemia in the polycythemic patients might indicate increased NO release on stimulation. Overall, these results strongly suggest appropriate adjustment of systemic arteries to chronic or acute WSS increases via appropriate basal and stimulated NO release in polycythemic patients.

Our findings are consistent with studies in experimental animals with markedly or moderately elevated hematocrit val-
ues, in which endothelial NO maintained normotension, pre-
vented cardiovascular dysfunction, and exerted a key influence on survival (20, 25, 26). They conflict, however, with results from patients with polycythemia vera (21), a myeloproliferative syndrome in which thromboembolism remains a major cause of mortality and morbidity despite therapeutic interventions to decrease red cell mass. Studies in patients with polycythemia vera found decreased FMD values despite normal-
ized hematocrit levels. These results suggest that thrombosis in polycythemia vera may be partly ascribable to mechanisms other than increased blood viscosity or altered NO bioavail-
ability, such as erythrocyte adhesion to endothelial cells and platelet function abnormalities (30).

To further evaluate vascular reactivity in polycythemic vs.
normocythemic patients, we measured FBF during infusions of endothelium- and non-endothelium-dependent vasodilators. We found that the vasodilator response to acetylcholine was markedly impaired in the polycythemic patients compared with the normocythemic patients, whereas the vasodilator responses to SNP and isoptin did not differ significantly between the two groups. An impaired vasodilator response to acetylcholine coexisting with a normal vasodilator response to SNP was recently reported in polycythemic adult patients with cyanotic congenital heart disease and was interpreted as indicating endothelial dysfunction (22). We previously reported profound impairment of the acetylcholine-induced vasodilator response.

Figure 5. FBF variations in response to L-NMMA infusion into the brachial artery in polycythemic and normocythemic patients. ANOVA detected no significant difference between the 2 groups. Polycythemic patients; O, normocythemic patients.
in the pulmonary circulation of polycythemic patients with COPD, contrasting with a normal vasodilator response to inhaled NO (11). These results indicate clearly that polycythemia per se dramatically reduces acetylcholine-induced vasodilation, regardless of whether the cause is cardiac or pulmonary. On the other hand, we and others previously reported a major increase in acetylcholine-induced vasodilation in patients with anemia due either to sickle cell disease (SCD) or to other causes (2, 16). Here and in our previous study in patients with SCD, acetylcholine-induced vasodilation correlated negatively with the hemoglobin level. Thus the hemoglobin level may exert a major effect on acetylcholine responses in humans but not on responses to other endothelium-dependent dilators such as bradykinin and substance P. Another possible interpretation is that polycythemia is associated with improvements in the endothelial function of large arteries, which is highly dependent on shear stress, and with impaired endothelial function in the microcirculation. However, it is difficult to reconcile the fact that polycythemia impairs endothelial function in the microcirculation and at the same time increases oxygen delivery to the tissues, as seen in athletes, in whom polycythemia markedly improves exercise performance. Our findings are consistent with other studies showing impaired vasodilation in response to acetylcholine, despite preserved vasodilation induced by substance P (7, 12, 17). The mechanisms underlying these effects can only be speculated from our results, which is a limitation of our study. Changes in vascular muscarinic receptors may have played a role, as previously reported in elderly individuals (3).

Several studies found low prevalences of coronary artery disease in adults with congenital cyanotic heart disease or secondary polycythemia (13, 22). In contrast, patients with COPD are at high risk for cardiovascular disease (6, 29). Although widespread use of supplemental oxygen therapy has decreased the severity of polycythemia in patients with chronic hypoxemic lung disease, hematocrit elevation remains common, especially when sleep-disordered breathing is present. In our study population, 33% of polycythemic patients had OSA, an independent risk factor for atherosclerosis. COPD and OSA, separately or in combination, are major risk factors for cardiovascular disease and often cause polycythemia. Our present findings suggest that, in patients with COPD or OSA, polycythemia may protect vascular function by increasing the vessel diameter, thereby normalizing shear stress and increasing blood supply in a situation of chronic hypoxia.

One limitation of our study is that most of our “polycythemic” patients had moderate polycythemia and that the vascular effects of hematocrit elevation may vary according to the magnitude of the increase. Exercise performance improves with hematocrit elevation in athletes and with hemodilution in patients with COPD (9). In mice chronically treated with phlebotomies, Hôpital Henri Mondor) for assistance in the pharmacological evaluation of forearm blood flow using venous occlusion plethysmography.

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