Putative endothelial progenitor cells are associated with flow-mediated dilation in refractory hypertensives

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Abstract
Background. Hypertension has been related to endothelial dysfunction. Patients with refractory hypertension (RH) have a reduced number of endothelial progenitor cells (EPCs).

Aim. To evaluate if blood EPC levels relate to endothelium-dependent vasodilation (ED-VD) in RH.

Methods. We analyzed 29 RH confirmed by 24-h ambulatory blood pressure monitoring and assessed complete clinical and laboratory evaluation. EPCs were isolated from peripheral mononuclear cells (MNC) by flow cytometry. ED-VD was determined measuring flow-mediated dilation (FMD) by venous occlusion plethysmography.

Results. Circulating EPCs/10^5 MNC (median [Q1–Q3]): 23.0 [4.5–53.8]. FMD (median [Q1–Q3]): 211.7 [79.5–365.8]%.

Significant correlations with log-FMD: EPCs ($r = 0.469; p = 0.018$) and homocysteine ($r = -0.414; p = 0.045$). There was no collinearity between EPCs and homocysteine. FMD did not correlate with age, gender, office BP, 24-h systolic blood pressure or 24-h diastolic blood pressure, laboratory parameters, C-reactive-protein, left ventricular-mass index, dyslipidaemia, smoking habit and statin or angiotensin system blockers treatment. Multiple linear regression analysis showed that after age-adjustment, EPC ($p = 0.027$) and homocysteine ($p = 0.004$) were the only variables that predicted FMD ($R = 0.740$). After dividing patients according to EPC number, patients in the lower tertile showed a significantly reduced FMD compared with those in the group of the two upper tertiles of EPC: log-FMD (mean ± SD): 4.7 ± 0.9 vs 5.6 ± 0.8, respectively ($p = 0.031$).

Conclusions. ED-VD independently correlates with circulating EPCs in RH. Homocysteine is also an independent predictor of lower FMD in such patients.

Key Words: Endothelial progenitor cells, endothelium-dependent vasodilation, flow-mediated dilation, reactive hyperaemia, refractory hypertension

Introduction
The endothelium is a major regulator of vascular tone. It has been suggested that in patients with hypertension there is an abnormal vasorelaxation (1–3) probably as a consequence of the imbalance between the endothelial production of vasodilating and vasoconstricting mediators. Endothelial function, as evaluated in peripheral conduit vessels, including the brachial, femoral and carotid arteries, correlates with measurements in the coronary arteries and is abnormal in subjects at risk for atherosclerosis and in patients with coronary artery...
disease (4). Vascular endothelium contributes to the regulation of arterial tone and to the adaptation of arterial diameter to changes in flow or humoral mediators, which characterizes the flow-mediated dilation (FMD) (5). Brachial artery FMD measurement is a validated, non-invasive method widely used to quantify endothelial function, consisting in the dilation of a vessel when its endothelium is subjected to an increase in blood flow (6–8). Moreover, brachial FMD has also been shown to predict incident cardiovascular events such as stroke, myocardial infarction, coronary microvascular dysfunction and increased arterial stiffness, especially in older patients (9), but also in some high-risk patients such as those with peripheral artery disease or cardiovascular disease (10,11).

On the other hand, bone marrow-derived endothelial progenitor cells (EPCs) appear to play a critical role in repair and maintenance of the endothelial monolayer (12). Reduced number and function of EPCs has been associated with several traditional cardiovascular risk factors, such as aging, diabetes, hyperlipidaemia or renal failure (13–16). Recently, our group has demonstrated a reduced number of circulating EPCs in patients with refractory hypertension (RH) (17) although this relationship is less clear in patients with a lower grade of hypertension (18). Various studies suggest an evolving role for EPCs in structural and functional endothelial changes, mostly in the setting of endothelium damage processes (13–15), although there is scarce reported information about the potential mechanisms. Some inflammatory markers appear to concern the regulation of haematopoiesis and EPC-endothelium interaction in vitro, mainly the endothelial E-selectin (19) and the monocyte chemotactic protein-1 (20).

We hypothesized that a reduced number of EPCs could account for the endothelial dysfunction observed in RH. We aimed to examine the relationship between brachial FMD and EPCs.

Patients and Methods

Study population

The local institutional ethics committee approved the study protocol. Written informed consent was obtained from all participants in the study. The investigation conforms with the principles outlined in the declaration of Helsinki. Thirty-seven patients with confirmed RH diagnosis were consecutively enrolled in this cross-sectional study. RH was defined according to current guidelines (21) as a state of hypertension in which lowering systolic (SBP) and diastolic blood pressure (DBP) to goal had failed with a therapeutic plan that included at least three drugs in adequate doses, one of them a diuretic, besides attention to lifestyle measures and after ruling out secondary hypertension. After 5 min of rest in the sitting position, BP was measured and considered as the average of three measurements spaced by 2 min with validated oscillometric semi-automatic device and appropriate size cuffs. Hypertension was defined as office BP values equal or higher than 140 and/or 90 mmHg. Twenty-four-hour ambulatory blood pressure monitoring was performed in all patients with a validated Spacelabs 90207 device in order to confirm the diagnosis of RH. RH was confirmed if hypertensive individuals had mean 24-h BP values of more than 130/80 mmHg and these subjects were enrolled in the study. Diabetic patients as well as subjects who had suffered a cardiovascular event in the last 6 months, or patients with concomitant malignant diseases or active inflammation were excluded.

Clinical evaluation and biochemical analysis

Demographic and anthropometric characteristics, cardiovascular risk factors and clinical associated conditions were recorded. Hyperlipidaemia was defined as serum cholesterol level greater than 220 mg/dl (5.7 mmol/l) and/or serum triglyceride level above 150 mg/dl (1.7 mmol/l) or if treatment with lipid-lowering drugs had been implemented. Plasma homocysteine (Hcy) was determined by electrochemoluminiscence immunoassay. Smokers were considered as those with an active smoking habit over the last year. All medications, including antihypertensive drugs, were maintained in this observational study.

Measurement of inflammatory biomarkers

Fasting venous blood samples were drawn between 07:00 and 09:00 h. Sera were obtained by centrifugation and stored at −70°C until analysis. High-sensitivity C-reactive protein (hs-CRP) was determined by immunonephelometry. Adhesion molecules, selectins and chemokines were determined in duplicate wells using commercially available ELISA assays from R&D Systems, Minneapolis, MN (soluble vascular cell adhesion molecule-type 1 [sVCAM-1], soluble intercellular adhesion molecule-type 1 [sICAM-1], E-selectin, P-selectin and monocyte chemotactic protein-type 1 [MCP-1]).

Identification and quantification of circulating putative EPCs

In all participants, the total number of EPCs was assessed by using an in vitro assay as described in
detail previously (16,17). In brief, mononuclear cells (MNC) were obtained from peripheral blood sample (20 ml) by Ficoll density-gradient centrifugation (Histopaque 1077, Sigma St Louis, MO). EPCs were identified by flow cytometry (22) and their phenotype was determined by fluorescence-activated cell-sorting (FACS) analysis after staining with fluorescein isothiocyanate (FITC)-conjugated CD45 (Becton Dickinson), phycoerythrin-Cy5 (PE-Cy5)-conjugated CD34 (Becton Dickinson PharMingen, San Diego, CA) and with PE-conjugated CD133 antibodies (Miltenyi Biotec GmbH, Germany). Firstly, 300,000 cells per tube were softly resuspended in 50 µl of FACS buffer (that is, phosphate-buffered saline with 1% albumin and 0.1% sodium acide) plus 10 µl of fetal calf serum for 10 min at room temperature. For the labelling reaction, 3 µl of each antibody (1 µl per 1 x 10^5 cells) were added and incubated at room temperature, light protected, for 30 min. Samples were stored in the dark at 4°C overnight before the FACS lecture. Progenitor cells were identified by CD45 expression and separated by flow cytometry with right-angle light scatter properties. Cells expressing CD34 and CD133 were identified and quantified out of the progenitor population. To assess background, cells were stained with isotype-identical non-specific antibodies as negative controls. We analysed a minimum of 2 x 10^5 CD45+ cells. Results were plotted against SS (side scatter- ing) to identify putative EPC population as high CD34, high CD133, low SS and low CD45. Thus, putative early circulating EPCs were enumerated as CD34+/CD133+/CD45dim and expressed in absolute number of cells per 1 x 10^5 MNC.

**Brachial artery FMD**

Endothelium-dependent vasodilation (ED-VD) was assessed measuring the brachial artery FMD, a composite measure of endothelial integrity, by non-invasive venous occlusion plethysmography. All conduit arterial dilatory capacity studies were performed by the same operator. These procedures have been previously described in detail (7,23). Briefly, subjects were examined in the morning after an overnight fast and tobacco abstinence and were studied in quiet conditions while in the supine position in a temperature-controlled room. A blood pressure cuff placed at the level of the wider region of the arm and connected to a mercury-filled Silastic strain-gauge plethysmograph (EC5R; D.E. Hokanson, Inc., Bellevue, WA, USA) was quickly inflated at a pressure of 40 mmHg in order to occlude venous outflow from the arm. A second cuff placed at the level of the wrist was inflated to suprasystolic pressure for exactly 60 s before each forearm blood flow measurement to exclude the vascular bed of the hand. Blood flow measurements with the upper arm congesting cuff were carried out for 7 s in each 15-s cycle and the output signal was transmitted to a recorder. Baseline flow was determined as the average of seven such measurements and is expressed as millilitres per minute per 100 ml of forearm tissue. Reactive hyperaemia was induced by inflation of the distal pneumatic cuff to suprasystolic pressure for 5 min followed by its deflation. Endothelium-dependent vasomotion was defined as the percentage increase in blood flow from baseline to maximum post-cuff deflation blood flow and was expressed as percent FMD (%FMD) computed with the formula: [(maximum blood flow—baseline blood flow) x 100]/baseline blood flow. Values of FMD were given as the average of two consecutive measurements performed in each patient (three if more than 5% variability was observed).

**Statistical analysis**

Data are expressed as mean±SD or median (interquartile range) when the continuous variables were not normally distributed. Univariate relationships were performed with the use of Student’s t-test, Mann–Whitney rank-sum test, Spearman’s correlation or Pearson’s correlation as appropriate. To identify determinants of flow-mediated brachial reactivity in a multivariate setting, we used multiple linear regression on those variables that significantly correlated with FMD in the univariate analysis. The values of FMD were log_{10}-transformed before inclusion in multivariate model because of skewed distribution. Statistical significance was assumed if a null hypothesis could be rejected at $p = 0.05$. Despite the scarce reported information about relationship between FMD and EPC, we believed that it could be at least 0.5. Therefore, we calculated that 31 patients would suffice to have a statistical power of 80% to find a significant correlation index $\geq 0.5$. We also added extra 20% patients considering hypothetical withdrawals from the study. Finally, we did not have complete data for eight patients because of an equipment technical failure; these were considered missing completely at random and were eliminated from the analyses. However, the final number of included patients fits that required according to the performed power calculation. All analyses were performed with statistical package SAS 9.0 (SAS Institute Inc., Cary, NC, USA).
Results

Study subjects

Twenty-nine out of the 37 initially recruited patients with RH had valid measurements of FMD after reactive hyperaemia and valid determinations of EPC concentration. Recorded demographic and anthropometric characteristics, cardiovascular risk factors and clinical associated conditions were the following: mean age: 58.3 ± 10.1 years; 62% female; body mass index: 31.0 ± 4.4 kg/m². In total, 66% of subjects had hyperlipidaemia and 14% were current smokers. Physical inactivity was reported by 72% of patients. Five patients (17%) had developed a cardiovascular event prior to entering the study (stroke in three patients and myocardial infarction in two other patients). Forty-five per cent of the subjects received a statin as part of their lipid-lowering treatment, and 90% out of all patients were treated with a renin–angiotensin system blocker. As for other antihypertensive drugs, 72% of patients were treated with a calcium-channel blocker, 35% of subjects received a beta-blocker and 30% of them an α1-adrenergic antagonist. According to the definition of RH, all of them were treated with a diuretic, mostly hydrochlorothiazide. In total, 40% of subjects were treated with four or more drugs. Attending to laboratory parameters and BP data, the baseline characteristics are shown in Table I.

Table I. Baseline laboratory and blood pressure data.

<table>
<thead>
<tr>
<th>Blood pressure data</th>
<th>n=29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office SBP (mmHg)</td>
<td>161.7 ± 19.3</td>
</tr>
<tr>
<td>Office DBP (mmHg)</td>
<td>92.6 ± 12.2</td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>143.6 ± 14.2</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>82.2 ± 11.7</td>
</tr>
<tr>
<td>Laboratory parameters</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>207.0 ± 23.7</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>131.5 ± 19.3</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>59.4 ± 17.1</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>101 [81.5–126.0]</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>100.6 ± 11.3</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (%)</td>
<td>4.7 [4.3–5.1]</td>
</tr>
<tr>
<td>hs-CRP (mg/dl)</td>
<td>0.40 [0.20–0.75]</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>9.3 ± 3.2</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.02 ± 0.14</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>67.8 ± 10.6</td>
</tr>
<tr>
<td>UACR (µg/mg)</td>
<td>6.5 [4.2–30.0]</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate by the MDRD (Modification of Diet in Renal Diseases study) equation; UACR, urinary albumin:creatinine ratio. Data are expressed as the mean value ± SD or median [quartile 1–3].

Circulating putative EPCs

Regarding the number of circulating putative EPCs, median [interquartile range] was 23.0 [4.5–53.8] EPCs per 10⁵ MNC, ranging between 2 and 107 EPCs per 10⁵ MNC.

ED-VD

FMD after reactive hyperaemia (shown as median [IQR]), was 211.7% [79.5–365.8]. We next assessed whether flow-mediated brachial reactivity correlated with the presence or absence of conventional and newer cardiovascular risk factors as well as the levels of circulating putative EPCs and inflammatory markers. The univariate analyses showed an inverse relationship between FMD and serum levels of MCP-1 (r = −0.52; p = 0.006), E-selectin (r = −0.57; p = 0.002) and P-selectin (r = −0.41; p = 0.041). Otherwise, no relationship was found between FMD and serum levels of hs-CRP, sVCAM-1 or sICAM-1. As for the remaining univariate analysis after age adjustment, the only significant correlations with FMD were circulating EPCs (r = 0.47; p = 0.018) (Figure 1) and plasma Hcy (r = −0.41; p = 0.045). Correlation with 24-h SBP was only marginally significant (r = −0.39; p = 0.05). Other correlations with FMD that proved to be non-significant in the univariate analysis were age, gender, body mass index, office BP, 24-h DBP, laboratory biochemistry parameters, dyslipidaemia, smoking habit, physical inactivity, previous cardiovascular diseases and statins or renin–angiotensin system blockers treatment (data not shown).

Independent predictors of ED-VD

To estimate the predictive value of EPC number in explaining variability of ED-VD measured by FMD, a multiple linear regression analysis was performed. In this analysis, we included those parameters known to influence vascular function and progression of atherosclerosis, which correlated with FMD in the univariate analyses. Thus, circulating EPC number, Hcy levels and serum levels of E-selectin were included as covariates. MCP-1 and P-selectin levels were not introduced in the model because they showed collinearity with E-selectin values. Twenty-four-hour SBP was also forced in the model because of its clinical relevance. As shown in Table II, the final model reveals that after age adjustment only the number of circulating EPCs and Hcy levels were independently associated with FMD (R = 0.740, p = 0.001). Interestingly, a reciprocal analysis that divided subjects into two groups according to EPC
number also demonstrated a striking relationship between the number of circulating EPCs and FMD. The subjects in the group with the lower tertile of EPCs, compared with those in the group of the two upper tertiles of EPCs, showed a significantly reduced FMD, while there were no differences between both groups as for known determinants of circulating EPC number such as age, 24-h SBP or plasma Hcy levels, nor with inflammatory biomarkers (Table III).

Discussion

In this study, we observed that circulating putative EPC number was associated with impaired conduit arterial dilatory capacity, assessed by brachial FMD, in subjects with RH. We have established this association in a well-characterized population of patients with RH, whereby potential confounders were discarded to be responsible for this relationship. This association has previously been reported in other populations, such as patients with coronary artery disease (24) and a small sample of healthy South Asian men (25), but not in patients with hypertension. On the other hand, in our study only plasma Hcy was also related to FMD, independently of circulating EPCs.

The most widely used method to evaluate endothelial function in humans has been blood flow study. This method permits the assessment of agonist-induced vasomotor reactivity or vasodilation in response to reactive hyperaemia, i.e. flow-dependent vasodilation (3,26). Although some invasive vasomotor techniques are considered the diagnostic standard for the evaluation of ED-VD, the non-invasive brachial artery FMD measurement is a broadly applicable method that is used for the examination of endothelial function (6–8).

Table II. Multivariate linear regression analysis: Independent factors associated with flow-mediated dilation.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Unstandardized coefficient (B) (95% CI)</th>
<th>Standardized coefficient (β)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine</td>
<td>−0.171 (−0.280 to −0.061)</td>
<td>−0.547</td>
<td>0.004</td>
</tr>
<tr>
<td>Circulating EPCs</td>
<td>0.012 (0.002 to 0.022)</td>
<td>0.385</td>
<td>0.027</td>
</tr>
</tbody>
</table>

EPCs, endothelial progenitor cells. Data are expressed as regression coefficients and p-values. Dependent variable: log-transformed flow-mediated dilation; R=0.740, p=0.001.
Endothelial progenitor cells and flow-mediated dilation

Table III. Characteristics of patients with refractory hypertension according to the number of endothelial progenitor cells (EPCs), comparing patients with EPC count within the lower tertile (15 EPC per 10^5 mononuclear cells is the cut-off value) with patients with EPC count within the two upper tertiles.

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n=29)</th>
<th>Lower EPC count (&lt;15)</th>
<th>Higher EPC count (&gt;16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>58.3 ± 10.1</td>
<td>60.1 ± 9.7</td>
<td>57.3 ± 10.7</td>
<td>0.49</td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>143.6 ± 14.2</td>
<td>146.6 ± 7.9</td>
<td>140.9 ± 16.3</td>
<td>0.23</td>
</tr>
<tr>
<td>MCP-1 (pg ml^{-1})</td>
<td>457.1 [332.0–531.9]</td>
<td>488.8 [351.4–562.6]</td>
<td>380.2 [309.9–521.6]</td>
<td>0.21</td>
</tr>
<tr>
<td>E-selectin (ng ml^{-1})</td>
<td>38.9 [22.3–77.8]</td>
<td>68.0 [22.0–81.2]</td>
<td>33.0 [21.5–73.9]</td>
<td>0.33</td>
</tr>
<tr>
<td>P-selectin (ng ml^{-1})</td>
<td>129.8 [110.9–171.4]</td>
<td>141.3 [125.7–185.0]</td>
<td>119.5 [103.7–164.6]</td>
<td>0.052</td>
</tr>
<tr>
<td>Homocysteine (µmol/l)</td>
<td>9.3 ± 3.2</td>
<td>10.6 ± 3.3</td>
<td>8.9 ± 2.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Log-FMD</td>
<td>5.2 ± 1.0</td>
<td>4.7 ± 0.9</td>
<td>5.6 ± 0.8</td>
<td>0.031</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; MCP-1, monocyte chemotactic protein type 1; log-FMD, log-transformed flow-mediated dilation. Data are expressed as the mean value ± SD or median [quartile 1–3]. p-value has been calculated through a t-test comparison of the highest and lowest cell-count groups.

Hypertension is associated with abnormal endothelial function in peripheral, coronary and renal circulation in most studies. This endothelial dysfunction has been suggested to represent an important mechanism, whereby hypertension promotes the development and progression of vascular disease. There is evidence that sustained arterial hypertension blunts FMD in conduit arteries in the peripheral and coronary circulation, although the pathogenesis of this association is not fully understood (27). Our results show an independent positive relationship between the number of circulating EPCs and brachial FMD, suggesting that the endothelial dysfunction observed in patients with RH could be related to an imbalance in the endothelial homeostasis, as a consequence of a downregulation of EPCs. Some other authors have approached this subject previously. Hill et al. (13) found similar results, but their study only included healthy male and only 22% of them were hypertensives. More recently, Heiss et al. (28) showed a significant relationship between FMD and migration and proliferation of EPCs in older healthy subjects without major cardiovascular risk factors with respect to younger, but FMD failed to correlate with circulating EPCs. We here demonstrate a significant correlation between FMD and the number of circulating EPCs, some markers of inflammation and plasma Hcy levels. In a multivariate model, both a reduced number of circulating EPCs and high plasma Hcy levels are independent predictors of impaired ED-VD. There was no collinearity between EPCs and Hcy, indicating that downregulation of EPCs in patients with RH may modify endothelial function through a different way from that related to Hcy. Although Zhu et al. (15) found an inverse correlation between EPCs and Hcy, our own previous study showed a reduced number of EPCs in refractory hypertensive patients with respect to healthy subjects with equivalent plasma Hcy levels (17). An impaired ED-VD has been shown in patients with even mild hyperhomocysteinemia and with no other identifiable vascular risk factors (29), but the underlying pathobiological mechanisms for this association are not entirely understood. The most important is thought to involve the influence of Hcy on the bioavailability of nitric oxide, a mediator of vasoreactivity (30). Hyperhomocysteinemia seems to lead to increased oxidative inactivation of nitric oxide by oxygen-derived free radicals. Additionally, elevated Hcy levels have been found to induce the expression of several chemokines and adhesion molecules (27). Our results show a relationship between FMD and some inflammatory markers such as E- and P-selectins and MCP-1. However, they lose significance in the multivariate analysis, whereby putative EPC number and Hcy remain as independent predictors of FMD, suggesting that they are more powerful markers of endothelial dysfunction and that the inflammatory markers may rely on one or both of them.

Although we previously demonstrated a reduced number and function of EPCs in patients with RH, we did not study the mechanisms of this relationship nor could we elucidate if hypertension was the cause or the consequence of this downregulation of stem cells of endothelium lineage (17). Anyway, downregulation of EPCs may be responsible for an imbalance between vascular injury and vascular repair, leading to atherosclerosis. Impaired endothelium-dependent dilatation measured by FMD may reflect atherosclerotic process in these patients with RH. Furthermore, this EPC downregulation-related impairment of arterial dilatory capacity may contribute to the known increased cardiovascular risk of these patients.

There are several limitations that should be pointed out. First of all, the size of our study may be responsible for the lack of a clear relationship...
between FMD and BP, although it must be emphasized that all patients have RH, so BP did not show wide differences in this sample population. Another possibility is the fact that if RH-related downregulation of EPCs is responsible for the impaired ED-VD, perhaps circulating EPC would be a more important surrogate marker than BP itself. Similarly, the nature of the study does not permit to establish a definitive cause-and-effect relation between a decrease in EPCs and an impaired flow-mediated brachial vascular repair, neither can we deduce the mechanisms for this relationship. As for the tool to measure FMD, we used reactive hyperaemia, perhaps not the most spread method but acceptable enough because it has been shown that peripheral flow reserve correlates with FMD (26).

We believe that EPCs could be a valuable surrogate marker of ED-VD. EPCs-downregulation may be responsible for an imbalance between vascular injury and vascular repair in patients with RH, leading to atherosclerosis and contributing to the known increased cardiovascular risk of these patients. Endothelium-dependent dilatation measured by FMD may reflect this atherosclerotic process. It still remains to be seen whether therapeutic approaches targeting EPCs can be translated into therapeutic benefit by improving endothelial function and preventing the vascular complications associated with the hypertensive process.

In conclusion, the data provided by our study show that ED-VD assessed by brachial artery FMD independently correlates with circulating putative EPCs in patients with RH. A reduction in circulating EPCs could account for the impaired endothelial function in RH patients. Hcy is also an independent predictor of lower FMD in them.

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Conflict of interest statement: None.

References


