Increased Circulating Inflammatory Endothelial Cells in Blacks With Essential Hypertension


Abstract—Morbidity and mortality attributable to hypertension are higher in black essential hypertensive (EH) compared with white EH patients, possibly related to differential effects on vascular injury and repair. Although circulating endothelial progenitor cells (EPCs) preserve endothelial integrity, inflammatory endothelial cells (IECs) detach from sites of injury and represent markers of vascular damage. We hypothesized that blood levels of IECs and inflammatory markers would be higher in black EH compared with white EH patients. Inferior vena cava and renal vein levels of CD34+/KDR+ (EPC) and VAP-1+ (IEC) cells were measured by fluorescence-activated cell sorting in white EH and black EH patients under fixed sodium intake and blockade of the renin–angiotensin system, and compared with systemic levels in normotensive control subjects (n=19 each). Renal vein and inferior vena cava levels of inflammatory cytokines and EPC homing factors were measured by Luminex. Blood pressure, serum creatinine, lipids, and antihypertensive medications did not differ between white and black EH patients, and EPC levels were decreased in both. Circulating IEC levels were elevated in black EH patients, and inversely correlated with EPC levels (R²=0.58; P=0.0001). Systemic levels of inflammatory cytokines and EPC homing factors were higher in black EH compared with white EH patients, and correlated directly with IECs. Renal vein inflammatory cytokines, EPCs, and IECs did not differ from their circulating levels. Most IECs expressed endothelial markers, fewer expressed progenitor cell markers, but none showed lymphocyte or phagocytic cell markers. Thus, increased release of cytokines and IECs in black EH patients may impair EPC reparative capacity and aggravate vascular damage, and accelerate hypertension-related complications. (Hypertension. 2013;62:585-591.) • Online Data Supplement

Key Words: blacks, hypertension, inflammation, progenitor cells

Black individuals have a higher incidence and prevalence of essential hypertension (EH) compared with whites.1 Furthermore, morbidity and mortality rates are higher in black EH compared with white EH patients.2,3 Over the past decade, factors suggested to explain this ethnic disparity included dietary habits, obesity, and insulin resistance.4 Augmented activity of the Na-K-2Cl cotransport in the thick ascending limb of Henle’s loop,5 a strong correlation between uric acid levels and total peripheral resistance,6 and increased activity of the amiloride-sensitive epithelial sodium channel7 have been also reported. Furthermore, studies using mapping by admixture disequilibrium have identified a strong association between gene variants in a region on chromosome 22 and the development of hypertensive renal disease in black individuals.8,9 Although the mechanisms responsible for the development of hypertension in black EH patients carrying these gene variants remain unknown,10 mutations in the nonmuscle myosin heavy chain gene (MYH)9 have been associated with disease syndromes that include leukocyte inclusion bodies, suggesting higher propensity for inflammation.11

In turn, inflammation plays a central role in mediating vascular dysfunction in hypertensive patients.12 For example, elevated circulating levels of transforming growth factor-β,13 interleukin-6, tumor necrosis factor (TNF)-α, and matrix metalloproteinase-2 directly correlate with the extent of pulse pressure in black EH patients.14 Furthermore, endothelial activation is higher in ethnic minority participants (comprising black, Hispanic Americans, and Asian Americans) than among European American populations, suggesting an important role of inflammation, endothelial activation, and matrix remodeling in the pathogenesis of hypertension and its complications in ethnic minorities.15

Circulating inflammatory endothelial cells (IECs), which detach from the vessel wall in sites of vascular injury, hold potential to become unique markers of endothelial inflammation. These cells are characterized by the expression of vascular adhesion protein (VAP)-1, a cell adhesion molecule that...
contributes to leukocyte extravasation and promotes formation of hydrogen peroxide, increasing oxidative stress and magnifying the inflammatory response. Importantly, an increased inflammatory status may impair repair mechanisms, such as recruitment of endothelial progenitor cells (EPCs) to the site of injury. These bone-marrow–derived cells have the ability to proliferate, migrate, and differentiate into mature endothelial cells, contributing to vascular repair. However, whether the number of circulating EPCs and IECs is altered in black EH patients remains unknown. Therefore, we hypothesized that greater levels of inflammation in black EH patients with relatively preserved renal function would be associated with elevated circulating IEC and lower EPC levels compared with healthy individuals. Moreover, considering their increased propensity for hypertensive renal injury, we also tested whether renal vein levels of inflammatory markers, EPCs, and IECs were particularly pronounced in black EH patients.

### Methods

This study was approved by the institutional review board at the Mayo Clinic. Black (n=19) and white (n=19) patients with diagnosis of EH were enrolled in the study from January 2008 to January 2012. The control group consisted of 19 age- and sex-matched normotensive healthy volunteers (HV), prospectively recruited through the Mayo Clinic Biobank.

In all patients, clinical and laboratory parameters were collected via the electronic medical records. Estimated glomerular filtration rate was calculated using the chronic kidney disease epidemiology collaboration formula. Urine protein levels (mg/24 hour) were measured by standard procedures in samples collected from white and black EH patients, and 16 consenting healthy age-matched potential kidney donors.

### Inflammatory Biomarkers and EPC Homing Factors

Peripheral blood (in HV), renal vein, and inferior vena cava (in EH patients) samples were collected. Renal vein and circulating levels of soluble E-selectin, soluble vascular cell adhesion molecule-1, intercellular adhesion molecule-1, myeloperoxidase, plasminogen activator-inhibitor-1, monocyte chemotractant protein-1, macrophage inflammatory protein-1, TNF-α, interleukin-6, adiponectin, matrix metalloproteinase-9, stromal cell–derived factor-1, and stromal cell factor-1 were measured by luminex. EPCs and IECs were isolated from fresh blood samples by the density-gradient method and subsequently characterized for antigen expression of EPC and IEC markers. Systemic and renal vein levels of CD34+/KDR+ (EPC) and VAP-1+ (IEC) cells were determined by fluorescence-activated cell sorting, as previously described. Results were expressed as percentage of EPCs or IECs (per 100 000 cell counts). To further characterize IECs, VAP-1+ cells were also stained with endothelial (CD31), lymphocyte (CD3 and CD45), monocyte/macrophage (CD16 and CD14), and progenitor cell (CD34 and CD133) markers and analyzed using fluorescence-activated cell sorting. For detailed Methods and statistical analysis see the online-only Data Supplement.

### Results

Table 1 shows clinical, laboratory, and demographic characteristics of the patients included in the study. Body mass index was higher in black EH compared with HV and white EH patients (both P<0.05). Systolic, diastolic, and mean blood pressures were similarly higher in both hypertensive groups compared with HV, whereas cholesterol fractions and triglyceride levels did not differ among the groups. Antihypertensive medications were similar between EH patients. C-reactive protein levels were similarly higher in both hypertensive groups compared with normal. Serum creatinine levels were similar among the groups, but estimated glomerular filtration rate was higher in black EH compared with HV and white EH patients. Urinary protein levels did not differ among the groups.

### Inflammatory Biomarkers and EPC Homing Factors

Systemic and renal vein levels of soluble E-selectin, soluble vascular cell adhesion molecule-1, and stromal cell–derived factor-1 were elevated in both hypertensive groups compared with HV, but higher in black EH compared with white EH.
patients (Table 2). In contrast, systemic and renal vein levels of myeloperoxidase, plasminogen activator inhibitor-1, monocyte chemoattractant protein-1, macrophage inflammatory protein-1δ, and stromal cell factor were only elevated in black EH patients (Table 2; \( P<0.05 \) versus HV and \( P<0.05 \) versus white EH). Contrarily, systemic and renal vein levels of soluble intercellular adhesion molecule-1, TNF-α, interleukin-6, and adiponectin were similar among the groups.

### EPCs and IECs

Systemic and renal vein EPC levels were both similarly lower in both hypertensive groups compared with HV, whereas IEC levels were higher in black EH compared with HV and white EH patients (Figure 1A).

Statistical differences in the levels of EPCs and IECs between the groups persisted after adjustment for body mass index and estimated glomerular filtration rate (Figure S1A–S1D in the online-only Data Supplement). Notably, an inverse correlation was found between the number of circulating EPCs and IECs in black EH patients (Figure 1B), but not in white EH patients (\( R^2=0.03; \ P=0.47 \)). Furthermore, systemic levels of the inflammatory cytokines E-Selectin, myeloperoxidase, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1δ in black EH patients correlated directly with IEC levels (Figure 2A, 2C, 2E, and 2G), but inversely with EPC levels (Figure 2B, 2D, 2F, and 2H). Renal vein levels of IECs and EPCs showed similar relationships (data not shown).

### IEC Characterization

A large fraction of VAP-1+ cells costained with CD31, but not with CD3, CD45, CD16, or CD14 (Figure S2A–S2E). Finally, a large fraction of VAP-1+ cells costained with CD31, but not with CD3, CD45, CD16, or CD14 (Figure S2A–S2E). An important proportion of VAP-1+ cells expressed CD34 and CD133 (Figure S2F and S2G). An important proportion of VAP-1+ cells expressed CD34 and CD133 (Figure S2F and S2G).

### Systemic and Renal Vein (Mean±SD) Cytokine Levels in HV, White EH, and Black EH Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HV</th>
<th>White EH</th>
<th>Black EH</th>
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<tr>
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<td>Systemic</td>
<td>Systemic</td>
<td>Renal Vein</td>
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<td><strong>Inflammatory markers</strong></td>
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<tr>
<td>sE-Selectin, pg/mL</td>
<td>15.2±8.3</td>
<td>27.8±14.7*</td>
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<td>sVCAM-1, pg/mL</td>
<td>683.8±299.7</td>
<td>1087.5±295.9*</td>
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<td>sICAM-1, pg/mL</td>
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<td>MPO, pg/mL</td>
<td>161.6±6.9</td>
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<td>PAI-1, pg/mL</td>
<td>22.9±20.8</td>
<td>33.2±29.8</td>
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<td>MCP-1, pg/mL</td>
<td>118.7±29.4</td>
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<td>MIP-1β, pg/mL</td>
<td>4109.9±3328.1</td>
<td>4777.2±2291.7</td>
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<td>TNF-α, pg/mL</td>
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<td>IL-6, pg/mL</td>
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<td>14.4±29.5</td>
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<td>Adiponectin, pg/mL</td>
<td>7012.7±854.0</td>
<td>7618.0±14868.6</td>
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<td>MMP-9, pg/mL</td>
<td>80.12±45.2</td>
<td>76.2±40.4</td>
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<td>SDF-1, pg/mL</td>
<td>1380.8±426.3</td>
<td>2108.8±1567.2</td>
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<tr>
<td>SCF, pg/mL</td>
<td>9.5±10.6</td>
<td>18.7±17.7*</td>
<td>19.1±19.5*</td>
</tr>
</tbody>
</table>

EH indicates essential hypertensive; EPC, endothelial progenitor cells; HV, healthy volunteers; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1β; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; PAI-1, plasminogen activator inhibitor-1; SDF, stromal cell factor; SDF-1, stromal cell–derived factor-1; sE-Selectin, soluble E-selectin; sVCAM-1, soluble vascular cell adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1; and TNF, tumor necrosis factor.

*\( P<0.05 \) vs HV.

†\( P<0.05 \) vs EH.

### Discussion

The current study shows elevated circulating and renal vein levels of IECs and inflammatory cytokines in black EH compared with white EH patients, despite controlled blood pressure and preserved renal function. Furthermore, we found reduced levels of renal vein and circulating EPCs in both hypertensive groups, which may suggest impaired reparative mechanisms. These observations may imply higher propensity for vascular injury in black EH compared with white EH patients and introduce IECs as novel markers of inflammatory endothelial injury in hypertensive patients. The current study may also suggest a differential mechanism of end-organ damage in black EH compared with white EH patients, which may have therapeutic implications.

Hypertension remains one of the major risk factors for cardiovascular and cerebrovascular diseases, which affects 1 in 3 US adults, with estimated annual costs of $50.6 billion.\(^1\) Importantly, recent projections show that an additional 27 million people could have hypertension by 2030, a 9.9% increase in prevalence from 2010.\(^2\) There is now compelling evidence that hypertension affects black individuals disproportionately, as their prevalence of hypertension remains one of the highest in the world.\(^3\) Furthermore, black EH patients develop hypertension at an earlier age and cardiac (left ventricular mass and posterior wall thickness) and renal hemodynamic involvement is more severe in these patients compared with white EH,\(^4\) suggesting greater end-organ damage.

Polymorphisms in several genes involved in blood pressure regulation have been associated with development of hypertension in black EH patients.\(^7,23,24\) For example, studies identified variants within a 60-kb region of chromosome 22 containing part of the apolipoprotein L1\(^9\) and MYH9\(^6\) genes (associated with increased risk of hypertensive renal disease) in blacks.
underscoring important contributions of genetic factors to the development of hypertension in black individuals. Furthermore, heterozygous mutations in the MYH9 have been associated with 4 autosomal-dominant clinical syndromes characterized by neutrophil inclusion bodies, implying increased susceptibility for systemic inflammation in these individuals.\textsuperscript{11}

Indeed, inflammation and endothelial activation play a critical role in impairing vascular function in hypertensive patients. De la Sierra et al\textsuperscript{12} reported increased levels of E-selectin, P-selectin, monocyte chemoattractant protein-1, and tissue inhibitor of metalloproteinases-1 in patients with impaired acetylcholine-dependent vasodilation. In turn, endothelial dysfunction is associated with a higher incidence of myocardial infarction, angina, coronary revascularization procedures, cerebrovascular events, and aortoiliac occlusive disease in hypertensive patients, underscoring the prognostic role of endothelial dysfunction in the development of cardiovascular complications.\textsuperscript{25} However, inflammatory markers alone may lack specificity in predicting disease progression in hypertensive patients,\textsuperscript{26} emphasizing the need for alternative markers of endothelial injury.

Figure 1. A, Representative flow cytometric dot plots for CD34+/KDR+ endothelial progenitor cells (EPCs) and VAP-1+ inflammatory endothelial cells (IECs) and quantification of their inferior vena cava (IVC) and renal vein (RV) levels in healthy volunteers (HV), white essential hypertensive (EH), and black EH patients. B, Systemic levels of IECs inversely correlated with circulating EPC levels in black EH patients (E). *$P \leq 0.05$ vs HV; and ‡$P \leq 0.05$ vs EH.
Circulating IECs, released from sites of vascular injury, might reflect the extent of endothelial injury in kidney and vascular diseases. These cells are characterized by expression of VAP-1, a cell adhesion molecule that mediates binding, rolling, and transmigration of lymphocytes to the endothelium. Moreover, VAP-1 is a semicarbazide-sensitive amine oxidase that catalyzes a reaction that promotes the formation of hydrogen peroxide and ammonium, increasing oxidative stress and enhancing the inflammatory response. Recent data suggest that VAP-1 may predict cardiovascular mortality in subjects with type II diabetes mellitus. Likewise, VAP-1 levels are elevated in chronic kidney disease and fall with time after kidney transplantation. However, whether circulating and renal vein levels of VAP–1–expressing cells are elevated in hypertensive individuals remained unknown.

This study shows higher renal vein and circulating IEC levels in black EH compared with white EH patients and HV, which may implicate these cells in the pathogenesis or sequelae of hypertension in black individuals. Importantly, neither body mass index nor estimated glomerular filtration rate alone seem to have accounted for the increase in IEC levels in our study because the differences persisted after adjustment for both measurements.

We also found that systemic and renal vein levels of multiple inflammatory biomarkers were elevated in black EH patients compared with HV and white EH patients, which may indicate increased systemic inflammation in this ethnic group. In particular, systemic levels of soluble E-selectin and soluble vascular cell adhesion molecule-1 were higher in black EH compared with EH patients and HV, suggesting higher endothelial activation in this ethnic group, as previously documented. Conversely, circulating and renal vein levels of several inflammatory markers remained within normal range in white EH patients, in agreement with previous observations from our group that showed similar levels of interferon-γ, TNF-α, TNF receptor-1, and macrophage inflammatory protein-1β between HV and white EH patients treated with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. Notably, the lack of gradient between circulating and renal vein cytokine or IEC levels might argue against the kidney as the main source of inflammation.

Figure 2. Systemic levels of soluble E-selectin (sE-Selectin), myeloperoxidase (MPO), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 δ (MIP-1δ) correlated directly with the number of circulating inflammatory endothelial cells (IECs; A, C, E, and G), but inversely with the number of circulating endothelial progenitor cells (EPCs; B, D, F, and H).
source of systemic inflammation in black EH patients, suggesting increased systemic vascular injury. This, in turn, may induce end-organ damage in black EH compared with white EH patients. In agreement, increased inflammation and oxidative stress in black EH patients is associated with diminished endothelial function, which may imply a critical role for these pathways in aggravating cardiovascular disease.

Importantly, systemic inflammation can trigger mobilization, homing, and transdifferentiation of EPCs, which play a major role in regulation and protection of the endothelium after vascular injury. Previous studies have shown that endothelial repair capacity of EPCs is reduced in prehypertensive patients, which might represent an early event in the development of hypertension. Similarly, we have previously shown that circulating EPC levels are lower in EH patients compared with HV, suggesting inadequate reparative capacity. In our study, renal vein and circulating EPC levels were similarly reduced in both hypertensive groups, which may argue against ethnic disparities in their mobilization. However, the inverse correlation between circulating IEC and EPC levels in black EH patients may suggest that IECs might interfere with the recruitment or functional capacity for endothelial repair by recruited EPCs.

In agreement with a previous study, IECs expressed endothelial markers, but not lymphocyte, monocyte, or macrophage markers. Interestingly, we found that an important fraction of these cells expressed the progenitor cell markers CD133 and CD34, suggesting that some of these cells might represent inflammatory progenitor cells. Whether these VAP-1+ progenitor cells have impaired reparative capacity or mediate vascular injury warrants further investigation.

Our study is limited by its cross-sectional nature and relatively small study population. In addition, treatment with calcium channel blockers, angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers, or statins might have masked elevation in some circulating cytokines or IECs in both black and white EH patients. Further studies are also needed to explore in more detail the precise cause and effect relationships between IEC levels and reparative EPC capacity in black EH patients.

**Perspectives**

Our results show that, despite preserved kidney function and controlled blood pressure, renal vein and circulating inflammatory markers were elevated in black EH patients and correlated with increased IECs and decreased EPC levels. Increased release of cytokines and IECs in black EH patients may impair EPC reparative capacity and predispose to hypertensive vascular injury. This process may aggravate vascular damage and accelerate hypertension-related morbidity and mortality rates in black EH patients. Therefore, future management strategies may need to consider the propensity for increased inflammation in black EH patients, which might be amenable to anti-inflammatory interventions.

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**Disclosures**

None.

**References**


12. de la Sierra A, Larrousse M. Endothelial dysfunction is associated with increased levels of biomarkers in essential hypertension. J Hum Hypertens. 2010;24:373–379.


20. Heidenreich PA, Trogdon JG, Khavjou OA, et al; American Heart Association Advocacy Coordinating Committee; Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Clinical Cardiology; Council on Epidemiology and Prevention; Council on Arteriosclerosis; Thrombosis and Vascular Biology; Council on Cardiopulmonary; Critical Care; Perioperative and Resuscitation; Council on Cardiovascular...
Inflammation in Essential Hypertension

What Is New?

- Our study implies higher propensity to vascular injury in black essential hypertensive compared with white essential hypertensive patients, associated with higher circulating levels of inflammatory endothelial cells.

What Is Relevant?

- Black individuals have a higher incidence and prevalence of essential hypertension compared with whites, possibly related to a differential effect on vascular injury and repair.

Summary

Inflammatory endothelial cells are novel markers of inflammatory endothelial injury in hypertensive patients, which may aggravate vascular damage and accelerate hypertension-related morbidity and mortality rates in black essential hypertensive patients.
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Increased Circulating Inflammatory Endothelial Cells in Blacks with Essential Hypertension

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Methods
This study was approved by the Institutional Review Board at the Mayo Clinic in adherence with the Declaration of Helsinki and the Health Insurance Portability and Accountability Act (HIPAA) guidelines. African American (n=19) and Caucasian (n=19) patients with a diagnosis of essential hypertension were enrolled in the study from January 2008 to January 2012. Patients with secondary hypertension were excluded. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, or diastolic blood pressure (DBP) ≥90 mmHg in supine position, after 20 min of rest on 2 separate days. Hypertensive subjects were studied during 150 mEq sodium intake during therapy with blockade of the renin-angiotensin system with angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) at standard recommended daily dose (equivalent: 40 mg lisinopril). We administered a single dose of furosemide (20 mg IV) to all hypertensive patients one day before renal vein sampling for other protocol studies1. The control group consisted of 19 age- and sex-matched healthy volunteers (HV, SBP<130 and DBP<80 mmHg), who were prospectively recruited through the Mayo Clinic Biobank. Exclusion criteria included uncontrolled hypertension (SBP >180 mmHg, despite antihypertensive therapy), serum creatinine >1.7 mg/dL, diabetes requiring insulin or oral hypoglycemic medications, recent cardiovascular events (myocardial infarction, stroke, congestive heart failure within 6 months), pregnancy, and kidney transplant.

In all patients, clinical and laboratory parameters were collected via the electronic medical records. Clinical parameters included: age, sex, height, weight, body mass index (BMI), SBP, DBP, mean arterial pressure (MAP), and use of concomitant medication. Laboratory parameters included: total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride, C-reactive protein (CRP, R&D systems, Cat# DCRP00), serum creatinine, 24 hr urinary protein concentration (Thermo Scientific, Cat#23236, Waltham, MA, USA), and estimated glomerular filtration rate (eGFR) calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula2.

Inflammatory biomarkers and EPC homing factors
Peripheral blood (HV), renal-vein and inferior-vena-cava (EH) samples were collected, centrifuged, and plasma aliquot stored at -80°C. For hypertensive patients, individual kidney (right and left) renal-vein levels were averaged. At the time of the assay, samples were centrifuged at 5000 rpm/5 min and samples (25 μl) incubated overnight at 4°C. Renal-vein and circulating levels of soluble E-selectin (sE Selectin), soluble vascular cell adhesion molecule (sVCAM-1), soluble intercellular adhesion molecule (ICAM)-1, myeloperoxidase (MPO), plasminogen activator inhibitor (PAI)-1, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein-(MIP)-18, tumor necrosis factor (TNF)-α, interleukin (IL)-6, adiponectin, matrix metalloproteinase (MMP)-9, stromal cell-derived factor (SDF)-1 and stromal cell factor (SCF) were measured by Luminex (Millipore, cat No: MPXHCYTO-60K; MPXHCYP2-62K; HCVD-67AK; and HSCR-32K)3.4.

EPC and IEC
Mononuclear cells were isolated from fresh blood samples by the density-gradient method and subsequently characterized for antigen expression of endothelial progenitor markers CD34 (Beckton-Dickinson), KDR (Santa Cruz), and vascular adhesion protein (VAP-1) (Lifespan biosciences). Systemic and renal-vein levels of CD34+/KDR+ (EPC) and VAP-1+ (IEC) were determined by fluorescence-activated cell sorting (FACS, Becton Dickinson, Calibur), as previously described. A total of 150,000 events/sample were counted using CellQuest software (Becton Dickinson). EPC and IEC levels were determined within the lymphocyte gate by using sequential gating strategies, as previously described. In order to eliminate bias secondary to variations attributed to body fluids and total blood cell counts, results were expressed as EPC or IEC % (per 100,000 cell counts). To further characterize IEC, VAP-1+ cells were stained with endothelial (CD31), lymphocyte (CD3 and CD45), monocyte/macrophage (CD16 and CD14) and progenitor cell (CD34 and CD133) markers and analyzed using FACS.

Statistical analysis
Statistical analysis was performed using JMP software package version 9.0.1 (SAS Institute Inc. Cary, NC). The sample size was estimated for detecting differences of 0.1% (per 100,000 cell counts) in VAP-1+ IEC between groups. By assuming an alpha risk of 0.05, a mean of 0.053%, and a standard deviation of 0.041%, at least 10 subjects per group were needed. We used the Shapiro-Wilk test to test for any deviation from normality. Normal distributed variables were expressed as mean±SD. Comparisons within groups were performed using the paired Student t-test/ANOVA or Wilcoxon/Kruskal Wallis tests were used when appropriate. Regressions were calculated by the least-squares fit. Statistical significance for all tests was accepted for p≤0.05.
References


Quantification of circulating EPC and IEC levels adjusted by body mass index (BMI, A-B) and estimated glomerular filtration rate (eGFR, C-D) in healthy volunteers (HV), white essential hypertensive (EH) and black EH patients. *p≤0.05 vs. HV, ‡ p≤0.05 vs. EH.
Representative flow cytometric plots showing double staining for VAP-1+ IEC cells and CD31 (A), CD3 (B), CD45 (C), CD16 (D), CD14 (E), CD34 (F), and CD133 (G).