Influence of Biochemical Alterations on Arterial Stiffness in Patients With End-stage Renal Disease

Jacques Blacher, Karine Demuth, Alain P. Guerin, Michel E. Safar, Nicole Moatti, Gérard M. London

Abstract—The incremental elastic modulus of the common carotid and radial arteries is increased in patients with end-stage renal disease (ESRD), independently of blood pressure, wall stress, and the presence of atherosclerotic alterations. Whether biochemical factors may be involved in the arterial changes and related to renal dysfunction remain largely ignored. To assess this question, we measured aortic (carotid-femoral), upper-limb (carotid-radial), and lower-limb (femoral-tibial) pulse wave velocity (PWV) in 74 ESRD patients undergoing hemodialysis in comparison with 57 control subjects similar in age, sex ratio, and mean blood pressure. We evaluated arterial blood pressure by sphygmomanometry, aortic calcifications and cardiac mass by echography, and routine biochemical parameters, total plasma homocysteine, and plasma endothelin levels by standard techniques. In the population of patients with ESRD, on the basis of multiple stepwise regression analysis, aortic PWV was positively and independently correlated with systolic blood pressure (P < .0001), age (P < .0001), prevalence of aortic calcification (P = .0004), and the prevalence of diabetes mellitus (P = .0043). Upper-limb PWV was influenced exclusively by mean blood pressure (P < .0001). Lower-limb PWV was positively and independently correlated with plasma total homocysteine (P = .0004) and plasma endothelin (P = .0187) only. At any vascular site, PWV was not independently correlated with tobacco consumption; plasma levels of cholesterol, triglyceride, fibrinogen, or hemoglobin; body mass index; or the presence of bilateral nephrectomy. Finally, plasma homocysteine was independently correlated with cardiac mass (P = .0022). This study provides evidence that in ESRD patients, the stiffness of the arterial wall and cardiac mass are strongly influenced by biochemical factors related to the kidney alterations and are independent of age and blood pressure level. Increased plasma homocysteine and homocysteine may be specifically involved in the vascular damage of lower limbs. (Arterioscler Thromb Vasc Biol. 1998;18:535-541.)

Key Words: end-stage renal disease ● pulse wave velocity ● homocysteine ● endothelin

The Einc of conduit arteries is a marker of the stiffness of wall material,1 and Einc is increased in patients with ESRD.2 Because the Einc of the CCA is increased independently of BP and wall stress, the role of mechanical factors in the mechanism of elevated Einc does not seem dominantly involved.2 Because higher values for Einc have been observed at the site of the radial artery, which is consistently devoid of atherosclerosis,3 the “accelerated atherosclerosis” described in ESRD patients4,5 does not seem responsible for this increase in Einc. Thus, it is relevant to determine in patients with ESRD whether biochemical and/or structural factors related to kidney alterations might influence the changes in Einc.

Because Einc requires measurement of the distensibility and the wall-to-lumen ratio of the vessel,1 its determination is difficult to perform routinely. However, for a given wall-to-lumen ratio, Einc is directly and strongly related to PWV.3 Thus, PWV may be used as an indirect index of the stiffness of the arterial wall material. PWV may be determined at various arterial sites, making it possible to evaluate whether biochemical and/or structural factors related to kidney alterations may differ according to the topography of arterial vessels.

Of the various abnormalities of kidney function in patients with ESRD, changes in sodium and water balance are two of the most constant characteristics.4 We have previously shown that interdialytic weight gain, a classic marker of sodium and water overload, is strongly associated with increased aortic PWV, independently of BP levels.8 However, in chronic renal failure, there are many other nonmechanical factors that may be related to the status of large arteries. In particular, increased levels of parathyroid hormone,9,10 ET,11,12 and homocysteine13,14 have been widely reported. However, their connection with the alterations in both the structure and function of conduit vessels has not yet been established.

In the present study, we investigated a large population of ESRD patients in whom cardiac mass and PWV, as measured in the aortic, upper-limb, and lower-limb territories, were determined in conjunction with several clinical and biochemical parameters related to renal alterations, namely, the...
presence of arterial calcifications and plasma ET and homocysteine levels. In this study, we show that some of these parameters are strongly correlated with PWV, indicating a possible influence of kidney-induced biochemical alterations on the observed changes in PWV.

Methods

Subjects

Seventy-four stable ESRD patients on hemodialysis for 105±85 months (mean±SD; range 6 to 312 months) were included. Fifty-seven nonuremic subjects served as the control group; we chose the nonuremic subjects in order to have no significant intergroup difference in terms of age, sex, and MBP. Patients or control subjects with acute myocardial infarction, valvular heart disease, cerebral vascular disease, CCA stenosis, or heart failure were excluded. Patients or control subjects taking vitamin supplements were also excluded. Fifty ESRD patients were being treated with regular recombinant human erythropoietin. Six control subjects and 9 ESRD patients were insulin-dependent diabetics. No control or ESRD patients had non-insulin-dependent diabetes mellitus. Nine control subjects and 6 ESRD patients were current smokers; the lifelong dose (in pack-years) was 9.7±12.0 for the control subjects and 10.7±17.4 for the ESRD patients (NS). Twenty-one control subjects and 42 ESRD patients had a past history of hypertension, the known duration of which was 6.7±6.0 years for the control subjects and 8.0±7.0 years for the ESRD patients. Fifteen control subjects and 22 ESRD patients were being treated with antihypertensive therapy, which was stopped 4 weeks prior to the study. The antihypertensive drugs included calcium-entry blockers, angiotensin-converting enzyme inhibitors, and β-blockers, either alone or in combination. In 7 ESRD patients, binephrectomy had been performed 168 to 300 months prior to the study. Patients were dialyzed with the use of an AN69 membrane (Hospal) and a bicarbonate dialysate. The duration of dialysis was individually tailored (4 to 6 hours three times weekly) to control body fluids and blood chemistry and to achieve a Kt/V of 1.5±0.4, 1.7±0.4, and 1.8±0.4 for upper- and lower-limb PWVs and 1.5±0.5 for aortic PWV. From a previous study including determinations of CCA Eicn and aortic PWV in patients with ESRD, we found a strong positive correlation between Eicn and PWV (r=0.72, P<.001).

As previously published, the presence of aortic plaques was assessed by echography by using a 3.5-MHz transducer (Sonel 300, Compagnie Générale de Radiologie). The presence of CCA plaques was also assessed by echography by using a high-resolution B-mode (7.5-MHz transducer) echo-tracking system (Wall-Track system). A localized echostructure encroaching into the vessel lumen was considered to be a plaque if the CCA intima-media thickness was >50% thicker than at neighboring sites. Cardiac measurements were performed in a blinded fashion by two physicians (G.M.L. and B. Pannier) according to the methods of the American Society of Echocardiography. M-mode measurements included LV posterior wall thickness, interventricular septal thickness, LV end-diastolic diameter, and LV end-systolic diameter. LV mass was calculated according to the Penn convention. LV hypertrophy was defined as an LV mass index >136 g/m² in men and >110 g/m² in women. The reproducibility of LV mass measurements has been previously published on the basis of a blinded study.

Biochemical Parameters

After BP determination and arterial and cardiac measurements, blood was obtained from the arteriovenous fistula of ESRD patients and from an antecubital vein for control subjects (after an overnight fast). The plasma or serum was separated without delay at 4°C in a refrigerated centrifuge and stored at 4°C (for the determination of routine chemistry profiles by standard methods) or at −20°C (for the determination of total plasma homocysteine and plasma ET) until analysis. Serum albumin and plasma fibrinogen levels were determined by the nephelometric method. Parathyroid hormone was determined by radioimmunoassay.

Total homocysteine, ie, the sum of the acid-soluble (that is, reduced homocysteine, homocystine disulfide, and homocysteine-cystine mixed disulfide) and protein-bound moieties, was determined in plasma by the fluorometric HPLC method originally described by Fortin and Genest. In brief, this assay involves the following steps: addition of N-acetyl-L-cysteine (Sigma Chemical Co) as an internal standard, reduction of the sample with tri-n-butylphosphine (Fluka Chemicals), and HPLC separation with fluorescence detection. Chromatography was carried out using a C-18 reverse-phase column (250×4-mm LiChrospher 100 RP-18, with a 5-μm end cap; Merck) at room temperature. The HPLC system consisted of a Beckman model 116 pump (Beckman), a 20-mL injection valve (model 7125, Rheodyne), a fluorescence HPLC monitor (RF 530, Shimadzu), and an electronic integrator (model HP 3395, Hewlett-Packard). An isotopic buffer consisting of a 0.1 mol/L acetate buffer (0.1 mol/L sodium acetate and 0.1 mol/L

Selected Abbreviations and Acronyms

CCA = common carotid artery
Eicn = incremental elastic modulus
ESRD = end-stage renal disease
ET = endothelin
LV = left ventricular
(M)BP = (mean) blood pressure

pressure waveform recordings were carried out simultaneously at the base of the neck over the CCA and the femoral artery in the groin and recorded with a Gould 8188 recorder (Gould Electronique) at a speed of 100 mm/s. The time delay (τ) was measured between the feet of the pressure waves recorded at these different points and was averaged over 10 to 15 beats. The distance traveled by the pulse wave was measured over the body surface as the distance between the two recording sites minus that from the suprasternal notch to the CCA (D). PWV was calculated as PWV=D/τ. Upper- and lower-limb PWVs were determined using the same method. To assess upper-limb PWV, the pressure waveform recordings were carried out simultaneously at the base of the neck over the CCA and the radial artery on the wrist; the distance traveled by the pulse wave was measured over the body surface as the distance between the two recording sites. To assess lower-limb PWV, the pressure waveform recordings were carried out simultaneously at the femoral artery in the groin and the posterior tibial artery on the ankle; the distance traveled by the pulse wave was measured over the body surface as the distance between the two recording sites. The reproducibility of the measurement was 4 ± 1% for aortic PWV, 6 ± 2% for upper-limb PWV, and 5.1 ± 1.5% for lower-limb PWV. From a previous study including determinations of CCA Eicn and aortic PWV in patients with ESRD, we found a strong positive correlation between Eicn and PWV (r=0.72, P<.001).

As previously published,17 the presence of aortic plaques was assessed by echography by using a 3.5-MHz transducer (Sonel 300, Compagnie Générale de Radiologie). The presence of CCA plaques was also assessed by echography by using a high-resolution B-mode (7.5-MHz transducer) echo-tracking system (Wall-Track system). A localized echostructure encroaching into the vessel lumen was considered to be a plaque if the CCA intima-media thickness was >50% thicker than at neighboring sites.18

Cardiac measurements were performed in a blinded fashion by two physicians (G.M.L. and B. Pannier) according to the methods of the American Society of Echocardiography. M-mode measurements included LV posterior wall thickness, interventricular septal thickness, LV end-diastolic diameter, and LV end-systolic diameter. LV mass was calculated according to the Penn convention. LV hypertrophy was defined as an LV mass index >136 g/m² in men and >110 g/m² in women. The reproducibility of LV mass measurements has been previously published on the basis of a blinded study.16

Biochemical Parameters

After BP determination and arterial and cardiac measurements, blood was obtained from the arteriovenous fistula of ESRD patients and from an antecubital vein for control subjects (after an overnight fast). The plasma or serum was separated without delay at 4°C in a refrigerated centrifuge and stored at 4°C (for the determination of routine chemistry profiles by standard methods) or at −20°C (for the determination of total plasma homocysteine and plasma ET) until analysis. Serum albumin and plasma fibrinogen levels were determined by the nephelometric method. Parathyroid hormone was determined by radioimmunoassay.5,10

Total homocysteine, ie, the sum of the acid-soluble (that is, reduced homocysteine, homocystine disulfide, and homocysteine-cystine mixed disulfide) and protein-bound moieties, was determined in plasma by the fluorometric HPLC method originally described by Fortin and Genest. In brief, this assay involves the following steps: addition of N-acetyl-L-cysteine (Sigma Chemical Co) as an internal standard, reduction of the sample with tri-n-butylphosphine (Fluka Chemicals), and HPLC separation with fluorescence detection. Chromatography was carried out using a C-18 reverse-phase column (250×4-mm LiChrospher 100 RP-18, with a 5-μm end cap; Merck) at room temperature. The HPLC system consisted of a Beckman model 116 pump (Beckman), a 20-mL injection valve (model 7125, Rheodyne), a fluorescence HPLC monitor (RF 530, Shimadzu), and an electronic integrator (model HP 3395, Hewlett-Packard). An isotopic buffer consisting of a 0.1 mol/L acetate buffer (0.1 mol/L sodium acetate and 0.1 mol/L...
TABLE 1. Clinical Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Subjects n=57</th>
<th>ESRD Patients n=74</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49±15</td>
<td>52±16</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>24/33</td>
<td>31/43</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26±5</td>
<td>24±4*</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.85±0.25</td>
<td>1.67±0.21*</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>145±15</td>
<td>149±30</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>85±15</td>
<td>80±14</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>104±14</td>
<td>104±17</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>59±15</td>
<td>69±24*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>63±8</td>
<td>70±9†</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(absent=1, present=2)</td>
<td>1.11±0.31</td>
<td>1.12±0.33</td>
</tr>
<tr>
<td>Smoking status, pack-years</td>
<td>10±12</td>
<td>11±17</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*P<.05, †P<.001.

CH₃COOH, pH 4.0 (containing 20 mL methanol per liter of buffer, filtered, and degassed on a 0.2-µm membrane), was used as previously described.²¹ The flow rate was 1.2 mL/min for 20 minutes. The fluorescence intensities were measured with excitation at 385 nm and emission at 550 nm. The interassay and intra-assay coefficients of variation were <8%. Normal values for plasma total homocysteine are below 16 mmol/L.²¹

Plasma ET determination was performed using an enzyme immunoassay kit (Cayman Chemicals Co) after an extraction procedure. In brief, plasma was acidified with 4% CH₃ COOH, and immunoreactive ET was extracted with a Sep-pac C-18 cartridge (Waters Associates). To determine the recovery rate, a sample was spiked with a known amount of ET, acidified, and then treated in series. Each column was pretreated by sequentially adding methanol, distilled water, and 4% CH₃ COOH; the acidified sample was then distilled water, and 4% CH₃ COOH; the acidified sample was then treated in series. After being washed with 25% ethanol, the adsorbed peptide was eluted with 86% ethanol in 4% CH₃ COOH. After evaporation of the eluate, the dry residue was dissolved in assay buffer and subjected to the enzyme immunoassays. The recovery rate was 60±5%. The immunometric assay is based on a double-antibody “sandwich” technique. Each well of the microtiter plate was coated with a monoclonal antibody specific for human ET (ET capture antibody). The second antibody was a monoclonal antibody labeled with acetylcholinesterase and is selective for a different epitope on the ET molecule. The concentration of the analyte is then determined by measuring the enzymatic activity of acetylcholinesterase with Ellman’s reagent. The assay for ET did not cross-react with big ET. Although the assay specificity is 100% for ET-1, ET-2, and ET-3, the plasma levels principally reflect plasma ET-1 concentrations.²³ The ET detection threshold is 0.1 pg/mL. The interassay and intra-assay coefficients of variation were <10%. Normal values for plasma ET are 1.5±0.5 pg/mL.

Statistical Analysis

Data were expressed as mean±SD. Student’s t test was used for comparison of control subjects and ESRD patients. Qualitative data were compared with the χ² test. Sex (1=male, 2=female) and code (1=control subject, 2=ESRD) were used as dummy variables. Multiple stepwise regression analysis was used to assess the correlations between arterial PWV, cardiac mass, determinants of biochemical and cardiovascular parameters, and their interactions. Statistical analysis was done with NCSS 5.0 software.²⁴ Repeatability and reproducibility of the methods were defined as recommended by the British Standard Institution.²⁵ A value of P<.05 was considered significant. All testing was two-sided.

Results

Description of the Population

Population characteristics and biochemical parameters are presented in Tables 1 and 2. The two groups were similar in age, sex ratio, systolic BP, MBP, and diastolic BP. Pulse pressure (P<.05) and heart rate (P<.001) were increased in ESRD patients. Body mass index and body surface area were decreased in ESRD patients (P<.05).

ESRD patients had lower total and HDL cholesterol values (P<.05 and P<.001, respectively) and increased triglyceride levels (P<.001). Serum albumin was reduced in ESRD patients (P<.001) and was negatively correlated with age (P<.001) and fibrinogen level (P<.01). Plasma fibrinogen level was increased in ESRD patients (P<.001) and was positively correlated with age (r=.49, P<.001). Parathyroid hormone was increased in ESRD patients (P<.001), but hemoglobin was lower in ESRD patients (P<.001).
Arterial and Cardiac Measurements

Arterial and cardiac measurements are presented in Table 3. ESRD patients had higher aortic and lower-limb PWVs than did control subjects (P<.01 for both). Upper-limb PWV was higher in ESRD patients, but the difference was not statistically significant. In ESRD patients, PWV was quite similar at the three vascular sites, whereas in control subjects, aortic PWV was lower than the upper- (P<.05) and lower- (P<.05) limb PWVs. The prevalences of carotid and aortic plaques were higher in ESRD patients than in control subjects (P<.01 for both). LV diameters, wall thickness, and cardiac mass were increased in ESRD patients (P<.001).

The factors influencing aortic, lower-, and upper-limb PWV in the entire study population (uremic plus nonuremic subjects) are presented in Table 4. Age, MBP, and code (1=control subject, 2=ESRD) were the only independent determinants of PWV at any vascular site.

Plasma Homocysteine and ET Levels

Plasma total homocysteine was increased for all ESRD patients but 1 (99%): mean±SD, 35.9±12.8 μmol/L; range, 12.8 to 79.9. Plasma homocysteine concentration was not correlated with serum albumin nor with any cardiovascular risk factor, such as smoking habits, BP, fibrinogen, age, sex, or any lipid fraction. Plasma ET was increased in ESRD patients (4.6±3.8 pg/mL; range, 0.2 to 18.7), and was positively related to age (P<.05). After adjustment for age, ET concentrations were not correlated with systolic or diastolic BP but were significantly correlated with pulse pressure (r=50, P<.001). Homocysteine and ET concentrations were similar in binephrectomized patients (n=7) and those with both kidneys intact (n=67) (37.2±17.5 versus 34.8±11.9 μmol/L and 4.2±2.1 versus 5.0±4.2 pg/mL, respectively; NS).

Multiple Regression Analysis of PWV and Cardiac Mass in ESRD Patients

Aortic PWV was positively and independently correlated with systolic BP (P<.0001), age (P<.0001), the presence of aortic calcifications (P=.0004), and the presence of insulin-dependent diabetes mellitus (P=.0043; Table 5). None of the other biochemical parameters, including plasma glucose, cholesterol, calcium, parathyroid hormone, homocysteine, or ET, significantly entered the model. The only determinant of upper-limb PWV in this population was MBP (P<.0001); data not shown.

Lower-limb PWV was positively correlated with plasma homocysteine (P=.0004), and ET (P=.0187; Table 6). No other clinical or biochemical parameters significantly entered the model, including age, tobacco consumption, BP level,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>β Coefficient</th>
<th>T Value</th>
<th>Single r²</th>
<th>Sequential r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable: aortic PWV, m/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>0.35</td>
<td>5.50</td>
<td>0.159</td>
<td>0.159</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.37</td>
<td>4.82</td>
<td>0.354</td>
<td>0.503</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Aortic calcifications (presence-absence)</td>
<td>0.29</td>
<td>4.42</td>
<td>0.384</td>
<td>0.579</td>
<td>.0004</td>
</tr>
<tr>
<td>Diabetes mellitus (presence-absence)</td>
<td>0.19</td>
<td>2.92</td>
<td>0.135</td>
<td>0.610</td>
<td>.0043</td>
</tr>
</tbody>
</table>

Total variance explained, 61%; RMSE, 98.6; F ratio, 37.33; P<.0001.
diabetes mellitus, aortic calcifications, or glucose, cholesterol, triglyceride, serum albumin, plasma fibrinogen, and parathyroid hormone levels.

LV mass was positively correlated with plasma homocysteine level ($r = .31, P < .01$); this correlation persisted even after adjustments for the usual determinants of LV mass in this population: body surface area, systolic BP, hematocrit, sex, and age (single $r^2 = .094, P = .0022$; Table 7). Aortic PWV and plasma lipids, glucose, and serum albumin did not significantly enter into the multivariate analysis. Statistically significant associations were also found between plasma homocysteine and LV posterior wall thickness, interventricular septal thickness, and LV end-diastolic diameter (data not shown; $P < .05$ for all).

### Discussion

The salient findings of this study are that (1) in patients with ESRD, increased aortic PWV is influenced by the presence of aortic calcifications and diabetes mellitus, independently of age and BP; (2) the main factors influencing lower-limb PWV are plasma concentrations of homocysteine and ET, two compounds commonly related to the status of renal function; and (3) independently of aortic PWV, plasma homocysteine is correlated with cardiac mass.

In the present study, we used PWV as a marker of the stiffness of wall material. Indeed, according to the Moens Korteweg equation, $Einc = ([PWV]^2 \times 2 \rho R)/h$, where $\rho$ is blood density (considered constant) and $R$ and $h$ are the radius and thickness, respectively, of the artery. In a previous investigation, we showed that the R-to-h ratio at the site of the CCA and the radial artery remained within the normal range in patients with ESRD. In this work, we have shown (see “Methods”) that the carotid $Einc$ is strongly and positively correlated with aortic PWV. Thus, PWV may be considered an adequate marker to evaluate the stiffness of wall material in this particular population.

In clinical studies, PWV is a highly reproducible index of arterial rigidity. Repeatability studies, checks using Bland and Altman diagrams, and modern computer technology have now made it quite feasible to investigate arterial stiffness in cardiovascular epidemiological studies. In a previous study, we showed that aortic, upper-, and lower-limb PWV were markedly increased in patients with ESRD. Furthermore, the factors modulating PWV were shown to differ markedly according to the site of measurement and the type of population studied (ESRD patients versus control subjects). In the present study, we determined in a first approach the factors influencing aortic, upper-, and lower-limb PWV in the entire population. We showed that the presence of ESRD markedly influenced the level of PWV regardless of the site of measurement. Thus, our main goal was not to perform a case-control study but rather to determine in ESRD patients the possible influence of ET, homocysteine, or other factors on arterial stiffness according to the site of PWV measurement. Although age, BP, or a combination of both represented a major component of aortic and limbs PWV variability, we showed that four parameters, ie, the presence of aortic calcification, the presence of diabetes mellitus, and increased plasma ET and homocysteine levels, constituted a substantial proportion of the PWV variability. For instance, for lower-limb PWV, plasma homocysteine represented 19.8% of variability.

In the ESRD population, it is noteworthy that PWV did not correlate with plasma total cholesterol, HDL cholesterol, glucose, calcium, and with tobacco consumption, ie, with most of the classic markers of cardiovascular risk in epidemiological studies. In contrast, PWV did correlate with several factors directly related to advanced renal failure. Indeed, in ESRD patients, aortic calcifications, which have a greater prevalence in uremic than nonuremic patients, are observed independently of age, hypertension, and atherosclerosis. They are often considered a consequence of an increased calcium phosphate product, in association with

### Table 6. Multivariate Relations of Lower-Limb PWV in ESRD Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\beta$ Coefficient</th>
<th>$T$ Value</th>
<th>Single $r^2$</th>
<th>Sequential $r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable: lower-limb PWV, m/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma homocysteine, $\mu$mol/L</td>
<td>0.51</td>
<td>3.85</td>
<td>0.198</td>
<td>0.198</td>
<td>.0004</td>
</tr>
<tr>
<td>Plasma endothelin, pg/mL</td>
<td>0.30</td>
<td>2.44</td>
<td>0.061</td>
<td>0.290</td>
<td>.0187</td>
</tr>
</tbody>
</table>

Total variance explained, 29%; RMSE, 148.2; $F$ ratio, 9.38; $P < .0001$.

### Table 7. Multivariate Relations of LV Mass in ESRD Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$\beta$ Coefficient</th>
<th>$T$ Value</th>
<th>Single $r^2$</th>
<th>Sequential $r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable: LV mass, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>0.35</td>
<td>3.83</td>
<td>0.273</td>
<td>0.273</td>
<td>.0003</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>0.27</td>
<td>3.49</td>
<td>0.112</td>
<td>0.373</td>
<td>.0008</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>−0.25</td>
<td>−3.21</td>
<td>0.036</td>
<td>0.437</td>
<td>.0020</td>
</tr>
<tr>
<td>Plasma homocysteine, $\mu$mol/L</td>
<td>0.25</td>
<td>3.17</td>
<td>0.094</td>
<td>0.499</td>
<td>.0022</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.22</td>
<td>2.79</td>
<td>0.082</td>
<td>0.540</td>
<td>.0068</td>
</tr>
<tr>
<td>Sex (1 = male, 2 = female)</td>
<td>−0.25</td>
<td>−2.76</td>
<td>0.211</td>
<td>0.579</td>
<td>.0074</td>
</tr>
</tbody>
</table>

Total variance explained, 57.91%; RMSE, 68.7; $F$ ratio, 16.50; $P < .0001$. 

---

secondary hyperparathyroidism or adynamic bone disease. On the other hand, diabetes is known to be frequently associated with ESRD. Diabetic patients with advanced renal failure have a mediocré vascular prognosis, related in particular to macroangiopathies and increased arterial stiffness. Finally, increased plasma levels of homocysteine and ET are a common feature in subjects with chronic renal failure. In the present study, such plasma levels did not differ in the presence or absence of native kidneys, indicating that the altered renal parenchyma did not contribute substantially to the increased plasma levels of both biochemical components. It seems more likely that the increased plasma levels were a consequence of the loss in renal function, with resulting consequences on homocysteine and ET metabolism. Taken together, all of these findings strongly suggest that the alterations of renal function itself may be at least partly responsible for the observed arterial abnormalities.

Altered arterial structure and function have been shown to be classic features in patients with ESRD. It seems that independently of age, BP, and atherosclerosis, the arterial wall involves histological changes, including hypertrophy, calcium deposition, and changes in the extracellular matrix, involving mainly collagen fibers. Such modification may be induced in the course of chronic renal failure in different ways. First, we have already noted the role of an increased calcium phosphate product in the mechanism of aortic calcifications. Second, in diabetic subjects, vascular lesions are related not only to the numerous metabolic anomalies linked to this pathological condition but also to the induction of tissue damage via slow, irreversible changes in extracellular molecules due to hyperglycemia, particularly covalent modifications and the resulting advanced glycation end products. Third, homocysteine has also been shown to enhance smooth muscle cell proliferation and even alter elastic tissue. Finally, ET is enhanced not only in the circulation but also mostly in terms of its expression in the arterial wall, with potential consequences on vascular smooth muscle proliferation. In addition, with respect to whichever biochemical components may be involved, endothelial function in ESRD patients is usually modified through these different mechanisms. More specifically, the formation and retention of the competitive inhibitor of NO synthase, asymmetric dimethyl-L-arginine, may favor a decrease in NO generation. NO donors are known to decrease arterial stiffening. Their blockade in turn may contribute to an increase in arterial rigidity through functional changes.

One of the dominant specificities of the present study was that the respective roles of aortic calcification, non-insulin-dependent diabetes, homocysteine, and ET differed markedly according to the site of PWV measurement. For aortic PWV, the particular role of diabetes and aortic calcification is not surprising. Aortic accumulations of collagen and calcium are common in central elastic arteries such as the thoracic aorta. For lower-limb PWV, epidemiological studies on hyperhomocysteinemia have previously reported that the odds ratio related to a 5 μmol/L increment is greater at the site of the lower limbs, with a lesser impact on the coronary and the supra-aortic circulation. Taylor et al found in a prospective study that the progression of symptomatic peripheral arterial disease was increased in patients with hyperhomocysteinemia. Nevertheless, the particular contribution of increased plasma ET to the increased PWV of the lower limbs is more difficult to explain. Clinical and experimental studies suggest that ET excess is strongly related to the alterations of blood flow and shear stress, a finding already reported in patients with ESRD. Atherosclerotic disease of the lower limbs is often detected in specific conditions involving elevation of limb blood flow, as observed during intermittent claudication. On the other hand, recent reports have shown that ET is observed in high concentration not only in the endothelium but also at the vascular smooth muscle level and/or within atherosclerotic plaques.

In the present study, the finding of a significant correlation between cardiac mass and homocysteine further confirmed that this biochemical factor had a heterogeneous effect on cardiovascular structure and function. Because plasma homocysteine is not correlated with aortic PWV, it does not seem likely that the cardiac mass–homocysteine correlation may be related to an effect of homocysteine on arterial impedance, with resulting consequences in cardiac structure and function. It seems more likely that a direct interaction on the heart should be involved.

In conclusion, the present study has shown that in patients with ESRD, the structural and functional alterations of conduit vessels are not necessarily related to age, BP level, and atherosclerotic disease. Biochemical components related to the kidney are strongly involved and differ according to the site of the vessel abnormalities. The observation of a positive association between cardiac mass and plasma homocysteine has not yet been published and requires further investigation.

Acknowledgments

This work was supported by GEPR (Groupe d’Etude de la Physiopathologie de l’Insuffisance Rénale) and HOSPAL SA (both grants to G.M.L.). The authors thank Wendy Kay Johnson for her invaluable and friendly assistance.

References


Influence of Biochemical Alterations on Arterial Stiffness in Patients With End-stage Renal Disease

Jacques Blacher, Karine Demuth, Alain P. Guerin, Michel E. Safar, Nicole Moatti and Gérard M. London

doi: 10.1161/01.ATV.18.4.535

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/4/535