Norepinephrine and Concentric Hypertrophy in Patients With End-Stage Renal Disease

Carmine Zoccali, Francesca Mallamaci, Giovanni Tripepi, Saverio Parlongo, Sebastiano Cutrupi, Francesco Antonio Benedetto, Alessandro Cataliotti, Lorenzo Salvatore Malatino,
on behalf of the CREED investigators

Abstract—We have recently observed that in patients with end-stage renal disease (ESRD) raised plasma norepinephrine (NE) is an independent predictor of incident cardiovascular events but that its prognostic power is reduced when this sympathetic marker is tested in statistical models including also left ventricular mass. Because left ventricular hypertrophy (LVH) may be a mechanism whereby NE contributes to the high rate of cardiovascular events in ESRD, we examined the relationship between plasma NE and echocardiographic parameters of left ventricle mass in a large group of ESRD patients. Mean wall thickness (MWT) was higher in patients in the third NE tertile than in the other 2 tertiles ($P=0.001$), and such an increase was paralleled by a rise in relative wall thickness (RWT) ($P=0.006$). Concentric LVH was more prevalent in patients in the third NE tertile (46%) than in the second (38%) and first (25%) NE tertiles. Multivariate regression analysis confirmed that the association of plasma NE with the muscular component of left ventricle (MWT) and with RWT was independent ($P=0.001$) of other cardiovascular risk factors, and in these models, plasma NE ranked as the second correlate of MWT and RWT. Similarly, multiple logistic regression analysis showed that the association of plasma NE with concentric LVH was strong and again independent of other risk factors ($P=0.003$). Plasma NE is associated to concentric LVH in ESRD patients. These observations constitute a sound basis for testing the effect of anti-adrenergic drugs on left ventricle mass and on cardiovascular outcomes in patients with ESRD. (Hypertension. 2002;40:41-46.)

Key Words: cardiovascular risk ▪ dialysis ▪ left ventricular hypertrophy ▪ norepinephrine ▪ renal failure ▪ sympathetic activity ▪ uremia

At least 3 prospective studies have coherently demonstrated that raised left ventricular mass (LVM) has a strong negative prognostic impact in patients with end-stage renal disease (ESRD).1-3 and on this basis, left ventricular hypertrophy (LVH) is now considered a major cardiovascular risk factor in these patients. The pathogenesis of LVH is multifactorial, and several causative factors have been identified—including hypertension, anemia, hyperparathyroidism, and chronic volume expansion4—but these factors only in part account for the high prevalence of this alteration in patients with ESRD. Sympathetic activity measured by sympathetic nerve microneurography is increased in patients with mild to moderate renal dysfunction5 and in those with ESRD,6 and high sympathetic tone is considered a major player in the pathogenesis of hypertension in renal diseases.7-8 Although sympathetic overactivity is being suspected as a likely mechanism responsible for the high cardiovascular morbidity and mortality in patients with chronic renal diseases, to our knowledge there is no study linking raised norepinephrine (NE) to alterations in left ventricular mass in these patients.

In a large cohort of dialysis patients, we have recently observed that raised plasma NE is a strong and independent predictor of incident cardiovascular events.9 Giving the importance of this sympathetic neurotransmitter in mechanisms regulating myocardial trophism and plasticity10 alterations in LVM may well represent a mechanism whereby NE contributes to the high rate of cardiovascular events in ESRD. Although modification of adrenergic activity in an intervention study remains the ultimate test for the hypothesis that high plasma NE is involved in LVH and cardiovascular complication in ESRD, studying the relationship between plasma NE and LVM and geometry may provide useful information for clarifying the issue. In the present study, we have therefore undertaken a detailed analysis of this relationship in patients who took part into the above-mentioned follow-up study.

Protocol
The protocol was in conformity to the ethical guidelines of our institutions, and informed consent was obtained from each partici-
### Study Cohort

One hundred and ninety-seven hemodialysis patients (110 men and 87 women) with ESRD who had been on regular dialysis treatment for at least 6 months (median duration of regular dialysis treatment, 43 months; interquartile range, 20 to 110 months) with left ventricular ejection fraction (LVEF) ≥ 35% and without clinical evidence of heart failure (defined as dyspnea in addition to 2 of the following conditions: raised jugular pressure, bibasilar crackles, pulmonary venous hypertension or interstitial edema on chest X-ray requiring hospitalization or extra ultrafiltration) were considered eligible for the study. All patients were virtually anuric (diuresis < 200 mL/day). One hundred and nine patients were on treatment with erythropoietin. The demographic, anthropometric, clinical, and biochemical characteristics of the patients are detailed in Table 1.

### Laboratory Measurements

Blood sampling was performed during a midweek nondialysis day after 20 to 30 minutes of quiet resting in semirecumbent position. Samples were taken into prechilled EDTA vacutainers, placed immediately on ice, and centrifuged within 30 minutes at 4°C. The plasma stored at −80°C until analyses.

Serum lipids, albumin, calcium, phosphate, and hemoglobin measurements were made using standard methods in the routine clinical laboratory. C-reactive protein levels were measured by a commercially available kit (Behring, Scoppito). Plasma homocysteine was quantified by a high-performance liquid chromatography method. Plasma concentration of NE was measured by a commercially available radioimmunoassay kit (Amicyl-test, Immunological Laboratory). The intraassay coefficient of variation was 7% to 15%. The upper limit of the normal range of plasma NE in our laboratory is 3.54 nmol/L, which is very close to the value (3.38 nmol/L) reported in a previous study.

### Table 1. Somatometric, Clinical, Biochemical, and Echocardiographic Data of the Study Population

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>1st Tertile (&lt;2.20 nmol/L)</th>
<th>2nd Tertile (2.20–4.42 nmol/L)</th>
<th>3rd Tertile (&gt;4.42 nmol/L)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57.1±16.6</td>
<td>55.5±14.5</td>
<td>63.3±12.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>27 (41.5%)</td>
<td>40 (61.5%)</td>
<td>43 (64.2%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetics, n (%)</td>
<td>14 (21.5%)</td>
<td>6 (9.2%)</td>
<td>8 (11.9%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>21 (32.3%)</td>
<td>30 (46.1%)</td>
<td>25 (37.3%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Patients with previous CV events, n (%)</td>
<td>25 (38.5%)</td>
<td>24 (36.9%)</td>
<td>34 (50.7%)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

**Antihypertensive therapy**
- ACE inhibitors/angiotensin II receptor antagonists: 13 (20%) 13 (20%) 7 (10%) 0.23
- Calcium antagonists: 13 (20%) 19 (29%) 20 (30%) 0.36
- Sympathomimetics and β-blockers: 9 (14%) 14 (22%) 7 (10%) 0.19

**Hemodynamic data**
- Systolic pressure, mm Hg: 142.7±25.7 141.6±24.0 137.8±24.8 0.49
- Diastolic pressure, mm Hg: 78.3±12.5 76.9±14.3 74.4±12.7 0.23
- Heart rate, bpm: 76.1±10.6 79.8±10.2 80.1±9.6 0.05

**Biochemical data**
- Hemoglobin, g/L: 105.8±16.5 107.3±19.2 106.2±20.6 0.89
- Serum albumin, g/L: 41.4±3.9 42.0±6.4 41.7±4.6 0.83
- Serum cholesterol, mmol/L: 5.09±1.27 5.33±1.45 5.61±1.59 0.11
- Serum calcium, mmol/L: 2.25±0.22 2.24±0.22 2.32±0.30 0.18
- Serum phosphate, mmol/L: 1.97±0.42 1.98±0.49 2.05±0.47 0.57
- Serum CRP, mg/L: 44.9 (3.4–15.7) 6.5 (3.4–18.0) 11.7 (4.0–16.5) 0.17
- Plasma homocysteine, μmol/L: 27.2 (19.2–43.7) 26.2 (20.9–42.5) 26.5 (17.3–38.5) 0.91
- Kt/V: 1.22±0.26 1.21±0.28 1.22±0.27 0.91
- LVMI, g/m²: 58.1±17.9 60.4±18.7 64.6±18.0 0.11

Data are means±SD, median (interquartile range), or % frequency, and comparisons between groups were made by 1-way ANOVA, Kruskal-Wallis Test, or χ² test, as appropriate.
Blood Pressure Measurements

Blood pressure was estimated by averaging all predialysis arterial pressure recordings during the month before the study (total of 12 measurements, ie, 3/wk).13

Echocardiography

These studies were performed during the dialysis interval within 2 hours after blood sampling. LVM was calculated according to the Devereux cube formula and indexed to height2 (LVM).14 LVH was defined by a LVMi of $47 \text{ g/m}^2$ in women or $>50 \text{ g/m}^2$ in men. The height-based indexing of LVM was specifically chosen to minimize any potential distortion attributable to extracellular volume expansion (surface area indexing being weight-sensitive).15 The relative wall thickness (RWT, $2 \times$ posterior wall thickness/left ventricular end diastolic diameter (LVEDD)) was also calculated, as an index of the left ventricular geometric pattern. Values indicative of concentric and eccentric left ventricular geometry were established on the basis of age-specific reference standards.16 Mean wall thickness (MWT) was calculated by the standard formula: MWT = [(interventricular septum+$\times$ posterior wall thickness)/2].

Statistical Analyses

Data are reported as mean±SD, median and interquartile range or as percentage frequency, and comparisons between groups were made by 1-way ANOVA, Kruskal-Wallis test, or $\chi^2$ test, as appropriate. Variables that did not show a Gaussian distribution were log-transformed before further analyses (log$_{10}$).

To test the independent relationship between plasma NE and the indicators of the muscular component of the left ventricle (MWT and RWT), we constructed multivariate models (either multiple linear or logistic regression analysis) based on plasma NE (expressed in tertiles) and on a series of traditional and nontraditional cardiovascular risk factors in dialysis patients (age, sex, diabetes, previous cardiovascular events, smoking, systolic pressure, serum cholesterol, plasma homocysteine, serum calcium and phosphate, serum HDL cholesterol, plasma homocysteine, serum calcium and phosphate, serum CRP, serum albumin, hemoglobin, and fractional urea clearance (Kt/V)) as well as on the use of sympatholytic agents or $\beta$-blockers, calcium antagonists, and ACE inhibitors/angiotensin II receptor antagonists. Significant independent variables were identified by a stepwise approach. Furthermore, to control for the potential confounding effect of covariates that differed in the 3 tertiles of plasma NE, we always forced such covariates ($P<0.20$) into the final models. By this approach, we constructed models of adequate statistical power (at least 15 subjects for each variable in the final model). Data are expressed as regression coefficient ($\beta$) or as odds ratio and 95% confidence interval (CI), as appropriate. All calculations were done using a standard statistical package (SPSS, version 9.0.1).

Results

Plasma NE concentration (median, 3.12 nmol/L; interquartile range, 1.78 to 5.70 nmol/L) was above the upper limit of the normal range (cut-off $>3.54$ nmol/L) in 91 dialysis patients (ie, 46%). One hundred and forty-seven patients displayed LVH on echocardiography (eccentric LVH: n=75, 38.1%; concentric LVH: n=72, 36.5%), and the remaining 50 patients (25.4%) had normal LVM.

As shown in Table 1 patients in the third NE tertile were older, had a greater prevalence of males and higher heart rate in comparison with those in the second and the first NE tertile. Fractional urea clearance (Kt/V) was very similar in the 3 groups. The proportion of patients on antihypertensive treatment (including sympatholytic agents or $\beta$-blockers) did not differ in the 3 groups. MWT was significantly higher in the third NE tertile than in the other 2 tertiles ($P=0.001$), and such an increase was paralleled by a rise in RWT ($P=0.006$). Because LVEDD was similar in the 3 NE tertiles ($P=0.77$), the difference in LVMI failed to achieve statistical significance ($P=0.11$). According to the findings that MWT and RWT increase in parallel in patients with high NE (while LVEDD remains unchanged), left ventricular concentric hypertrophy was more prevalent in patients in the third NE tertile (46%) than in the second (38%) and first (25%) NE tertiles.

Multivariate linear regression analysis confirmed that the association of plasma NE with the muscular component of left ventricle (MWT) and with RWT was independent of other cardiovascular risk factors and of antihypertensive treatment (Table 2). Indeed, in these analyses, plasma NE ranked as the second correlate of these echocardiographic parameters of left ventricular geometry. By the same token, multiple logistic regression analysis confirmed that the association of plasma NE with concentric hypertrophy was strong and again independent of other risk factors (Table 3). In this model patients in the third tertile of plasma NE had an odds ratio for concentric hypertrophy that was 3.81 (95% CI, 1.56 to 9.27) times higher than in those in the first tertile ($P=0.003$).

**TABLE 2. Multiple Regression Models**

<table>
<thead>
<tr>
<th>Significant Correlates</th>
<th>$\beta$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>−0.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma NE (tertiles)</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.06</td>
<td>0.37</td>
</tr>
<tr>
<td>CRP, lg$_{10}$</td>
<td>0.05</td>
<td>0.45</td>
</tr>
<tr>
<td>Use of ACE inhibitors/angiotensin II receptor antagonists</td>
<td>0.02</td>
<td>0.79</td>
</tr>
<tr>
<td>Male sex</td>
<td>−0.02</td>
<td>0.79</td>
</tr>
<tr>
<td>Age</td>
<td>−0.01</td>
<td>0.86</td>
</tr>
<tr>
<td>Use of sympathicolitic agents or $\beta$-blockers</td>
<td>0.009</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Significant predictors | $\beta$ | $P$  |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>−0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma NE (tertiles)</td>
<td>0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.21</td>
<td>0.006</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Calcium</td>
<td>−0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>CRP, lg$_{10}$</td>
<td>0.09</td>
<td>0.19</td>
</tr>
<tr>
<td>Age</td>
<td>0.09</td>
<td>0.21</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.07</td>
<td>0.32</td>
</tr>
<tr>
<td>Use of calcium antagonists</td>
<td>−0.07</td>
<td>0.32</td>
</tr>
<tr>
<td>Use of ACE inhibitors/angiotensin II receptor antagonists</td>
<td>0.04</td>
<td>0.65</td>
</tr>
<tr>
<td>Calcium</td>
<td>−0.006</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Data are expressed as regression coefficient ($\beta$) and $P$ values.
Sympathetic activity as assessed by the measurement of plasma NE is independently related to the muscular component of the left ventricle and to concentric LVH in patients with ESRD.

NE and Sympathetic Activity in ESRD

Plasma NE in ESRD patients was found to be high in most studies performed between the 1970s and 1980s, and on the basis of these studies, chronic renal failure was considered a situation characterized by enhanced sympathetic activity. The interpretation of plasma NE as a marker of sympathetic activity is complex in patients with renal failure because circulating NE represents only a small proportion of the neurotransmitter amount secreted from adrenergic nerve terminals and because these patients display metabolic alterations that may alter the plasma concentration of this substance. Measurements of plasma NE in no way can be considered a substitute to sympathetic microneurography, which is the undisputed standard for the assessment of sympathetic activity. This technique has convincingly demonstrated that sympathetic activity in dialysis patients is increased and that this increase is even more consistent than it emerged from early studies based on plasma NE measurements. In this study, we found that plasma NE level is above the upper limit of the normal range in a substantial proportion of patients. Of note, the close similarity of fractional urea clearance in the 3 NE tertiles indicates that high plasma NE in ESRD is not paralleled by the accumulation of metabolic waste products.

NE and Echocardiographic Parameters of LVM

LVH is an adaptive process aimed at minimizing ventricular wall stress. Several factors, including hypertension and altered circadian arterial pressure profile, may influence this process in patients with renal diseases. The evidence that the sympathetic system is involved in the progression of cardiovascular structural alterations such as LVH and arterial remodeling in the general population is now substantial. NE promotes myocardial cell hypertrophy in vitro, and in vivo sustained sympathetic activity generates myocardial hypertrophy by mechanisms that are only in part dependent on raised arterial pressure. In line with observations suggesting that hypertension only in part accounts for raised LVM, we found that the link between the muscular component of LVM (mean wall thickness) and plasma NE was largely independent of arterial pressure and other risk factors. Concentric hypertrophy in dialysis patients entails an ominous prognosis, even worse than that of eccentric hypertrophy. Studies by London et al indicate that arterial rigidity is perhaps the major factor triggering concentric LVH in these patients. The sympathetic nervous system exerts a marked tonic restraint of arterial distensibility in man. We speculate that in addition to high calcium phosphate product and endothelial dysfunction, high NE may be another mechanism linking raised NE, reduced arterial distensibility, and concentric hypertrophy in dialysis patients.

Study Limitations

An important limitation of peripheral venous NE is that it may not reflect local sympathetic activity in the heart. High...
peripheral sympathetic drive (by sympathetic microneurography) has been recently associated to LVH in essential hypertension. Although the issue deserves further study, it is worth noting that increased peripheral NE is associated with relatively higher LVM and that coronary venous plasma concentration of NE is higher in essential hypertensives with LVH than in those without LVH. Furthermore NE spillover is distinctly increased in physiological LVH in athletes. There are no studies exploring regional (cardiac) sympathetic activity in patients with renal failure. However, cardiac metiodobenzylguanidine clearance is rapid in patients with chronic renal failure on dialysis, particularly in those with left ventricle dysfunction or LVH, suggesting cardiac sympathetic over-activity in these patients. Our study had a power greater than 95% to detect a correlation coefficient (for the relationship between NE and echocardiographic parameters of LVM) equal to 0.28 with a P<0.01. Thus, the statistical strength of our study explains why, notwithstanding the weaker reproducibility of plasma NE measurements in comparison to more refined techniques, we have been able to capture an association between this neurotransmitter and the muscular component of the left ventricle in dialysis patients.

A second limitation of our study derives from its cross-sectional design. As previously discussed, raised sympathetic activity may be causally involved in the pathogenesis of concentric LVH in dialysis patients; yet, the opposite viewpoint is in theory possible. In other words, it can also be hypothesized that raised NE is a consequence of concentric LVH because stimulation of cardiac afferent fibers may be altered in the hypertrophied ventricle. The same problem applies to left ventricular dysfunction because raised NE may be a cause or an effect of this alteration. The issue of causality cannot be resolved on the basis of the present cross-sectional data.

Finally, it is important noting that because circulating NE is a less reproducible marker of sympathetic function than sympathetic microneurography, due to regression dilution bias, our study may substantially underestimate the association between sympathetic activity and echocardiographic parameters of the left ventricle in dialysis patients.

Perspectives

High-circulating NE is associated to concentric LVH in patients with ESRD. Increased norepinephrine is an important cardiovascular risk factor in these patients. The cardiovascular mortality in ESRD is exceedingly high, and controlling this ‘cardiovascular epidemics’ is considered an absolute priority. Our data lend support to the hypothesis that LVH may represent an intermediate mechanism mediating the adverse effect of circulating NE on the heart in these patients. This is important because the effects of raised NE can be modified by appropriate therapeutic interventions. Clinical trials with antiadrenergic drugs will establish whether the link between plasma NE and alterations in LVM and function is a causal one.

CREED Investigators

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References

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