Vascular Function and Mild Renal Impairment in Stable Coronary Artery Disease

Pim van der Harst, Tom D.J. Smilde, Hendrik Buikema, Adriaan A. Voors, Gerjan Navis, Dirk J. van Veldhuisen, Wiek H. van Gilst

Objective—In patients with coronary artery disease, the concomitant presence of renal function impairment is associated with decreased survival. We aimed to assess whether in coronary artery disease patients renal function impairment is associated with systemic vascular function, functional parameters of the renin-angiotensin system, or inflammation as potential mediators for cardiovascular risk.

Methods and Results—We studied 125 patients, 87% male, with a mean age of 62.2±8.2 years; 72% had 3-vessel disease, and mean renal function was 74±13 mL/min per 1.73 m². Internal thoracic artery rings were sampled during coronary bypass surgery and used for in vitro vascular measurements. We could not establish an association between endothelium-dependent vasorelaxation (response to methacholine) and renal function. In addition, vascular response to potassium chloride, phenylephrine, and angiotensin II were not associated with renal function. Finally, serum angiotensin-converting enzyme (ACE) activity, usage of ACE inhibitors, C-reactive protein, and soluble intercellular adhesion molecule 1 were not related to renal function.

Conclusions—In coronary artery disease patients, mild renal function impairment is not associated with systemic vasomotor responsiveness, inflammation, or functional systemic parameters of the renin-angiotensin system. The relation between systemic endothelial dysfunction and mild renal insufficiency might be more complicated than previously thought. (Arterioscler Thromb Vasc Biol. 2006;26:379-384.)

Key Words: vascular function ■ coronary artery disease ■ endothelium ■ renal function angiotensin II

The concomitant presence of renal function impairment in patients with various forms of cardiovascular disease is associated with a worse prognosis.1–5 Limited data are available on the pathophysiologic mechanisms linking mild renal function impairment to the marked increase in cardiovascular events. A recent study in essential hypertension demonstrated an association between mild renal function impairment and endothelial dysfunction, suggesting that renal function impairment might be a marker of generalized vascular dysfunction, as a possible mediator of cardiovascular risk.6 However, because hypertension can be expected to elicit endothelial and renal dysfunction this assumption needs to be tested in a nonessential hypertensive population as well. Other factors affecting the prognosis of coronary artery disease involve alterations of the renin-angiotensin system and inflammation.7–9 However, whether these factors are associated with renal function remains to be determined.

In this report, we analyzed the potential relationship between renal function and endothelial dependent vasodilation in subjects with severe coronary artery disease. Second, we assessed whether renal function was associated with the vascular responses of several vasoconstrictors, systemic parameters of the renin-angiotensin system, and systemic markers of inflammation.

Methods

Study Population
Patients in the present study participated in the QUinapril On Vascular Ace and Determinants of Ischemia Study (QUO VADIS). A total of 187 patients were included into QUO VADIS, and methods and results of this study were published elsewhere.10 We studied all 125 subjects undergoing coronary artery bypass grafting (CABG), of whom endothelium-dependent vasodilatation or vascular responsiveness to angiotensin II was assessed. The glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease (MDRD) equation11 and, additionally, the Cockcroft-Gault (corrected for body surface area).12 The Institutional Review Board approved this study, and written informed consent was obtained from each subject.

Organ Chamber Experiments
During CABG, segments of internal thoracic arteries were collected as excess graft material and transported immediately to the laboratory, where in vitro vascular measurements were performed within 6
hours of harvesting as published previously.10,11 Endothelial dependent relaxation was studied by cumulative concentrations of methacholine. Endothelium-independent relaxation was studied by the response to a single high concentration of sodium nitrite (NaNO2) (10 mmol/L; yields ~10 mmol/L nitric oxide).12 Vascular responsiveness to angiotensin II was studied with cumulative concentrations as described previously.10,12 Maximal vasoconstriction was evoked by stimulation with 60 mmol/L potassium chloride.

**Angiotensin-Converting Enzyme Activity Determination**

Plasma angiotensin-converting enzyme (ACE) activity was measured 1 day before CABG as described previously.15 Briefly, using 35-times diluted plasma, 10 minutes of incubation with 7 mmol/L hippuryl-l-histidyl-l-leucine (Hip-His-Leu) at 37°C, the production of hippuric acid (nM His-Leu/min per mL) was measured spectrophotometrically.

**Inflammatory Markers**

Of our 125 patients, inflammatory markers were determined in a subset of 62 patients, solely defined by the availability of a stored (at −80°C) blood sample taken before surgery.16 This subgroup was comparable to the whole study population with regard to the variable measurements. As shown in Table 1, soluble intercellular adhesion molecule-1 (sICAM) and C-reactive protein (CRP) were determined by enzyme-linked immunosorbent assay (ELISA) in duplicate as described previously.16

**Statistical Analysis**

Data are expressed as mean±SD or presented as frequency, unless indicated different. Non-normally distributed variables were log-transformed for statistical comparisons. Comparisons between groups were made by 1-way ANOVA or the χ² test, as appropriate. Relationships between paired parameters were analyzed by Pearson product moment correlation coefficient. Dose-response curves of methacholine and angiotensin II were performed of each of the GFR tertiles and the differences of these curves were tested using repeated measures analysis of variance test. All calculations were made with a standard statistical package (SPSS for Windows version 12.0.1).

**Results**

The population consisted of predominantly (87%) male patients; 72% had 3 diseased coronary arteries. The estimated mean renal function was 74±13 mL/min per 1.73 m² (range 34 to 112). At time of CABG, 58% of the patients were undergoing ACE inhibitor therapy, 75% were using aspirin, and 40% were using lipid-lowering medication.

All patients showed normal dilatory response to endothelium-independent vasodilatation (sodium nitrite). On the basis of maximal response to methacholine, patients were grouped into 3 tertiles. As shown in Figure 1, there was a considerable difference among the tertiles of endothelium-dependent vasodilatation. As shown in Table 1, patients in the first tertile (ie, those with less endothelium-dependent vasodilatation) were older, with a lower prevalence of males than

### Table 1. Clinical and Biochemical Data of Patients Divided on the Basis of 3 Tertiles of Endothelium-Dependent Vasodilatation*

<table>
<thead>
<tr>
<th>Tertile</th>
<th>Tertile 1 (n=37)</th>
<th>Tertile 2 (n=38)</th>
<th>Tertile 3 (n=37)</th>
<th>P*</th>
<th>R (P†)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.2±6.9</td>
<td>60.9±7.9</td>
<td>60.3±9.6</td>
<td>0.09</td>
<td>0.21 (&lt;0.05)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>28 (76)</td>
<td>34 (89)</td>
<td>35 (95)</td>
<td>0.05</td>
<td>0.30 (&lt;0.05)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (10)</td>
<td>2 (5)</td>
<td>1 (3)</td>
<td>0.36</td>
<td>0.10 (0.29)</td>
</tr>
<tr>
<td>3-vessel disease, n (%)</td>
<td>26 (70)</td>
<td>25 (66)</td>
<td>30 (81)</td>
<td>0.32</td>
<td>−0.15 (0.11)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7±3.5</td>
<td>26.5±2.9</td>
<td>27.2±2.7</td>
<td>0.58</td>
<td>−0.06 (0.53)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>144.9±22.3</td>
<td>138.2±18.7</td>
<td>137.4±21.6</td>
<td>0.24</td>
<td>−0.04 (0.51)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80.5±7.8</td>
<td>80.8±8.4</td>
<td>80.7±7.0</td>
<td>0.99</td>
<td>−0.04 (0.67)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>72.0±15.3</td>
<td>70.1±13.5</td>
<td>70.3±13.2</td>
<td>0.82</td>
<td>−0.02 (0.81)</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>4 (11)</td>
<td>5 (13)</td>
<td>5 (14)</td>
<td>0.93</td>
<td>0.05 (0.64)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.3±1.2</td>
<td>6.0±1.1</td>
<td>6.2±1.5</td>
<td>0.66</td>
<td>0.11 (0.27)</td>
</tr>
<tr>
<td>sICAM, μg/L</td>
<td>176±211</td>
<td>142±40</td>
<td>124±63</td>
<td>0.47</td>
<td>0.18 (0.19)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.60 [0.50–4.28]</td>
<td>2.50 [1.10–5.35]</td>
<td>1.55 [0.70–5.20]</td>
<td>0.36</td>
<td>−0.07 (0.64)</td>
</tr>
</tbody>
</table>

*On the basis of maximal response to ME, patients were ranked and grouped into 3 tertiles (first tertile, lower response; second tertile, intermediate response; third tertile, high response).

†Correlation coefficient R and P value of relationship between maximal response to ME (as a continuous variable) and each variable listed in the Table.

C-reactive protein is expressed as median [interquartile range], all other data are expressed as mean±SD or as percent frequency, and comparisons among groups were made by 1-way ANOVA or χ² test, as appropriate.

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Figure 1. Three tertiles of endothelium-dependent vasodilatation response to methacholine. Data (mean±SEM) are expressed as percentage of phenylephrine (PE) precontraction. Differences among groups are significant (P<0.001).
for patients in the other 2 tertiles. The 3 tertiles did not differ with regard body mass index, arterial pressure and heart rate, serum cholesterol, diabetes, or the proportion of smokers.

Serum creatinine was not different among the tertiles of endothelium-dependent vasodilatation, neither was (MDRD) estimated GFR. Univariate linear regression analysis, using continues variables, confirmed maximal endothelium-dependent vasorelaxation was not to be associated with serum creatinine or estimated GFR (Figure 2). Because diabetic patients with relative glomerular hyperfiltration are likely to mask these associations, we repeated additional analyses with the diabetic patients excluded. However, this did not change our results (data not shown). A posteriori, our computation showed statistical power to detect a correlation of $r^2 \leq 0.07$ (based on 2-sided test; n=112; $P<0.05$; power=0.80).17 Additional analysis with correction for maximal achieved vasodilatation with sodium nitrite (%SN) instead of phenylephrine (%PE) did not change these findings (data not shown).

Next, we studied the vascular responsiveness to the vasoconstrictors phenylephrine, potassium chloride and to angiotensin II. We did not observe a relation between these vasoconstrictors and estimated GFR (Table 2). To further examine the possible involvement of the systemic renin-angiotensin system, we considered the relation between serum ACE activity and usage of ACE inhibitors with renal function. ACE activity and ACE inhibitor usage were also not associated with differences in renal function. Furthermore, performing stratified analysis for ACE inhibitor therapy did not reveal an association between vascular function and renal function parameters (data not shown). Finally, we evaluated the possible role of inflammatory markers with renal function. Both CRP and soluble intercellular adhesion molecule-1 were not related with estimated GFR (Figure I, available online at http://atvb.ahajournals.org).

**TABLE 2. Correlation Coefficients for Renal Function**

<table>
<thead>
<tr>
<th>Variable</th>
<th>$R$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial-dependent vasodilatation</td>
<td>-0.049</td>
<td>0.61</td>
</tr>
<tr>
<td>Endothelial-independent vasodilatation</td>
<td>-0.113</td>
<td>0.24</td>
</tr>
<tr>
<td>Phenylephrine contraction</td>
<td>0.089</td>
<td>0.35</td>
</tr>
<tr>
<td>Potassium chloride contraction</td>
<td>0.071</td>
<td>0.43</td>
</tr>
<tr>
<td>Renin-Angiotensin system parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II contraction</td>
<td>0.011</td>
<td>0.91</td>
</tr>
<tr>
<td>Serum ACE activity</td>
<td>0.186</td>
<td>0.21</td>
</tr>
<tr>
<td>ACE inhibitor usage</td>
<td>0.014</td>
<td>0.88</td>
</tr>
<tr>
<td>Inflammatory parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.058</td>
<td>0.66</td>
</tr>
<tr>
<td>sICAM</td>
<td>0.009</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Correlation coefficient $R$ and $P$ value of relationship between estimated renal function (as a continuous variable) and each variable listed in the Table.
To evaluate to possible the relation of mild–moderate renal function impairment with vascular function, we grouped patients according to the renal and, in addition, 2 clinical relevant renal function categories (estimated GFR <90 versus GFR >90, and GFR <60 versus GFR >60 mL/min per 1.73m²).18 Dose-response curves for endothelial-dependent relaxation and vascular responsiveness to angiotensin II for these renal function categories were constructed (for median, see Figure 3). Repeated measurement analysis did not reveal any differences among these categorizations.

Discussion

In these patients with severe coronary artery disease, we observed a wide range of endothelium dependent vasodilatation of the internal thoracic artery. Compromised endothelium results in the loss of a powerful defense mechanism against vascular and renal damage.19–21 Although the differences in endothelial function were considerable, it did not enable us to observe any associations with serum creatinine or estimated GFR. Furthermore, we did not find significant associations between the response to the potent vasoconstrictors phenylephrine, potassium chloride, or angiotensin II with serum creatinine or estimated GFR. In addition to vascular response to angiotensin II, the other parameters of the RAS serum ACE activity and use of ACE inhibitors were also not associated with renal function. Additionally, inflammatory markers were not related to renal function in our patients. Overall, this study did not demonstrate a relation between systemic vascular measurements and renal function in CABG patients.

The present study has a number of strengths. We performed detailed vascular measurements consisting of a 9-point dose-response curve for endothelium-dependent vasodilatation and angiotensin II responsiveness in >100 patients. The range of endothelium-dependent vasodilatation was considerable, enhancing the detection of potential differences in renal function parameters. In addition to vasodilatation, we assessed several potent vasoconstrictors to assess smooth muscle cell properties. The vascular measurements were all performed within one structured protocol by experienced biotechnicians. The a posteriori computation of statistical power showed that this study could detect a correlation with an r² of 0.07, ie, a relationship in which ≈7% of the variability in renal function was explained by endothelial function. Thus, our negative findings are unlikely to be caused by a lack of power. Most previous studies focused on the relation between renal and endothelial function in patients with chronic renal failure or severe renal function impairment.22–24 Only recently, Perticone et al studied untreated essential hypertensive patients with normal to mild renal function. In that study, endothelial-dependent vasodilatation was correlated with serum creatinine levels and estimated renal function (r²=0.10 and r²=0.04, respectively, both P<0.001). In addition, they observed a relation between serum CRP with estimated GFR. We studied patients with similar renal function, performed similar statistics and grouping of patients, and estimated GFR with the same formula. In addition, we performed more and detailed vascular measurements. In vitro assessment of vessels allowed us to determine, in addition to endothelium dependent and independent relaxation, the vascular responsiveness to potent vasoconstrictors. In contrast to the study of Perticone, the present study demonstrated no association of endothelial-dependent vasodilatation on serum creatinine levels or estimated renal function. Furthermore, we did not observe a relation between CRP and estimated GFR. Several factors might explain these apparently discrepant findings. One of the major differences is the difference in the study population. Although both studies focused on patients in which endothelial function plays a crucial pathophysiological role, the origin of endothelial function impairment was dissimilar.25–26 Whereas Perticone studied uncomplicated untreated essential hypertensive patients, we studied patients with severely diseased coronary arteries with a lower incidence of hypertension and lower blood pressure levels. Of note, the kidney is a well-known target of hypertension. The previously reported association between endothelial function and renal function therefore might not result from one another, but instead from the common denominator hypertension. Although some adjustments were made for blood pressure levels in the study of Perticone, other hypertension characteristics known to influence renal function were not taken into account, ie, the duration of hypertension. In addition, the individual susceptibility for renal insufficiency might be traced back to disturbances in the way the kidney autoregulates. Increased susceptibility for impaired renal autoregulation might merely be the renal manifestation of a more widespread abnormality in endothelial function. In that case, the kidney is less well-protected from sustained elevations in arterial pressure, potentially making some subjects more vulnerable to subtle...
acquired injury, particularly in the setting of hypertension. Together, the study by Perticone, and the present study are suggesting that hemodynamic, ie, blood pressure, may lead to renal injury instead of systemic endothelial dysfunction per se. The influence of atherosclerosis on renal function is not fully elucidated and remains to be established. Systemic endothelial dysfunction associated with atherosclerosis might not be a critical determinant contributing to mild renal dysfunction, in contrast to essential hypertension.

The current study provides 2 new pathophysiological insights into the relation between vasomotor function and renal function impairment in coronary artery disease patients. First, in atherosclerotic patients, endothelium-dependent dilatory function of the internal thoracic artery is not associated with renal function. Second, vasoconstrictor responses to phenylephrine and potassium chloride and, in addition, several markers of the RAS, namely vascular responsiveness to angiotensin II, serum ACE activity, and use of ACE inhibitors, were also not associated with renal function in these patients. Thus, our findings suggest that in CABG patients renal function is, in contrast to hypertensive patients, not related to the functional integrity of the systemic endothelium.

The present study has its limitations. This study provides cross-sectional observational data and therefore can only be used to generate new hypotheses. We were unable to determine local renal vascular responses to an nitric oxide agonist or antagonist and angiotensin II. Endothelial function has been demonstrated to be parallel in various vascular beds and endothelial dysfunction is considered a generalized condition. However, the functional heterogeneity of the vascular bed is also well-established. Thus, we cannot exclude that the lack of association between our endothelial function estimates and renal function is due to functional disparity between the internal thoracic artery and the renal vascular bed.26 We studied vascular function in an ex vivo set-up. An advantage of this set-up is that it is not influenced by the sympathetic nervous system or by circulating neurohumoral factors or factors influencing vascular function originating from or cleared by the kidney. However, this can also be viewed as a limitation because a possible role of these factors cannot be analyzed and thus will remain undetected. For example, the endogenous L-arginine analogue asymmetrical dimethylarginine concentrations are raised in patients with renal dysfunction. Because asymmetrical dimethylarginine inhibits endothelial nitric oxide synthase, it can diminish endothelium-dependent vasodilatation.26 We cannot exclude that our estimation of GFR was not precise enough to find a correlation with vascular function. The shortcomings of the MDRD formula, as well as other renal function equations, in populations with only mildly impaired renal function is increasingly recognized.26 However, the association between mild renal function impairment and cardiovascular risk was established in several studies that also used the MDRD or Cockcroft Gault equation, and considering the lack of a generally accepted better estimate of renal function it would be best to comply with common practice in the literature for sake of comparability.1,6,31

Furthermore, no urine samples were taken, so we have no information on urinary albumin excretion. Urinary albumin excretion is considered a more sensitive measure of early renal damage than glomerular filtration rate.32,33 Thus, the current study cannot exclude a possible association between urinary albumin excretion and systemic vascular function in this population, but this does not affect the conclusion on absence of a relationship between reduced glomerular filtration rate and endothelial function.

Nevertheless, the present data are unique in their kind and provide novel pathophysiological insights. We are not aware of a larger series of detailed vascular assessment performed within a single structured protocol.

In conclusion, this is the first study to study the relation between vascular function and renal function in patients with severe coronary artery disease. We did not found a relation between diminished endothelium-dependent relaxation and the presence of mild to moderate reduced renal function impairment. In addition, functional parameters of the systemic renin–angiotensin system, smooth muscle properties and inflammatory markers were not associated with renal function. The relation between vascular function and mild renal function impairment might be more complicated than previously thought.

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References
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Figure I

A.) Relationship between C-reactive protein (CRP) and estimated GFR. B.) Relationship between soluble intercellular adhesion molecule (sICAM) and estimated GFR.