C-Reactive Protein and Soluble Vascular Cell Adhesion Molecule-1 Are Associated With Elevated Urinary Albumin Excretion but Do Not Explain Its Link With Cardiovascular Risk

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Abstract—An elevated urinary albumin excretion rate (UAER) is associated with an increased risk of cardiovascular mortality, but the pathophysiological mechanism underlying this association is poorly understood. To investigate the role of endothelial dysfunction, leukocyte adhesion, and low-grade inflammation (1) in the development of elevated UAER (study I) and (2) in linking elevated UAER with risk of cardiovascular mortality (study II), we performed a prospective study in an age–, sex–, and glucose tolerance–stratified sample of a population-based cohort aged 50 to 75 years. High levels of von Willebrand factor, soluble vascular cell adhesion molecule-1 (sVCAM-1), and C-reactive protein (CRP) were used as markers of endothelial dysfunction, leukocyte adhesion, and low-grade inflammation, respectively. For study I, subjects who had normal UAER at baseline (n = 316 subjects, 66 with type 2 diabetes) were reexamined after a mean follow-up of 6.1 years. The development of elevated UAER was defined as a mean albumin-to-creatinine ratio >2.0 mg/mmol at follow-up. Age–, sex–, and glucose tolerance–adjusted logistic regression analyses showed the development of elevated UAER to be significantly associated with levels of sVCAM-1 and CRP (odds ratio 1.14 [95% CI 1.02 to 1.27] per 10% increase of sVCAM-1 and odds ratio 1.17 [95% CI 1.04 to 1.32] per 50% increase of CRP). The results were not materially different after additional adjustment for hypertension, body mass index, cardiovascular disease, and creatinine clearance or stratification by the presence of diabetes. For study II, the vital status of all subjects (n = 575) was determined after a mean follow-up of 6.6 years. Eighty-one of 575 subjects died (30 died of cardiovascular disease). The presence of elevated UAER at baseline was associated with a 4.1-fold (1.94 to 8.73) increased risk of cardiovascular death after adjustment for age, sex, and glucose tolerance status. Adjustment for levels of von Willebrand factor, sVCAM-1, or CRP did not materially affect the results, nor did additional adjustment for the presence of hypertension, retinopathy, and cardiovascular disease and for levels of homocysteine, triglycerides, and high density lipoprotein cholesterol. Leukocyte adhesion (sVCAM-1) and low-grade inflammation (CRP) are determinants of the development of elevated UAER. However, these determinants do not explain the association between elevated UAER and cardiovascular mortality. (Arterioscler Thromb Vasc Biol. 2002;22:593-598.)

Key Words: elevated urinary albumin excretion rate ■ von Willebrand factor ■ C-reactive protein ■ soluble vascular cell adhesion molecule-1 ■ cardiovascular mortality

Elevated urinary albumin excretion rate (UAER) is associated with an increased risk of cardiovascular disease among individuals with and without type 2 diabetes. The pathophysiological mechanism linking elevated UAER to cardiovascular disease is unknown; the most commonly held view is that elevated UAER reflects a pathophysiological process predisposing an individual to atherothrombosis. Atherothrombosis is a low-grade inflammatory disease of the vessel wall characterized by endothelial dysfunction and an increased transendothelial passage of leukocytes. Therefore, these features could be the pathogenic factor linking elevated UAER to cardiovascular disease.

In support of this hypothesis, increased plasma levels of von Willebrand factor (vWF), a marker of endothelial dysfunction, have been associated not only with an increased risk of cardiovascular events but also with the development of elevated UAER. In addition, increased plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), an
adhesion molecule that reflects the recruitment of leukocytes into the vessel wall, have been associated with the risk of cardiovascular death on one hand and of elevated UAER on the other. Finally, increased levels of C-reactive protein (CRP), an acute-phase reactant, reflect inflammatory activity and are associated with an increased risk of cardiovascular mortality. Acute inflammation is associated with increased urinary protein excretion and elevated UAER. Conversely, the presence of elevated UAER is associated with increased levels of proinflammatory cytokines.

In view of these considerations, we performed a prospective population-based cohort study to investigate the role of endothelial dysfunction, leukocyte adhesion, and low-grade inflammatory activity (1) in the development of elevated UAER (study I) and (2) in linking elevated UAER with the risk of cardiovascular mortality (study II). We used increased levels of vWF, sVCAM-1, and CRP as markers of endothelial dysfunction, leukocyte adhesion, and low-grade inflammatory activity, respectively. For study II, we updated and extended previously published analyses.

Methods

The Hoorn Study is a prospective cohort study of disturbances of glucose tolerance and cardiovascular disease in a general white population aged 50 to 75 years, but still randomly selected, to measure the urinary albumin-to-creatinine ratio (ACR). Of all the urine samples, 32 were excluded because of the use of an ACE inhibitor. In a representative random sample of 174 subjects, 2 urine collections were available; therefore, the presence of (micro)albuminuria for these subjects was based on the mean ACR of the 2 urine collections.

We obtained data on blood pressure, weight, height, and waist and hip circumference, data on fasting and 2-hour postload glucose, creatinine, homocysteine, total cholesterol, HDL cholesterol, and triglyceride levels, and data on vWF, sVCAM-1, and CRP, and we assessed an ankle-brachial pressure index, a resting ECG, ophthalmoscopy, and/or fundus photography. Current smoking was defined as currently smoking cigarettes and/or cigars. Other definitions are described in Results.

Baseline Examination (Studies I and II)

We obtained an early-morning, first-voided, spot urine sample to measure the urine albumin-to-creatinine ratio (ACR). Of all the urine samples, 32 were excluded because of the use of an ACE inhibitor. In a representative random sample of 174 subjects, 2 urine collections were available; therefore, the presence of (micro)albuminuria for these subjects was based on the mean ACR of the 2 urine collections.

We obtained data on blood pressure, weight, height, and waist and hip circumference, data on fasting and 2-hour postload glucose, creatinine, homocysteine, total cholesterol, HDL cholesterol, and triglyceride levels, and data on vWF, sVCAM-1, and CRP, and we assessed an ankle-brachial pressure index, a resting ECG, ophthalmoscopy, and/or fundus photography. Hypertension was defined as diastolic pressure ≥95 mm Hg, systolic pressure ≥160 mm Hg, and/or the use of antihypertensive drugs in accordance with guidelines in use at the time the study was designed. Current smoking was defined as currently smoking cigarettes and/or cigars. Other definitions are described in Results.

Follow-Up Study I

For the analyses in study I, we focused only on those subjects who, at baseline, were normoalbuminuric and did not use ACE inhibitors. All participants were asked to hand in an overnight, first-voided, untimed spot urine sample. In a representative sample (n = 161), subjects were asked to hand in a second set of urine samples within 4 weeks. Of all 340 participants, 4 collected no urine samples, 18 collected 1 sample, 168 collected 2 samples, 10 collected 3 samples, and 140 collected 4 samples. The urinary ACRs were determined, and the mean ACR was calculated. Subjects were classified as having normoalbuminuria when the mean ACR was ≤2.0 mg/mmol and as having (micro)albuminuria when the mean ACR was >2.0 mg/mmol.

Follow-Up Study II

Data on the vital status and date of death for each subject were collected from the mortality register of the municipalities of Hoorn or other local municipalities. For all subjects who had died, the cause of death was classified according to the ninth edition of the International Classification of Diseases. Cardiovascular mortality was defined as codes 390 to 459. Information on the cause of death could not be obtained for 10 (12%) of the deceased subjects, and 1 subject was lost to follow-up.

Statistical Analyses

Differences between the 2 groups were tested with the Student t test, the Mann-Whitney test, and the χ² test, as appropriate. To assess whether determinants were independently associated with the development of (micro)albuminuria, logistic regression analyses were primarily adjusted for all variables that were statistically significant in the initial analyses, secondarily adjusted for creatinine clearance, and, finally, adjusted for other variables of interest. To investigate whether endothelial dysfunction, leukocyte adhesion, or low-grade inflammation could be the pathogenic link between cardiovascular mortality and (micro)albuminuria, regression analyses were performed with adjustment for the levels of vWF, sVCAM-1, and CRP.

For further information, please see the online supplement, which can be accessed at http://www.atvb.ahajournals.org.

Results

Study I: Determinants of the Development of Elevated UAER

The mean duration of follow-up was 6.1 years (standard deviation 0.7 years, range 4.4 to 7.7 years). The cumulative incidence of elevated UAER was 14.0% (95% CI 9.7 to 19.3) among nondiabetic subjects and 22.7% (95% CI 12.9 to 32.5) among type 2 diabetic patients. The cumulative incidence of elevated UAER increased with tertiles of sVCAM-1 and CRP level but not with tertiles of vWF level (Figure 1).

Subjects who died during the follow-up period, compared with those who participated in the follow-up examination, were older, more obese, and more often smokers; those who died, compared with those who survived, also more often had type 2 diabetes, hypertension, and cardiovascular disease and higher levels of homocysteine, triglycerides, vWF (1.60 versus 1.29 IU/mL, respectively; P = 0.01), and CRP (2.30 versus 1.48 mg/mL, respectively; P = 0.047) at baseline (other data not shown). The nonresponders (Figure 2) were not materi-
nonresponders were older (65 versus 63 years, $P<0.01$). Subjects who developed elevated UAER, compared with those who did not, were significantly older and more obese; they had higher systolic blood pressure and higher homocysteine, sVCAM-1, and CRP levels; and they more often had hypertension, cardiovascular disease, and retinopathy at baseline (Table 1). Age-adjusted, sex-adjusted, and glucose tolerance status–adjusted logistic regression analyses showed the development of elevated UAER to be significantly associated with age, male sex, systolic blood pressure, presence of hypertension, body mass index, levels of homocysteine, sVCAM-1, and CRP, and the presence of cardiovascular disease (Table 1). Analyses performed in nondiabetic and diabetic subjects separately gave similar results (data not shown). Levels of vWF in the upper 10% of the distribution, n = 483, and analyses after exclusion of subjects with impaired glucose tolerance status (a determinant of vWF levels, 7 data not shown).

In multiple regression analyses, the risk of developing elevated UAER associated with high levels of sVCAM-1 and CRP was independent of other determinants (Table 2). Additional adjustment for homocysteine level did not materially change the results (data not shown). Substituting systolic blood pressure or diastolic blood pressure for hypertension did not materially change the results either (data not shown). Analyses in nondiabetic and diabetic subjects separately showed somewhat higher, but not significantly different, odds ratios of developing elevated UAER associated with sVCAM-1 and CRP levels among nondiabetic than among diabetic subjects (probability values for interaction 0.10 and 0.12, respectively; data not shown).

The correlation between vWF and logarithmically transformed CRP, it was 0.10 ($P=0.10$); and between logarithmically transformed sVCAM-1 and logarithmically transformed CRP, it was 0.11 ($P=0.06$). The risk of developing elevated UAER associated with sVCAM-1 levels was not materially changed after adjustment for vWF or CRP levels (Table 2, models 5 and 7). Similarly, the risk of developing elevated UAER for CRP levels was not materially changed after adjustment for vWF or sVCAM-1 levels (Table 2, models 5 and 6).

Additional Analyses

The following additional analyses did not materially affect our results: analyses with elevated UAER defined as an ACR >3.0 instead of >2.0 mg/mmol, analyses with elevated UAER defined as an ACR >2.5 mg/mmol for men and >3.5 mg/mmol for women, analyses with elevated UAER defined on the basis of the overnight or spot samples only, analyses with elevated UAER defined on the basis of the median ACR, analyses after exclusion of subjects who developed “macroalbuminuria” (as defined by an ACR >30 mg/mmol, n = 3), analyses after exclusion of urine samples with a positive dipstick test for leukocytes and/or nitrite at baseline (n = 49) and/or at follow-up (n = 47), analyses among nondiabetic subjects that excluded subjects with impaired glucose tolerance status (n = 84), analyses after exclusion of subjects with CRP levels >10.0 mg/L (n = 10), analyses after exclusion of subjects with sVCAM-1 levels >1890 ng/mL (upper 10% of the distribution, n = 31), and analyses that included blood groups (a determinant of vWF levels, data not shown).

Study II: Can Markers of Endothelial Dysfunction, Leukocyte Adhesion, and Inflammatory Activity Explain the Association Between Elevated UAER and Cardiovascular Mortality?

After 6.6 years (standard deviation 1.4 year, range 0.5 to 8.2 years) of follow-up, 14% (81, with 35 type 2 diabetic subjects) of the 574 subjects had died, of whom 37% (30, with 15 type 2 diabetic subjects) had died of cardiovascular disease (Figure 2). Subjects who died, compared with those who survived, more often had elevated UAER (23.5% versus 9.5%, respectively; $P=0.001$) and had higher levels of vWF (1.57 versus 1.33 IU/mL, respectively; $P=0.005$), sVCAM-1 (1383 versus 1305 ng/mL, respectively; $P=0.07$), and CRP (2.24 versus 1.62 mg/L, respectively; $P=0.005$).

In the entire group, elevated UAER was associated with an approximately 4-fold increased risk of cardiovascular death after adjusting for age, sex, and glucose tolerance status (Table 3). Adjustment for levels of vWF, sVCAM-1, or CRP did not materially change the results. Analyses in nondiabetic and diabetic subjects separately showed somewhat higher, but not significantly different, relative risks of cardiovascular mortality associated with elevated UAER among diabetic than among nondiabetic subjects ($P=0.07$ for interaction between type 2 diabetes and elevated UAER, data not shown); additional adjustment for levels of vWF, sVCAM-1, or CRP again did not materially change the results.

In previous analyses, we showed age, current smoking, levels of homocysteine, triglycerides, and HDL cholesterol, and the presence of hypertension, type 2 diabetes mellitus, retinopathy,
and cardiovascular disease to be associated with cardiovascular mortality.\textsuperscript{16,21} Additional adjustment for these risk factors somewhat decreased the relative risks of cardiovascular mortality for elevated UAER (eg, relative risk among all subjects 2.97 [1.32 to 6.69]) but did not affect the results of the analyses with vWF, sVCAM-1, and CRP added (data not shown).

Additional analyses analogous to those performed in study I showed that the presence of elevated UAER was associated with a 4-fold increased risk of cardiovascular mortality. This risk estimate was not materially affected by adjustment for levels of vWF, sVCAM-1, and CRP, which argues against the hypothesis that endothelial dysfunction, leukocyte adhesion, or low-grade inflammation is the pathogenic link between elevated UAER and the risk of cardiovascular mortality.

High levels of vWF and CRP among subjects who are not acutely ill are thought to be reasonably specific markers of endothelial dysfunction and low-grade inflammation, respectively.\textsuperscript{5,22} In contrast, the interpretation of high sVCAM-1 levels is less clear.\textsuperscript{18} High levels of sVCAM-1 among subjects who are not acutely ill are thought to reflect increased levels of advanced glycation end products,\textsuperscript{26,27} and cardiovascular disease to be associated with cardiovascular mortality.\textsuperscript{16,21} Additional adjustment for these risk factors somewhat decreased the relative risks of cardiovascular mortality for elevated UAER (eg, relative risk among all subjects 2.97 [1.32 to 6.69]) but did not affect the results of the analyses with vWF, sVCAM-1, and CRP added (data not shown). Additional analyses analogous to those performed in study I (see above) did not materially affect the results (data not shown).

**Discussion**

We showed that high levels of sVCAM-1 and CRP were independently associated with the development of elevated UAER. These data suggest that leukocyte recruitment into the vessel wall and low-grade inflammation play a pathogenic role in the development of elevated UAER. We further showed that the presence of elevated UAER was associated with a 4-fold increased risk of cardiovascular mortality. This risk estimate was not materially affected by adjustment for levels of vWF, sVCAM-1, and CRP, which argues against the hypothesis that endothelial dysfunction, leukocyte adhesion,
proinflammatory cytokines, as reflected by increased acute-phase reactants such as CRP, may cause elevated UAER through renal and nonrenal vascular mechanisms.

Increased vWF levels were not associated with the development of elevated UAER, which is in contrast with some but not all studies. There are several possible explanations for these discrepant findings. First, studies that found high vWF levels to be associated with the development of elevated UAER had a shorter duration of follow-up (3.1 to 5.3 years) than did studies that did not find this association (Yokoyama et al and the present study, 6.6 to 10.0 years). Late-onset elevated UAER may have a different pathogenesis than does early-onset elevated UAER. Second, 50 of the 509 subjects investigated at baseline died during follow-up. We have previously shown in this population that mortality risk is related to having elevated UAER and high vWF levels. Indeed, subjects who died had higher vWF levels at baseline than did those who survived. These data suggest that compared with subjects who survived, subjects who died may have been at increased risk of developing elevated UAER. Therefore, we may have underestimated the association between increased vWF levels and incident elevated UAER.

The associations between incident elevated UAER and sVCAM-1 and CRP levels appeared stronger among nondiabetic than among diabetic subjects. We emphasize that this may just be the play of chance, although we clearly cannot exclude the possibility that with regard to the processes reflected by high sVCAM-1 and CRP levels, the pathogenesis of elevated UAER differs between nondiabetic and diabetic subjects.

The present data confirm that elevated UAER is strongly and independently associated with cardiovascular mortality. We investigated 3 possible pathophysiological processes that might explain this association. We found no clear evidence that this link was explained by endothelial dysfunction, leukocyte adherence, or low-grade inflammation. The most important assumption in this conclusion is that these processes are reflected by high sVCAM-1 and CRP levels, with sufficient accuracy. The most important assumption in this conclusion is that these processes are reflected by high sVCAM-1 and CRP levels, with sufficient accuracy. The most important assumption in this conclusion is that these processes are reflected by high sVCAM-1 and CRP levels, with sufficient accuracy.

What then could explain the link between elevated UAER and risk of cardiovascular mortality? One possibility is that elevated UAER reflects a prothrombotic state. Alternatively, elevated UAER may reflect a certain susceptibility to the vascular adverse effects of a variety of cardiovascular risk factors. This concept is supported by the observation that determinants of the development of elevated UAER, such as diabetes, hypertension, and the processes reflected by high sVCAM-1 and CRP levels, do not appear to confound the elevated UAER–cardiovascular disease link (Dinneen and Gerstein, Yudkin et al, Ridker et al, and the present findings).

In conclusion, we have shown that high levels of sVCAM-1 and CRP are associated with the development of elevated UAER. Therefore, we may have underestimated the association between increased vWF levels and incident elevated UAER.

### TABLE 2. ORs of Developing Elevated UAER According to sVCAM-1 and CRP Level After Adjustment for Potentially Confounding Variables (Study I)

<table>
<thead>
<tr>
<th>Model</th>
<th>Added Variables</th>
<th>sVCAM-1, ng/mL</th>
<th>CRP, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age, sex, impaired glucose tolerance, and type 2 diabetes mellitus</td>
<td>1.14 (1.02–1.27)</td>
<td>1.17 (1.04–1.32)</td>
</tr>
<tr>
<td>2</td>
<td>Model 1 and hypertension, body mass index, cardiovascular disease</td>
<td>1.14 (1.01–1.29)</td>
<td>1.14 (1.01–1.30)</td>
</tr>
<tr>
<td>3</td>
<td>Model 2 and creatinine clearance</td>
<td>1.14 (1.01–1.29)</td>
<td>1.15 (1.01–1.31)</td>
</tr>
<tr>
<td>4</td>
<td>Model 2 and waist-to-hip ratio, current smoking, and retinopathy</td>
<td>1.16 (1.03–1.31)</td>
<td>1.16 (1.01–1.32)</td>
</tr>
<tr>
<td>5</td>
<td>Model 1 and vWF</td>
<td>1.14 (1.02–1.27)</td>
<td>1.17 (1.04–1.32)</td>
</tr>
<tr>
<td>6</td>
<td>Model 1 and sVCAM-1</td>
<td>...</td>
<td>1.16 (1.03–1.30)</td>
</tr>
<tr>
<td>7</td>
<td>Model 1 and CRP</td>
<td>1.12 (1.00–1.25)</td>
<td>...</td>
</tr>
</tbody>
</table>

There were 316 subjects (50 cases). A case is a subject who developed elevated UAER. ORs and 95% CIs were according to logistic regression analyses for development of elevated UAER associated with a 10% increase in sVCAM-1 levels or with a 50% increase in CRP levels.

*Highest vs 2 lower tertiles.
†Described in legend to Table 1.
‡Highest vs the 2 lower tertiles.
§Logarithmically transformed.

### TABLE 3. Relative Risk of Cardiovascular Mortality Associated With the Presence of Elevated UAER After Adjustment for Levels of vWF, CRP, and sVCAM-1 (Study II)

<table>
<thead>
<tr>
<th>Model</th>
<th>Added Variables</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age, sex, impaired glucose tolerance, and type 2 diabetes mellitus</td>
<td>4.12 (1.94–8.73)</td>
</tr>
<tr>
<td>2</td>
<td>Model 1 and levels of vWF‡</td>
<td>3.92 (1.85–8.29)</td>
</tr>
<tr>
<td>3</td>
<td>Model 1 and levels of sVCAM-1</td>
<td>3.36 (1.55–7.26)</td>
</tr>
<tr>
<td>4</td>
<td>Model 1 and levels of CRP§</td>
<td>3.71 (1.74–7.92)</td>
</tr>
</tbody>
</table>

There were 574 subjects; 66 subjects had elevated UAER (ACR>2.0 mg/mmol). Relative risk of cardiovascular mortality was associated with elevated UAER analyzed by Cox multiple regression analyses. Model 1 consists of stratification variables.

*Highest vs 2 lower tertiles.
UAER. Furthermore, elevated UAER is associated with a 4-fold increased risk of cardiovascular mortality, which is not materially affected by adjustment for levels of vWF, sVCAM-1, and CRP. This may be of clinical relevance, because sVCAM-1 and CRP levels can be decreased by drug interventions and because prophylactic administration of aspirin has been found to reduce the risk of cardiovascular events, particularly among men with the highest baseline levels of CRP.

Acknowledgment

Prof Dr V. W. M. van Hinsbergh and Dr J. J. Emeis were supported by a grant of the Praventiefonds (28-1622-1).

References