C-Reactive Protein Modifies the Relationship Between Blood Pressure and Microalbuminuria

Erik M. Stuveling, Stephan J.L. Bakker, Hans L. Hillege, Johannes G.M. Burgerhof, Paul E. de Jong, Reinold O.B. Gans, Dick de Zeeuw, for the PREVEND Study Group

Abstract—C-reactive protein (CRP) and microalbuminuria reflect intimately related components of the atherosclerotic disease process. Epidemiological studies found only modest associations between CRP and microalbuminuria. Blood pressure, one of the components of the metabolic syndrome in the general population, is the main determinant of microalbuminuria in diabetes and hypertension. We questioned whether CRP modifies the relationship of blood pressure and other cardiovascular risk factors with microalbuminuria in a cross-sectional study in 8592 inhabitants from Groningen, The Netherlands. The crude data showed an increase in the prevalence of microalbuminuria with increasing CRP quartiles (4.8, 9.6, 14.5, and 18.6%, \( P<0.0001 \)). On stratification for cardiovascular risk factors, the data revealed a significant and positive interaction between mean arterial pressure (MAP) and quartiles of CRP with respect to the risk of microalbuminuria (Wald statistic 9.2, \( P=0.03 \)). In subjects with a MAP <90 mm Hg, a nonsignificant trend in the association between CRP quartiles and microalbuminuria was found (prevalence: 3.9%, 5.8%, 6.6%, 8.7%; \( P=0.11 \)). This trend was much steeper and significant in subjects with an MAP >90 mm Hg (prevalence: 6.7%, 13.6%, 20.4%, 25.1%; \( P<0.0001 \)). Controlling for other risk factors in multivariate analyses, the positive interaction persisted (\( P=0.0004 \)). No significant interactions between other risk factors and CRP with respect to the risk of microalbuminuria were encountered. Thus, CRP modifies the relation between blood pressure and microalbuminuria. (Hypertension. 2004;43:791-796.)

Key Words: cardiovascular diseases ■ blood pressure ■ risk factors ■ kidney ■ albuminuria

C-reactive protein (CRP), a sensitive marker of (sub)clinical inflammation, is thought to represent the state of chronic low-grade inflammation of the arterial vessel wall at atherosclerotic sites.\(^1\) CRP consistently predicts cardiovascular (CV) outcome.\(^2\) Microalbuminuria is also an established risk marker for future cardiovascular events in various populations.\(^5\)\(^6\) The appearance of low levels of albumin in the urine is thought to be the consequence of generalized endothelial damage along the vascular tree, including the glomerulus.\(^7\)

Blood pressure has been shown to be the main determinant of microalbuminuria in diabetes and hypertension.\(^8\) Many other CV risk factors, which cluster within the metabolic syndrome, have been shown to relate to microalbuminuria in the population.\(^9\)\(^10\) Because CRP and microalbuminuria reflect closely related components of the same disease processes, one might anticipate a strong relationship between them. However, studies that investigated this relationship in large populations only found weak associations.\(^11\)\(^12\) Interdependence between CRP and other cardiovascular risk factors with the risk of microalbuminuria may have been overlooked.

We questioned whether elevated serum CRP levels modify the relation of blood pressure and other CV risk factors with microalbuminuria.

Methods

Study Design

This study is part of the PREVEND study (Prevention of REnal and Vascular ENdstage Disease) in Groningen, The Netherlands. PREVEND investigates the natural course of microalbuminuria in relation to renal and cardiovascular morbidity and mortality in the general population. All inhabitants aged 28 to 75 years (\( n=85,421 \)) were asked to send in a morning urine sample and to complete a brief questionnaire on demographics and renal and cardiovascular morbidity. A total of 40,856 subjects responded. Pregnant women and subjects using insulin were excluded. All subjects with an albumin concentration of >10 mg/L in their morning urine sample plus a random sample of the population with a morning urine albumin excretion <10 mg/L were invited to our outpatient clinic. A detailed overview of this protocol is described elsewhere.\(^13\) All subjects completed an extensive questionnaire on demographics, renal and cardiovascular history, and information on pharmacy data. Furthermore, they underwent investigations in 2 visits: anthropometry, automated blood pres-
Laboratory Measurements
Urinary volume and albumin were measured in each collection. Urinary albumin concentrations were determined by nephelometry with a threshold of 2.3 mg/L and intra-assay and interassay coefficients of variation of <2.2 and <2.6, respectively (Dade Behring Diagnostic, Marburg, Germany). High sensitive CRP was also determined by nephelometry with a threshold of 0.175 mg/L and intra-assay and interassay coefficients of variation of <2.3 mg/L and intra-assay and interassay coefficients of variation of <2.6 mg/L. The logits of the remaining variables were linear. To test linearity in the logit of continuous variables, we used the Box-Tidwell transformation, which adds a term of the form $x \ln(x)$ to the model, where $x$ represents a continuous variable. If the coefficient for this variable is significant, then there is evidence for nonlinearity in the logit. If nonlinearity was present, we divided this variable into deciles and subsequently selected the scale of the independent variable. We assured linearity of the $\beta$ coefficients by log-transformation of CRP. The logits of the remaining variables were linear.

Calculations, Definitions, and Exclusion:
Response Variable
The albumin levels (mg) of the two 24-hour urine collections were measured. Microalbuminuria was defined as a mean urinary albumin level of 30 to 300 mg per 24 hours.

Predictor Variables
The last 2 blood pressure values of both visits were averaged; thus, blood pressure data are based on the average of 4 blood pressure values. Mean arterial pressure (MAP) was calculated as one third of the average systolic blood pressure plus two thirds of the average diastolic blood pressure (mm Hg). Body mass index (BMI) was calculated as the ratio of weight (kg) and height squared (m$^2$). Smoking was defined as never (reference category), current, and past smoking. Other independent variables were age, gender, waist circumference, serum cholesterol, plasma glucose, and antihypertensive and/or lipid-lowering therapy.

Exclusion Criteria
We excluded 451 subjects with leukocyturia and/or erythrocyturia, 304 subjects with type 2 diabetes (fasting glucose >7.0 mmol/L or nonfasting glucose >11.1 mmol/L or use of antidiabetic drugs), 55 subjects with renal disease, 89 subjects with macroalbuminuria (>300 mg/24 h), and 76 subjects with a CRP level >20 mg/L. The data set contained missing data on diabetes (n = 73) and CRP (n = 360). Thus, 7184 subjects were eligible for analyses. A total of 900 subjects were identified as having microalbuminuria.

Statistical Methods
Continuous data are reported as mean±SD. Differences between groups were assessed with $\chi^2$ analysis or ANOVA. Because our cohort is a sample of the general population with a supplement of microalbuminuric subjects, and because microalbuminuria is the outcome variable of interest, the results of our analyses cannot be simply extrapolated to the general population. To make such extrapolation possible, we performed design based logistic regression analyses, taking into account unequal probability of selection to ensure unbiased estimates. We used STATA 7.0 (Stata Statistical Software) to perform these “complex survey analyses.” To test linearity in the log of continuous variables, we used the Box-Tidwell transformation, which adds a term of the form $x \ln(x)$ to the model, where $x$ represents a continuous variable. If the coefficient for this variable is significant, then there is evidence for nonlinearity in the logit. If nonlinearity was present, we divided this variable into deciles and subsequently selected the scale of the independent variable. We assured linearity of the $\beta$ coefficients by log-transformation of CRP. The logits of the remaining variables were linear.

In multivariate analyses, including interactions, continuous variables were centralized to their mean values. A $P<0.05$ (2-sided) was used for all variables and interaction terms as the nominal level of statistical significance.

The following procedures were conducted to validate the robustness of the model. The 24-hour urine collections are prone to collection errors; thus, misclassification of subjects having either no microalbuminuria or microalbuminuria could have occurred. Therefore, we also analyzed our data with exclusion of subjects with $>$20% difference in 24-hour urinary creatinine excretion levels between the 2 collections. In a separate analysis, we analyzed the data defining the occurrence of microalbuminuria when present in both urine collections.
Results

Detailed characteristics of our study cohort are given in Table 1. Age, MAP, BMI, waist circumference, glucose, and cholesterol increased significantly with increasing CRP levels \((P<0.0001)\). The proportion of current and past smokers increased with increasing CRP levels as compared with never-smokers \((P<0.0001)\). More subjects used antihypertensive or lipid-lowering therapy with increasing CRP levels \((P<0.0001)\). More female subjects were present in the highest CRP quartile \((P<0.0001)\).

Among subjects with a blood pressure below the median population level, the trend in the association between CRP and microalbuminuria was not significant \((P=0.11)\), whereas this trend was significant in subjects with high MAP \((P<0.0001)\) (Table 2). All other risk factors showed a significant trend in the association between CRP and albuminuria in both strata (low versus high, no use versus use). Compared with subjects with a MAP below the median level (90 mm Hg), subjects with a MAP above the median level were more likely to have microalbuminuria in presence of an elevated CRP (Wald statistic for the interaction 9.2, \(P=0.03)\). In subjects with a low MAP, the prevalence of microalbuminuria increased \approx 2-fold (from 3.9% to 8.7%), whereas in subjects with an elevated MAP the prevalence of microalbuminuria increased \approx 4-fold (from 6.7% to 25.1%). Stratification for other cardiovascular risk factors did not yield significant interactions with CRP for the presence of microalbuminuria. Table 3 provides the multivariate design-based logistic regression model, in which the association of the studied risk factors and interactions with the presence of microalbuminuria is shown. Factors, which significantly increased the odds of having microalbuminuria, were age, waist circumference, cholesterol (negatively), lipid-lowering therapy, and current smoking. MAP and CRP were found interdependently; the interaction between CRP and MAP contributed significantly to the multivariate model \((P=0.0004)\). Thus, blood pressure positively modified the association be-

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>C-Reactive Protein (mg/L)</th>
<th>(P) for Trend*</th>
<th>Wald Statistic†</th>
<th>(P) Value</th>
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<td>9.6</td>
<td>14.5</td>
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<td>6.2</td>
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<tr>
<td>Gender</td>
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<tr>
<td>(F)</td>
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<td>6.4</td>
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<td>(M)</td>
<td>6.1</td>
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</table>

Prevalence of microalbuminuria (%) according to strata of risk factors and quartiles of C-reactive protein. Strata of continuous variables are based on their median level. M, indicates male; F, female.

* \(P\) for trend indicates the significance level of the increase in the prevalence of microalbuminuria with increasing CRP protein levels in one stratum of each risk factor.

† Wald statistic indicates the strength of the interaction between C-reactive protein and risk factors on the risk of having microalbuminuria, including the corresponding \(P\) value of the interaction.
Discussion

Our study shows that CRP modifies the relation between blood pressure and microalbuminuria. CRP did not modify the relation between other cardiovascular risk factors and microalbuminuria.

Most scientific reports on the association between inflammatory parameters (fibrinogen, sialic acid) and microalbuminuria come from small studies of subjects with type 1 and 2 diabetes⁰¹,¹² who encounter an increased risk for diabetic nephropathy and/or cardiovascular disease. Data on the association between CRP, as measured by highly sensitive assays, and microalbuminuria are scarce at the level of the general population. The Insulin Resistance Atherosclerosis Study (IRAS) only found a modest correlation (adjusted r=0.06) between CRP and microalbuminuria in a large nondiabetic cohort. In a multivariate analysis, CRP did not significantly increase the odds of having microalbuminuria.¹² The only study that found a significant association between CRP and microalbuminuria, also after controlling for other risk factors, was performed by Pannacciulli et al.¹¹ This study was conducted, however, in a selected population consisting of 103 obese, nondiabetic, and premenopausal women. In one prospective study, a 50% increase in baseline levels of CRP predicted a 17% increase in risk for microalbuminuria in the general population.¹⁸ Possible effect modification was not investigated in the latter 2 studies. The IRAS investigators did study possible interaction. However, the relation between CRP and microalbuminuria was found similar in strata of gender, ethnicity, or diabetic status. It is uncertain whether the investigators performed studies of potential interactions with other risk factors. Our data show that elevated CRP enhances the relationship between blood pressure and microalbuminuria. This interaction was found independently of other co-variates and interactions in the multivariate analysis.
Systemic blood pressure is the most important determinant of microalbuminuria in diabetes and hypertension. Functional abnormalities, indicative of impaired glomerular autoregulation, and structural changes in the glomerulus can be observed in diabetes and hypertension. Albuminuria may therefore occur as a result of direct transmission of raised systemic pressure to the glomerulus and/or permselectivity changes of the glomerular filter. Blood pressure is already known to relate to albuminuria in the population, a fact that we confirmed in the present study. A new finding of our study shows that the relation was more pronounced in subjects with an elevated CRP level. Inflammation is implicated in early endothelial dysfunction and more advanced atherosclerosis. Therefore, CRP may be a marker of vascular disease, which indicates impaired autoregulation of glomerular pressure and/or dysfunction of the glomerular endothelium. Both of these factors may enhance microalbuminuria.

The associations of microalbuminuria with age, blood pressure, lipid-lowering therapy, and CRP suggest the presence of the metabolic syndrome in subjects with microalbuminuria. CRP has been shown to be associated with measures of insulin resistance, like fasting hyperinsulinemia and impaired glucose disposal rate during a euglycemic hyperinsulinemic clamp. In diabetes and hypertension, insulin resistance has also been related to microalbuminuria although not in all studies. Moreover, CRP and microalbuminuria both predict incident cases of type 2 diabetes, which underlines their role in insulin resistance. Because we did not measure insulin sensitivity or insulin concentrations, we cannot exclude that our findings relate more directly to insulin resistance than to CRP.

Some methodological issues have to be addressed. First, our data are of cross-sectional nature, which makes temporal and causal inferences impossible. Second, we have to discuss possible selection bias. Our cohort consists of a selected sample of subjects with an elevated urinary albumin excretion and a randomly selected sample of control subjects. This sampling affects absolute values and point estimates; therefore, the generalizability of our findings for the general population may be questioned (internal validity). However, to control for our sampling procedure, we conducted design-based analyses, which allow generalization of the outcome data to the population at large. Third, misclassification of subjects having microalbuminuria could occur because of partly collected samples. To control for the effect of collection errors, we have validated the data in various ways as mentioned in the statistical methods section. These validation procedures did not affect the outcome of the analyses. We also validated our data by exclusion of subjects with suspected clinical inflammation (CRP>10 mg/L). This also did not affect the results.

In our study, all participants collected two 24-hour urine samples to assess urinary albumin excretion. Other studies used urinary albumin/creatinine ratio (A/C ratio), determined in a morning urine sample, to assess the presence of microalbuminuria. Additional studies were Colin et al, which also used two 24-hour urine collections. Assessment of 24-hour urinary albumin excretion is considered the preferred method. Although the A/C ratio is accepted as an alternative to detect the presence of microalbuminuria, urinary creatinine excretion depends on the amount of muscle mass, which is variable for several biological parameters (gender, age, body weight, exercise). This could be a reason that these studies did not find an interaction of CRP and blood pressure, which is also related to these parameters, with the risk of microalbuminuria.

We used MAP as the blood pressure variable of interest. We also calculated our data including both systolic and diastolic blood pressure in the analyses. Interestingly, the interaction of blood pressure with CRP on the risk of microalbuminuria was equally found for systolic as well as diastolic blood pressure (data not shown).

**Perspectives**

Our study shows the novel finding of effect modification by CRP of the relation between blood pressure and microalbuminuria. The observed interaction is important for our understanding of the pathophysiology of increased urinary albumin excretion in the general population. Elevated CRP levels indicate systemic low-grade inflammation. Inflammation apparently increases the likelihood of increased glomerular leakage of albumin in response to blood pressure. This glomerular leakage may involve either increased transmission of systemic blood pressure or decreased barrier function of the glomerulus.

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