Diverse Associations of Microalbuminuria With C-Reactive Protein, Interleukin-18 and Soluble CD 40 Ligand in Male Essential Hypertensive Subjects

Costas Tsioufis, Kyriakos Dimitriadis, Efstathios Taxiarchou, Carmen Vasiliadou, George Chartzoulakis, Dimitrios Tousoulis, Athanasios Manolis, Christodoulos Stefanadis, and Ioannis Kallikazaros

Background: Microalbuminuria (MA) and low-grade inflammation constitute emerging markers of subclinical atherosclerosis. We investigated whether urinary albumin excretion, expressed as the albumin-to-creatinine ratio (ACR), is associated with high sensitivity C-reactive protein (hs-CRP), interleukin (IL)-18, and soluble CD40 ligand (sCD40L), in hypertensive subjects.

Methods: The study population consisted of 108 non-diabetic male patients with newly diagnosed untreated stage I to II essential hypertension (aged 44.6 years, office blood pressure [BP] 148/95 mm Hg). According to ACR values determined as the average of two nonconsecutive overnight spot urine samples, subjects were divided into microalbuminurics (n = 28) (mean ACR = 30 to 300 mg/g) and normoalbuminurics (n = 80) (mean ACR < 30 mg/g).

Results: Although microalbuminurics as compared to normoalbuminuric hypertensives had greater hs-CRP levels (2.55 ± 1.18 v 1.45 ± 0.52 mg/L, P < .0001), independently of confounding factors, these two groups did not differ regarding IL-18 and sCD40L values (P = not significant [NS] for both cases). In the entire population, ACR exhibited a positive correlation with hs-CRP (r = 0.623, P < .0001), whereas there was no association with both IL-18 and sCD40L (P = NS for both cases). When multiple linear regression analysis was performed, it was revealed that age, body mass index, office systolic BP, total cholesterol, and hs-CRP levels were significant independent predictors of the ACR (P < .05).

Conclusions: In essential hypertensive subjects, MA is accompanied by elevated hs-CRP levels, but not by augmented IL-18 and sCD40L concentrations, suggesting activation of different inflammatory pathways in the progression of renal and cardiovascular atherosclerotic disease. The pathophysiologic mechanisms of these associations remain to be further elucidated in future studies. Am J Hypertens 2006;19:462–466 © 2006 American Journal of Hypertension, Ltd.

Key Words: Kidney, microalbuminuria, inflammation, hypertension, atherosclerosis.
possible associations between MA and low-grade inflammation may provide a new insight into the pathophysiological mechanisms linking early renal impairment with elevated cardiovascular risk.\textsuperscript{1,17–20}

On the basis of these observations, we examined whether urinary albumin excretion, expressed as the albumin-to-creatinine ratio (ACR), is associated with hs-CRP, IL-18, and sCD40L in newly diagnosed nondiabetic male essential hypertensive subjects.

**Methods**

**Study Population**

The study population consisted of 149 consecutive men with newly diagnosed (within the past 2 years) untreated stage I–II essential hypertension, aged between 30 and 65 years, who were referred to the outpatient hypertension unit within a period of 6 months.\textsuperscript{6} Secondary forms of hypertension were excluded, according to established diagnostic methods.\textsuperscript{6,7}

Exclusion criteria included heart failure, atherosclerotic cardiovascular disease, diabetes mellitus or glucose intolerance, familial hypercholesterolemia, augmented serum creatinine concentration or overt proteinuria, and any other clinically significant concurrent medical conditions. None of the participants had any clinical and laboratory evidence of inflammation or underwent any medical or dental treatment during the past month before entry into the study. Moreover, to enhance the robustness of the findings, we limited analyses to nonsmokers and to subjects who had a body mass index (BMI) of less than 30 kg/m\textsuperscript{2}. Finally, 108 essential hypertensives fulfilling all these inclusion criteria were selected for participation.

The study protocol complies with the Declaration of Helsinki and was approved by our institutional ethics committee and all participants gave written informed consent.

**Procedures**

Office blood pressure (BP) measurements were obtained, according to the recent guidelines.\textsuperscript{6,7} In all subjects, ACR was determined as the average of two nonconsecutive overnight spot urine samples by using a quantitative assay (DCA 2000, Bayer Diagnostics Europe, Dublin, Ireland), with a coefficient of variation of 3.6%. More specifically, to account for the intrapatient variability of ACR, we limited analyses to nonsmokers and to subjects who had a body mass index (BMI) of less than 30 kg/m\textsuperscript{2}. Finally, 108 essential hypertensives fulfilling all these inclusion criteria were selected for participation.

Baseline plasma sCD40L levels were estimated by ELISA (Bender MedSystems, Austria), as previously described,\textsuperscript{10,11} with a coefficient of variation of 4.0%. In addition, serum IL-18 was measured with an ELISA method (Biosource International, Nivelles, Belgium), with a coefficient of variation of 6%.

All participants underwent echocardiographic examination, by a senior echocardiographer, who was blind to the clinical status of the examined subject, in line with the recommendations of the American Society of Echocardiography.\textsuperscript{23,24}

**Statistical Analysis**

Data are expressed as mean values ± SD. Significant differences between the study groups were determined using the Student independent samples $t$ test or the $\chi^2$ test where appropriate. Relations between variables were determined by linear regression analysis. Stepwise multiple regression analysis was applied to test the independent relation of ACR, with demographic and laboratory variables. An analysis of covariance was performed to detect significant differences of hs-CRP between the group of microalbuminurics and the group of normoalbuminurics, after the adjustment for a number of covariates. All tests were considered to be significant at the level of $P < .05$.

**Results**

On the basis of the ACR the study population ($n = 108$) was divided into microalbuminurics (ACR = 30 to 300 mg/g) ($n = 28$) and normoalbuminurics (ACR <30 mg/g) ($n = 80$). Microalbuminuric compared to normoalbuminuric subjects, were older, had greater BMI, longer duration of essential hypertension (1.6 ± 0.2 v 0.6 ± 0.4 years, $P = .026$), higher systolic BP values (Table 1), greater left ventricular mass index (114 ± 15 v 100 ± 18 g/m\textsuperscript{2}, $P = .031$) and relative wall thickness (0.44 ± 0.06 v 0.39 ± 0.03, $P = .01$). In addition, microalbuminurics as compared to normoalbuminurics were characterized by significantly greater levels hs-CRP concentrations (2.55 ± 1.18 v 1.45 ± 0.52 mg/L, $P ≤ .0001$), whereas these two groups did not differ regarding fasting glucose, hemoglobin A\textsubscript{1c}, lipid values, IL-18 (245 ± 87 v 241 ± 73 pg/mL, $P = .367$), and sCD40L levels (2.46 ± 0.8 v 2.41 ± 0.4 ng/mL, $P = .184$) (Table 2).

**Correlations**

There was a positive correlation of ACR with age ($r = 0.525, P < .0001$), BMI ($r = 0.482, P < .0001$), systolic BP ($r = 0.296, P < .0001$), total cholesterol ($r = 0.485, P < .0001$), and hs-CRP ($r = 0.623, P < .0001$). In addition, the hs-CRP exhibited positive relationships with BMI ($r = 0.235, P = .036$), waist-to-hip ratio ($r = 0.270, P = .018$), duration of hypertension ($r = 0.563, P < .0001$), systolic BP ($r = 0.333, P = .003$), total cholesterol ($r = 0.277, P = .015$), and low-density lipoprotein ($r = 0.319, P = .006$). Regarding IL-18, it was only negatively
related with HDL (r = 0.245, P = .04), whereas sCD40L was not associated with any of the evaluated demographic and laboratory parameters.

When multiple linear regression analysis was performed in the study group, with the ACR as the dependent variable and demographic and laboratory parameters as independent variables, it was revealed that age, BMI, systolic BP, total cholesterol, and hs-CRP were significant independent predictors of the ACR (Table 3). By applying analysis of covariance, it was shown that hs-CRP levels were significantly different between the normoalbuminuric and microalbuminuric group, after adjusting for age, BMI, systolic BP, total cholesterol, IL-18, and sCD40L levels (P < .05).

**Discussion**

The main finding of our study is that urinary albumin excretion, expressed as the ACR, exhibits a close association with hs-CRP, but not with IL-18 and sCD40L in newly diagnosed male essential hypertensive patients. Consequently, although MA is accompanied by heighted levels of an established inflammatory marker, such as hs-CRP, both IL-18 and sCD40L concentrations are not increased in the same setting.

In line with recently reported data, we showed that in the specific clinical setting of untreated and nondiabetic essential hypertension, male subjects with MA compared to those with normal ACR values are characterized by higher levels of hs-CRP. This could be attributed to the fact that subclinical inflammation by causing injury to the kidney may directly alter glomerular function, leading to the development of MA. Alternatively, increased ACR and elevated levels of low-grade inflammatory markers may simply be two facets of the same underlying biological mechanism that is associated with diffuse atherosclerotic disease.

In an attempt to shed more light on the inter-relationships between early renal dysfunction and alternative cascades of proinflammatory activation in essential hypertensives, we reported for the first time that microalbuminurics as compared to normoalbuminurics exhibited no difference in sCD40L and IL-18 values. In previous works, high levels of sCD40L are associated with the presence of high-risk atherosclerotic lesions in elderly subjects with carotid atherosclerosis, and higher concentrations of IL-18 mRNA are identified in unstable atherosclerotic plaques, denoting the interdependencies of these cytokines with diffuse vascular disease. It could be supported that the potential sources for sCD40L (ie, T-lymphocytes, mononuclear phagocytes, and endothelial cells) are perhaps not stimulated up to a sufficient degree in the microalbuminuric state of nondiabetic essential hypertension, whereas

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**Table 1.** Demographic and clinical data for the groups of normoalbuminuric and microalbuminuric hypertensives

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normoalbuminuric hypertensives (n = 80)</th>
<th>Microalbuminuric hypertensives (n = 28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>42.7 ± 5.6</td>
<td>50.2 ± 8.7</td>
<td>.041</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 3</td>
<td>27.51 ± 1.6</td>
<td>.02</td>
</tr>
<tr>
<td>Waist/hip</td>
<td>0.82 ± 0.09</td>
<td>0.86 ± 0.06</td>
<td>.136</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>146 ± 8</td>
<td>153 ± 10</td>
<td>.004</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>95 ± 4</td>
<td>94 ± 5</td>
<td>.618</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>70 ± 6</td>
<td>73 ± 4</td>
<td>.154</td>
</tr>
</tbody>
</table>

BMI – body mass index; BP – blood pressure. Values are mean ± standard deviation.

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**Table 2.** Laboratory data for the groups of normoalbuminuric and microalbuminuric hypertensives

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normoalbuminuric hypertensives (n = 80)</th>
<th>Microalbuminuric hypertensives (n = 28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>89 ± 4</td>
<td>92 ± 3</td>
<td>.366</td>
</tr>
<tr>
<td>Hb A1c (%)</td>
<td>5.0 ± 0.4</td>
<td>5.3 ± 0.2</td>
<td>.211</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>238 ± 42</td>
<td>242 ± 46</td>
<td>.130</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>120 ± 43</td>
<td>119 ± 39</td>
<td>.763</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>50.7 ± 10</td>
<td>45.5 ± 11.2</td>
<td>.123</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>150.1 ± 38.4</td>
<td>162.8 ± 37.5</td>
<td>.541</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>16.85 ± 5.92</td>
<td>38.44 ± 6.81</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>1.45 ± 0.52</td>
<td>2.55 ± 1.18</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>IL-18 (pg/mL)</td>
<td>241 ± 74</td>
<td>245 ± 87</td>
<td>.367</td>
</tr>
<tr>
<td>sCD40L (ng/mL)</td>
<td>2.41 ± 0.4</td>
<td>2.46 ± 0.8</td>
<td>.184</td>
</tr>
</tbody>
</table>

Hb A1c = hemoglobin A1c; BMI = body mass index; ACR = albumin-to-creatinine ratio; hs-CRP = high-sensitivity C-reactive protein; IL-18 = interleukin-18; sCD40L = soluble CD40 ligand. Values are mean ± standard deviation.
both sCD40L and IL-18 exhibit no correlation with hs-CRP-associated inflammatory pathway, as other investigators have previously suggested. Although IL-18 was negatively associated with HDL levels, confirming previous data, the similar values of the latter inflammatory mediator and sCD40L in the microalbuminuric and the normoalbuminuric group could be attributed partially to the rather non-high lipid profile observed in both groups. Consequently, both sCD40L and IL-18 could mirror more progressed inflammatory and atherosclerotic involvement than hs-CRP and MA, in this clinical setting. Moreover, our results support the notion that MA could be considered as an index of endothelial dysfunction and low-grade inflammation, at least of the cascade reflected by augmented plasma levels of hs-CRP. Taking this hypothesis even further, we may propose that in the vicious circle connecting essential hypertension, microalbuminuric state, and atherosclerotic cardiovascular events, apart from the mainstay role of both the renin-angiotensin-aldosterone system and endothelial dysfunction, subclinical inflammation participates through different pathways to all stages of this deleterious process.

From a clinical point of view, ACR determination in this setting may ameliorate cardiovascular risk stratification by identifying patients with a cluster of modifiable risk factors, who might benefit from early intervention. Models that include hs-CRP and ACR may predict cardiovascular events in a more accurate way than those models with only hs-CRP. In contrast, measurement of IL-18 and sCD40L at this stage of essential hypertension may be of no additional clinical value, because they may identify an inflammatory and atherogenic milieu, distinct from that associated with early stages of essential hypertension and MA.

These speculations should be considered with the potential limitations of the study, such as, the small number of the participants, the single measurements of IL-18 and sCD40L, and the established effect of BP status on both ACR and proinflammatory processes. Patients with cardiovascular risk factors that affect significantly the levels of ACR and inflammatory markers, such as smoking, obesity, and diabetes mellitus were excluded, limiting the generalization and the cost effectiveness of the study. Furthermore, because the genders differ with respect to prevalence and clinical implications of MA and subclinical inflammation, the fact that only male subjects participated in our cohort, renders important the confirmation of our findings by using female hypertensives.

In conclusion, MA is accompanied by increased levels of hs-CRP, but not by augmented IL-18 and sCD40L concentrations in essential hypertensive patients. These novel findings, although needing further validation, may support the notion of the activation of different inflammatory pathways in the progression of renal and cardiovascular atherosclerotic disease.

### References


### Table 3. Multiple linear regression analysis for ACR values in the study population

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Change in ACR (mg/g) per unit change in predictor</th>
<th>95% Confidence interval lower bound</th>
<th>95% Confidence interval upper bound</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−74.688</td>
<td>−107.854</td>
<td>−41.482</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.529</td>
<td>0.290</td>
<td>0.768</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.919</td>
<td>0.397</td>
<td>1.441</td>
<td>.001</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>0.237</td>
<td>0.056</td>
<td>0.419</td>
<td>.011</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>0.05314</td>
<td>0.015</td>
<td>0.092</td>
<td>.008</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>4.377</td>
<td>2.257</td>
<td>6.496</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>IL-18 (pg/mL)</td>
<td>0.01484</td>
<td>−0.038</td>
<td>0.008</td>
<td>.206</td>
</tr>
<tr>
<td>sCD40L (ng/mL)</td>
<td>−0.666</td>
<td>−2.974</td>
<td>1.643</td>
<td>.565</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 2.


