Structural Alterations of Subcutaneous Small-Resistance Arteries May Predict Major Cardiovascular Events in Patients With Hypertension

Carolina De Ciuceis, Enzo Porteri, Damiano Rizzoni, Nicola Rizzardi, Silvia Paiardi, Gianluca E. M. Boari, Marco Miclini, Francesca Zani, Maria Lorenza Muiesan, Francesco Donato, Massimo Salvetti, Maurizio Castellano, Guido A. M. Tiberio, Stefano M. Giuliani, and Enrico Agabiti Rosei

Background: Structural alterations in the microcirculation may be considered an important mechanism of organ damage. An increased tunica media to internal lumen ratio of subcutaneous small-resistance arteries (M:L) may predict the development of cardiovascular events in a high-risk population. However, it is not known whether structural alterations of small arteries may also predict major cardiovascular events.

Methods: Three hundred three subjects were included in the present study. There were 65 normotensive subjects, 111 patients with essential hypertension (33% of them with diabetes mellitus), 109 patients with secondary forms of hypertension, and 18 normotensive diabetic patients. Small-resistance arteries were dissected from subcutaneous fat biopsies and mounted on an isometric myograph, and the M:L was measured. Subjects were reevaluated after an average follow-up time of 6.9 years to assess the occurrence of cardio-cerebrovascular events.

Results: Eleven subjects died of a fatal cardio-cerebrovascular event (FCE), 14 had a major, nonfatal cardiovascular event (stroke or myocardial infarction) (SMI), 23 had a minor cardiovascular event (MCE), and 255 had no cardiovascular event (NCE). A significant difference was observed in M:L and in event-free survival between patients with FCEs + SMIs + MCEs and those with NCE and between patients with FCEs + SMIs and those with NCE. Similar results were obtained by restricting the analysis to patients with essential hypertension.

Conclusions: Structural alterations of small-resistance arteries may predict FCE and SMI. The prognostic role of small-resistance artery structure also applies to medium-risk patients with essential hypertension, at least when MCEs are included in the analysis. Am J Hypertens 2007;20:846–852 © 2007 American Journal of Hypertension, Ltd.

Key Words: Vessels, prognosis, arteries, remodeling, hypertension, hypertrophy.

Essential hypertension is characterized by increased peripheral vascular resistance to blood flow, which occurs mostly as a result of energy dissipation in small arteries with a lumen diameter of 100 to 350 μm and in smaller arterioles.\(^1\) The increased peripheral resistance may be ascribed, in large part, to the consequences of structural and functional alterations in the resistance vasculature. It is now widely accepted that structural abnormalities of microvessels, usually defined as vascular remodeling, are common alterations associated with chronic hypertension.\(^1,4\) In the last several years, the presence of structural alterations in subcutaneous and omental small-resistance arteries, dissected from biopsies performed in patients with essential hypertension, was confirmed by direct investigation using micromyographic methods.\(^5\) Patients with essential hypertension presented an increased tunica media to lumen ratio (M:L). An important consequence of the presence of structural alter-
ations in small-resistance arteries may be an impairment of the vasodilator reserve.\textsuperscript{8,9} In fact, remodeling of small-resistance arteries is characterized by a narrowing of the lumen, which leads to an increase in vascular resistance even at full dilatation, ie, in the absence of vascular tone. A significant correlation between coronary flow reserve and subcutaneous small-resistance artery remodeling was detected in hypertensive patients, thus suggesting that structural alterations in small-resistance arteries may be present at the same time in different vascular districts.\textsuperscript{10}

Alterations in small-resistance artery morphology may represent the most prevalent and perhaps the earliest form of preclinical target-organ damage in essential hypertension.\textsuperscript{11} Recently, we observed that the presence of an increased wall to lumen ratio in the subcutaneous resistance arteries is associated with a worse prognosis in hypertensive patients, and that structural alterations in the microcirculation are probably the most potent predictor of cardiovascular events,\textsuperscript{12} probably because they are associated with similar changes in the coronary, renal, and cerebral microcirculation. The prognostic relevance of vascular structural alterations in the microcirculation was partly confirmed in a different vascular district, ie, the retinal circulation.\textsuperscript{13–15}

However, in our previous study,\textsuperscript{12} major and minor cardiovascular events were considered together. In addition, the patients investigated were at high cardiovascular risk (5.43 events/100 patients per year). Therefore, it is not known whether structural alterations of small arteries may also predict mortality or major cardiovascular events, and whether the prognostic relevance of an increased M:L of subcutaneous small arteries is also present in patients at medium or low risk. The present study therefore aimed to investigate these aspects in a population much larger than that previously evaluated.\textsuperscript{12}

\textbf{Methods}

From January 1992 to July 2005, >350 subjects and patients were submitted to an evaluation of small-resistance artery structure: 303 of those for whom follow-up data were available were included in the present study. They included 65 normotensive subjects, 111 patients with essential hypertension, 29 patients with pheochromocytoma, 46 patients with primary aldosteronism, 25 patients with renovascular hypertension, 18 normotensive patients with noninsulin-dependent diabetes mellitus (NIDDM), and 9 patients with acromegaly. Their age range was 20 to 83 years. The presence of hypertension was established according to guidelines of the World Health Organization.\textsuperscript{16} The presence of NIDDM was established according to the guidelines of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.\textsuperscript{17} The diagnosis of secondary forms of hypertension was made on the basis of an indication for renal-artery revascularization or adrenal-tumor resection, after proper investigation by imaging techniques and humoral assessments. The majority of hypertensive patients had been previously treated for short periods of time (<6 months) with calcium-channel blockers, angiotensin-converting enzyme inhibitors, diuretics, or \(\beta\)-blockers. Treatment was withdrawn at least 3 weeks before the procedure, which is usually long enough to avoid any further hemodynamic effect of antihypertensive drugs. The protocol of the study was approved by the Ethics Committee of the Medical School of the University of Brescia (Brescia, Italy), and informed consent was obtained from each participant. The procedures followed were in accordance with institutional guidelines.

Venous blood samples were taken with participants in supine position, after a washout period of at least 3 weeks, for standard hematology and serum biochemistry tests (including triglycerides and total cholesterol). In a subset of subjects (\(n = 136\)), a standard echocardiographic evaluation was performed. Left-ventricular internal dimensions, left-ventricular posterior wall thickness, and interventricular septum thickness were measured according to the recommendations of the American Society of Echocardiography and the Penn Convention.\textsuperscript{12} Left-ventricular hypertrophy was considered present if the left-ventricular mass index exceeded 125 g/m\(^2\) in both sexes. For further technical details, see Rizzoni et al.\textsuperscript{12}

All subjects included in our previous study (\(n = 151\))\textsuperscript{12} are also included here. Their data about cardiovascular events were updated in only a small number of them, because our major effort was to gather data on the remaining 152 subjects in whom we were originally unable to collect such information. Thirty-seven of 111 essential hypertensive patients had NIDDM (33%). Of the remaining 74 patients, about 50% had dyslipidemia; therefore, a proper analysis of patients with essential hypertension and without additional risk factors was not possible.

\textbf{Micromyography}

All subjects underwent a biopsy of subcutaneous fat from the gluteal or anterior abdominal region. The biopsy of abdominal subcutaneous fat was taken during a surgical procedure (usually cholecystectomy in normotensive and essential hypertensive patients and adrenalectomy or vascular surgical intervention in the renal arteries for patients with secondary hypertension), while in the remaining cases, a standard skin biopsy of the gluteal region (3 cm long, 0.5 cm wide, and 1.5 cm deep) was performed.\textsuperscript{6,12} Small arteries (about 100 to 280 \(\mu\)m average diameter in relaxed conditions, 2 mm long) were dissected from the subcutaneous fat of the biopsies and mounted as a ring preparation on an isometric myograph (410 A; Danish Myo-Technology, Aarhus, Denmark), by threading them onto two stainless steel wires (40-\(\mu\)m diameter). Media cross-sectional area, wall thickness, and media thickness, as well as the M:L of blood vessels in normalized condition, were measured. Details of the micromyographic technique of evaluation of small-artery morphology were
Statistical Analysis

All data are expressed as mean ± SD, unless otherwise stated. One-way analysis of variance and χ² statistics (Fisher’s exact test and Yates correction) were used to evaluate differences between groups. The Kaplan-Meier method was used to analyze event-free survival, and groups were compared using the Mantel-Cox and Breslow tests. The relative importance of each prognostic factor, adjusted for the others, was assessed using the Cox proportional hazards model. All statistical tests were two-tailed, and P < .05 was considered statistically significant. All analyses were carried out with the BMDP statistical package (BMDP Statistical Software, Inc., Los Angeles, CA).

Results

In our population (n = 303), we observed 11 fatal cardiovascular events (FCEs); 14 major, nonfatal cardiovascular events (SMIs), ie, stroke or myocardial infarction; 23 minor cardiovascular events (MCEs); and 255 subjects with no cardiovascular events (NE). The incidence of total (major and minor) cardiovascular events was 2.3/100 patients per year (intermediate cardiovascular risk). A similar incidence was observed in patients with essential hypertension (n = 111, FCEs + SMIs = 9, FCEs + SMIs + MCEs = 22, or 2.8 events/100 patients per year). The average follow-up time of our subjects was 6.9 years (range, 0.6 to 13.9 years) for a total of 2091 patient-years. Patients with cardiovascular events had higher systolic and pulse pressure (Table 1).

Taking into account the whole population (n = 303), in the subcutaneous small-resistance arteries of subjects with major fatal and nonfatal cardiovascular events (FCEs + SMIs), compared with NE, a smaller internal diameter and a greater M:L was observed (Table 1). A similar finding was obtained by evaluating only SMIs versus NE (M:L, 0.112 ± 0.044 v 0.088 ± 0.028, respectively, P < .05). As expected, subjects with major + minor cardiovascular events (FCEs + SMIs + MCEs) had a greater M:L compared with those with NE (0.111 ± 0.031 v 0.088 ± 0.028, respectively, P < .00001). Restricting the analysis to patients with essential hypertension (n = 111), patients with FCEs + SMIs + MCEs had a greater M:L compared with those with NE (0.113 ± 0.021 v 0.10 ± 0.032,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With major fatal and nonfatal cardiovascular events (n = 25)</th>
<th>With minor cardiovascular events (n = 23)</th>
<th>Without cardiovascular events (n = 255)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>62 ± 11</td>
<td>62 ± 8</td>
<td>55 ± 14</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>18 M, 7 F</td>
<td>16 M, 7 F</td>
<td>120 M, 135 F</td>
</tr>
<tr>
<td>Systolic arterial pressure (mm Hg)</td>
<td>161 ± 21*</td>
<td>148 ± 19</td>
<td>144 ± 19</td>
</tr>
<tr>
<td>Diastolic arterial pressure (mm Hg)</td>
<td>92 ± 16</td>
<td>89 ± 12</td>
<td>88 ± 12</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>69 ± 12*</td>
<td>59 ± 13</td>
<td>56 ± 14</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>102 ± 34</td>
<td>108 ± 31</td>
<td>113 ± 43</td>
</tr>
<tr>
<td>Percentage of diabetic patients</td>
<td>23%</td>
<td>40%</td>
<td>34%</td>
</tr>
<tr>
<td>Percentage of smokers or previous smokers</td>
<td>14%</td>
<td>13%</td>
<td>25%</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>203 ± 59</td>
<td>20 ± 65</td>
<td>210 ± 46</td>
</tr>
<tr>
<td>Percentage with dyslipidemia</td>
<td>50%</td>
<td>52%</td>
<td>62%</td>
</tr>
<tr>
<td>Left-ventricular mass index (g/m²) (n = 86)</td>
<td>139 ± 40</td>
<td>128 ± 37</td>
<td>121 ± 38</td>
</tr>
<tr>
<td>Percentage with left-ventricular hypertrophy</td>
<td>46%</td>
<td>44%</td>
<td>42%</td>
</tr>
<tr>
<td>Subcutaneous small arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media thickness (µm)</td>
<td>22.3 ± 5.8</td>
<td>23.7 ± 4.36</td>
<td>22.9 ± 5.23</td>
</tr>
<tr>
<td>Wall thickness (µm)</td>
<td>40.9 ± 10.3</td>
<td>43.6 ± 7.27</td>
<td>41.7 ± 9.58</td>
</tr>
<tr>
<td>Internal diameter (µm)</td>
<td>234 ± 75*</td>
<td>213 ± 50*</td>
<td>273 ± 86</td>
</tr>
<tr>
<td>Media:lumen ratio</td>
<td>0.106 ± 0.04*</td>
<td>0.116 ± 0.02*</td>
<td>0.088 ± 0.03</td>
</tr>
<tr>
<td>Media cross-sectional area (µm²)</td>
<td>18,760 ± 10,252</td>
<td>18,076 ± 6509</td>
<td>21,470 ± 10,615</td>
</tr>
</tbody>
</table>

* P < .001 v no cardiovascular events.
respectively, \( P < .05 \)). No statistically significant difference was observed in this subgroup when taking into account FCEs + SMIs or SMIs alone, due to the low number of events observed in this subgroup (two FCEs and seven SMIs).

To evaluate the prognostic role of the M:L of subcutaneous small-resistance arteries, in the previous study, \(^{12}\) we subdivided subjects into two groups, according to the presence of M:L above or below the mean (and median) value observed in the whole population (0.098) or according to the presence of M:L >0.11 or <0.11, ie, the value corresponding to two standard deviations above the mean of normal control subjects. We used the same cutpoints in the present analysis, for consistency. We evaluated event-free survival (major cardiovascular events = FCEs + SMIs) in the whole population \((n = 303)\) according to M:L ≥0.11 or <0.11. Life-table analyses showed a significant difference in event-free survival among the two subgroups \((P = .013\) by Mantel-Cox test, \(P = .008\) by Breslow test) (Fig. 1). Then, we evaluated event-free survival (major and minor events = FCEs + SMIs + MCEs) in the whole population \((n = 303)\) according to M:L ≥0.11 or <0.11. Again, a significant difference was observed between groups \((P < .0001\) by Mantel-Cox test, \(P < .0001\) by Breslow test) (Fig. 2). In addition, event-free survival (major and minor events = FCEs + SMIs + MCEs) in the whole population \((n = 303)\) was evaluated according to the second cutpoint (M:L ≥0.098 or <0.098) and was significantly different between groups \((P < .001\) by Mantel-Cox test, \(P < .002\) by Breslow test) (Fig. 3). Then we restricted the analysis to patients with essential hypertension \((n = 111)\). The event-free survival (major and minor events = FCEs + SMIs + MCEs) according to M:L ≥0.11 or <0.11 was significantly different between groups \((P < .034\) by Mantel-Cox test, \(P < .041\) by Breslow test) (Fig. 4).

The relative importance of known prognostic factors at baseline or at follow-up, such as age; sex; clinic systolic, diastolic, or pulse pressure; dyslipidemia; the presence of diabetes; and smoking status; together with baseline diagnosis (essential hypertension, pheochromocytoma, primary aldosteronism, renovascular hypertension, and normotensive hypertension)
NIDDM) and the M:L of subcutaneous small arteries, was evaluated in the whole population, and the association of these variables with cardiovascular risk was assessed by using the Cox proportional hazards model. When major cardiovascular events (FCEs + SMIs + MCEs) were considered in the whole population, the predictive variables were M:L (P = .017) and systolic blood pressure (P = .007). When we considered all major and minor cardiovascular events (FCEs + SMIs + MCEs) in the whole population, predictive variables were M:L (P < .0001), systolic blood pressure (P = .05), age (P = .029), and male sex (P = .026). Finally, when the analysis was restricted to patients with essential hypertension, considering all major and minor cardiovascular events (FCEs + SMIs + MCEs), predictive variables were systolic blood pressure (P = .03) and M:L (P = .05), while age was of borderline statistical significance. The predictive power of M:L increased when considered as a dichotomic variable (≥0.11 or <0.11): P = .028. When we used the 0.098 cutpoint, the difference in event-free survival was no longer statistically significant, probably because of the relatively low number of patients evaluated.

We also repeated the statistical calculations using the present median value of M:L observed in the whole population (.090). Results were superimposable in respect to those obtained with the .098 cutpoint, both in the whole population and in patients with essential hypertension (data not shown).

We also evaluated possible differences in the prognostic value of small-artery structure in vessels taken from an abdominal (n = 140) or gluteal (n = 264) location. No significant difference was observed, although the statistical significance of differences between subjects with high or low M:L was much lower in the former group, because of the relative low number of subjects (data not shown).

**Discussion**

We previously demonstrated, in 151 subjects and patients, that the M:L of subcutaneous small arteries, an index of structural alteration in the microcirculation, is the most potent predictor of cardiovascular events in a selected high-risk population. The prognostic role of M:L was demonstrated to be independent of other known cardiovascular risk factors, as documented by Cox analysis of covariance. In fact, only pulse pressure provided a further contribution in the prediction of cardiovascular events. The prognostic relevance of vascular structural alterations in the microcirculation was partly confirmed in a different vascular district, ie, the retinal circulation. In fact, Wong et al observed that, in a population-based cohort study of 9648 subjects (the Atherosclerosis Risk in Communities Study), a retinal arteriolar narrowing, as evaluated by retinal photographs, was associated with a higher risk of coronary artery disease in women but not in men. In particular, for each decrease of one standard deviation of the retinal arteriole to venule ratio (AVR), a relative risk of coronary artery disease of 1.37 and of myocardial infarction of 1.50 occurs. A similar evaluation was performed in another general-population cohort of 4926 subjects in Wisconsin (the Beaver Dam Study); no relationship between smaller AVR and 10-year mortality was observed.

However, in the same cohort, the presence of retinal disease (retinal microaneurysms, blot hemorrhages, cotton-wool spots, hard exudates, venous bleeding, new vessels on the disk, and preretinal or vitreous hemorrhages) was associated with increased cardiovascular mortality, with an odds ratio of 1.814 and with a higher prevalence of cerebral white-matter lesions and stroke. On the other hand, in a different study, lower grades of hypertensive retinopathy were not associated with more prominent cardiac or extracardiac target-organ damage. Narrowed retinal arterioles or changes in retinal-vessel architecture are associated with an increased incidence or prevalence of hypertension and diabetes, and, therefore, the quantification of these changes, although sometimes complex, may be a useful approach to the assessment of target-organ damage in hypertension.

The available data suggest, therefore, a relevant prognostic role for structural changes, especially in the subcutaneous microcirculation, in high-risk patients with hypertension and other cardiovascular risk factors, probably because these changes reflect a reduction in flow reserve and an impairment of tissue perfusion in the heart, kidney, or brain. Structural alterations in resistance arteries may be closely related to target-organ damage, especially at the cardiac level. In fact, a linear relationship between the media to lumen ratio of subcutaneous small-resistance arteries and left-ventricular mass index or relative wall thickness was detected in patients with hypertension. This relationship with left-ventricular geometry was more evident in patients with activation of the renin-angiotensin-aldosterone system. Therefore, alter-
ations in the microcirculation may play an important role in the development of organ damage in hypertension. It is also possible that different processes that lead to an increase in M:L, eg, rearrangement of the same wall material around a narrowed lumen (eutrophic remodeling) or vascular smooth muscle cell growth (hypertrophic remodeling), may differently affect a prognosis, although this aspect needs to be further investigated. The novelty of the present study involves the demonstration that structural alterations in the subcutaneous microcirculation may predict major cardiovascular events (death, stroke, and myocardial infarction), and that a relevant prognostic role of M:L is present not only in high-risk patients but also in patients at lower cardiovascular risk, including patients with essential hypertension. A controversial point is represented by the time course of the development of vascular structural changes in hypertension and by their role in promoting hypertension. It was suggested that an increase in M:L is most prevalent and probably the earliest form of target-organ damage in mild essential hypertension. However, no evidence of vascular structural alterations was observed in normotensive subjects with a positive family history of hypertension. Therefore, there is no clear evidence about the possible role of vascular structural changes in initiating hypertension. In the present study, we also tested other indicators of small-artery structure, such as wall cross-sectional area to lumen cross-sectional area ratio, wall thickness to internal diameter ratio, or internal diameter (data not shown). The M:L was a superior predictor of cardiovascular events compared with all other indicators of vascular morphology.

Limitations of the Study

In our study, all patients with essential hypertension had other cardiovascular risk factors. Therefore, a weakness of the study is that there were not enough patients with essential hypertension without other risk factors to determine if altered resistance vessel structure is a predictor in such a group. Another crucial point is represented by the blood-pressure control and the antihypertensive drugs used during follow-up. Possible differences in the number or types of antihypertensive medication, or in the blood-pressure control between groups, cannot be ruled out. However, similar limitations apply to almost all studies about the prognostic significance of intermediate end points in hypertension.

In addition, as previously pointed out, the invasiveness of the current method for the evaluation of subcutaneous small-artery structure may limit its application to large populations. However, it is possible that noninvasive techniques for the investigation of the microcirculation (presently under evaluation and validation) may provide, in the near future, important contributions in this regard.

The presence of structural alterations in the microcirculation may have an important role in the development of ischemic heart disease, heart failure, cerebral ischemic attacks, and renal failure. Therefore, it is conceivable that vascular structural changes in small-resistance arteries may be considered, in the future, as an important intermediate end point for evaluating the benefits of antihypertensive therapy, although this point needs to be demonstrated by specific intervention studies. In conclusion, our results indicate, in a larger population than previously investigated, that structural alterations of small-resistance arteries may predict fatal cardiovascular events, stroke, and myocardial infarction. In addition, the prognostic role of small-resistance artery structure is also present in medium-risk patients with essential hypertension, at least when minor cardiovascular events are included in the analysis.

References


