Prognostic Significance of Small-Artery Structure in Hypertension

Damiano Rizzoni, MD; Enzo Porteri, MD; Gianluca E.M. Boari, MD; Carolina De Ciucnis, MD; Intissar Sleiman, MD; Maria Lorenza Muiesan, MD; Maurizio Castellano, MD; Marco Miclino, MD; Enrico Agabiti-Rosei, MD

Background—The presence of structural alterations in the microcirculation may be considered an important mechanism of organ damage; however, it is not currently known whether structural alterations of small arteries may predict fatal and nonfatal cardiovascular events.

Methods and Results—One hundred twenty-eight patients were included in the present study. There were 59 patients with essential hypertension, 17 with pheochromocytoma, 20 with primary aldosteronism, 12 with renovascular hypertension, and 20 normotensive patients with non–insulin-dependent diabetes mellitus. All subjects were submitted to a biopsy of subcutaneous fat. Small resistance arteries were dissected and mounted on an isometric myograph, and the tunica media–to–internal lumen ratio (M/L) was measured. The subjects were reevaluated after an average follow-up time of 5.4 years. Thirty-seven subjects had a documented fatal or nonfatal cardiovascular event (5.32 events/100 patients per year). In the subcutaneous small arteries of subjects with cardiovascular events, a smaller internal diameter and a clearly greater M/L was observed. Our subjects were subdivided according to the presence of an M/L greater or smaller than the mean and median values observed in the whole population (0.098) or mean value +2 SD of our normal subjects (0.11). Life-table analyses showed a significant difference in event-free survival between the subgroups. Cox’s proportional hazard model, considering all known cardiovascular risk factors, indicated that only pulse pressure (P=0.009) and M/L (P<0.0001) were significantly associated with the occurrence of cardiovascular events.

Conclusions—Our results strongly indicate a relevant prognostic role of structural alterations in small resistance arteries of a high-risk population. (Circulation. 2003;108:●●●-●●●.)

Key Words: vessels ▪ prognosis ▪ arteries ▪ remodeling ▪ hypertension

Microcirculation may be defined as that part of the vasculature that regulates flow and pressure.1,2 Energy dissipation (necessary to overcome vascular resistance), evidenced by a steep decline in blood pressure, occurs primarily in the precapillary arterioles and small arteries,3,4 that is, the arteries with diameters <350 μm.

The increased peripheral resistance that is the hallmark of hypertension in animals and humans may be ascribed in large part to the consequences of structural and functional alterations in the resistance vasculature.1,5 A thickened arterial wall together with a reduced lumen, with a consequently higher media-to-lumen ratio (M/L), may play an important role in the peripheral vasoconstriction and may also be an adaptive response to the increased hemodynamic load.1,5

The presence of structural alterations in the microcirculation may be considered an important link between hypertension and ischemic heart disease, heart failure, cerebral ischemic attacks, and renal failure. Therefore, it may represent an important mechanism of organ damage, possibly explaining the subsequent development of overt cardiovascular events. However, no data are currently available about a possible prognostic significance of the M/L of small resistance arteries in addition to other known cardiovascular risk factors.

Evaluation of the structure of small resistance arteries in humans is not an easy task because it usually implies invasive procedures, such as surgical biopsies of suitable tissues. More than 15 years ago, a reliable and minimally invasive technique for evaluation of the structure of subcutaneous small arteries was introduced and then widely applied, ie, a biopsy of subcutaneous fat from the gluteal or the anterior abdominal region with subsequent micromyographic evaluation.6,7 The aim of the present study was to evaluate the incidence of cardiovascular events during an average follow-up period >5 years in a large number of subjects submitted to the evaluation of the structure of subcutaneous small resistance arteries in our institution.

Methods

From January 1992 to June 2000, 151 subjects were submitted to an evaluation of the structure of small resistance arteries and were

Received February 26, 2003; de novo received May 14, 2003; revision received July 16, 2003; accepted August 1, 2003.

From the Department of Medical and Surgical Sciences, University of Brescia, Italy.

Correspondence to Enrico Agabiti-Rosei, Chair of Internal Medicine, Department of Medical and Surgical Sciences, University of Brescia, c/o 2° Medicina Spedali Civili di Brescia, Piazza Spedali Civili 1, 25100 Brescia, Italy. E-mail rizzoni@med.unibs.it

© 2003 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/01.CIR.0000095031.51492.C5
including the present study. They were 23 normotensive subjects, 59 patients with essential hypertension, 17 with pheochromocytoma, 20 with primary aldosteronism, 12 with renovascular hypertension, and 20 normotensive patients with non–insulin-dependent diabetes mellitus (NIDDM). Their age range was 20 to 81 years. The presence of hypertension was established according to International Society of Hypertension/World Health Organization Guidelines.8 The presence of NIDDM was established according to the Guidelines of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.9 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.

All hypertensive patients had been treated previously for short periods of time with calcium channel blockers, ACE inhibitors, diuretics, or β-blockers. Treatment was withdrawn at least 3 weeks before the procedure. There was no statistically significant difference in the therapeutic regimens of the different hypertensive groups. The protocol of the study was approved by the Ethics Committee of our institution (Medical School, University of Brescia), and informed consent was obtained from each participant. The procedures followed were in accordance with institutional guidelines.

Venous blood samples were taken with the participants in the supine position, after a washout period of ≥2 weeks, for standard hematology and serum biochemistry tests (including triglycerides and total cholesterol). In a subset of subjects (n=93), a standard echocardiographic evaluation was performed. Left ventricular internal dimensions and left ventricular posterior wall and interventricular septum thicknesses were measured according to the recommendations of the American Society of Echocardiography and the Penn convention.10 Left ventricular hypertrophy was considered present if the left ventricular mass index exceeded 125 g/m² in both sexes. For further technical details, see Muiesan et al.11

**Micromyography**

All subjects were then submitted to a biopsy of subcutaneous fat from the gluteal or the anterior abdominal region. The biopsy of the abdominal subcutaneous fat was taken during a surgical procedure (usually cholecystectomy) in normotensives and essential hypertensives and adenectomy or vascular surgical intervention on the renal arteries in patients with secondary hypertension), whereas in the remaining patients, a standard skin biopsy of the gluteal region (3 cm long, 0.5 cm wide, 1.5 cm deep) was performed.6,7 Small arteries (~100 to 280 μm 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 AJP Trading), by threading onto 2 stainless steel wires (40 μm in diameter). Media cross-sectional area, wall and media thicknesses, and the M/L of blood vessels in normalized condition were measured. Details about the micromyographic technique of evaluation of small-artery morphology were reported previously.12-14 Follow-up information was obtained by telephone interviews or by reevaluating subjects in the outpatient clinic. We considered cardiovascular events to be the following conditions: sudden death, ischemic or hemorrhagic stroke, transient cerebral ischemic attack, myocardial infarction, new-onset angina requiring hospitalization, progressive heart failure requiring hospitalization, coronary artery bypass or angioplasty, renal failure requiring dialysis, implantation of a pacemaker device, development of symptomatic lower-limb atherosclerosis, or surgical intervention for aortic aneurysms. Hospital records and all other available source documents were collected and reviewed by all authors of the study. The attribution of cardiovascular events to patients was decided, on the basis of the available documentation, by 2 medical members of the local Ethics Committee who were unaware of the vascular morphological data recorded.

**Statistical Analysis**

All data are expressed as mean±SD unless otherwise stated. One-way ANOVA and χ² statistics (Fisher exact test and Yates correction) were used to evaluate differences between groups. The Kaplan-Meier method was used to analyze event-free survival, and the groups were compared by use of the Mantel-Cox and Breslow tests. The relative importance of each prognostic factor, adjusted for the others, was assessed by use of the Cox proportional hazards model. All the statistical tests were 2 tailed. A value of P<0.05 was considered statistically significant. All analyses were carried out with the BMDP statistical package (BMDP Statistical Software Inc).

**Results**

We have observed cardiovascular events in 5 of 23 normotensive subjects and in 37 of 128 patients with hypertension or NIDDM. Demographic characteristics of the whole population and of the subjects with and without cardiovascular events are reported in Table 1. Because of a possible selection bias, we did not include the normotensive group in the following statistical analysis.

The average follow-up time of our subjects was 5.35 years (range, 2.6 to 9.93 years) for a total of 685 patient-years. Thirty-seven of 128 subjects had a documented fatal (n=4) or nonfatal (n=33) cardiovascular event (Table 2). Because of the elevated prevalence of hypertension, dyslipidemia, NIDDM, and cigarette smoking, the population included in the study may be considered at high cardiovascular risk, as documented by a prevalence of cardiovascular events of 28.9% and an incidence of 5.43 per 100 patients per year. Patients with cardiovascular events were older, with higher systolic and pulse pressure (Table 2). We observed cardiovascular events in 20 of 59 essential hypertensive patients, in 4 of 17 patients with pheochromocytoma, in 3 of 20 patients with primary aldosteronism, in 6 of 12 patients with renovascular hypertension, and in 4 of 20 normotensive patients with NIDDM (Figure 1).

In the subcutaneous small resistance arteries of subjects with cardiovascular events, compared with those without events, a smaller internal diameter and a greater M/L (P=0.000006) were observed (Table 1). To evaluate the prognostic role of the M/L of subcutaneous small resistance arteries in our study population, we subdivided our subjects into 2 groups according to the presence of an M/L above or below the mean (and median) value observed in the whole population (0.098) (Figure 2). Life-table analyses showed a significant difference in event-free survival between the 2 subgroups (P=0.015 by the Mantel-Cox test, P=0.036 by the Breslow test). The relative risk related to the presence of an M/L greater than 0.098 was 2.31 (95% CI, 1.15 to 4.64).

In addition, we subdivided our subjects according to the presence of an M/L greater or smaller than 0.11, that is, the value corresponding to 2 SD above the mean of our normal control subjects (n=23). Again, life-table analyses showed a significant difference in event-free survival between the 2 subgroups (P<0.00001 by the Mantel-Cox and Breslow tests) (Figure 2). The relative risk related to the presence of an M/L greater than 0.11 was 4.16 (95% CI, 2.13 to 8.11). The differences in event-free survival were statistically significant (P=0.009) even when patients with secondary forms of hypertension were excluded from the analysis (Figure 3).

The relative importance of known prognostic factors at baseline or at follow-up, such as age; sex; clinic systolic, diastolic, or pulse pressure; dyslipidemia; presence of diabetes; smoking status; and baseline diagnosis (essential hypertension, pheochromocytoma, primary aldosteronism, reno-
vascular hypertension, normotensive NIDDM) and the M/L of subcutaneous small arteries, was evaluated in the whole population, and the association of those variables with cardiovascular risk was assessed by the Cox proportional hazard model. Only pulse pressure \((P=0.009)\) and M/L of subcutaneous small arteries \((P<0.0001)\) were found to be significantly associated with the occurrence of cardiovascular events. The same results have been obtained when the analysis was restricted to the patients in whom data about left ventricular mass were available \((n=86)\). Also in this subgroup, the M/L of subcutaneous small arteries was the most potent predictor of cardiovascular events \((P=0.002)\), and left ventricular mass index did not enter the model. Surprisingly, age never entered the model, probably because part of the importance of age was eliminated in the model by pulse pressure (correlation coefficient between the 2 variables, \(r=0.37, P<0.001\)). Similarly, left ventricular mass did not contribute to the model. In this case also, a possible explanation is the presence of a correlation with systolic \((r=0.38, P<0.001)\), diastolic \((r=0.43, P<0.001)\), and pulse pressure \((r=0.22, P<0.05)\) and with M/L of small resistance arteries \((r=0.24, P<0.05)\). No significant correlation was observed between M/L of small arteries and pulse pressure.

### TABLE 1. Demographic Variables and Vascular Morphological Data of the Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Patients ((n=128))</th>
<th>With Cardiovascular Events ((n=37))</th>
<th>Without Cardiovascular Events ((n=91))</th>
<th>Normotensive Subjects ((n=23))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57±13</td>
<td>61±11*</td>
<td>55±13</td>
<td>59±14</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>74/54</td>
<td>25/12</td>
<td>49/42</td>
<td>13/10</td>
</tr>
<tr>
<td>Systolic arterial pressure, mm Hg</td>
<td>147±20</td>
<td>153±26*</td>
<td>145±18</td>
<td>125±9</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mm Hg</td>
<td>89±12</td>
<td>89±14</td>
<td>89±11</td>
<td>77±5</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>58±14</td>
<td>64±16†</td>
<td>56±12</td>
<td>48±8</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>6.87±2.85</td>
<td>6.75±2.68</td>
<td>6.87±2.96</td>
<td>5.58±1.34</td>
</tr>
<tr>
<td>Diabetic patients, %</td>
<td>37</td>
<td>38</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Smokers or previous smokers, %</td>
<td>37</td>
<td>43</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>5.43±1.24</td>
<td>5.09±1.66</td>
<td>5.60±1.06</td>
<td>5.12±1.50</td>
</tr>
<tr>
<td>Dyslipidemia, %</td>
<td>58</td>
<td>46</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td>Left ventricular mass index, g/m², (n=86)</td>
<td>117±34</td>
<td>122±29</td>
<td>115±36</td>
<td>94±16</td>
</tr>
<tr>
<td>Left ventricular hypertrophy, %</td>
<td>38</td>
<td>40</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Subcutaneous small arteries: media cross-sectional area, (\mu m²)</td>
<td>19 530±8527</td>
<td>18 038±8202</td>
<td>20 137±8626</td>
<td>18 088±7812</td>
</tr>
<tr>
<td>Media thickness, (\mu m)</td>
<td>22.5±5.04</td>
<td>23.3±5.04</td>
<td>22.1±5.03</td>
<td>18.8±4.76</td>
</tr>
<tr>
<td>Wall thickness, (\mu m)</td>
<td>41.4±8.89</td>
<td>42.7±8.28</td>
<td>40.9±9.12</td>
<td>35.4±8.14</td>
</tr>
<tr>
<td>Internal diameter, (\mu m)</td>
<td>247±76</td>
<td>215±64†</td>
<td>260±78</td>
<td>281±75</td>
</tr>
<tr>
<td>M/L</td>
<td>0.098±0.029</td>
<td>0.116±0.034</td>
<td>0.091±0.024</td>
<td>0.070±0.020</td>
</tr>
</tbody>
</table>

\* \(P<0.05\), † \(P<0.01\), ‡ \(P<0.001\) vs without cardiovascular events.

### TABLE 2. Cardiovascular Events in the Study Population \((n=37/128)\)

<table>
<thead>
<tr>
<th>Event</th>
<th>Occurring in Patients With M/L &lt;0.11 ((n=16/92))</th>
<th>Occurring in Patients With M/L &gt;0.111 ((n=21/36))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Sudden death or cardiac arrest</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Cardiac pacemaker device implant+ TIA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Atrial fibrillation requiring hospitalization</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Lower-limb atherosclerosis with intermittent claudication</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Surgical intervention for aortic aneurism</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>TIA</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Stroke</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Heart failure requiring hospitalization</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Angina pectoris requiring hospitalization</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

M/L indicates M/L of subcutaneous small arteries, 0.11: 2 SD above the mean value of our normal reference subjects; and TIA, transient ischemic attack.
We also examined the antihypertensive therapy that the subjects received during the follow-up period. There were no qualitative or quantitative differences between those with a high or low M/L. There was no qualitative difference in the cardiovascular events observed in patients with high or low M/L of subcutaneous small arteries (Table 2).

Discussion

For the first time, the results obtained in our study have demonstrated 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. Its prognostic role is independent of other known cardiovascular risk factors, as demonstrated by Cox ANCOVA; in fact, only pulse pressure provided a further contribution in the prediction of cardiovascular events. A possible explanation may be that vascular structure is so potent a predictor of outcome that it may carry a relevant part of the contribution of other known risk factors, at least in our population. Our results point strongly toward a relevant prognostic role of vascular structural alterations in high-risk patients, although this evidence cannot yet be further extended to the general population. Cardiovascular events were qualitatively similar but quantitatively different in the subgroups with high or low M/L, suggesting that an altered vascular structure may uniformly increase the risk of cardiac, cerebral, and renal events.

**Figure 1.** Plot of M/L of subcutaneous small resistance arteries against incidence of cardiovascular (CV) events in different groups of patients. Filled diamonds indicate patients with essential hypertension; filled squares, patients with pheochromocytoma; filled circles, patients with primary aldosteronism; open squares, patients with renovascular hypertension; and open circles, normotensive patients with NIDDM.

**Figure 2.** Top left, event-free survival (Kaplan-Meier method) in group of patients with an M/L of subcutaneous small arteries ≥0.098 (mean and median values observed in whole population) (n=64, solid line) or <0.098 (n=64, dotted line). Mantel-Cox test between curves, P=0.015; Breslow test between curves, P=0.036. Bottom left, event-free survival in group of patients with an M/L of subcutaneous small arteries ≥0.11 (2 SD above mean value of our normal reference subjects) (n=36, solid line) or <0.11 (n=92, dotted line). Mantel-Cox test and Breslow test between curves, P<0.00001. Top and bottom right, incidence of cardiovascular (CV) events in subgroups of patients.
A possible bias of our study could have been the presence of a surgical correction of secondary hypertension, which could have reduced the incidence of cardiovascular events of treated patients. However, as is evident in Figure 1, this was not the case for our population. In particular, patients with renovascular hypertension and pheochromocytoma were all submitted to a surgical intervention or a renal angioplasty, but their average M/L of small arteries and their incidence of cardiovascular events were clearly different. In addition, no qualitative or quantitative difference in the pharmacological treatment between patients with high or low M/L of small arteries was detected; therefore, it is highly unlikely that follow-up treatment may have influenced outcome.

The wire micromyographic technique permits the direct evaluation of the morphofunctional characteristics of small arteries in vitro ex vivo.\textsuperscript{12,13,15} The presence of structural alterations in subcutaneous and omental small resistance arteries from essential hypertensive patients has been demonstrated with this approach.\textsuperscript{6,14,15} The mechanisms leading to vascular remodeling are currently unknown, although it has been suggested that vascular wall stress, neurohormonal environment, and changes in extracellular matrix proteins\textsuperscript{16} may have a crucial role.

It is not clear whether vascular structural changes are an important factor in initiating hypertension.\textsuperscript{17} Some experimental studies and theoretical analyses have suggested that narrowing of the lumen and increase of the M/L may be responsible for the so-called vascular amplifier mechanisms, whereby the increase in vascular resistance per unit of constrictor stimulus is enhanced in the hypertensive circulation.\textsuperscript{18}–\textsuperscript{20} However, these conclusions have not been confirmed by others.\textsuperscript{21,22}

An important consequence of the presence of structural alterations in small resistance arteries and arterioles may be an impairment of the vasodilator reserve. Remodeling of small resistance arteries is characterized by narrowing of the lumen, which may increase vascular resistance even at full dilatation, i.e., in the absence of vascular tone. In fact, a significant correlation between coronary flow reserve and subcutaneous small resistance artery remodeling was detected in hypertensive patients, suggesting that structural alterations in small resistance arteries may be present simultaneously in different vascular districts, and changes in morphology of the subcutaneous vasculature may reflect concomitant clinically important alterations in the coronary vessels.\textsuperscript{23} The extent of structural alterations in small resistance vessels is more pronounced in hypertensive patients with diabetes mellitus compared with those observed in nondiabetic hypertensives or with normotensive diabetics, suggesting that clustering of risk factors may have synergistic deleterious effects on the vasculature.\textsuperscript{24} Recent data suggest that in humans as well, alterations in small resistance artery morphology may represent the most prevalent and perhaps the earliest form of target organ damage in essential hypertension.\textsuperscript{25} In addition, structural alterations in resistance arteries may be closely related to target organ damage, especially at the cardiac level. In fact, a linear relation between M/L of subcutaneous small resistance arteries and left ventricular mass index or relative wall thickness has been detected in hypertensive patients; this relation with left ventricular mass and geometry was more evident in patients with activation of the renin–angiotensin–aldosterone system.\textsuperscript{26} It is interesting to note that several studies have demonstrated that the effect of different antihypertensive drugs on vascular structure is not the same, being clearly more effective for those drugs that interfere with the renin–angiotensin system\textsuperscript{27} and calcium antagonists\textsuperscript{30} than for β-blockers.

The presence of structural alterations in the microcirculation may thus 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 intermediate end point for the evaluation of the benefits of antihypertensive therapy, although this point needs to be demonstrated by specific intervention studies.

The current method for the evaluation of the structure of subcutaneous small arteries is minimally invasive, but nevertheless, the invasiveness may limit its applicability to large populations. It is possible, however, that noninvasive techniques for investigation of the microcirculation that are currently still under evaluation and validation, such as acoustic\textsuperscript{31,32} confocal,\textsuperscript{33,34} fluorescence,\textsuperscript{35} or intravital\textsuperscript{36} microscopy, may in the near future provide important information to achieve a better diagnostic and therapeutic approach in hypertensive patients.
References

Prognostic Significance of Small-Artery Structure in Hypertension
Damiano Rizzoni, Enzo Porteri, Gianluca E.M. Boari, Carolina De Ciuceis, Intissar Sleiman, Maria Lorenza Muiesan, Maurizio Castellano, Marco Miclini and Enrico Agabiti-Rosei

Circulation. published online October 13, 2003;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/early/2003/10/13/01.CIR.0000095031.51492.C5.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/