Endothelial Microparticles and Renal Disease

In Vivo Shear Stress Determines Circulating Levels of Endothelial Microparticles in End-Stage Renal Disease

Chantal M. Boulanger, Nicolas Amabile, Alain P. Guérin, Bruno Pannier, Aurélie S. Leroyer, Clément Nguyen, Ziad Mallat, Alain Tedgui, Gérard M. London

Abstract—Shear stress is a major determinant of endothelial apoptosis, but its role in the in vivo release of shed membrane microparticles by endothelial cells remains unknown. Thus, we sought to evaluate the possible relationship between circulating endothelial microparticle levels and laminar shear stress in end-stage renal disease patients with high cardiovascular risk, whose levels of endothelial microparticles are elevated. In 34 hemodialyzed patients, we analyzed the relationships between brachial artery and aortic shear stress and circulating microparticles levels. Only endothelial microparticles were inversely correlated with laminar shear stress values ($P<0.0001$) or its components shear rate and whole blood viscosity, independent of age or arterial blood pressure. Changes in hematocrit resulting from hemodilutional hemoconcentration or erythropoietin anemia improvement induced a significant increase in whole blood viscosity and shear stress and were associated with a significant decrease in endothelial microparticles with a significant and inverse correlation with changes in hematocrit/hemoglobin or laminar shear stress. These results demonstrate that, in end-stage renal disease patients, laminar shear stress is an important determinant of plasma levels of endothelial microparticles. Anemia as an important determinant of whole blood viscosity and shear stress, contributes to endothelial apoptosis, and could play an indirect role in the pathogenesis of accelerated arteriosclerosis in this high-risk population. (Hypertension. 2007;49:902-908.)

Key Words: microparticles ■ ESRD ■ endothelium ■ whole blood viscosity ■ shear stress ■ hemodialysis

Microparticles (MPs) are submicron vesicles shed from plasma membranes after cell activation or apoptosis.1 MPs of different cellular origin are found in the plasma of healthy subjects, and their numbers increase in patients with cardiovascular diseases.2–6 Surprisingly, the mechanisms leading to the in vivo formation and release of MPs by endothelial cells remain obscure. Yet, apoptotic stimuli are extensively used in vitro to generate MPs from cultured cells.6 Shear stress (SS) is a major determinant of endothelial apoptosis, and physiological laminar fluid SS promotes endothelial cell survival and quiescence.7 Maintenance of a physiological laminar SS is crucial for normal vascular structure and function and exerts atheroprotective effects in vivo through the release of substances that promote anticoagulation, inhibit inflammation, and induce vasodilation.7–10 In association with anemia-related low whole blood viscosity (WBV), SS is reduced in patients with end-stage renal disease (ESRD),11 and this abnormality is associated with arterial outward remodelling, increased arterial stiffness, and reduced flow-mediated dilation.12 Increased SS because of partial anemia correction was associated with reduced arterial stiffness and improved flow-mediated dilation.12 Plasma levels of endothelial MPs in patients with ESRD predict the severity of these arterial and endothelial dysfunctions independent of other classical risk factors,5 but the eventual role of SS alterations on the in vivo formation and release of MPs by endothelial cells remains unknown. Thus, in the present study we sought to evaluate the relationship between circulating endothelial MPs levels and SS and its determinants in ESRD patients known to represent a high cardiovascular risk population, with high levels of circulating endothelial MPs,5 and to evaluate the effects of acute and chronic anemia correction-related SS changes on circulating platelets and endothelial MPs.

Methods

An expanded Methods section is available in an online supplement available at http://hyper.ahajournals.org. Thirty-four ESRD patients on hemodialysis (HD) for ≥3 months were included. Inclusion criteria were as follows: (1) no clinical cardiovascular complication, (2) absence of aortic valves stenosis, and (3) agreement to participate in the study, which was approved by our institutional review board and adhered to the principles of the Declaration of Helsinki. Patients were dialyzed for 4 to 6 hours 3 times weekly on high-flux hemodialyzers with a synthetic biocompatible membrane (Nephral 500 Hospal Merieux). Twenty ESRD patients received antihypertensive therapy, which was stopped 10 days before the study. Patients received sevelamer as a phosphate binder and, if necessary, were on...
erythropoietin (EPO) and intravenous iron therapy to maintain hemoglobin level ≥110g/L. Twenty-five matched healthy control subjects were included.

Arterial Hemodynamic Measurements
Experiments were carried out as reported previously.\textsuperscript{13–16}

MP Isolation and Cytofluorometry Analysis
Circulating MPs isolated from venous citrated blood after 3 days off dialysis were analyzed by flow cytometry\textsuperscript{5,6,17–19} (Figure S1). Platelet- and endothelial-derived MPs were defined as CD41\textsuperscript{+}CD31\textsuperscript{+} and CD41\textsuperscript{−}CD31\textsuperscript{+} MPs, respectively. Endothelial MPs were also identified as CD144\textsuperscript{+} MPs.

Results

Characteristics of Study Populations
Table S1 includes blood chemistry characteristics of ESRD patients. Abnormalities were those classically observed in ESRD, that is, lower hemoglobin, lower WBV, high parathormone, and serum phosphates. Blood lipids, serum albumin, and serum total or ionized calcium were in the reference range. C-reactive protein was slightly above the normal values (2 mg/L), but microinflammation was mild. All of the studied MPs were elevated in comparison with values in the control population (Table S1).

Hemorheologic Characteristics and Relationships With MPs
Brachial artery characteristics determined by direct measures are given in Table S2. ESRD patients were characterized by larger brachial artery diameter, lower mean flow velocity, and lower shear rate. Lower shear rate and lower WBV were responsible for decreased SS in ESRD patients, but the values were still within the typically observed range (5 to 20 dynes/cm\textsuperscript{2}).

In univariate correlation analysis, endothelial CD144\textsuperscript{+} MPs were significantly correlated only with age ($r=0.597; P<0.01$), systolic blood pressure (BP; $r=0.618; P<0.001$), and mean SS ($r=-0.770; P<0.0001$). The correlation of CD144\textsuperscript{+} MPs with peak SS was also significant but weaker ($r=-0.565; P<0.01$). In multivariate analyses, circulating CD144\textsuperscript{+} MPs were strongly and inversely correlated with brachial artery SS or its 2 determinants, that is, shear rate, and WBV, that is, hematocrit (Figure 1), and positively correlated with age and systolic BP (Table 1). Shear rate and aortic SS, which was computerized from stroke volume, aortic diameter, and WBV, were lower in ESRD patients and were significantly correlated with directly measured parameters in brachial artery. Furthermore, circulating CD144\textsuperscript{+} MPs were strongly and inversely correlated with aortic SS (Figure 2). In univariate correlation analysis, endothelial CD41\textsuperscript{−}CD31\textsuperscript{+} MPs only correlated with systolic BP ($r=0.718; P<0.0001$) and mean SS ($r=-0.565; P<0.005$). Multivariate analyses of variables associated with endothelial CD41\textsuperscript{−}CD31\textsuperscript{+} MPs are shown in Table 1. An independent and significant positive correlation was observed between CD41\textsuperscript{−}CD31\textsuperscript{+} MPs and systolic BP. Circulating CD41\textsuperscript{−}CD31\textsuperscript{+} MPs were strongly

Figure 1. Correlations between brachial artery SS (expressed as dyne/cm\textsuperscript{2}) and CD144\textsuperscript{+} MPs (A), CD41\textsuperscript{−}CD31\textsuperscript{+} MPs (B), CD41\textsuperscript{−}CD31\textsuperscript{+} MPs (C), and annexin V\textsuperscript{+} MPs (D).
and inversely correlated with SS. In univariate and multivariate correlation analysis, circulating platelets MPs were associated with plasma fibrinogen \((P=0.048)\) and no other parameter.

**Effect of Increasing SS on Circulating MPs**

The effects of acute systolic BP and rheology changes on circulating MPs were analyzed after HD ultrafiltration-induced hemoconcentration in 25 patients, including 14 patients with repeated brachial artery hemodynamics (Table 2). Hemodialysis induced a significant decrease in systolic BP \((P<0.0001)\) and increased brachial artery SS \((P<0.0001)\) associated with increased WBV and hematocrit \((P<0.0001)\) and increased shear rate \((P<0.01)\). Changes in systolic BP were significantly and inversely correlated with changes in BA shear stress \((r=-0.619; P=0.014)\). HD induced a significant decrease in CD41−CD31+ MPs with no effects on CD41+CD31+ MPs or annexin V+ MPs. In 14 patients with repeated measurement of SS, changes in CD41−CD31+ MPs were positively correlated with changes in systolic BP \((r=0.454; P<0.01)\) and negatively correlated with changes in SS \((r=-0.643; P<0.01)\; Figure 3). When analyzed in shear stress components, the decrease in CD41−CD31+ MPs was inversely associated with changes in WBV \((P=0.015)\) and with changes in shear rate \((P=0.050)\).

In univariate analysis concerning all 25 of the patients, changes in CD41−CD31+ MPs were inversely correlated with increased hematocrit/WBV \((r=-0.579; P<0.001)\) and positively correlated with changes in systolic BP \((r=0.440; P<0.01)\). Because of significant inverse correlation between changes in systolic BP and brachial artery SS \((P<0.01)\) in multivariate analysis, only changes in hematocrit/WBV were inversely correlated with changes in MPs \((r=-0.513; P<0.01)\; Figure 4). Changes in CD41−CD31+ MPs were not correlated with changes in blood chemistries.

Association between long-term hematocrit–hemoglobin–WBV changes and MPs was studied in 18 patients, including 10 patients with repeated BA hemodynamics, after treatments with EPO and/or intravenous iron. During this period \((15±4\ weeks)\) hemoglobin and WBV increased in all but 2 of the patients \((in 2\ iron-depleted\ patients,\ the\ hemoglobin\ decreased)\). Although CD41−CD31+ MPs significantly decreased, the CD41+CD31+ population did not change significantly. BA hemodynamics were repeated in 10 subjects.

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**Table 1. Multiple Correlation Report for CD144+ and CD41−CD31+ Endothelial MPs as a Dependent Variable in ESRD Patients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD144+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>2.051</td>
<td>0.049</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>2.243</td>
<td>0.0321</td>
</tr>
<tr>
<td>Brachial artery SS, dynes/cm²</td>
<td>−3.730</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD41−CD31+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>4.009</td>
<td>0.0004</td>
</tr>
<tr>
<td>Brachial artery SS, dynes/cm²</td>
<td>−3.416</td>
<td>0.0019</td>
</tr>
</tbody>
</table>

\(R^2 = 0.687; P<0.0001\) for the CD144+ model. \(R^2 = 0.666; P<0.0001\) for the CD41−CD31+ model.

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**Figure 2.** Correlations between brachial artery and ascending aortic SS (A), between brachial artery and aortic shear rates (B), and between aortic SS and CD144+ MPs (C).

The shear rate was unaffected, and increased SS \((P<0.05)\) was entirely associated with increased WBV (Table 3). A significant inverse relationship was observed between changes in circulating CD41−CD31+ endothelial MPs and changes in hemoglobin (Figure 4) or hematocrit and WBV (data not shown). No correlation was observed between the EPO dose and endothelial \((r=0.067; P=0.819)\) or platelet MPs. BP, CD41+CD31+ MPs, and blood chemistries (data not shown) were unchanged.

**Discussion**

The present study is the first to identify a robust relationship between in vivo measured shear stress level and circulating...
endothelial MPs, independent of age and BP. The inverse relationship between shear stress and MPs concerned more specifically endothelial MPs and was not observed with platelet MPs.

Several recent studies support the concept that plasma levels of endothelial MPs represent a surrogate marker of endothelial cell damage. Endothelium-derived MPs impair endothelial function in vitro, and recent studies in patients with ESRD or acute myocardial infarction have demonstrated a strong and independent association between circulating endothelial MPs with several indexes of arteriosclerosis and decreased flow-mediated arterial dilation. Maintenance of physiological laminar fluid SS is crucial for normal vascular function and structure. Laminar SS affects multiple endothelial functions, such as proliferation, apoptosis, migration, permeability, and remodelling, as well as gene expression. Hence, our results show an inverse correlation between circulating endothelial MPs and baseline SS values, suggesting that both enhanced productions of CD144+ and CD41−CD31+ endothelial MPs result from increased apoptosis of the endothelium, triggered by low laminar stress on the vessel wall. Moreover, previous results in ESRD populations showed that circulating levels of endothelial MPs are associated with alteration of mechanical properties of arteries and decreased endothelial flow-mediated dilation and that increased SS because of partial anemia correction was associated with reduced arterial stiffness and improved flow-mediated dilation. The major determinants of in vivo SS are blood viscosity, blood flow, and arterial diameter. Arterial enlargement with outward remodeling is well documented in ESRD patients. These changes are already observed in patients with mild-to-moderate chronic kidney disease in the

<table>
<thead>
<tr>
<th>Variables</th>
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<th>After HD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>72.3±14</td>
<td>69.6±13.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>142±32</td>
<td>125±28</td>
<td>0.001</td>
</tr>
<tr>
<td>Whole blood viscosity, cPoise</td>
<td>3.00±0.52</td>
<td>3.21±0.60</td>
<td>0.001</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>115±13</td>
<td>128±9.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>35±3.6</td>
<td>38.0±3.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>CD41−CD31+ MP, ev/µL</td>
<td>1356±1066</td>
<td>678±473</td>
<td>0.0001</td>
</tr>
<tr>
<td>CD41−CD31+ MP, ev/µL</td>
<td>7702±11894</td>
<td>4780±10610</td>
<td>NS</td>
</tr>
<tr>
<td>Annexin V+ MP, ev/µL</td>
<td>4661±9203</td>
<td>4934±12303</td>
<td>NS</td>
</tr>
<tr>
<td>Brachial artery shear rate, s⁻¹*</td>
<td>31±18</td>
<td>47±25</td>
<td>0.01</td>
</tr>
<tr>
<td>Brachial artery SS, dynes/cm²*</td>
<td>8.9±6.1</td>
<td>15.6±10.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values as means±SD.

*P<0.05.
absence of anemia. Hematocrit is a major determinant of blood viscosity, and modest decreases in hemoglobin concentration have been shown to impair flow-mediated dilatation in the human brachial artery of normal subjects. The present study indicates that the degree of anemia as a determinant of SS is independently and inversely associated with values of endothelial circulating MPs and the degree of endothelium injury.

Endothelial dysfunction is observed in patients with ESRD and is attributed to the presence of NOS inhibitors and accumulation of uremic toxins. In agreement with a previous study, the present results suggest that, other than the role of metabolic disorders, anemia, indirectly through its influence on SS, could play a role in the development of endothelial and arterial dysfunctions observed in ESRD patients. We analyzed the possibility that an increase in SS induced by increased WBV and increased hematocrit could be associated with decreased MP concentrations. We repeated the study following acute and long-term changes in SS obtained by HD ultrafiltration/hemoconcentration or long-term hemoglobin/hematocrit increase. Hemodialysis induced a significant decrease of endothelial-derived MPs associated with an increase in SS but also with decreased systolic BP, both factors associated with MPs levels. Because of a significant correlation between HD-induced changes in SS and BP, the separate effect of these changes on MP variation is difficult to analyze. In a multivariate model including the respective roles of systolic pressure and WBV changes on endothelial MPs, only changes in blood viscosity and or hematocrit were significantly associated with MP variations. Faure et al have shown recently that, in vitro, the uremic toxins p-cresol and indoxyl sulfate increase endothelial MP release from cultured endothelial cells, and the present study cannot rule out the possibility that the observed decreased MP after HD results from the removal of uremic toxins. Nevertheless, no association between changes in MPs and changes in HD-induced biochemistry, including urea removal rate and an index of dialysis adequacy (Kt/V), were observed. Hemodialysis induced a significant decrease of endothelial-derived MPs but has no effect on platelet-derived MPs. Our results differ from those of Daniel et al, who observed a significant increase of circulating platelet MPs during HD. The difference could be because of the high use of cellulosic membranes in this study, whereas only biocompatible synthetic membranes were used in our study.

Long-term anemia and hematocrit changes were associated with changes in SS because of variations in WBV but not because of changes in shear rate. Changes in hemoglobin/hematocrit and WBV were only associated with reciprocal variations in circulating endothelial, but not platelet, MPs. Conditions of the measurements before and after the long-term hematocrit changes were identical, that is, before HD.

### Table 3. Effect of Long-Term Hemoglobin Changes on CD41–CD31+ Endothelial and CD41+CD31+ Platelet MPs (n=18) and on Brachial Artery SS (n=10)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before</th>
<th>After</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>140±30</td>
<td>142±25</td>
<td>NS</td>
</tr>
<tr>
<td>Whole blood viscosity, cPoise</td>
<td>2.92±0.20</td>
<td>3.16±0.23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>106±13</td>
<td>113±9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>32.7±3.8</td>
<td>35.6±3.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Erythropoietin, units per wk</td>
<td>5824±4290</td>
<td>6470±6324</td>
<td>NS</td>
</tr>
<tr>
<td>Microparticles CD41–CD31+ , ev/μL</td>
<td>1730±1039</td>
<td>835±657</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Microparticles CD41+CD31+ , ev/μL</td>
<td>4110±2531</td>
<td>3085±1722</td>
<td>NS</td>
</tr>
<tr>
<td>Brachial artery shear rate, s⁻¹</td>
<td>32±16</td>
<td>36±22</td>
<td>NS</td>
</tr>
<tr>
<td>Brachial artery SS, dynes/cm²</td>
<td>7.5±4.2</td>
<td>12±6.4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean±SD. NS indicates not significant.

**Figure 4.** Correlation between changes in CD41–CD31+ MPs and hemodialysis-induced hematocrit (A), hemodialysis-induced SS (B), or long-term hemoglobin changes (EPO dose adjustments; C).
and with similar blood chemistries and unchanged BP. The observed decrease in endothelial MPs could result from the protective effect of higher SS or from the effect of EPO used to correct anemia. Treatment with a long-acting EPO analogue enhances endothelial progenitor cell proliferation and differentiation in renal patients and confers vascular protection.31,32 The role of EPO cannot be excluded, but the concentrations used were not related to MP levels, and, in some patients, the anemia improvement was achieved only by intravenous iron.

Platelet MPs were also increased in ESRD patients, but their concentration was not associated with prevailing SS. Although SS in ESRD patients was lower than in control subjects, it was still within the usual range observed in large conduit arteries.8 Activation of platelets MPs is induced by high SS largely over the physiological range,33 which is not the situation observed in the present study.

Limitations
This study has a limitation concerning aortic SS, which was not directly measured but computerized from the Hagen–Poiseuille equation. The limitation is principally because of uncertainty about the laminar flow regime and the flow unidirectional pattern in the proximal ascending aorta. We presented the results concerning aortic shear stress to illustrate that SS differences in ESRD patients are systemic and not only limited to the brachial artery, as well as to illustrate that the relationships between MPs and SS are present in local and systemic circulation. Nevertheless, because brachial artery SS was directly measured and not dependent on the Hagen–Poiseuille formula, this study focused mainly on brachial artery rheology. Another limitation concerns the measurement of the wall shear rate, which is underestimated in large arteries when assuming a parabolic velocity profile, although this is less pronounced for brachial arteries.15,16,34

Perspectives
Our study demonstrates for the first time the relation between in vivo circulating endothelial MPs and mechanic hemorheological parameters in hemodialyzed patients, depicting SS as a major determinant for endothelial cell injury and vasculature. Because changes in SS appear to be related also to the low hematocrit value in our population, and because changes in hemoglobin–hematocrit–WBV are independently associated with changes in endothelial MPs, the present results suggest that anemia and resulting alterations in SS increased endothelial apoptosis and could indirectly contribute to the high prevalence of arterial diseases and cardiovascular events in an ESRD population. This might also explain the poor survival rate observed in ESRD patients with cardiovascular diseases and low hematocrit.35

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Disclosures
None.

References


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