Soluble fms-Like Tyrosine Kinase-1 and Endothelial Adhesion Molecules (Intercellular Cell Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1) as Predictive Markers for Blood Pressure Reduction After Renal Sympathetic Denervation

Oliver Dörr, Christoph Liebetrau, Helge Möllmann, Luise Gaede, Christian Troidl, Johannes Rixe, Christian Hamm, Holger Nef

Abstract—Renal sympathetic denervation (RSD) is a treatment option for patients with resistant arterial hypertension, but in some patients it is not successful. Predictive parameters on the success of RSD remain unknown. The angiogenic factors soluble fms-like tyrosine kinase-1 (sFLT-1), intercellular cell adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) are known to be associated with endothelial dysfunction, vascular remodeling, and hypertension. We evaluated whether sFLT-1, ICAM-1, and VCAM-1 are predictive markers for blood pressure reduction after RSD. Consecutive patients (n=55) undergoing renal denervation were included. Venous serum samples for measurement of sFlt-1, ICAM-1, and VCAM-1 were collected before and 6 months after RSD. A therapeutic response was defined as an office systolic blood pressure reduction of >10 mmHg 6 months after RSD. A significant mean office systolic blood pressure reduction of 31.2 mmHg was observed in 46 patients 6 months after RSD. Nine patients were classified as nonresponders, with a mean systolic blood pressure reduction of 4.6 mmHg. At baseline, sFLT-1 levels were significantly higher in responders than in nonresponders (P<0.001) as were ICAM-1 (P<0.001) and VCAM-1 levels (P<0.01). The areas under the curve for sFLT-1, ICAM-1, and VCAM-1 were 0.82 (interquartile range, 0.718–0.921; P<0.001), 0.754 (0.654–0.854; P<0.001), and 0.684 (0.564–0.804; P=0.01), respectively, demonstrating prediction of an RSD response. Responders showed significantly higher serum levels of sFLT-1, ICAM-1, and VCAM-1 at baseline compared with nonresponders. Thus, this study identified for the first time potential biomarkers with a predictive value indicating a responder or nonresponder before renal denervation. (Hypertension. 2014;63:984-990.)

Key Words: biological markers ▼ hypertension

Article—Arterial hypertension (HT) represents a major risk factor for cardiovascular morbidity and mortality.1 HT is associated with endothelial dysfunction that aggravates progressive vascular damage and atherosclerosis.2–4 Endothelial dysfunction can be indicated by elevated serum concentrations of endothelial adhesion molecules, including intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).5,6 These adhesion molecules play important roles in accelerating atherosclerosis by attaching inflammatory cells to the vascular endothelial wall and promoting their subsequent migration through the endothelium.

The vascular endothelial growth factor and its soluble receptor fms-like tyrosine kinase-1 (sFLT-1) are also associated with endothelial dysfunction, vascular remodeling, and endothelial repair and regeneration mechanisms.7–11 Hypoxia and endothelial shear stress in HT are known to be major stimuli for sFLT-1 expression and release.12–16 Furthermore, sFLT-1 is stimulated by the vascular sympathetic nervous system and has been reported to be elevated in patients with HT. The persistence of hypertensive blood pressure (BP) in patients with resistant hypertension is partially regulated by the renal sympathetic nerve system.17–21 Renal sympathetic denervation (RSD) is an interventional treatment option for patients who have resistant hypertension, despite optimal medical treatment.22,23 However, RSD is not effective in ≈8% to 12% of treated patients.22,23 Predictive parameters regarding the response or nonresponse to RSD have not yet been described.

We postulated a relationship among successful RSD-related BP reduction, endothelial dysfunction, and activated endothelial cells as indicated by ICAM-1, VCAM-1, and sFLT-1, specific markers for endothelial cell activity and hypertension-related endothelial shear stress. Therefore, the primary aim of the present study was to examine the predictive value of sFlt-1, ICAM-1, and VCAM-1 before renal denervation.
VCAM-1 concentrations in patients undergoing RSD on the success of RSD-related BP reduction after 6 months of follow-up.

Methods

Patients and Treatment

Fifty-five consecutive patients with resistant hypertension who were to undergo RSD at 2 clinical centers were included in the study. Clinical history, physical examination, and laboratory tests were assessed for all patients.

At baseline and at 6-month follow-up, office blood pressure measurements were performed in accordance with the Guidelines of the European Society of Cardiology for the management of arterial hypertension. The final diagnosis of resistant hypertension was made according to these guidelines that are based on a systolic BP >150 mmHg, despite treatment with ≥3 antihypertensive medications of different classes, including diuretics, at the maximum tolerated doses. Patients with secondary origins of hypertension, as well as patients with known peripheral arterial disease, were excluded.

Angiography was performed in all patients before RSD to exclude renal artery stenoses. All patients received 500 mL of 0.9% NaCl IV before and after RSD. RSD was performed according to standard clinical practice with the Symplicity-Catheter system (Medtronic/Ardisain Inc) using a transfemoral approach with a 6F sheath via a femoral artery. Patients were followed for 6 months. All patients provided written informed consent for their participation in the study, and approval of the Institutional Review Board of the University of Giessen (230/11) was obtained. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Laboratory Assessment

Venous blood samples for determination of sFlt-1, ICAM-1, and VCAM-1 were collected before and at 6 months after RSD. Samples were processed immediately and frozen at −80°C until assayed.

Soluble FLT-1 was measured in serum using an immunnoassay (Quantikine Human sFlt-1 Immunoassays, R&D Systems, MN). The minimum detectable concentration of this assay is 0.6 ng/mL, and the coefficient of variation is 5.5%.

Soluble ICAM-1 and VCAM-1 concentrations were measured in serum by immunoassays (Quantikine Human sICAM-1 and sVCAM-1 Immunoassays, R&D Systems, MN). The minimum detectable concentrations of the VCAM-1 and ICAM-1 assays are 0.6 and 0.096 ng/mL, respectively. The coefficient of variation is 5.5% for VCAM-1 and 4.4% for ICAM-1.

Statistical Analysis

All data for continuous variables are expressed as mean±SD or as median and interquartile range (IQR), as appropriate. Categorical variables are reported as number and percentage. After testing for normal distribution, values were compared by unpaired Student’s t test or Mann–Whitney test, as appropriate. A Fisher exact test or a χ² test was used for categorical variables with nominal scales. To evaluate test performance of sFlt-1, ICAM-1, and VCAM-1 as predictors for response to RSD, the area under the curve of the receiver operating characteristic curve was plotted. The cutoff values for response to RSD were evaluated by the Youden index. A correlation analysis was performed to assess the correlation between baseline concentration and RSD-related BP reduction. All statistical tests were performed 2-tailed, and a significance level of P <0.05 was considered to indicate statistical significance. For all statistical analyses, the statistical software SPSS 20.0 (Statistical Package for the Social Sciences, Chicago, IL) for Windows was used.

Results

A total of 55 consecutive patients (28 men [51%]; mean age [SD], 67.7 [10.5] years) were included in the present study. Clinical and procedural characteristics of all patients enrolled in the study are shown in Table 1. In the present study, there were no differences in systolic BP values at baseline, baseline characteristics, procedural data, or antihypertensive medications between the groups (Table 1). Subjects received an average of 5.4 (±1.4) antihypertensive drugs in different substance classes (Table 1). All RSD procedures were performed bilaterally in a single-session procedure with 5.7±1.5 ablation points at the right renal artery and 5.2±1.4 ablation points at the left renal artery (Table 1). The antihypertensive medical treatment remained unchanged during the follow-up period to avoid bias of the biomarker levels by modification of the antihypertensive drugs. If necessary, the medical treatment was modified after the 6-month follow-up time point.

Six months after RSD, a significant mean office systolic BP reduction of 31.2 mmHg (172.6±11.6 mmHg at baseline versus 141.4±12.6 mmHg at follow-up; P<0.001) could be
observed in 46 patients (83.6%). Nine patients (16.4%) were classified as nonresponders, with a mean systolic BP reduction of <10 mm Hg (4.67 mm Hg). The systolic BP before RSD was not significantly different in responders and nonresponders (172.6 versus 170.1±12.6 mm Hg; P=0.86; Table 1; Figure 1).

The concentrations of sFlt-1, ICAM-1, and VCAM-1 according to the prespecified time points are shown in Table 2. At baseline, there were no sex-specific differences about the biomarker levels in responders and nonresponders. The proportion of patients with type 2 diabetes mellitus was higher in nonresponders (Table 2). However, there were no differences between patients in the baseline concentrations of the 3 specified biomarkers in responders and nonresponders.

In addition, there were no differences in patients with hypercholesterolemia about baseline concentrations of the specified biomarkers; also, the treatment with statins did not influence the levels of the specified markers in responders and nonresponders. Measurement of sFlt-1 revealed significantly higher serum concentrations in RSD responders compared with nonresponders (149.9 [IQR, 126.7–177.9] versus 110.2 pg/mL [IQR, 77.4–151.1]; P<0.001) or nonresponders (110.2 [IQR, 77.4–151.1] versus 138.6 pg/mL [IQR, 112.9–161.6]; P=0.10) or nonresponders (110.2 [IQR, 77.4–151.1] versus 132.8 pg/mL [IQR, 104.0–167.1]; P=0.09; Table 2).

Measurement of the adhesion molecule ICAM-1 showed significantly higher baseline concentrations in responders compared with nonresponders (370.6 [IQR, 262.9–544.5] versus 240.6 ng/mL [IQR, 207.6–324.5]; P<0.001; Table 2; Figure 3). Serum concentrations of VCAM-1 before RSD were also significantly higher in responders compared with nonresponders (1011.0 [IQR, 804.0–1326.0] versus 815.0 ng/mL [IQR, 673.7–999.8]; P<0.001; Table 2; Figure 4). Neither responders nor nonresponders displayed significant changes in ICAM-1 or VCAM-1 concentrations after 6 months of follow-up (Table 2). The correlation analyses revealed a correlation of RSD-related BP reduction with ICAM-1 (r=−0.46; P=0.02), VCAM-1 (r=−0.39; P=0.04), and sFlt-1 (r=−0.51; P=0.07; Table 3). Receiver operating characteristic analysis was performed to assess the predictive value of baseline sFlt-1, ICAM-1, and VCAM-1 serum levels as markers for response to RSD. The areas under the curve for s-Flt-1, ICAM-1, and VCAM-1 were 0.82 (IQR, 0.718–0.921; P<0.001), 0.754 (IQR, 0.654–0.854; P<0.001), and 0.684 (IQR, 0.564–0.804; P=0.01), respectively (Table 4; Figure 5). The cutoff values, evaluated by the Youden index, for sFlt-1, ICAM-1, and VCAM-1 were 125 pg/mL, 341 ng/mL, and 1005 ng/mL, respectively (Table 4). The positive predictive values of the specified biomarkers for levels higher than the respective cutoff levels were sFlt-1: 0.91, ICAM-1: 0.93, and VCAM-1: 0.93. The negative predictive values were sFlt-1: 0.44, ICAM-1: 0.66, and VCAM-1: 0.64. Furthermore, the positive predictive values and negative predictive values, on condition that the levels of sFlt-1, ICAM-1, and VCAM-1 were higher than the specified cutoff levels, were positive predictive values (sFlt-1: 0.44, ICAM-1: 0.66, VCAM-1: 0.64), and negative predictive values (sFlt-1: 0.91, ICAM-1: 0.93, VCAM-1: 0.93).

**Discussion**

Published data have illustrated the prognostic effects of elevated BP on morbidity and mortality in patients with HT. Although RSD is an effective therapy option for resistant hypertension, some patients do not respond to the procedure. Importantly, this study identified for the first time potential biomarkers that predict whether a patient will be a responder or nonresponder to RSD-related BP reduction. Compared with nonresponders, responders showed

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**Table 2. Laboratory Measurements**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Level at Baseline, ng/mL, median (IQR)</th>
<th>Level at 6-Mo Follow-up, ng/mL, median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1</td>
<td>370.6 (262.9–544.5)</td>
<td>240.4 (207.6–324.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>1011.0 (804.1–1326.0)</td>
<td>815.55 (673.78–999.85)</td>
<td>0.01</td>
</tr>
<tr>
<td>sFlt-1</td>
<td>149.90 (126.70–177.90)</td>
<td>138.60 (112.97–161.57)</td>
<td>0.01</td>
</tr>
<tr>
<td>Creatinine levels</td>
<td>0.94±0.3</td>
<td>0.9±0.4</td>
<td>0.36</td>
</tr>
<tr>
<td>Glomerular filtration rate at baseline, mL/min per 1.73 m²</td>
<td>84.7±29.7</td>
<td>80.6±25.0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

ICAM-1 indicates intercellular cell adhesion molecule-1; IQR, interquartile range; sFlt-1, soluble fms-like tyrosine kinase-1; and VCAM-1, vascular cell adhesion molecule-1.
significantly elevated serum levels of the angiogenic factor sFLT-1 and the adhesion molecules ICAM-1 and VCAM-1 before RSD. Previous studies focused exclusively on clinical parameters for a response to or being affected by RSD.\textsuperscript{22,23} This study is the first to evaluate potential biomarkers with predictive value in RSD.

Our data demonstrate that sFlt-1 concentrations at baseline were significantly higher in patients who underwent successful RSD, although there were no significant differences in systolic BP at baseline between responders and nonresponders in this study. sFlt-1 is known to be upregulated during angiogenesis under physiological and pathological conditions. sFLT-1 levels are higher in hypertensive versus normotensive patients; it was previously described as a biomarker for early microvascular damage and to be essential in repair processes in hypertension.\textsuperscript{9–11,24} Furthermore, sFlt-1 is a well-known predictive biomarker for preeclampsia in pregnancy.\textsuperscript{25}

Our results indicate that responders may be more affected by hypertension-related shear stress and endothelial dysfunction, an assumption that stays in line with the knowledge that hypoxia is a major stimulus for the release of sFlt-1 and is associated with hypertension-related oxidative stress.\textsuperscript{24} Several observations have suggested a role for sFlt-1 in promoting and maintaining sympathetic innervation in blood vessels and stimulating the sympathetic vascular nervous system.\textsuperscript{4–6} Vascular sympathetic innervation is an important determinant of BP and blood flow; alterations in the vascular sympathetic innervation have been implicated in the development and maintenance of cardiovascular disease and hypertension.\textsuperscript{15,16}

Cell adhesion molecules play important roles in accelerating the process of atherosclerosis. Soluble ICAM-1 and VCAM-1 have also been explored in the context of essential hypertension.\textsuperscript{26–31} In this study, ICAM-1 and VCAM-1 concentrations before RSD were significantly higher in patients who responded to RSD. Shear stress and endothelial cell activation stimulate the expression of ICAM-1 and VCAM-1 during essential hypertension, suggesting that the endothelium is more activated in responders than in nonresponders, although baseline systolic BP did not significantly differ between responders and nonresponders.

In both responders and nonresponders, no significant changes in sFLT-1, ICAM-1, and VCAM-1 concentrations were detected 6 months after RSD relative to baseline values. These findings are inconsistent with previous data in which elevated sFLT-1, ICAM-1, and VCAM-1 concentrations in patients with hypertension decreased after drug treatment–related BP reduction.\textsuperscript{32} In these studies, patients were without antihypertensive treatment or did not receive optimal medical treatment. sFlt-1, ICAM-1, and VCAM-1 concentrations decreased after antihypertensive treatment with BP normalization (systolic <140 mmHg). In contrast, patients included in the present study were hypertensive, despite optimal medical
treatment with an average of 5 antihypertensive substances. Our data demonstrate that RSD is an effective treatment option in patients with resistant hypertension.

But even with a significant RSD-related BP decrease, the patients in this study remained hypertensive (mean systolic BP >140 mm Hg) 6 months after RSD. Therefore, previously reported data are not entirely comparable with the data from the patient population of the present study. Nevertheless, these results do not specifically provide information on the reduction of endothelial cell activation during the follow-up period after RSD. Prolonged endothelial remodeling processes in atherosclerotic lesions and changes in endothelial structure as well as endothelial repair processes might cause the persistence of increased biomarker concentrations.

As previously shown, some patients undergoing RSD do not respond with adequate BP reduction. Recently published data showed that the only parameter for prediction of RSD-related BP reduction was elevated systolic BP values at baseline. No further predictive parameters have yet been identified. To date, little is known about predicting RSD-related success, in particular in identifying nonresponders or pseudoresistant patients with hypertension. Data from a recent meta-analysis provide additional information on high variability in individual response to RSD. This further confirms the need for expanded preinterventional screening. Thus, RSD should remain an interventional treatment option in well-selected patients with real resistant hypertension that is unresponsive to medical treatment.

Therefore, to supplement the clinical parameters, the present study was undertaken to analyze possible biomarkers that could be predictive about RSD-related success. Biomarker assessment might be an option for the prediction of RSD-related BP reduction and is of major clinical and economical interest. The predictive value of the specified markers in this study showed promising results. In particular, the combined use of sFlt-1, ICAM-1, and VCAM-1 indicated an improvement of the positive predictive values and negative predictive values for response to RSD. Identifying nonresponders before RSD would lead to higher procedure-related success rates and would avoid unnecessary invasive treatment, although BP at baseline did not significantly differ between responders and nonresponders.

The results of our study underline the possible relationship between RSD treatment success and resistant hypertension because of an overactivated sympathetic nervous system, reflected by higher concentrations of sFlt-1, ICAM-1, and VCAM-1. In addition, it has been reported that RSD reduces the renal sympathetic efferent activity and the sympathetic nerve traffic to the skeletal muscle vasculature as measured by muscle sympathetic nerve activity. Therefore, the combined analysis of the sympathetic activity and measurement of ICAM-1, VCAM-1, and sFlt-1 may be advantageous in phenotyping responders and nonresponders; this needs to be studied in further clinical trials.

To the best of our knowledge, this is the first study that has identified potential biomarkers with a predictive value.

**Table 3. Correlation Analysis**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Correlation Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-FLT125 (ng/mL)</td>
<td>-0.51</td>
<td>0.07</td>
</tr>
<tr>
<td>ICAM341 (ng/mL)</td>
<td>-0.46</td>
<td>0.02</td>
</tr>
<tr>
<td>VCAM1005 (ng/mL)</td>
<td>-0.39</td>
<td>0.04</td>
</tr>
</tbody>
</table>

ICAM-1 indicates intercellular cell adhesion molecule-1; sFlt-1, soluble fms-like tyrosine kinase-1; and VCAM-1, vascular cell adhesion molecule-1.

**Table 4. ROC Analysis; Youden Index**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Area Under the Curve (IQR)</th>
<th>Cutoff</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sFlt-1</td>
<td>0.82 (0.718–0.921)</td>
<td>125 pg/mL</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>0.754 (0.654–0.854)</td>
<td>341 mg/mL</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>0.684 (0.564–0.804)</td>
<td>1005 ng/mL</td>
<td>0.01</td>
</tr>
</tbody>
</table>

ICAM-1 indicates intercellular cell adhesion molecule-1; IQR, interquartile range; ROC, receiver operating characteristic; sFlt-1, soluble fms-like tyrosine kinase-1; and VCAM-1, vascular cell adhesion molecule-1.

**Table 5. PPV and NPV**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV (sFlt-1)</td>
<td>0.91</td>
<td>0.44</td>
</tr>
<tr>
<td>PPV (ICAM-1)</td>
<td>0.93</td>
<td>0.66</td>
</tr>
<tr>
<td>PPV (VCAM-1)</td>
<td>0.93</td>
<td>0.64</td>
</tr>
<tr>
<td>PPV (sFlt-1, ICAM-1, VCAM-1)</td>
<td>0.94</td>
<td>0.71</td>
</tr>
</tbody>
</table>

ICAM-1 indicates intercellular cell adhesion molecule-1; IQR, interquartile range; NPV, negative predictive values; PPV, positive predictive values; ROC, receiver operating characteristic; sFlt-1, soluble fms-like tyrosine kinase-1; and VCAM-1, vascular cell adhesion molecule-1.
indicating response to RSD. These results reveal significantly higher concentrations of sFlt-1, ICAM-1, and VCAM-1 in patients who responded after RSD. However, these data require further validation in large-scale studies.

**Limitation**

The small number of nonresponders is a major limitation that must be considered. Furthermore, large-scale studies are required to confirm the results of the present study.

**Conclusions**

This study identified for the first time potential biomarkers with a predictive value indicating a responder or nonresponder of RSD treatment. By the assessment of sFLT-1, ICAM-1, and VCAM-1 as predictive biomarkers, the identification of nonresponders to RSD might become possible.

**Perspectives**

RSD is a treatment option for patients with resistant HT, but in some patients this treatment is unsuccessful. To the best of our knowledge, this study identified for the first time potential biomarkers with a predictive value indicating a responder or nonresponder before RSD. Biomarker assessment might be an option for the prediction of RSD-related BP reduction and is of major clinical and economical interest. The identification of nonresponders before RSD would lead to higher procedure-related success rates and would avoid unnecessary invasive treatment.

**Disclosures**

None.

**References**


Novelty and Significance

What Is New?

- This is the first study to identify biomarkers with a predictive value indicating a responder or nonresponder before renal denervation.
- A relationship among successful renal denervation–related blood pressure reduction, endothelial dysfunction, and activated endothelial cells was indicated by biomarkers.

What Is Relevant?

- Prediction of successful renal denervation–related blood pressure reduction is of major clinical and economical interest.
- Identifying nonresponders before renal denervation would lead to higher procedure-related success rates and would avoid unnecessary invasive treatment.

- Biomarker assessment is an option for the prediction of renal sympathetic denervation–related blood pressure reduction.

Summary

Responders showed significantly increased serum levels of soluble fms-like tyrosine kinase-1, intercellular cell adhesion molecule-1, and vascular cell adhesion molecule-1 at baseline compared with nonresponders. This study identified for the first time potential biomarkers with a predictive value indicating a responder or nonresponder before renal denervation.
Soluble fms-Like Tyrosine Kinase-1 and Endothelial Adhesion Molecules (Intercellular Cell Adhesion Molecule-I and Vascular Cell Adhesion Molecule-I) as Predictive Markers for Blood Pressure Reduction After Renal Sympathetic Denervation

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