Vitronectin Concentrations Predict Risk in Patients Undergoing Coronary Stenting

Wolfgang Derer, MD; Elliot S. Barnathan, MD; Erdal Safak, MD; Prasheen Agarwal, PhD; Harald Heidecke, PhD; Martin Möckel, MD; Michael Gross, MD; Cemil Oezcelik, MD; Rainer Dietz, MD; Ralf Dechend, MD

Background—Vitronectin is a multifunctional protein with a multiple binding domain that interacts with a variety of plasma and cell proteins. Vitronectin binds multiple ligands, including the soluble vitronectin receptor. Abciximab binds equally well to soluble vitronectin receptor and glycoprotein IIb/IIIa, because both share the β3 subunit. We tested whether vitronectin concentrations correlate with adverse outcomes in acute coronary syndrome patients.

Methods and Results—Baseline serum samples \((n=233)\) from a randomized, placebo-controlled trial of abciximab plus stenting (Evaluation of Platelet IIb/IIIa Inhibitor for Stenting EPISTENT) were retrospectively analyzed. We stratified vitronectin concentrations into the 3 lower quartiles \((n=178; <49.7 \, \mu\text{g/mL})\) versus the fourth upper quartile \((n=55; \geq 49.7 \, \mu\text{g/mL})\). The end point was a major adverse cardiovascular event defined as death, myocardial infarction or urgent revascularization at 30 days and 6 months. A higher proportion of patients with baseline vitronectin \(\geq 49.7 \, \mu\text{g/mL}\) had major adverse cardiovascular event than patients with baseline vitronectin \(<49.7 \, \mu\text{g/mL}\) at 30 days \((18.2\% \) versus \(5.6\%; \, P=0.01\)) and 6 months \((20.0\% \) versus \(6.2\%; \, P=0.006\)). When baseline variables not predictive of major adverse cardiovascular event \((\text{eg, troponin positive, history of congestive heart failure, diabetes, history of hypertension, smoking status})\) were excluded from the multivariate model, only baseline vitronectin \(\geq 49.7 \, \mu\text{g/mL}\) (at 30 days: OR, 3.23; 95% CI, 1.23, 8.49; at 6 months: OR, 3.36; 95% CI, 1.33, 8.52) and history of myocardial infarction \((30 \text{ days: OR, 5.02; 95% CI, 1.41, 17.9; at 6 months: OR, 3.99; 95% CI, 1.28, 12.43})\) remained. No interaction occurred between abciximab and vitronectin.

Conclusions—Our findings indicate that vitronectin may be an independent predictor of adverse cardiovascular outcomes following acute stenting. \((\text{Circ Cardiovasc Intervent. 2009;2:14-19.})\)

Key Words: risk factors vitronectin stents glycoproteins IIb-IIIa

A\acute{c}ute coronary syndromes feature platelet activation and aggregation, thrombus formation, and infarction.1–3 C-reactive protein, sCD40, myeloperoxidase, and decreased concentrations of interleukin-10 are also associated with increased cardiovascular mortality in acute coronary syndrome patients.4–7 Treatment with glycoprotein IIb/IIIa (GP IIb/IIIa) antagonists is effective.8,9 GP IIb/IIIa and the soluble vitronectin receptor (sVNR) share a common β subunit and are from the same integrin subfamily. Abciximab, a GP IIb/IIIa antagonist approved for use in patients with an acute coronary syndrome or undergoing elective percutaneous coronary intervention with stenting, also binds to the vitronectin (αvβ3) receptor on platelets and smooth muscle cells.10 Data indicate that the extraplatelet actions of abciximab may be at least in part responsible for its observed treatment benefit11,12; however, direct associative data are not available.

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Vitronectin is present in plasma, the extracellular matrix, and in the granules of blood platelets. It belongs to the group of adhesive glycoproteins that is involved in various functions including complement activation, blood coagulation, binding to proteoglycans, and modification of the matrix.13 Vitronectin plays a key role in the attachment of cells to their matrix and is involved in the regulation of cell differentiation, proliferation, migration, and morphogenesis.14–16 Vitronectin and sVNR are present in human atheromatous plaques, suggesting that they may be implicated in atherosclerosis and restenosis.17–20 Plasma vitronectin levels were significantly increased in patients with coronary artery diseases, showing a positive correlation with severity of the disease.21 Using data from a randomized, placebo-controlled trial of abciximab in patients undergoing percutaneous coronary intervention, we tested the hypothesis that patient serum concentrations of vitronectin and sVNR correspond with cardiovascular outcomes at 30 days and 6 months following revascularization.

Methods

This is a retrospective analysis of patient data from the Evaluation of Platelet IIb/IIIa Inhibitor for Stenting (EPISTENT) trial. The primary
trial results have been previously reported. In the primary trial, patients scheduled to undergo coronary stenting were randomly assigned to placebo with standard-dose heparin or abciximab (Centocor and Eli Lilly, Indianapolis, Ind) with low-dose heparin. Heparin was administered at a standard dose of 100 U/kg (maximum 10,000 U) or at a low dose of 70 U/kg (maximum 7000 U) with abciximab administered at 0.25 mg/kg up to 60 minutes before intervention, followed by 0.125 µg/kg/min to 60 minutes before intervention, followed by 0.125 µg/kg/min (maximum 10 µg/kg/ min) for 12 hours. The primary end point was the composite of all-cause mortality, myocardial infarction or reinfarction, or severe myocardial ischemia requiring urgent coronary-artery bypass surgery or revascularization through 30 days and 6 months following intervention.

Our sample subgroup population was selected from the first 899 consecutively randomized EPISTENT patients who participated in the Angiographic Substudy, who were the only patients with baseline blood samples stored for the analysis of predictive biomarkers. We took a random sample (n = 234) of these 899 patients who had blood samples available for analysis for the determination of vitronectin and sVNR serum concentrations. Among these patients, 233 had vitronectin data available and 231 also had sVNR data available. The institutional review board or independent ethics committee for each trial site approved the protocol for the EPISTENT trial, including the collection of all patient blood samples for biomarker analysis. All patients provided written informed consent.

Baseline patient sera were collected before study treatment and were stored at the EPISTENT trial central laboratory. For this study, these samples were obtained and measured by ELISA (CellTrend, Luckenwalde, Germany) for serum concentrations of vitronectin and sVNR. Serum samples were diluted and then measured in the linear range of the ELISAs. The limit of detection was 10 ng/mL for vitronectin and 7 ng/mL for sVNR, and the intraassay variation was 3.6% and 4.2%, respectively. The ELISA for vitronectin uses the antibody clones VN58–1 (No. M017) and VN49–1 (No. M016), which are both directed against the N-terminal region of vitronectin (amino acids 1 to 130) and bind to both free and bound vitronectin. The recovery rate of free vitronectin added to serum is between 89% and 118%, suggesting that the epitope of the antibodies used in the ELISA is not masked by the binding sites for other molecules. The ELISA for sVNR is a competitive ELISA. The first antibody is directed against the β3 subunit, and the second one against the α5 subunit of the receptor. Cross-reactivity to related integrin receptors was excluded. All analyses were performed at the research laboratory of the Charité, Campus-Buch (Berlin, Germany) under blinded conditions.

**Statistical Analysis**

To distinguish between patients with different degrees of cardiac risk, we used an exploratory approach. No formal multivariate testing was performed. We performed logistic regression analysis for dichotomous variables. Wald confidence intervals (95%) for odds ratios were constructed where appropriate. For time-to-event data, survival curves were estimated using the Kaplan–Meier product-limit method. The log-rank test was used for treatment comparisons. S-Plus 6.1 Professional or the SAS System 8.02 was used for all analyses.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

At baseline, serum concentrations of vitronectin and sVNR were not associated with hypertension, diabetes, serum troponin concentration, or unstable angina; the only statistically significant difference observed was that patients aged <60 years had higher baseline vitronectin concentrations than patients aged 70 to 79 years (P < 0.001; Table 1). The median serum concentration was 38.2 µg/mL (range 7.5 to 101.8 µg/mL) for vitronectin and 1.4 µg/mL (range 0.4 to 25.2 µg/mL) for sVNR (Table 2). At 30 days, major adverse cardiovascular event (MACE) rates in the placebo plus stent and abciximab plus stent groups for this subpopulation (11.3% versus 5.5%, P = 0.159) were similar to those for the overall EPISTENT population (10.8% versus 5.3%, P < 0.001). The observed early divergence suggests that the differential patient outcomes were related to increased MACE rates shortly after intervention.

To test for possible associations between vitronectin and MACE and between sVNR and MACE, we first explored baseline serum concentrations of vitronectin and sVNR as

<table>
<thead>
<tr>
<th>Table 1. Baseline sVNR and Vitronectin Serum Concentrations in Relation to Other Baseline Patient Characteristics</th>
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<tbody>
<tr>
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<tr>
<td></td>
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<tr>
<td>With unstable angina within 48 hours of intervention</td>
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<tr>
<td>Without unstable angina within 48 hours of intervention</td>
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<tr>
<td>P value</td>
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<tr>
<td>Troponin ≥0.1 ng/mL</td>
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<tr>
<td>Troponin &lt;0.1 ng/mL</td>
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<tr>
<td>P value</td>
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<tr>
<td>With a history of diabetes</td>
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<tr>
<td>Without a history of diabetes</td>
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<td>P value</td>
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<tr>
<td>With a history of hypertension</td>
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<tr>
<td>Without a history of hypertension</td>
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<tr>
<td>P value</td>
</tr>
<tr>
<td>Age &lt;60 years</td>
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<tr>
<td>Age 60–69 years</td>
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<tr>
<td>P value (&lt;60 vs 60–69 years)</td>
</tr>
<tr>
<td>Age 70–79 years</td>
</tr>
<tr>
<td>P value (&lt;60 vs 70–79 years)</td>
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</tbody>
</table>
Figure 1. Percentage of patients with MACE at 6 months, stratified by baseline vitronectin serum concentration at the median (left) or the third quartile (right).

Table 2. Baseline Patient Demographics and Disease Characteristics, Presented by Baseline Vitronectin Serum Concentration

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitronectin &lt;49.7 μg/mL</th>
<th>Vitronectin ≥49.7 μg/mL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>178</td>
<td>55</td>
<td>233</td>
</tr>
<tr>
<td>Sex, female</td>
<td>42 (23.6)</td>
<td>14 (25.5)</td>
<td>56 (24.0)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>60.6 (0.80)</td>
<td>55.7 (1.33)</td>
<td>59.4 (0.7)</td>
</tr>
<tr>
<td>Range</td>
<td>35–85</td>
<td>36–77</td>
<td>35–85</td>
</tr>
<tr>
<td>Vitronectin, μg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>33.1 (10.4)</td>
<td>62.5 (12.2)</td>
<td>40.0 (16.5)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>34.8 (7.5–49.6)</td>
<td>58.4 (49.9–101.8)</td>
<td>38.2 (7.5–101.8)</td>
</tr>
<tr>
<td>sVNR, μg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.9 (2.3)</td>
<td>2.1 (1.4)</td>
<td>1.9 (2.1)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.3 (0.4–25.2)</td>
<td>1.7 (0.5–6.4)</td>
<td>1.4 (0.4–25.2)</td>
</tr>
<tr>
<td>Current smoker or quit within the previous year</td>
<td>59/177 (33.3)</td>
<td>26/55 (47.3)</td>
<td>85/232 (36.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>91 (51.1)</td>
<td>29 (52.7)</td>
<td>120 (51.5)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>36 (20.2)</td>
<td>12 (21.8)</td>
<td>48 (20.6)</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>87 (48.9)</td>
<td>36 (65.5)</td>
<td>123 (52.8)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>8 (4.5)</td>
<td>1 (1.8)</td>
<td>9 (3.9)</td>
</tr>
<tr>
<td>Troponin ≥0.1 ng/mL</td>
<td>32/170 (18.8)</td>
<td>10/45 (22.2)</td>
<td>42/215 (19.5)</td>
</tr>
</tbody>
</table>

Data are presented as n (%) unless noted otherwise.

Continuous variables. The results from this analysis were similar and not significant. Cutpoints at the median and the third quartile were also explored. Baseline sVNR serum concentrations were not associated with 6-month MACE in patients for the median (<1.35 μg/mL versus ≥1.35 μg/mL; 8.0% versus 11.0%) or third quartile cutpoints (<2.21 μg/mL versus ≥2.21 μg/mL; 9.4% versus 9.8%), and were excluded from further analysis. Although baseline serum concentrations of vitronectin were associated with 6-month MACE with both cutpoints, we chose to use the cutpoint at the third quartile for vitronectin (<49.7 μg/mL versus ≥49.7 μg/mL) because it provided the better dichotomous boundary of the cutpoints we explored (Figure 1). Subsequent analyses of this patient subpopulation were stratified using this cutpoint for baseline vitronectin serum concentration. Baseline characteristics were generally similar across this cutpoint, and no differences between patients with < 49.7 μg/mL and those with ≥49.7 μg/mL baseline serum vitronectin were statistically significant (Table 2).

Univariate analysis demonstrated that patients with baseline vitronectin ≥49.7 μg/mL were significantly more likely than patients with baseline vitronectin <49.7 μg/mL to experience MACE at 30 days (18.2% versus 5.6%; P=0.01) and 6 months (20.0% versus 6.2%; P=0.006). A multivariate logistic regression model demonstrated that baseline vitronectin ≥49.7 μg/mL was significantly associated with an increased risk of MACE at 30 days (OR, 3.94; 95% CI, 1.37, 11.33; P=0.011) whereas treatment with abciximab tended to reduce MACE (OR, 0.37; 95% CI, 0.11, 1.23; P=0.105) (Figure 2A). Other variables such as baseline troponin, history of congestive heart failure, and diabetes were not significant predictors of MACE. When these were excluded from the model, only baseline vitronectin ≥49.7 μg/mL (OR, 3.23; 95% CI, 1.23, 8.49) and history of myocardial infarction remained (OR, 5.02; 95% CI, 1.41, 17.9) (Figure 2B). Baseline vitronectin ≥49.7 μg/mL (OR, 3.36; 95% CI, 1.33, 8.52; P=0.0104) and history of myocardial infarction (OR, 3.99; 95% CI, 1.28, 12.43; P=0.0172) were similarly predictive of the risk of MACE at 6 months (data not shown).

The Kaplan–Meier estimates of the 6-month MACE rates following intervention are shown in Figure 3, stratified by baseline vitronectin serum concentration and treatment. The log rank test (P=0.013) revealed a statistically significant difference between the placebo and abciximab/stent treatment groups stratified by vitronectin (<49.7 μg/mL; ≥49.7 μg/mL). A greater treatment benefit for the MACE rates was observed in the abciximab/stent group over
the placebo group in those patients with low vitronectin (<49.7 μg/mL) (Figure 4). Because abciximab directly binds to the vitronectin receptor with high affinity, we investigated if the effects of abciximab on cardiovascular outcomes after percutaneous coronary intervention were dependent on basal vitronectin levels. Interactions between vitronectin and treatment with abciximab were not significant at the 5% level.

**Discussion**

The major finding of our study is that vitronectin was an independent risk factor for adverse cardiovascular events in patients with ischemic heart disease undergoing percutaneous interventions with stenting in the EPISTENT trial. Baseline vitronectin serum concentrations were independent from other cardiovascular risk factors, such as hypertension and diabetes. Furthermore, the presence of an acute coronary syndrome did not influence the observed baseline vitronectin serum concentrations.

We raised this hypothesis because of the pivotal role of the vitronectin/vitronectin receptor system in atherosclerosis and endothelial dysfunction.15 The therapeutic activity of GP IIb/IIIa antagonists depend on the ability to block platelet
Abciximab/Stent

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vitronectin were found to be increased in a small cohort of 62 disorders in which these receptors play a role.28

tor raises the possibility that abciximab, or other agents that interactions. The reactivity of abciximab with the vitronectin rece-
bind to the vitronectin receptor.15 The vitronectin receptor has such as fibrinogen, thrombospondin, and prothrombin also
microvessels of atherosclerotic plaques exhibit high vitronec-
ton receptor expression. Besides vitronectin, other ligands

circulation of patients with multiple sclerosis, reflecting pathophysiology and aid in the diagnosis and management of
patients with cardiovascular disease.36

Vitronectin can be expressed and generated in the vessel wall, particularly in atherosclerotic lesions.17 Several studies have found a regulatory function for vitronectin in the hemostatic response to vascular injury.14,25 Plasma levels of vitronectin were found to be increased in a small cohort of 62 patients with coronary artery disease when compared with controls. Thus vitronectin may serve as a marker for coronary artery disease and elevated levels may indicate its role in the genesis and/or progression of coronary artery disease.14,25

The vitronectin α3β1 receptor is not only widely expressed on endothelial and smooth muscle cells, but also on platelets, macrophages, and neutrophils.26 Endothelial cells in the microvessels of atherosclerotic plaques exhibit high vitronectin receptor expression. Besides vitronectin, other ligands such as fibrinogen, thrombospondin, and prothrombin also bind to the vitronectin receptor.15 The vitronectin receptor has important physiological functions including bone resorption, tumor invasion, metastasis, cell adhesion, and spreading.

Data from animal studies support the utility of vitronectin receptor blockade in restenosis.27 Thus, the vitronectin receptor is an attractive candidate for coronary disease interventions. The reactivity of abciximab with the vitronectin receptor raises the possibility that abciximab, or other agents that inhibit the receptor, may be useful in preventing or treating disorders in which these receptors play a role.28

In our retrospective analysis, we measured vitronectin and sVNR in the serum of patients enrolled in the EPISTENT trial. We assume that the vitronectin receptor exists in serum in a soluble form as microparticles because of receptor shedding from the surface of platelets, neutrophils, and endothelial cells.29 Minagar et al.30 found sVNR in the circulation of patients with multiple sclerosis, reflecting chronic inflammation. Serebruany et al31 studied the effect of soluble platelet biomarkers, including sVNR, and receptor platelet expression in 41 randomized patients with myocardial infarction and found that tenecteplase seemed to have an advantage over alteplase in deactivating platelets. The underlying mechanism leading to sVNR detection in the serum is not understood. Detached endothelial cells and microparticles from activated endothelial cell monolayers may be involved in this process. However, we did not find any change in sVNR in our cohort.

Although several studies have shown a prognostic value of biomarkers in patients with chronic stable angina,32,33 in general these patients have not been well characterized by biomarkers assessing prognosis, selection of therapeutic approaches, or titration of therapeutic agents.33–35 Our study found that measuring vitronectin serum concentrations enabled the identification of a group of patients at particularly high risk of MACE after coronary intervention with stenting. This finding suggests that vitronectin could serve as a biomarker of coronary risk following stenting. Although we were unable to compare vitronectin with other markers such as C-reactive protein or sCD40 ligand, vitronectin was not associated with troponin T concentrations or risk factors such as hypertension or diabetes. As a new biomarker, especially one that may not be duplicative of established biomarkers, vitronectin could provide important new insights into the pathophysiology and aid in the diagnosis and management of patients with cardiovascular disease.36

Whether vitronectin is solely a marker of disease activity or also has direct consequences for our patients remains uncertain. Although the interaction term between treatment and vitronectin was not significant, it is likely that this analysis was underpowered as the trial from which our sample population was selected was not designed to study this possible relationship. As studies aimed for detecting interaction between treatment and event rates should ideally be strongly powered and comprise a large sample of patient population, the results of our study should be interpreted with caution given our sample size of only 233 patients and the low MACE rates observed at 30 days (8.6%) and 6 months (9.4%). Finally, the observed lower relative percent reduction in MACE in abciximab-treated patients in the high baseline vitronectin subgroup compared with the low baseline vitronectin subgroup could represent an interference with abciximab efficacy, but this would need to be confirmed in a much larger, more appropriately powered study. Likewise, the EPISTENT trial was not designed or powered to explore the relationship between possible baseline markers of disease activity and event rates; consequently, these respective findings should also be interpreted with caution.

We believe our findings indicate that vitronectin may be clinically relevant as a biomarker for adverse cardiovascular outcomes in patients with ischemic heart disease undergoing coronary intervention. Vitronectin was an independent risk predictor in this substudy of patients in the EPISTENT trial and may be a potential target for agents directed against GP IIb/IIIa receptors. Moreover, vitronectin could have active consequences for patients with acute coronary syndromes and may provide an additional pathogenic pathway that has not...
yet been investigated. Further studies are warranted to explore these possibilities.

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Disclosures

Drs. Barnathan and Agarwal are employees of Centocor Research and Development, Inc. Dr. Heidecke is an employee of CellTrend.

References

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