Circulating P-selectin and the risk of recurrent venous thromboembolism

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Summary
The clinical relevance of high P-selectin levels in venous thrombosis is unknown. We prospectively followed 544 patients with first unprovoked venous thromboembolism (VTE) after cessation of anticoagulation and evaluated P-selectin as a risk factor of recurrent VTE. VTE recurred in 63 (12%) patients. Patients with recurrence had significantly higher P-selectin levels than those without (45.8 mg/dl ± 16.4 vs. 40.1 mg/dl ± 13.3; p = 0.006). After four years, the probability of recurrence was 20.6% (95% confidence interval [CI] 12.6–28.5) among patients with P-selectin values above the 75th percentile of the patient population and was 10.8% (95% CI 7.2–14.3) among patients with lower values (p = 0.046). Compared to patients with low P-selectin, adjusted risk of recurrence was 1.7-fold (95% CI 1.0–2.9, p = 0.045) increased among patients with P-selectin levels exceeding the 75th percentile. We conclude that high circulating P-selectin is a risk factor of recurrent VTE.

Keywords
Venous thromboembolism, P-selectin, recurrence, risk factor

Introduction
P-selectin mediates adhesion and migration of leukocytes at sites of inflammation and platelet-leukocyte interaction, and supports fibrin formation and thrombus growth (1, 2). Infusion of anti-P-selectin antibodies into baboons inhibited fibrin deposition onto thrombogenic grafts (3). In a venous stasis model, P-selectin-deficient mice had less fibrin and reduced thrombus growth than wild-type controls (4). After laser-induced microvascular injury, mice lacking P-selectin or PSGL-1, or mice infused with an anti-P-selectin antibody produced platelet-rich thrombi with minimal tissue factor (TF) and fibrin (5). Conversely, mice with high plasma levels of soluble P-selectin had short clotting times and increased fibrin formation in an ex-vivo perfusion chamber and in a model of deep-vein thrombosis (6, 7). Compared to controls, these mice had a two-fold increase in microparticles and depletion of microparticles by ultracentrifugation resulted in prolonged clotting times (6). Of interest, labelled microparticles injected into mice specifically accumulated at the thrombus site (8). In another study, fluorescently labelled monocyte-derived microparticles that expressed TF and PSGL-1 were infused into mice and localized within the developing thrombus (5).

Increased levels of soluble P-selectin were found in patients with coronary or peripheral arterial disease (9–11). Elevated P-selectin is associated with increased risk of future cardiovascular events (12, 13). However, the role of P-selectin in venous thromboembolism (VTE) is unknown.

We followed 544 patients with first unprovoked VTE for an average of 35 months and studied the relationship between P-selectin and recurrence.

Patients and methods
The Austrian Study on Recurrent Venous Thrombosis (AUREC) is an on-going prospective cohort study (14). To enter the study, patients have to be older than 18 years and had to be treated with anticoagulants for at least three months after a first unprovoked VTE (established by venography, color duplex sonography, ventilation-perfusion scanning, or spiral computed tomography [CT]). The ethics committee of the Medical University of Vienna approved the study. Patients requiring long-term anticoagu-
lation such as patients with lupus anticoagulant, factor VIII > 230 IU/dl, antithrombin, protein C or protein S deficiency, homozygous or combined congenital clotting defects, cancer patients or patients requiring long-term antithrombotic therapy for other reasons were excluded. Patients entered the study at the time of withdrawal of anticoagulation. They were observed at three-month intervals for the first year and every six months thereafter. Patients were provided with written information on the symptoms of VTE, and were instructed to report to us in case of symptoms.

Outcome measures
The study endpoint was recurrent symptomatic VTE. Recurrence was confirmed by duplex sonography (in case of venous thrombosis in the contralateral leg), venography, perfusion/ventilation lung scan, and/or spiral-CT as described in detail elsewhere (14).

Laboratory analysis
Laboratory assays were performed in blood obtained 3–12 weeks after withdrawal of anticoagulation. Antithrombin, protein C or protein S, factor VIII clotting activity and the diagnosis of a lupus anticoagulant were determined by routine laboratory methods, as were factor V Leiden and factor II G20210A (14). Plasma for determination of soluble P-selectin was stored at −80°C and batch-analysed at a later time-point by ELISA technique (human sP-selectin, R&D Systems, Inc., Minneapolis, MN, USA).

Statistical analysis
Times to recurrence (uncensored observations) or follow-up times in patients without recurrence (censored observations) were analysed using survival time methods. The probability of recurrence was estimated according to the Kaplan-Meier method (15). To test for homogeneity between strata, we applied the log-rank and the generalized Wilcoxon rank sum test. Categorical data were checked for homogeneity using contingency table analyses (Chi²-test), whereas the Mann-Whitney test was used for linear data. A Cox proportional-hazard model was used to analyse the association between risk for recurrent VTE and P-selectin levels. Adjustments were made for sex, age, F V Leiden, F II G20210A, duration of anticoagulation and site of VTE. All data is given as mean ± standard deviation (SD) unless otherwise indicated.

Table 1: Thrombotic risk factors of recurrent venous thromboembolism (VTE) and hazard ratios among 544 patients with first unprovoked VTE by quartiles of P-selectin.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>P-selectin, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 25th percentile</td>
</tr>
<tr>
<td>Factor V Leiden (%)</td>
<td>34</td>
</tr>
<tr>
<td>Factor II G20210A (%)</td>
<td>9</td>
</tr>
<tr>
<td>No. of recurrences</td>
<td>12</td>
</tr>
</tbody>
</table>

Discussion
There is evidence from laboratory studies and animal experiments that increased levels of P-selectin are of particular importance for thrombus formation under conditions of minimal en-
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According to the concept put forward by Cambien and Wagner, P-selectin under these circumstances supports platelet rolling, recruits platelets onto vascular injury sites and induces generation of leukocyte-derived TF-bearing microparticles (16). These procoagulant microparticles are then incorporated into the thrombus via PSGL-1/P-selectin binding thereby amplifying thrombin generation and fibrin formation (16). Once a layer of platelets and fibrin has formed, the growing thrombus might form a barrier between blood and vessel wall, and thrombus growth will become at least in part dependent upon TF from fluid phase (17).

Here we report the first clinical evidence of an association between increased P-selectin levels and venous thrombus formation in man. In a large prospective cohort study, we followed patients with a first episode of unprovoked VTE for an average of almost three years after cessation of anticoagulant therapy. Patients with P-selectin levels exceeding the 75th percentile of the patient population had a significant 1.7-fold higher risk of recurrent VTE compared to those with lower values, and a graded increase in the recurrence risk in relation to P-selectin rather than a threshold level was apparent. The recurrence risk conferred by high P-selectin was independent of age, sex and other risk factors such as factor V Leiden, the prothrombin mutation, duration of anticoagulation and site of VTE.

The importance of microparticles for thrombus growth and fibrin formation in animal models of venous stasis has already been addressed. Their relevance in the pathogenesis of venous thrombosis in man is still obscure. Our study provides the first, albeit indirect, hint that indeed microparticle formation could play a role in human venous clot formation. However, for practical as well as financial reasons, it is beyond the scope of this large clinical study of more than 500 patients to further investigate into this issue.

Some limitations have to be addressed. AUREC is a hypothesis-generating cohort study, which precludes predefinition of certain cut-off values. Therefore, our observation should serve as the basis for validation in a prospective interventional outcome trial. AUREC is one of the largest prospective studies to investigate the natural course of venous thrombosis. Clearly, important candidate risk factors of recurrent VTE emerge during the course of the study and hence have to be determined in retrospect. In our patients, plasma for P-selectin determination was obtained 3–12 weeks after study entry, stored at –80°C and batch-analysed at a later time-point. We therefore adjusted relative risk of recurrence for storage times and found no influence (data not shown).

Successful strategies against VTE recurrence include the use of vitamin K antagonists, heparin therapy and administration of novel compounds directed against F Xa or thrombin (18). There is now evidence from animal experiments that administration of an oral P-selectin inhibitor produced a significant reduction in thrombus weight and also affected vein wall injury (19, 20). Our finding that high P-selectin confers an increased risk of recurrent venous thrombosis should stimulate researchers to investigate the effects of P-selectin inhibition in human subjects.

Acknowledgements

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References


