Soluble Vascular Endothelial Growth Factor (sVEGF) and the Risk of Venous Thromboembolism in Patients with Cancer: Results from the Vienna Cancer and Thrombosis Study (CATS)

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Abstract

Purpose: Cancer patients are at an increased risk of venous thromboembolism (VTE). VEGF promotes the growth of highly thrombogenic tumor vessels. Here, we explored the utility of soluble plasma VEGF-A (sVEGF) as a biomarker for the prediction of VTE in patients with cancer.

Experimental Design: Eight hundred four patients with newly diagnosed cancer or progression after remission were prospectively followed for 2 years until the occurrence of VTE or death (tumor sites: brain (n = 87), breast (n = 137), lung (n = 120), gastrointestinal (n = 143), pancreas (n = 53), prostate (n = 95), kidney (n = 22), myeloma and lymphoma (n = 99), and others (n = 48)). Primary endpoint was symptomatic or fatal VTE. sVEGF was measured by immunoassay in baseline plasma.

Results: Fifty-five patients developed VTE (6.8%) and 364 patients (45.3%) died. Five-hundred and forty-two (68.3%) participants had sVEGF levels above the detection limit of 0.5 pg/mL. The median sVEGF level (25th–75th percentile) was 8.1 pg/mL (0–17.7). The cumulative 2-year incidence of VTE was 10.2% [95% confidence interval (CI), 6.4–14.9] in patients with sVEGF greater than the 75th percentile of the sVEGF distribution (Q3, cutoff: 17.7 pg/mL), and 5.9% (95% CI, 4.2–7.9) in patients with lower levels (P = 0.03). The corresponding 2-year risk of death was 52.8% (95% CI, 46.0–60.0) and 43.9% (95% CI, 40.0–48.0), respectively (P = 0.02). In univariable time-to-VTE regression, elevated sVEGF was associated with VTE [subhazard ratio (SHR) per 10 pg/mL increase, 1.04; 95% CI, 1.00–1.09; P = 0.04]. The association between sVEGF and risk of VTE prevailed after multivariable adjustment for high-risk tumor sites, age, gender, factor VIII, thrombin generation potential, and soluble P-Selectin (adjusted SHR, 1.04; 95% CI, 1.00–1.09, P = 0.05).

Conclusion: Elevated sVEGF is associated with an increased risk of VTE in patients with cancer.

Introduction

Venous thromboembolism (VTE) is a frequent complication and leading cause of death in cancer patients (1). Several individual risk factors related to patient-, tumor- and treatment characteristics determine the substantially increased overall risk of VTE in the cancer population (2). While primary thromboprophylaxis conceptually appears as a meaningful strategy to reduce the burden of cancer-associated VTE, its implementation is hampered by the great variation of VTE risk between patients (3). In this respect, recent progress has been made via the development of risk-scoring models that facilitate the identification of patients at a high risk of VTE (4, 5). Importantly, it has been demonstrated that the addition of selected biomarkers to preexisting risk-scoring models can increase the prognostic performance of these models (6). Further improvement of such scores by the identification of new prognostic biomarkers for VTE is believed to facilitate targeting thromboprophylaxis to the patients with the greatest net clinical benefit while sparing low-risk patients from potential bleeding complications (7).

VEGF-A is a key regulator and stimulating agent of angiogenesis (8), which promotes the formation of highly thrombogenic and leaky tumor vessels that are necessary for maintaining oxygen and energy supply at the site of the tumor lesion (9). VEGF further induces the expression of tissue factor (TF), the main initiator of the coagulation cascade in vivo, on endothelial cells (10). TF activity has been shown to be increased more than 100-fold after the exposition to VEGF and TNFα (11). Elevated levels of VEGF are present in the bloodstream of patients with highly prothrombotic malignancies, such as ovarian and renal carcinomas (12), and have been reported to be associated with primary resistance to chemotherapy and increased mortality (13–15). Monocytes,
Translational Relevance

Venous thromboembolism (VTE) is a frequent complication and leading cause of death in patients with cancer. Prophylactic anticoagulation (PA) has been shown to reduce the risk of cancer-associated VTE by half; but comes at the cost of an increased risk of bleeding. Biomarkers hold great promise to identify cancer patients with the highest VTE risk and net clinical benefit of PA, while sparing low-VTE-risk cancer patients from PA-associated bleeding complications. In this study, we report the identification of a novel biomarker for cancer-associated VTE, soluble VEGF-A (sVEGF). We observed a significant association between elevated sVEGF and an excessively increased risk of VTE during a 2-year prospective follow-up of 804 cancer patients. This prognostic relationship was independent of major other prognostic factors for VTE, such as tumor type and stage. We propose sVEGF as a candidate biomarker for the personalization of VTE prophylaxis in patients with malignant diseases.

Outcome Measures

The primary endpoint of this study was symptomatic nonfatal and fatal VTE. Diagnosis of VTE had to be confirmed by objective methods [(duplex ultrasound or venography for deep vein thrombosis (DVT), ventilation/perfusion scan or computed tomography (CT) of the chest for non-fatal pulmonary embolism (PE), and autopsy records for fatal PE)]. VTE events were independently adjudicated by an external committee consisting of experts in diagnostic radiology and vascular medicine. No routine screening for VTE was performed. Incidentally discovered VTE (e.g., PE on restaging CT scans) was counted as an event given the independent adjudication committee deemed the VTE event to be of clinical significance. Secondary endpoint was death from any cause.

Laboratory analysis

Baseline venous blood samples were obtained by atraumatic antecubital venipuncture for laboratory analysis. Biomarker measurements (sVEGF and others) were performed in platelet-poor citrated plasma, which was obtained by drawing baseline blood into citrate vacuum tubes [Vacuette; Greiner-Bio-One; 9 parts blood and 1 part sodium citrate (concentration: 0.129 mmol/L)] and centrifuged at 3,000 g for 10 minutes to obtain platelet-poor plasma. Plasma aliquots were stored at −80°C until testing was performed in series. sVEGF was measured by a multiplex immunoassay [(xMAP technology from Luminex; sVEGF detection limit = 0.5 pg/mL)]. Specific anti-VEGF antibodies as well as recombinant growth factors were obtained from R&D Systems. The biomarkers D-Dimer, soluble P-Selectin (sP-Selectin), peak of thrombin generation, prothrombin fragment 1.2, and coagulation factor VIII activity (FVIII) were measured as described in previous reports (18–22).

Statistical analysis

All statistical analyses were performed using Stata (Windows version 13, Stata Corp.) and R (Version 3.1.1., The R core development team, Vienna, Austria). Continuous variables were summarized with medians (25th–75th percentile), whereas categorical data were described by absolute frequencies and percentages. The correlation between two continuous variables was evaluated using linear regression with log(10)–transformed sVEGF as the dependent variable. For estimation of VTE risk, competing risk cumulative incidence estimators according to Marubini and Valsecchi (Stata module stcompt) were implemented (23). In all analyses, death from any cause except fatal VTE (which is counted as a VTE event) was defined as the competing event of interest. To study the association between VEGF and the rate of VTE, we performed a cause-specific analysis of VTE hazards using log-rank tests and Cox regression (24). In contrast, the association between VEGF and the absolute risk of VTE was studied with a subdistribution approach using Gray test (R library cmprsk) and Fine and Gray regression (Stata program stcrreg; ref. 24). For multivariable adjustment, six covariates were selected as priori, namely age and sex (as demographic variables of general interest), tumor type (as the strongest known determinant of VTE risk), soluble P-Selectin (as a biomarker for VTE, and platelet and endothelial

Materials and Methods

Study population and design

In this study, we analyzed 804 patients from the Vienna Cancer and Thrombosis Study (CATS), an ongoing cohort study to investigate prognostic factors and biomarkers for VTE in cancer patients. The 804 patients in this analysis were enrolled between October 2003 and March 2008. The exact inclusion and exclusion criteria of CATS were described extensively in previous reports (18, 19). Briefly, patients 18 years or older with newly diagnosed malignancy or disease progression after complete or partial remission were recruited after written informed consent and followed-up for 2 years until the occurrence of VTE, death, or censoring alive. The following tumor sites were included: blood (myeloma and lymphoma), brain, breast, bronchus, colorectal, kidney, pancreas, prostate, and “selected others” (mainly sarcomas and gynecologic tumors). Exclusion criteria were clinically overt infection, thromboembolic events (both arterial and venous) within the last 3 months, and continuous anticoagulation. Patients that underwent surgery or radiotherapy within the past 2 weeks before recruitment, and/or chemotherapy within the past 3 months were ineligible. At baseline, study patients underwent a detailed interview and chart review to ascertain clinicopathological variables, and venous blood samples were taken for storage in our biobank. The study protocol was approved by the local Institutional Review Board before any patient-related activities took place (EC number: 126/2003, ethik-kom@meduni-wien.ac.at).

endothelial cells, lymphocytes, and granulocytes also express VEGF on their surface (16). Activated platelets, whose role in promoting thrombogenesis and tumor growth in malignancy is well established, release soluble VEGF from their α-granules (17). Hence, it is conceivable that VEGF might play a role in the pathogenesis of cancer-associated VTE, and represent a candidate prognostic biomarker. Here, we have investigated the association between soluble VEGF (sVEGF) in the plasma of cancer patients and the risk of VTE and death within a prospective, observational cohort study.
Table 1. Distribution of baseline variables overall and by sVEGF levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall (n = 804)</th>
<th>VEGF ≤ Q3 (n = 607)</th>
<th>VEGF &gt; Q3 (n = 197)</th>
<th>ρc</th>
<th>Rho (p)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry, y</td>
<td>804 (0.0%)</td>
<td>63.1 (54.2–69.2)</td>
<td>63.0 (54.3–68.7)</td>
<td>63.3 (53.5–71.8)</td>
<td>0.77</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>802 (0.3%)</td>
<td>25.0 (22.3–28.1)</td>
<td>24.9 (22.4–28.1)</td>
<td>25.1 (21.8–28.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Female gender</td>
<td>804 (0.0%)</td>
<td>433 (53.9%)</td>
<td>338 (55.7%)</td>
<td>95 (48.2%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Tumor site</td>
<td>804 (0.0%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.60</td>
</tr>
<tr>
<td>Brain</td>
<td>—</td>
<td>87 (10.8%)</td>
<td>66 (10.9%)</td>
<td>21 (10.7%)</td>
<td>—</td>
</tr>
<tr>
<td>Breast</td>
<td>—</td>
<td>137 (17.0%)</td>
<td>101 (16.6%)</td>
<td>36 (18.3%)</td>
<td>—</td>
</tr>
<tr>
<td>Bronchus</td>
<td>—</td>
<td>120 (14.9%)</td>
<td>84 (13.8%)</td>
<td>36 (18.3%)</td>
<td>—</td>
</tr>
<tr>
<td>Colorrect</td>
<td>—</td>
<td>105 (13.1%)</td>
<td>82 (13.5%)</td>
<td>23 (11.7%)</td>
<td>—</td>
</tr>
<tr>
<td>Kidney</td>
<td>—</td>
<td>22 (2.7%)</td>
<td>16 (2.6%)</td>
<td>6 (3.1%)</td>
<td>—</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>—</td>
<td>84 (10.5%)</td>
<td>68 (11.2%)</td>
<td>16 (8.1%)</td>
<td>—</td>
</tr>
<tr>
<td>Melanoma</td>
<td>—</td>
<td>15 (1.9%)</td>
<td>13 (2.1%)</td>
<td>2 (1.0%)</td>
<td>—</td>
</tr>
<tr>
<td>Other site</td>
<td>—</td>
<td>48 (6.0%)</td>
<td>36 (5.9%)</td>
<td>12 (6.1%)</td>
<td>—</td>
</tr>
<tr>
<td>Pancreas</td>
<td>—</td>
<td>53 (6.6%)</td>
<td>36 (5.9%)</td>
<td>17 (8.6%)</td>
<td>—</td>
</tr>
<tr>
<td>Prostate</td>
<td>—</td>
<td>95 (11.8%)</td>
<td>77 (12.7%)</td>
<td>18 (9.1%)</td>
<td>—</td>
</tr>
<tr>
<td>Stomach</td>
<td>—</td>
<td>38 (4.7%)</td>
<td>28 (4.6%)</td>
<td>10 (5.1%)</td>
<td>—</td>
</tr>
<tr>
<td>High-risk or very-high-risk tumor sitec</td>
<td>804 (0.0%)</td>
<td>546 (32.1%)</td>
<td>411 (32.2%)</td>
<td>135 (31.5%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Localized disease</td>
<td>804 (0.0%)</td>
<td>467 (58.1%)</td>
<td>363 (59.8%)</td>
<td>104 (52.8%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Newly diagnosed cancer</td>
<td>804 (0.0%)</td>
<td>596 (74.1%)</td>
<td>453 (74.6%)</td>
<td>143 (72.6%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Tumor staged</td>
<td>684 (12.0%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.215</td>
</tr>
<tr>
<td>Stage I</td>
<td>—</td>
<td>79 (11.6%)</td>
<td>65 (12.7%)</td>
<td>14 (8.2%)</td>
<td>—</td>
</tr>
<tr>
<td>Stage II</td>
<td>—</td>
<td>158 (23.1%)</td>
<td>120 (23.4%)</td>
<td>38 (22.2%)</td>
<td>—</td>
</tr>
<tr>
<td>Stage III</td>
<td>—</td>
<td>89 (13.0%)</td>
<td>68 (13.3%)</td>
<td>21 (12.3%)</td>
<td>—</td>
</tr>
<tr>
<td>Stage IV</td>
<td>—</td>
<td>357 (52.3%)</td>
<td>259 (50.6%)</td>
<td>98 (57.3%)</td>
<td>—</td>
</tr>
<tr>
<td>Tumor gradef</td>
<td>690 (14.2%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.757</td>
</tr>
<tr>
<td>G1</td>
<td>—</td>
<td>51 (7.4%)</td>
<td>41 (7.9%)</td>
<td>10 (5.8%)</td>
<td>—</td>
</tr>
<tr>
<td>G2</td>
<td>—</td>
<td>332 (45.2%)</td>
<td>233 (45.1%)</td>
<td>79 (45.7%)</td>
<td>—</td>
</tr>
<tr>
<td>G3</td>
<td>—</td>
<td>262 (38.0%)</td>
<td>193 (37.3%)</td>
<td>69 (39.9%)</td>
<td>—</td>
</tr>
<tr>
<td>G4</td>
<td>—</td>
<td>65 (9.4%)</td>
<td>50 (9.7%)</td>
<td>15 (8.7%)</td>
<td>—</td>
</tr>
<tr>
<td>Use of erythropoietin-stimulating agents</td>
<td>—</td>
<td>37 (4.6%)</td>
<td>25 (4.1%)</td>
<td>12 (6.1%)</td>
<td>0.251</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; ref, reference category; PAI-1, plasminogen activator inhibitor 1.

*Categorical variables are reported as absolute frequencies and percentages (round brackets), continuous data as medians with Q1-Q3 (square brackets with Q1-Q3 representing the first and third quartiles of the sVEGF distribution).

The cutoff for Q3 is set at 17.65 pg/mL.

ρ values are from χ² tests for categorical variables, and t tests (when appropriate with correction for heteroscedasticity) for continuous variables.

Rho (p) indicates the Spearman rank correlation coefficient with P value (no adjustment for multiplicity done).

High-risk or very-high-risk tumor sites are defined according to Khorana et al. with extensions according to Ay et al. (high-risk sites: lung, colon, kidney, myeloma, lymphoma, gynecologic; very-high-risk: brain, stomach, pancreas).

Localized disease is defined as the absence of clinically overt metastatic disease at baseline (with brain tumors being per se defined as localized).

Staging cannot be provided for the remaining 15% of patients because no UICC-conforming staging system (i.e., stages I, II, III, and IV) is defined for some tumor entities (glioblastoma (n = 87), multiple myeloma (n = 15), selected types of lymphoma (such as chronic lymphocytic leukemia (1.9%))).

Grading cannot be provided for the remaining patients because a specific grading system (i.e., G1–G4) is not defined for some tumor entities (multiple myeloma and lymphoma).

activation), peak of thrombin generation (as an indicator of coagulation activation), and FVIII (as a hemostatic marker of inflammation and the acute-phase reaction). We examined interactions between sVEGF and selected covariates (tumor type, D-Dimer, C-reactive protein) by including sVEGF, the respective covariate and an interaction term between the covariate and sVEGF into a multivariable model. To compare time-to-VTE between patients with high and low sVEGF, sVEGF was dichotomized into a binary variable at the 75th percentile of its distribution. To appreciate the VTE risk "effect size" of sVEGF in relation to the presently established VTE biomarkers sP-Selectin, D-Dimer, FVIII, peak thrombin Generation, and prothrombin fragment 1,2,
we Z-standardized these biomarkers and compared their univariable subhazard ratios (SHR). Mortality data were analyzed with Kaplan–Meier product-limit-estimators, the log-rank test, and uni- and multivariable Cox-Models. No evidence for nonproportional hazards according to sVEGF emerged in any of the models for both the VTE and mortality endpoint.

Results

Baseline analysis

At baseline, the median age (Q1-Q3) of the study population was 63.1 (54.2–69.2), and 433 (53.9%) of the 804 patients were female (Table 1). The majority of patients suffered from newly diagnosed malignancy (n = 596, 74.1%), and had no clinical evidence for metastatic disease (n = 467, 58.1%).

Five hundred forty-nine (68.3%) out of 804 patients had sVEGF levels above the detection limit of 0.5 pg/mL (Table 2). A group of patients with elevated baseline sVEGF (n = 197) was defined by dichotomizing sVEGF into a binary variable at the 75th percentile of its distribution (Q3, cutoff: 17.65 pg/mL; >Q3 defines the “elevated sVEGF” group). On average, patients with elevated sVEGF had higher platelet counts, lower hemoglobin, as well as higher levels of D-Dimer and peak thrombin generation (Table 1). Weak evidence was found for an association between localized disease and lower VEGF (P = 0.08). The distribution of other baseline variables appeared to be comparable between the two sVEGF groups.

Weak positive correlations were observed between sVEGF and leukocyte count, platelet count, D-Dimer, thrombin generation, fibrinogen, von Willebrand factor (vWF), lactate dehydrogenase (LDH), and C-reactive protein (CRP; all rho >0.16 and all P < 0.01, Table 1). A negative correlation was found between sVEGF and hemoglobin (rho = -0.17, P < 0.001). A multiple linear regression model with sVEGF as the dependent variable and high- and very-high-risk tumor sites, sex, age, BMI, and thrombocyte and leukocyte count as independent variables was performed to study the amount of variation in sVEGF that may be explained by these markers (“Multiple Model 1,” Supplementary File S1). R², the coefficient of determination of this model, was 0.03, suggesting that these variables explain only around 3% of the variation in sVEGF. A corresponding model with CRP, platelet count, D-Dimer, fibrinogen, peak of thrombin generation, LDH, and vWF as the independent variables explained 7% of the variation in sVEGF (“Multiple Model 2,” Supplementary File S1).

sVEGF and the risk of VTE

In the overall study population, the cumulative incidence of VTE at 6, 12, and 24 months of follow-up was 5.0%, 6.2%, and 6.9%, respectively. Patients with elevated sVEGF (i.e., >Q3) had a higher rate of VTE than patients with sVEGF<Q3 (log-rank test P = 0.02). The cumulative 6-, 12-, and 24-month incidences of VTE (95% CI) were 8.6% (5.2–13.1), 10.2% (6.4–14.9), and 10.2% (6.4–14.9) in the VEGF>Q3 group, and 3.8% (2.5–5.6), 5.0%...
sVEGF was associated with a 4% increase in the risk of VTE [SHR, 1.18 (1.00–1.39); 0.03 (1.00–1.09)] in univariable analysis (Table 4). In univariable cause-specific analysis, a 10-pg/mL increase in sVEGF was associated with a 5% increase in the rate of VTE (HR, 1.05; 95% CI, 1.00–1.09; P = 0.03). After multivariable adjustment for selected clinical and laboratory parameters (*multivariable model*), the associations between sVEGF and the risk and rate of VTE prevailed (Table 3). In the analysis of potential interactions between sVEGF and selected covariates (tumor type, D-Dimer, and CRP), the association between sVEGF and VTE appeared to be modified by D-Dimer, and vice versa (SHR for interaction term = 1.01, 95% CI, 1.00–1.01, P = 0.001).

Comparing the increase in VTE risk for 1 SD increase in sVEGF with the already established VTE biomarkers D-Dimer, sP-Selectin, peak of thrombin generation, prothrombin fragment 1.2, and FVIII, the univariable SHR for sVEGF was comparable with the SHR for D-Dimer, and smaller than the SHRs of the other markers (Table 4).

Patients with undetectable sVEGF (i.e., sVEGF = 0 pg/mL) had a significantly lower rate of VTE (HR, 0.48; 95% CI, 0.24–0.96; P = 0.04).

**sVEGF and the risk of death**

In the overall study population, the cumulative probability of death at 6, 12, and 24 months of follow-up was 16.2%, 29.7%, and 46.1%, respectively. Patients with elevated sVEGF (i.e., >Q3) had a higher risk of death than patients with sVEGF ≤Q3 (log-rank test P = 0.01). The 12-month risk of death was 27.9% (95% CI, 24.5–31.6) in the VEGF ≤Q3 group, and 35.6% (95% CI, 29.4–42.8) in the VEGF >Q3 group (HR, 1.34; 95% CI, 1.06–1.68, P = 0.01; Fig. 2). This association prevailed after multivariable adjustment for high- and very-high-risk tumor types, localized versus distant disease, D-Dimer, and leukocyte and platelet count (HR, 1.33; 95% CI, 1.05–1.68, P = 0.02; full model shown in Supplementary File S1). We did not observe any evidence for interactions between sVEGF and tumor type, D-Dimer, and C-reactive protein.

**Discussion**

In this prospective cohort study, we have provided evidence for an association between elevated levels of sVEGF and a higher risk of VTE in patients with cancer. Furthermore, we could confirm the association of elevated sVEGF and poor overall survival.

**sVEGF and the pathogenesis of VTE in cancer**

Using a sVEGF multiplex immunoassay, we found that sVEGF was present in the bloodstream of approximately 70% of the investigated patients. Interestingly, the other 30% of patients with undetectable sVEGF experienced only half the risk of developing VTE. It was well established in experimental studies that endothelial cells and several subsets of leukocytes can express sVEGF on their surface (10, 16), and that activated platelets release VEGF from their α-granules (17). Surprisingly, we found that leukocyte and platelet count explained less than 3% of the variation in sVEGF, which increased to 7% when we considered vWF and LDH, among others, as independent variables. Given that vWF and sP-Selectin are contained in α-granules (25), and LDH is secreted by degranulating platelets (26), the weak correlations between sVEGF, LDH, sP-Selectin, and vWF may indicate that at least some of the amount of sVEGF originates from the α-granules of activated platelets. Furthermore, vWF and sP-Selectin are also released from Weibel-Palade bodies of activated endothelial cells (25). The observed correlations between sVEGF, vWF, and sP could thus also be indicative of endothelial activation, a process that is a well-established hallmark of cancer-associated hypercoagulability and inflammation (27).

Some of the sVEGF in the bloodstream may originate directly from tumor cells. The weak correlation between sVEGF and LDH, an intracellular enzyme that is released by decaying tumor cells, released from their α-granules (17). Surprisingly, we found that leukocyte and platelet count explained less than 3% of the variation in sVEGF, which increased to 7% when we considered vWF and LDH, among others, as independent variables. Given that vWF and sP-Selectin are contained in α-granules (25), and LDH is secreted by degranulating platelets (26), the weak correlations between sVEGF, LDH, sP-Selectin, and vWF may indicate that at least some of the amount of sVEGF originates from the α-granules of activated platelets. Furthermore, vWF and sP-Selectin are also released from Weibel-Palade bodies of activated endothelial cells (25). The observed correlations between sVEGF, vWF, and sP could thus also be indicative of endothelial activation, a process that is a well-established hallmark of cancer-associated hypercoagulability and inflammation (27).

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Furthermore, correlations between sVEGF and inflammatory markers like CRP and leukocyte count were statistically significant but weak. Although correlated with sVEGF, biomarkers of endothelial and platelet activation such as vWF and sP-Selectin explained only a limited amount of variation in sVEGF when considered jointly in a multivariable regression analysis.

Previous clinical studies in cancer patients have shown that elevated markers of coagulation activation, such as D-Dimer, are associated with both an increased risk of VTE and mortality (18, 20). In this study, D-Dimer and thrombin generation were higher in patients with elevated VEGF, which may suggest a pathogenetic connection between the presence of sVEGF in the bloodstream and the induction of a prothrombotic state in malignancy. Importantly, our analysis of cause-specific VTE hazards identified a temporal relationship between increased sVEGF at baseline and an increased rate of VTE during a follow-up period of 2 years. This association was independent of other known risk factors for VTE, such as high-risk tumor types, as well as a panel of biomarkers indicative of in vivo hypercoagulability. This supports the concept of a pathogenetic connection between sVEGF and cancer-associated VTE.

We further observed an association between increased sVEGF and death that was robust to the multivariable correction for other prognostic variables, such as high-risk tumor sites, metastatic disease, and D-Dimer. The connection between sVEGF and mortality in cancer has already been described in previous studies, and we can reconfirm these results (13, 14).

**sVEGF and its potential utility as a biomarker for VTE prediction in cancer patients**

We investigated the relationship between sVEGF and the absolute risk of VTE using competing risk analysis. In univariable analysis, we found an association between increased sVEGF and a higher risk of VTE. The prognostic relationship between sVEGF and VTE risk prevailed after multivariable adjustment for established VTE risk factors and candidate biomarkers for cancer-associated VTE, such as high-risk tumor types and sP-Selectin. To compare the ‘effect sizes’ on VTE between sVEGF and other biomarkers, we brought all markers on a common scale (i.e., Z-standardization). This allowed us to appreciate how much the risk of VTE changes for 1 unit (i.e., 1 SD) increase in the biomarkers. Here, the risk ratios of sVEGF and D-Dimer were very similar, and higher for peak of thrombin generation, prothrombin fragment 1.2, and sP-Selectin. On the basis of these standardized comparisons, we cannot conclusively answer the question whether, for example, sP-Selectin is a better VTE biomarker than sVEGF or D-Dimer, as this would necessitate further elaborate analyses that are beyond the scope of this manuscript. Interestingly, we observed a positive interaction between sVEGF and D-Dimer. This could indicate that the predictive potential of sVEGF for VTE could be greater in patients with elevated D-Dimer, and vice versa.

**Limitations of the study**

Previous reports in cancer patients have described a relationship between increased sVEGF and disease progression (12, 29). Besides its adverse impact on survival, disease progression is also a strong risk factor for VTE (1). Unfortunately, no data are available on disease progression in our study. Furthermore, due to the relatively low VTE event rate (n = 55 VTE events), we had to refrain from extensive multivariable selection and model building. Finally, the cutoff for elevated sVEGF at the 75th percentile was chosen arbitrarily. However, as no data are currently available on plasma sVEGF reference ranges or clinically validated cutoffs in the cancer population, we pragmatically prespecified this cutoff at the 75th percentile, which is frequently used in studies where validated cutoffs are unavailable, and did not investigate any other cutoffs in order to preserve the type I error rate.
Conclusion

We demonstrated a prospective association between elevated plasma sVEGF and an increased risk of VTE in patients with cancer. This association was independent of major known prognostic factors, such as tumor type and biomarkers indicative of a prothrombotic state. Furthermore, we confirmed the role of increased sVEGF as an adverse prognostic factor for mortality. In conclusion, we propose sVEGF as a novel biomarker for cancer-associated VTE.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: F. Posch, J. Thaler, C. Zielinski, I. Pabinger, C. Ay
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Thaler, G-J. Zlabinger, O. Königshögbürg, C. Zielinski, C. Ay
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Posch, J. Thaler, G-J. Zlabinger, O. Königshögbürg, S. Koder, I. Pabinger, C. Ay
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Posch, J. Thaler, S. Koder, C. Zielinski, C. Ay
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