Biomarkers for prediction of venous thromboembolism in cancer

Ingrid Pabinger *, Johannes Thaler *, Cihan Ay *

* Clinical Division of Haematology and Haemostaseology, Department of Medicine I, Medical University of Vienna, Austria

Correspondence: Prof. Dr. Ingrid Pabinger
Medical University of Vienna, Department of Medicine I,
Clinical Division of Haematology and Haemostaseology
Waehringer Guertel 18-20, A-1090 Vienna, Austria
Phone number: +43 1 40400 4448
Fax number: +43 1 40400 4030
e-mail: ingrid.pabinger@meduniwien.ac.at

Key words: Cancer, venous thromboembolism, biomarkers, D-Dimer, microparticles
Short title: Biomarkers for cancer-associated thrombosis
Abstract

Cancer patients are at increased risk of deep vein thrombosis (DVT) and pulmonary embolism (PE). The incidence among different groups of cancer patients varies considerably depending on clinical factors, the most important being tumour entity and stage. Biomarkers have been specifically investigated for their capacity of predicting venous thromboembolism (VTE) during the course of disease. Parameters of blood count analysis (elevated leukocyte and platelet-count, decreased haemoglobin) have turned out to be useful in risk prediction. Associations between elevated levels and future VTE have been found for D-Dimer, prothrombin fragment 1+2 and soluble P-selectin and also for clotting factor VIII and the thrombin generation potential. The results for tissue factor (TF)-bearing microparticles are heterogeneous, an association with occurrence of VTE in pancreatic cancer might be present, whereas in other cancer entities, such as glioblastoma, colorectal or gastric carcinoma this could not be confirmed. Risk assessment models (RAM) were developed that include clinical and laboratory markers. In the high risk categories patient groups with up to >20% VTE rate within 6 months can be identified. A further improvement in risk stratification would allow better identification of patients for primary VTE prevention using indirect or novel direct anticoagulants.
Introduction

In the 19th century the association between thrombosis and malignancy was described already in 1823 Bouillaud \(^1\) and then in 1865 by Armand Trousseau, now often referred to as Trousseau syndrome\(^2\). A few years later, in 1878, Theodor Billroth found histological evidence for tumor cells within a thrombus and suggested a close interrelation between metastasis formation and activation of the hemostatic system\(^3\). To date, it is well established that cancer patients are at increased risk of developing venous thromboembolism (VTE).

Thromboprophylaxis is still challenging in cancer patients. On the one hand the incidence of VTE is not equally distributed among cancer patients, starting from about 1% in certain cancer types and reaching up to 20% or even more in the most prothrombotic malignancies like pancreatic cancer and malignant gliomas \(^4\). On the other hand, the benefit of anticoagulation has to be weighed against the substantially increased risk of bleeding complications. From a clinical perspective it would be extremely helpful to have biomarkers that enable early identification of cancer patients at risk of VTE and to target anticoagulation for primary prevention of VTE based on risk stratification.

In this review we will discuss data from studies that investigated biomarkers for prediction of VTE in cancer patients. We will also discuss risk assessment models, which incorporate clinical parameters and biomarkers for the stratification of cancer patients into groups according to their risk of developing VTE. We will mainly focus our review on patients with solid tumours and will not cover specific hematologic malignancies, such as myeloproliferative disorders or leukaemias.
Potential predictive biomarkers

Blood count parameters

Blood count parameters are of particular interest, because they are assessed at regular intervals in most cancer patients and methods of determination are highly standardized. First, Khorana et al. found an association between pre-chemotherapy leukocytosis and thrombocytosis and the risk of VTE in cancer patients who were included in the Awareness of Neutropenia in Chemotherapy (ANC) Study Group Registry \textsuperscript{5,6}.

Khorana et al. found in the ANC Study Group Registry that cancer patients with leukocytosis had a 2-fold increased risk of VTE \textsuperscript{6}. In a subsequent more in-depth analysis of the same cohort of patients it was shown that those with persistent leukocytosis after a first cycle of chemotherapy had a significantly higher incidence of VTE (3%) compared to patients in whom baseline leukocytosis resolved (1.7%) and those with normal leukocyte count (1.2%). Moreover, in this study elevated absolute neutrophil and absolute monocyte count but not lymphocytosis were associated with increased risk of VTE \textsuperscript{7}. In the RIETE registry that included 3805 cancer patients with acute VTE, those with leukocytosis had a 1.6-fold increased risk of recurrent VTE. \textsuperscript{8}.

The exact causative role of leukocytosis in cancer-related VTE remains to be elucidated. It was shown that tumours release inflammatory cytokines into the circulation that activate tissue factor expressed on monocytes and endothelial cells \textsuperscript{9-13}. Consistently, fibrin deposition is found in close proximity to mononuclear cells within the tumor stroma \textsuperscript{14}. Moreover, cancer cells enhance expression of P-selectin, a mediator of platelet adhesion that also induced TF expression on monocytes \textsuperscript{15}.

The role of high platelet count in the occurrence of VTE is supported by several studies. In a retrospective case control study Zakai et al. investigated risk factors for VTE in medical inpatients, of whom 24% had cancer. They found that those with high
platelet count (above $350 \times 10^9$ /L) had a 2.5-fold increased risk of developing VTE during hospitalization \textsuperscript{16}. In the ANC Study Group Registry 22\% of cancer patients had a platelet count above $350 \times 10^9$ /L prior to initiation of chemotherapy and the rate of VTE was 2.8-fold increased in these patients \textsuperscript{5}. In the analysis of the prospective Vienna Cancer and Thrombosis Study (CATS) comprising 665 cancer patients with solid tumours, the association between thrombocytosis and VTE could be confirmed \textsuperscript{17}. The latter study used a cut-off that represented the 95th percentile (above $443 \times 10^9$ /L) of the patient cohort. Those with platelet counts above this cut-off had a 3.5-fold increased risk of VTE in multivariate analysis \textsuperscript{17}. Interestingly, in the CATS cohort a cut-off of $350 \times 10^9$ /L was not significantly associated with future VTE (unpublished data) but turned out to be highly useful for prediction if the Khorana score was applied (Ay, 2010). We are convinced that setting the cut-off at higher levels leads to a better prediction with regard to an increased incidence in these patients, which on the other hand would be a disadvantage for the negative predictive value. We suggest that platelet count not be used as a single parameter for risk prediction, but rather ought to be used within risk assessment models.

The underlying mechanisms that link high platelet counts to cancer-related VTE are yet unclear. The interaction of tumor cells with the host haemostastic system may lead to the production of cytokines like IL-6 or angiogenesis-stimulating factors like VEGF that induce thrombocytosis and platelet activation \textsuperscript{18}. A role for increased thrombopoietin levels was discussed \textsuperscript{19,20}, but not found in CATS. \textsuperscript{17}. In a retrospective analysis of an interventional study of 187 cancer patients who received an erythropoiesis-stimulating agent and were randomized to intravenous, oral or no iron substitution, an increased thrombocyte count was associated with a higher frequency of VTE. Interestingly, thrombocytosis and VTE were less likely in those
with iron substitution compared to those without \textsuperscript{21}. Whether administration of iron could indeed reduce VTE in cancer patients must be addressed in appropriately designed clinical trials.

Furthermore, Khorana et al. found that cancer patients with pre-chemotherapy \textbf{hemoglobin} below 10 g/dl and/or the use of erythropoiesis-stimulating agents had a 1.8-fold increased risk of developing VTE in multivariate analysis \textsuperscript{5}.

Mandala et al also investigated blood count parameters and found only elevated platelet count to be independently associated with VTE during the course of disease of cancer patients \textsuperscript{22}.

Results on the association of biomarkers from prospective cohort studies are summarized in table 1.

\textbf{Markers of platelet- and clotting activation}

Activated platelets release a vast number of mainly prothrombotic molecules from their alpha- and dense-granules and synthesize thromboxane A2. Activated platelets change their shape and express prothrombotic phospholipids on their surface \textsuperscript{23}.

Moreover, activated platelets emit small vesicles called microparticles (MPs) from their surface, which have been reported to be 50- to 100-fold more prothrombotic than the equivalent area on activated platelets \textsuperscript{24}.

Tumor cells interact via multiple pathways with platelets and thereby promote metastasis formation \textsuperscript{25-27}, angiogenesis \textsuperscript{28,29}, protection from immune surveillance \textsuperscript{30}, tumor growth and invasion \textsuperscript{18}.

\textit{Soluble P-selectin}

P-selectin is a member of the selectin family of cell adhesion molecules that is released from the alpha-granules of activated platelets and from Weibel-Palade
bodies of endothelial cells. P-selectin plays a crucial role in thrombogenesis and induces a prothrombotic state. There is also evidence that P-selectin mediates the adhesion of platelets and leukocytes to cancer cells. Levels of soluble P-selectin (sP-selectin) were elevated in patients with acute VTE and were associated with risk of VTE in patients without cancer. In a prospective study of patients with cancer, elevated levels of sP-selectin predicted a 2.6-fold increased risk of developing VTE. The cumulative probability of VTE was 12% in patients with sP-selectin levels above the 75th percentile of the total study population compared to 4% in those with lower levels.

**D-dimer and prothrombin fragment 1+2**

D-dimer is a global indicator of coagulation activation and fibrinolysis. A close interrelation between cancer progression and activation of the coagulation system has been suggested. Several studies indicate that D-Dimer is associated with the risk of VTE in patients with cancer (Table 1). Also in CATS D-dimer was shown to be a valuable biomarker for prediction of VTE in cancer patients (Figure 1). In this latter evaluation the 75th percentile (1.44 μg/mL) of the cancer population was chosen as cut off. D-Dimer as predictive parameter was validated in an independent cohort of CATS patients (Ay et al, Blood 2010).

Prothrombin fragment 1+2 (F1+2) is released when activated factor X cleaves prothrombin to thrombin. In CATS elevated levels of F1+2predicted a 2-fold increased risk of VTE. Interestingly, the highest hazard ratio (HR) for VTE was found in patients who had both, elevated D-dimer and elevated F1+2 (HR: 3.6), corresponding to a cumulative VTE incidence of 15% after 6 months of study inclusion compared to 5% in patients with non-elevated D-dimer and F1+2.
Apparently both biomarkers reflect a hypercoagulable state in cancer patients and complement each other in the identification of patients at high risk of VTE. D-Dimer may even be a valuable biomarker for prediction of recurrent VTE in cancer patients after discontinuation of anticoagulation 44.

**Thrombin generation potential**

Thrombin is a key enzyme in the coagulation process. An individual's endogenous thrombin generation (TG) potential reflects the composite of multiple factors that influence blood coagulation. After addition of TF and phospholipids to plasma, in-vitro TG can be quantified with assays that measure the conversion of prothrombin to thrombin over time resulting in a thrombin generation curve 45. The most important parameters deduced from the thrombin generation curve are the lag time (time until thrombin burst occurs), the peak thrombin generation [PTG], and the area under the curve (reflecting the total amount of thrombin generated).

Associations between elevated TG and increased risk of VTE have been found in several clinical studies of non-cancer patients 32. In CATS we determined the PTG in 1033 cancer patients 46. Four percent of patients without elevated PTG developed VTE during follow-up compared to 11% of patients with elevated PTG, corresponding to a HR of 2.1 (Table 1). These results indicate that cancer patients at increased risk of VTE can be identified with a relatively simple assay that globally reflects an individual's coagulation potential.

**Microparticles and tissue factor**

MPs are negatively charged membrane vesicles that are most commonly defined by their procoagulant phosphatidylserine (PS)-rich surface and a size ranging between 0.1 and 1 µm 47. MPs in plasma are mainly derived from platelets 48, erythrocytes 49,
monocytes 50, leukocytes 51, and smooth muscle cells 52. In malignancy MPs may also originate from cancer cells 53. In 1983 Dvorak et al. first demonstrated that highly procoagulant TF-bearing MPs are shed from cultured cancer cells 54. Other well established sources of TF-bearing MP are monocytes and endothelial cells 55. Recently, we quantified the overall levels of procoagulant MPs with a chromogenic prothrombinase assay in 728 cancer patients in the framework of the prospective CATS 56. No significant association between procoagulant MPs and occurrence of VTE was present, but MP levels were significantly higher in patients with cancer than in age- and sex-matched healthy controls. In a small cross-sectional study, Tesselaar et al. detected highly elevated MP-associated TF (MP-TF) activity levels in seven breast- and pancreatic cancer patients with acute VTE compared to controls 57. This finding was confirmed in a case-control study by Manly et al. that included patients with different malignancies 58. In both studies it was suggested that elevated MP-TF activity might play a determining role in the development of cancer-related VTE. Results from prospective studies that investigated TF-bearing MPs as a biomarker for VTE in cancer are inconsistent 59,60, 61,62. In a case series of eleven pancreatic cancer patients consecutive blood samples were drawn at regular intervals 59. In two of these patients continuously rising MP-TF activity- and TF antigen levels were found. Intriguingly, both patients developed VTE, which was fatal in one patient. Zwicker et al determined TF-bearing MPs with impedance-based flow cytometry in 60 patients without VTE 60. Over a period of two years four out of 16 patients (25%) with measureable TF-bearing MPs at study inclusion developed VTE compared to 1 out of 44 patients (2%) without measureable TF-bearing MPs. Auwerda et al. measured MP-TF activity levels in 122 newly diagnosed multiple myeloma patients before and after induction of chemotherapy 61. They did not find higher MP-TF activity levels in patients who developed VTE during follow-up compared to those without VTE.
Interestingly, MP-TF activity remained elevated after chemotherapy only in patients with VTE during follow-up. In the prospective CATS, MP-TF activity was measured in patients with four different tumour entities, including 119 brain-, 60 pancreatic-, 126 colorectal-, and 43 gastric cancer patients. Applying Cox regression analysis and competing risk analysis we found no association between MP-TF activity and risk of VTE in brain, colorectal and gastric cancer patients. In pancreatic cancer patients an association in Cox-regression analysis was revealed, which was weakened when the competing risk analysis (death as competing variable) was applied. High MP-TF activity clearly increased risk of mortality in pancreatic cancer patients. In a subsequent study of pancreatic cancer patients we detected a highly elevated MP-TF activity only in patients with non-resectable metastatic and poorly differentiated adenocarcinomas. Strikingly, MP-TF activity was particularly high in patients with poorly differentiated tumors that were likely to infiltrate peripancreatic vessels. Therefore, it could be hypothesized that elevated MP-TF activity might reflect an aggressive, poorly differentiated and invasive pancreatic cancer phenotype. This hypothesis is supported by a growing body of experimental evidence, originating from in vitro- and animal model experiments, which demonstrate a crucial role of TF in pancreatic cancer cell invasion and metastasis formation.

The efficacy of primary thromboprophylaxis for the prevention of VTE in patients with advanced cancer and elevated levels of TF-bearing MPs was investigated in a recently published interventional study that applied impedance-based flow cytometry for determination of TF-bearing MPs. Twenty-three patients with elevated levels of TF-bearing MPs were randomized to a daily prophylactic dose of enoxaparin and eleven patients were followed without thromboprophylaxis. The cumulative incidence of VTE demonstrated by screening was 5.5% in patients with thromboprophylaxis versus 27.2% in those without (p=0.058). There was only one symptomatic event.
Unfortunately this study precludes definitive conclusions, as it was underpowered due to the lower rate of VTE events than anticipated in the power calculation (40% VTE events were expected in patients with elevated TF-bearing MPs without prophylaxis).

**Clotting factors (Fibrinogen, Factor XIII, Factor VIII) and C-reactive protein**

Elevated levels of fibrinogen were shown to be associated with arterial thrombosis and VTE. In CATS neither elevated nor decreased levels, which might potentially have been attributed to chronic DIC, were associated with VTE. In addition to the determination of fibrinogen levels, a polymorphism in the promoter region known to impact fibrinogen levels (-455G>A SNP) in the fibrinogen β-gene was investigated. Also for this polymorphism no association with VTE was found.

Polymorphisms in the factor XIII gene were reported to be associated with DVT and PE in non-cancer patients, however, no association was detected in CATS.

Elevated factor VIII (FVIII) is a well-established risk factor for first manifestation and for recurrence of VTE in non-cancer patients. Only few studies have investigated FVIII as a risk factor for VTE in cancer patients. Two small studies revealed discrepant results. In one retrospective cross-sectional study in patients with various cancer types, those with VTE had higher FVIII levels than those without VTE. In a prospective study of patients with multiple myeloma no association was found, however, further details were not given. Within the framework of CATS FVIII was also investigated. A strong and independent association was found in 840 patients of whom 62 developed VTE. Interestingly, this association was age-dependent. In 40 year-old patients a 20% increase in FVIII doubled the risk for VTE (HR 2.0 [95% CI]: 1.5-2.7), whereas the risk was attenuated in older patients (in a 60 year-old patient...
the HR for 20% increase was 1.4 [95% CI: 1.2-1.6]. The cumulative probability of development of VTE after 6 months was 4% in patients with normal FVIII levels compared to 14% in those with a FVIII level above 232%, which corresponds to the 95th percentile of the normal Austrian population.

C-reactive protein (CRP), which is an acute phase protein, induces TF expression on monocytes, smooth muscle cells and endothelial cells. In a retrospective study of 507 cancer patients CRP was identified as possible risk marker for VTE. In CATS CRP levels were predictive of VTE in univariate analysis (hazard ratio [HR]: 1.2 per doubling) but in multivariate analysis (including chemotherapy, surgery, radiotherapy, metastasis, cancer site and soluble P-selectin) the association with VTE was no longer observed.

**Risk assessment models for the prediction of VTE in cancer patients**

The use of risk assessment models (RAMs) for the stratification of cancer patients according to their propensity to develop VTE is a novel and promising approach for the identification of cancer patients who are likely to benefit from primary thromboprophylaxis. The first published and hitherto single well-validated RAM for prediction of cancer-related VTE was developed by Khorana et al. (We will refer to this RAM as “Khorana score”). In a recent review the Khorana score and studies that validated this RAM were discussed extensively. Parameters, determined prior to initiation of chemotherapy, that were included in the Khorana Score are listed in table 2. Numerical values (0-2) were assigned to each covariate and patients were stratified into three discrete categories according to the total score. Patients assigned to the low risk group (score 0) had a VTE risk of 0.3%
during 2.5 months of follow-up, those in the intermediate risk group (score 1-2) had a risk of 2% and those in the high risk group (≥3) of 6.7%.

This risk scoring model was validated in the cohort of patients included in CATS 84. An expanded RAM (Vienna prediction score) by incorporating additional biomarkers, namely soluble P-selectin and D-dimer84 (table 2) was proposed by Ay et al (Blood 2010). With this expanded RAM, the prediction of VTE was considerably improved, as patients with a score of 4 had a cumulative probability of developing VTE of 20.4% and those with a score ≥ 5 had a 35% probability.

Future interventional trials are needed to investigate whether cancer patients assigned to high risk groups, according to the Khorana score or other RAMs, benefit from primary thromboprophylaxis in terms of VTE incidence reduction. Moreover, as mechanisms leading to VTE may differ in specific types of malignancies, it also remains to be elucidated, whether RAMs specifically designed for certain cancer types further improve VTE risk assessment.

**Conclusions and future perspectives**

A number of biomarkers are associated with the occurrence of VTE in cancer patients. These encompass biomarkers reflecting activation of the blood clotting system, such as D-dimer or sP selectin, or an increase in the inflammatory potential, e.g. CRP, leukocyte and platelet count or haemoglobin levels. Part of the biomarkers and scores that were derived by using biomarkers have so far been sufficiently validated, such as blood count parameters or D-dimer levels 84 and have revealed very promising results with regard to the possibility of stratifying patients into those with low or high risk. Especially D-dimer and sP-selectin seem to be robust biomarkers for predicting the risk of VTE, as these biomarkers remained
independently associated with VTE in recent evaluations (Ahlbrecht et al JCO and Dickmann Hameatologica).

Presently, D-dimer seems to be one of the most promising candidates to gain a role for prediction of VTE in cancer patients, with the aim to identify patients who in all probability would benefit most from thrombosis prophylaxis. We suggest using a cut off that has been validated in an independent cohort in CATS (Ay, Blood 2010), which is the 75th% of the cancer population (1.44 μg/mL), of course further studies have to be performed to validate such a cut off in other patient cohorts. D-dimer testing is widely available and assays have been sufficiently validated. This biomarker is recommended and used in daily practice for exclusion of VTE (Goldhaber and Bounameaux, Lancet 2012, siehe offene Fragen). In addition examples of the usefulness of D-dimer measurement for prediction of VTE exist, namely for recurrence 85 86, being most successful in combination with easy-to-determine clinical parameters 87. This usefulness of D-dimer for the management of VTE patients was already demonstrated in an interventional trial in non-cancer patients 88. Combining D-dimer with other routinely available biomarkers, such as platelets, leukocytes or haemoglobin, and with clinical parameters (e.g. tumour type) would provide an easy-to-determine score for VTE risk prediction in cancer patients that could be broadly applied. Patients who are at a yet to be defined increased risk would then be subjected to thrombosis prophylaxis.

Although F1+2 was also found to be predictive, this parameter has never been validated in clinical studies and is not widely available, moreover, might not add much to the determination of D-Dimer. Therefore this biomarker is not amongst the candidates to be first choice for usage in daily practice. The open questions with regard to validation and availability are similar for thrombin generation, although the
predictive potential in CATS was comparable to other parameters like D-Dimer (see Table 1). The value of procoagulant MPs and TF-expressing MP-subpopulations as biomarkers for cancer-related VTE must currently be assessed as low due to the lack of standardization and large differences found between various tumour entities. Some promising data for TF-expressing MPs were revealed by small studies, one larger study revealed a trend in pancreatic cancer patients, whereas absolutely no association was found in glioblastoma, colorectal and gastric carcinoma. It will be interesting to investigate more sensitive assays for quantification of microparticles in larger patient series.

Up to now, the large interventional trials with primary thrombosis prophylaxis in ambulatory cancer patients have been performed in non-selected patients which revealed an advantage in terms of a decrease in VTE in patients receiving low molecular weight heparin, however, also patients without prophylaxis had a very low probability of VTE (less than 5% after 4 months) and prophylaxis is therefore not recommended in unselected ambulatory cancer patients. There is consensus that future trials should focus on high-risk-patients, and biomarkers could play a major role in identifying them. So far one feasibility study using microparticles has been performed (Zwicker 2013), which is described above. Another study applies the Khorana score and is still recruiting (ClinicalTrials.gov NCT00876915). Of course it would also be very important to have further studies on high risk patients initiated, probably using direct oral anticoagulants.

Although it was not the aim of this review to cover the aspect of the association of biomarkers with prognosis in cancer patients, this most interesting aspect should be mentioned, as it underlines the close connection between increased risk of VTE and higher aggressiveness of the tumour. D-dimer, high platelet counts and TF-
positive microparticles \textsuperscript{62} have been shown to be significantly associated with prognosis of cancer.

There are limitations for some of these biomarkers due to various reasons: (1) They are dependent on factors other than the tumour itself, e.g. additional inflammation due to infection, or coagulation activation by surgery or other invasive procedures. (2) The sensitivity and specificity of single biomarkers are low. The specificity is considerably improved combining clinical factors with biomarkers by creating “scores”. Only the Khorana score has been sufficiently validated in several cohort studies (Zitat 83). Also in two large prospective interventional trials this score turned out to be predictive in patients who had received placebo. In the SAVE-ONCO study patients on placebo and a low score (0) had an incidence of 1.3% compared to those with a score > 3, who had an incidence of 5.4% (George 2011, Blood ASH Abstract, Zitat see “Offenen Fragen unten). Also in the PROTECHT- study the score was validated, 3% VTE in the low-intermediate risk group (score 0-2) versus 11.1% in those with a score > 3 (Verso et al, see offene fragen, genaues Zitat). However, this score still has a low specificity. This means that by applying the score and taking a cut off of 3 a high percentage of patients who will develop VTE will still be missed \textsuperscript{96}. The inclusion of two additional biomarkers increased the specificity considerably (Table 3; data of the CATS study were derived from the patient cohort published by Ay et al. \textsuperscript{84}). Another disadvantage of some of the investigated biomarkers is the fact that they are not sufficiently standardized. This is specifically the case with microparticles, but also with the endogenous thrombin generation potential. On the other hand, determination of D-dimer is a very good example of how this particular problem can be overcome. Although many different assays for D-dimer are used, these assays are sufficiently validated to allow the daily application for the exclusion of VTE \textsuperscript{97}. 
In conclusion, biomarkers are a promising tool for risk prediction of VTE in patients with malignant disorders, especially when used in combination with clinical parameters. Although improved risk stratification has been achieved, the positive predictive value could still be enhanced to capture the majority of patients who are expected to experience VTE in order to allow targeted thromboprophylaxis in these patients.

Acknowledgments

The authors thank Tanja Altreiter for proofreading of the manuscript.

Authorship

Ingrid Pabinger, Johannes Thaler and Cihan Ay performed the literature research, wrote the manuscript and approved the final version.

The authors have no conflicts of interest to declare.
References


10. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood. 1993;82(2):513-520.


Table 1. Prospective studies investigating potential predictive biomarkers of venous thromboembolism (VTE) in cancer patients

<table>
<thead>
<tr>
<th>First author (ref.)</th>
<th>Variable</th>
<th>Cancer entity</th>
<th>Total number of patients</th>
<th>Cut-off</th>
<th>HR / OR for VTE during follow-up</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khorana a (5)</td>
<td>Leukocyte count</td>
<td>various</td>
<td>2701</td>
<td>&gt;11 x 10^9/L</td>
<td>2.2**</td>
<td>1.2-4.0</td>
</tr>
<tr>
<td>Khorana a (5)</td>
<td>Platelet count</td>
<td>various</td>
<td>3003</td>
<td>≥ 350 x 10^9/L</td>
<td>2.8**</td>
<td>1.1-3.2</td>
</tr>
<tr>
<td>Simanek b (16)</td>
<td>Platelet count</td>
<td>various</td>
<td>665</td>
<td>&gt; 443 x 10^9 /L c</td>
<td>3.5'</td>
<td>1.5-8.1</td>
</tr>
<tr>
<td>Khorana a (5)</td>
<td>Hemoglobin and/or erythropoiesis stimulating agents</td>
<td>various</td>
<td>3003</td>
<td>&lt; 10 g/dl</td>
<td>1.8'</td>
<td>1.1-3.1</td>
</tr>
<tr>
<td>Ay b (14)</td>
<td>Soluble P-selectin</td>
<td>various</td>
<td>687</td>
<td>≥ 53.1 ng/mL e</td>
<td>2.6'</td>
<td>1.4-4.9</td>
</tr>
<tr>
<td>Ay b (43)</td>
<td>D-dimer</td>
<td>various</td>
<td>821</td>
<td>≥ 1.44 µg/mL e</td>
<td>2.2'</td>
<td>1.3-3.6</td>
</tr>
<tr>
<td>Apaia (38)</td>
<td>D-dimer</td>
<td>various</td>
<td>124</td>
<td>&gt; 0.65 µg/mL e</td>
<td>4.0'</td>
<td>1.2-13.3</td>
</tr>
<tr>
<td>Stender (39)</td>
<td>D-dimer</td>
<td>colorectal</td>
<td>176</td>
<td>&gt; 0.3 µg/mL</td>
<td>6.5'</td>
<td>1.6-27</td>
</tr>
<tr>
<td>Kodama (40)</td>
<td>D-dimer</td>
<td>gynaecologic</td>
<td>291</td>
<td>&gt; 5 µg/mL</td>
<td>1.2'</td>
<td>1.0-1.4</td>
</tr>
<tr>
<td>Ferroni (41)</td>
<td>D-dimer</td>
<td>lung</td>
<td>108</td>
<td>&gt; 1.5 µg/mL</td>
<td>11'</td>
<td>2.6-46</td>
</tr>
<tr>
<td>Ay b (43)</td>
<td>Prothrombin fragment 1 + 2</td>
<td>various</td>
<td>821</td>
<td>≥ 358 pmol/L e</td>
<td>2.2'</td>
<td>1.3-3.6</td>
</tr>
<tr>
<td>Ay b (46)</td>
<td>Peak thrombin generation</td>
<td>various</td>
<td>1033</td>
<td>≥ 611 nM thrombin e</td>
<td>2.1'</td>
<td>1.3-3.3</td>
</tr>
<tr>
<td>Thaler b (56)</td>
<td>Procoagulant microparticles</td>
<td>various</td>
<td>728</td>
<td>≥ 4.62 nM phosphatidylserine equivalent d</td>
<td>1.0'</td>
<td>0.6-1.6</td>
</tr>
<tr>
<td>Thaler b (62)</td>
<td>Microparticle-associated tissue factor activity</td>
<td>pancreas</td>
<td>60</td>
<td>none (per doubling)</td>
<td>1.5'</td>
<td>1.0-2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>brain</td>
<td>119</td>
<td>none (per doubling)</td>
<td>1.0'</td>
<td>0.8-1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stomach</td>
<td>43</td>
<td>none (per doubling)</td>
<td>0.7'</td>
<td>0.4-1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>colorectal</td>
<td>126</td>
<td>none (per doubling)</td>
<td>0.9'</td>
<td>0.6-1.6</td>
</tr>
<tr>
<td>Zwicker (60)</td>
<td>Tissue factor bearing microparticles</td>
<td>various</td>
<td>60</td>
<td>&gt; 1x10^4/µl</td>
<td>3.7''</td>
<td>1.2-11.8</td>
</tr>
<tr>
<td>Tiedje b (68)</td>
<td>Fibrinogen</td>
<td>various</td>
<td>1079</td>
<td>none (continuous)</td>
<td>1.1'</td>
<td>0.8-1.3</td>
</tr>
<tr>
<td>Vormittag b (78)</td>
<td>Factor VIII activity</td>
<td>various</td>
<td>840</td>
<td>&gt; 232% e</td>
<td>2.8'</td>
<td>1.7-4.6</td>
</tr>
<tr>
<td>Kanz b (82)</td>
<td>C-reactive protein</td>
<td>various</td>
<td>705</td>
<td>None (per doubling)</td>
<td>1.0'</td>
<td>0.9-1.2</td>
</tr>
<tr>
<td>Mandala (21)</td>
<td>Homocysteine</td>
<td>various</td>
<td>381</td>
<td>none (continuous)</td>
<td>0.9''</td>
<td>0.9-1.0</td>
</tr>
<tr>
<td></td>
<td>Leukocyte count</td>
<td>various</td>
<td>381</td>
<td>none (continuous)</td>
<td>0.9''</td>
<td>0.7-1.1</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin</td>
<td>various</td>
<td>381</td>
<td>none (continuous)</td>
<td>1.1''</td>
<td>0.8-1.5</td>
</tr>
<tr>
<td></td>
<td>Platelet count</td>
<td>various</td>
<td>381</td>
<td>none (continuous)</td>
<td>1.6''</td>
<td>1.0-2.5</td>
</tr>
<tr>
<td></td>
<td>Protein S</td>
<td>various</td>
<td>381</td>
<td>none (continuous)</td>
<td>1.0''</td>
<td>1.0-1.0</td>
</tr>
<tr>
<td></td>
<td>Protein C</td>
<td>various</td>
<td>381</td>
<td>none (continuous)</td>
<td>1.0''</td>
<td>1.0-1.0</td>
</tr>
</tbody>
</table>
a. Patients included in the Awareness of Neutropenia in Chemotherapy (ANC) Study Group Registry
b. Patients included in the Vienna Cancer and Thrombosis Study (CATS)
c. 95th percentile of the total study population
d. 95th percentile of healthy controls
e. 75th percentile of the total study population
f. All hazard ratios are given for multivariate analyses except for the study by Mandala et al. (ref. 21)

Abbreviations: CI, Confidence interval; HR, hazard ratio; OR, odds ratio; ref., reference; VTE, venous thromboembolism
Note: Citations are given in parenthesis
Table 2: Two different risk models for identification of cancer patients at high risk of VTE

<table>
<thead>
<tr>
<th>Khorana VTE Risk Assessment Score (5)</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site of cancer:</strong></td>
<td></td>
</tr>
<tr>
<td>very high risk:</td>
<td></td>
</tr>
<tr>
<td>stomach, pancreas</td>
<td>2</td>
</tr>
<tr>
<td>high risk:</td>
<td></td>
</tr>
<tr>
<td>lung, lymphoma, gynaecologic, blader, testicular</td>
<td>1</td>
</tr>
<tr>
<td><strong>Platelet count</strong></td>
<td></td>
</tr>
<tr>
<td>≥ 350 x 10⁹ /l</td>
<td>1</td>
</tr>
<tr>
<td><strong>Hemoglobin and/or use of erythropoiesis-stimulating agents</strong></td>
<td>1</td>
</tr>
<tr>
<td>&lt; 10 g/dl</td>
<td>1</td>
</tr>
<tr>
<td><strong>Leukocyte count</strong></td>
<td></td>
</tr>
<tr>
<td>&gt; 11 x 10⁹ /l</td>
<td>1</td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td></td>
</tr>
<tr>
<td>≥ 35 kg/m²</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vienna VTE Risk Assessment Score (84), addition of:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D-Dimer</strong></td>
<td>1</td>
</tr>
<tr>
<td>≥ 1.44 μg/ml</td>
<td></td>
</tr>
<tr>
<td><strong>sP-selectin</strong></td>
<td>1</td>
</tr>
<tr>
<td>≥ 53.1 mg/ml</td>
<td></td>
</tr>
</tbody>
</table>

Note: In the Vienna Cancer and Thrombosis Study (CATS) brain tumours (high-grade glioma) were allocated to the “very high risk” sites of cancer. Citations are given in parenthesis.
Table 3: Rate of correctly identified patients (those who developed VTE) using 2 different risk models (Khorana or Vienna model) for patients with a Khorana or a Vienna score of > 3

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk model</th>
<th>Patient number</th>
<th>N'/N''</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protecht 91,92,</td>
<td>Khorana6</td>
<td>381</td>
<td>5/15</td>
<td>33%</td>
</tr>
<tr>
<td>Save-Onco91,92,</td>
<td>Khorana6</td>
<td>1583</td>
<td>15/54</td>
<td>28%</td>
</tr>
<tr>
<td>CATS84</td>
<td>Khorana6</td>
<td>819</td>
<td>16/61</td>
<td>26%</td>
</tr>
<tr>
<td>CATS84</td>
<td>Vienna84</td>
<td>819</td>
<td>33/61</td>
<td>54%</td>
</tr>
</tbody>
</table>

Abbreviations: N' number of patients with score > 3/N'' number of all patients with VTE
Note: Only patients without thrombosis-prophylaxis were included in this analysis
Legend to Figure 1. Kaplan-Meier analysis of the risk of venous thromboembolism in patients with cancer according to elevated and non-elevated D-dimer (cut-off level 1.44 µg/ml, representing the 75th percentile of the total study population)
Biomarkers for prediction of venous thromboembolism in cancer

Ingrid Pabinger, Johannes Thaler and Cihan Ay