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Biomolecular markers of cancer-associated thromboembolism

Diana L. Hanna^a, Richard H. White^b, and Ted Wun^{a,c,d,*}

^aDivision of Hematology and Oncology, UC Davis Cancer Center, Sacramento, CA 95817, United States

^bDivision of General Internal Medicine, UC Davis School of Medicine, Sacramento, CA 95817, United States

^cClinical and Translational Sciences Center, UC Davis School of Medicine, Sacramento, CA 95817, United States

^dSection of Hematology and Oncology, VA Northern California Health Care System, Sacramento, CA 95655, United States

Abstract

Venous thromboembolism (VTE; deep venous thrombosis and pulmonary embolism) is associated with a poor prognosis in most malignancies and is a major cause of death among cancer patients. Universal anticoagulation for primary thromboprophylaxis in the outpatient setting is precluded by potential bleeding complications, especially without sufficient evidence that all patients would benefit from such prophylaxis. Therefore, appropriately targeting cancer patients for thromboprophylaxis is key to reducing morbidity and perhaps mortality. Predictive biomarkers could aid in identifying patients at high risk for VTE. Possible biomarkers for VTE include C-reactive protein, platelet and leukocyte counts, D-dimer and prothrombin fragment 1+2, procoagulant factor VIII, tissue factor, and soluble P-selectin. Evidence is emerging to support the use of risk assessment models in selecting appropriate candidates for primary thromboprophylaxis in the cancer setting. Further studies are needed to optimize these models and determine utility in reducing morbidity and mortality from cancer-associated thromboembolism.

Keywords

Biomarkers; Cancer-associated thromboembolism; Leukocytosis; Risk models; Thrombocytosis; Thromboprophylaxis

1. Introduction

The impact of cancer-associated venous thromboembolism (VTE) on morbidity and mortality is well established [1–7]. Approximately 20% of patients experiencing a first

*Corresponding author: Ted Wun, MD, Division of Hematology and Oncology, UC Davis Cancer Center, 4501 X Street, Sacramento, CA 95817, United States; Tel.: +1 916 734 3772; fax: +1 916 734 7946. twun@ucdavis.edu.

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occurrence of VTE have cancer [8], and patients who have cancer have a 7-fold increased risk of developing VTE [9,10]. The risk of recurrent VTE during anticoagulant therapy is 3- to 4-fold higher for patients with active malignancy than for patients without cancer, and this figure triples with metastatic disease [11]. After an initial VTE event, the cumulative 1-year incidence of recurrent VTE is about 21% among patients with cancer compared with 7% among patients without cancer [11]. One population study [5] showed that patients with cancer diagnosed during the year after a thromboembolic event had lower 1-year survival rates than did cancer patients who never developed thrombotic disease. Such compromised survival persists even after adjustment for tumor stage and demographic risk factors (e.g., age, race) [1]. Thus, VTE is an independent risk factor for decreased survival.

The pathogenesis of cancer-associated thrombosis is influenced by patient characteristics, tumor histology and stage, and treatment-related factors [4,12]. The presence of chronic medical comorbidities is a particularly important patient characteristic. When analyzed as a composite risk index, the number of chronic comorbidities such as hypertension, congestive heart failure, liver and renal disease, obesity, and lung disease is a strong independent predictor of VTE developing in patients with regional or metastatic disease [4,9,12]. Tumor-related factors that predict the likelihood of VTE include cancer type and stage [1,9,13,14]. Data from the California Cancer Registry and a large Dutch study show that metastatic cancers of the brain, lung, uterus, ovary, pancreas, bladder, stomach, bone, and kidney are associated with a higher incidence of VTE, whereas prostate, liver, and breast cancers have a much lower incidence [1,9]. Overall, adenocarcinoma of the pancreas is associated with a 10-fold higher rate of VTE than is prostate cancer [9]. Other tumor characteristics correlating with VTE risk include advanced stage at presentation, shorter predicted survival time, and time from initial cancer diagnosis (with the highest risk within the first few months) [1,9]. Cytotoxic chemotherapy, high-dose corticosteroids, and antiangiogenic agents are treatment-related factors that contribute to VTE development, and adjunctive myeloid and erythropoiesis-stimulating agents (erythropoietin, darbepoietin) are also associated with an increased incidence of cancer-associated VTE [13,15–20].

Epidemiologic studies have shown that the relationship between cancer surgery and VTE is complex. In patients with high-grade glioma, neurosurgery may be associated with a high risk of developing VTE in the weeks after surgery [21], whereas in patients with colon cancer, surgery is associated with a lower risk of VTE even after adjusting for age, race, number of comorbidities, and cancer stage at diagnosis [4]. It is not clear whether this reduced incidence of VTE is due to the selection bias of operating on only the healthiest patients or to the use of postoperative thromboprophylaxis. It is possible that surgical excision of malignant masses diminishes the thrombotic triggers associated with the cancer. Other studies show a neutral or even inverse relationship between VTE and surgery [22].

Because VTE is predictive of increased mortality and a common proximate cause of death in patients with cancer [7], identifying risk factors for VTE could help clinicians stratify these patients and identify those who might benefit from primary prevention. This article describes the potential mechanisms of VTE in cancer and discusses patient, disease, treatment characteristics, and biomarkers that could be used to identify patients who require thromboprophylactic therapy. An overview of current risk assessment models is also provided.

2. Pathophysiology of cancer-associated thromboembolism

Although the strong association between cancer and thrombosis development is well recognized, the mechanisms underlying this paradigm are complex and not well understood. Virchow [23] is credited with first describing how venous thrombosis is orchestrated by a

combination of blood stasis or skeletal immobility, a vessel wall insult, and blood hypercoagulability. Subsequent studies have delineated many of the factors thought to be instrumental in this triad. Physiologic coagulation is initiated when tissue factor (TF) and factor VIIa mediate the activation of factor X. Malignant cells have been shown to produce a cysteine protease, known as cancer procoagulant, which cleaves factor X to activated factor Xa [24–26]. Factor Xa then forms a complex with factor Va on the platelet surface and converts prothrombin to thrombin [8]. Immunohistochemical studies have shown that TF occurs in two forms, one associated with microparticles and another non-microparticle-associated form, both expressed by platelets, leukocytes, and endothelial cells [27,28]. Microparticles are submicrometer-sized vesicles composed of proteins and phospholipids that express phosphatidylserine on their surface and can mediate coagulation [29]. These microparticles, formed from membrane vesiculation during cellular activation, are found at low steady-state concentrations in healthy individuals and at increased levels in inflammatory states [30]. It is proposed that microparticles traverse from tumor to bloodstream through leaky vessels or, alternatively, that cancers indirectly stimulate host cells to produce more vesicles [31].

C-reactive protein (CRP), which is elevated in many cancer patients, may contribute to cancer-associated thrombosis. In vitro and in vivo studies demonstrate the ability of CRP to induce TF expression from monocytes and endothelial cells [32–34]. Microparticles carrying TF then express platelet selectin (P-selectin) glycoprotein ligand-1 (PSGL-1), which binds P-selectin on the surface of activated endothelial cells. By mediating this receptor-ligand interaction, endothelial cells serve as a catalytic surface for TF expression [35,36], phosphatidylserine exposure, and ultimately thrombin formation resulting in fibrin deposition and platelet recruitment [8,37]. CRP has also been shown to promote P-selectin production, leading to platelet stabilization and clotting in a concentration-dependent manner [38,39]. Platelet adhesion is further mediated by glycoproteins Ib and IIb/IIIa, both expressed by platelets and tumor cells and thought to stimulate tumor angiogenesis, metastasis, and perhaps coagulation [25,40]. Defects within fibrinolytic pathways in cancer cells may further promote clot formation [41].

3. Candidate biomarkers in cancer-associated thrombosis

The elaborate pathophysiologic mechanisms of VTE suggest several potential biomarkers that can serve as clinically relevant risk prediction tools for cancer patients. An ideal biomarker is easily measurable, standardized, and has high sensitivity, specificity, and predictive value for subsequent thrombosis. It also remains a strong predictor over time, even in a patient receiving antithrombotic agents. The molecules discussed here serve in multiple pathways of inflammation and infection. Thus, diminished specificity remains a major challenge in identifying suitable biomarkers. One possible means of overcoming this challenge is to design a composite risk assessment tool using multiple clinical indicators to enhance predictive value.

3.1 C-reactive protein

CRP, a nonspecific marker of inflammation, is induced in tumor tissues by infiltrating lymphocytes and monocytes; it is proposed to be an opsonin and an activator of the complement system [34,38]. CRP levels have been correlated with tumor mass because they decrease after tumor resection [42]. Although initial studies showed a correlation between CRP and spontaneous VTE in patients without cancer, this relationship was lost when the data were adjusted for body mass index (BMI) [43]. In contrast, the utility of CRP as a biomarker for cancer-associated VTE has shown some promise in preliminary investigation [44]. Kroger and colleagues [44] conducted a prospective observational study in 507 patients (predominantly with lung and gastrointestinal malignancy) to assess risk factors present

within 30 days preceding a diagnosis of acute VTE. Predictive factors included exposure to various chemotherapies, central venous catheters, need for major surgery, and serum hematocrit (0.3675 L/L), lactic acid dehydrogenase ($>240 \text{ U/L}$), platelet count ($<50/\text{mm}^3$ or $>400/\text{mm}^3$), antithrombin III ($<70\%$), fibrinogen ($>400 \text{ mg/dL}$), and CRP ($>5 \text{ mg/L}$). Among these, only CRP was significantly elevated in the patients who developed VTE. Limitations of this study included a wide between-patient variation in follow-up time, the absence of screening for asymptomatic VTE (i.e., potential omitted cases), and lack of information regarding specific types of cancer [44].

The Vienna Cancer and Thrombosis Study (CATS) [38] is an ongoing prospective, observational investigation being conducted at the Medical University of Vienna to identify biomarkers predictive of VTE in cancer patients. In unadjusted analysis, CRP and soluble P-selectin (sP-selectin) levels were measured in 700 patients with solid tumors. Patients with high CRP levels ($>1.8 \text{ mg/dL}$ or above the 75th percentile in this cohort) had a higher risk of developing VTE within 12 months than did those with lower CRP levels (11.7% vs. 4.9%; $p=0.03$). However, when chemotherapy, radiation, surgery, tumor stage, and sP-selectin levels were analyzed together in a multivariate model, CRP was no longer an independent predictor of VTE. sP-selectin remained a significant predictor in this adjusted VTE risk model (hazard ratio [HR] 3.8; 95% confidence interval [CI] 2.0–7.4; $p<0.0001$), and CRP and sP-selectin levels did not correlate with each other. Interestingly, higher CRP levels correlated with tumor type and decreased survival; patients with lung and pancreatic malignancies had the highest levels, and patients with breast and prostate cancer had the lowest levels. Kaplan-Meier analysis showed that patients with elevated CRP levels ($>1.8 \text{ mg/dL}$) had significantly lower 6- and 12-month survival rates (66% and 43%, respectively) than did patients with lower CRP levels (90% and 82%); this trend was consistent across all tumor types. As a continuous variable, CRP was associated with a HR of 1.3–1.4 for overall survival, a relationship that persisted after incorporating age, gender, BMI, soluble P-selectin levels, tumor type, presence of distant metastases, and presence of newly diagnosed tumors. Data show that whereas CRP may not be an independent predictor of VTE, it may predict decreased survival and is closely related to sP-selectin in malignant thromboembolism [38].

3.2 D-dimer and prothrombin fragment 1+2

D-dimer is a fibrin degradation product. Because it has strong negative predictive value (NPV), a normal serum level indicates a low pretest probability of developing a VTE. D-dimer testing is widely used to exclude VTE. Another marker, prothrombin fragment 1+2 (F 1+2), is released once factor Xa cleaves prothrombin to thrombin, signaling in vivo thrombin generation [45]. Thus, D-dimer is a measure of fibrinolysis and F 1+2 is a measure of coagulation activation [45]. Elevated levels of these markers have been reported in patients with breast, prostate, gynecologic, and lung cancer without clinical thrombosis [46–49]. Whereas the correlation between normal D-dimer levels and absence of active thrombosis is strong, the relationship between F 1+2 levels and thrombotic events is less firmly established [50–52]. Direct measurement of thrombin activity may serve as a better means to predict VTE, as demonstrated in an analysis of the CATS data, which showed significantly higher cumulative rates of developing VTE at 6 months in patients with various malignancies [53]. However, this theory needs further investigation.

In the CATS studies [45], 821 patients with newly diagnosed cancer or progression after a treatment response were followed for a median of 501 days to assess the relationship between D-dimer and F 1+2 levels and VTE. VTE developed in 62 patients (7.6%) during the observation period. Median baseline D-dimer and F 1+2 levels were significantly higher in patients with VTE than in those without VTE ($p=0.013$, $p=0.003$, respectively). A statistically significant correlation was noted between D-dimer and F 1+2 levels ($r=0.5$;

$p < 0.001$). A 2-fold increase in D-dimer levels was associated with a 30% increased risk of developing VTE (HR 1.3; 95% CI 1.2–1.6; $p < 0.001$). This association persisted after adjusting for age, sex, surgery, chemotherapy, and radiotherapy. A 2-fold increase in F 1+2 levels indicated a significant increase in VTE risk (univariate HR 1.8; multivariate HR 2.1). Kaplan-Meier analysis revealed a greater risk of VTE in the first 6 months of follow-up among patients who had elevated levels of D-dimer and F 1+2 (15.2%; ~4-fold risk) compared with patients who had elevated D-dimer levels (4.6%) alone or F 1+2 levels (1.2%) alone or who had normal values for both markers (5.0%; $p < 0.001$). Unlike other biomarkers, D-dimer and F 1+2 were highest in patients with pancreatic cancer and lowest in patients with prostate cancer. This finding suggests a pathophysiologic mechanism that may explain the relatively higher incidence of VTE among patients with pancreatic cancer (~10-fold difference) [9].

3.3 P-selectin

A member of the selectin family of cell adhesion molecules, P-selectin (CD62P) is expressed on platelet and endothelial cells and mediates heterotypic cell-cell interactions. It may also mediate cancer cell adhesion as well as inflammation, cancer growth and metastasis, and thrombosis [54–56]. P-selectin interacts with the PSGL-1 receptor on the surface of monocytes, resulting in cell surface TF expression and the release of the TF-bearing microparticles thought to support fibrin and then thrombus formation [35,57].

In the CATS study [54], sP-selectin was studied as a biomarker of cancer-associated VTE. In the study cohort, 44 patients (6.4%) developed VTE within 415 days of follow-up. sP-selectin levels were significantly higher among the cancer patients who developed VTE ($p = 0.025$). In a proportional hazard model adjusting for age, sex, previous chemotherapy, surgery, and radiotherapy, each 10 ng/mL increase in sP-selectin was associated with a HR of 1.2 (95% CI 1.1–1.4; $p = 0.005$). The HR was more pronounced when patients were stratified into quartiles of sP-selectin ranges. Patients with sP-selectin values greater than the 75% quartile had a HR of 2.5 (95% CI 1.3–4.8; $p = 0.008$) compared with patients in the lower quartiles. The cumulative incidence of new-onset VTE during the first 6 months of observation was more than 3-fold greater among patients with sP-selectin values in the highest quartile (11.9% vs. 3.7%). Further, multivariate analysis revealed that significant predictors of VTE included elevated sP-selectin (HR 2.6; 95% CI 1.4–4.9; $p = 0.003$), surgery (HR 3.9; 95% CI 1.8–8.5; $p < 0.001$), and radiotherapy (HR 2.9; 95% CI 1.4–5.8; $p = 0.003$). Even after adjusting for other predictors of VTE, including platelet count, tumor type, and cancer stage, sP-selectin was still a significant predictor of VTE within 1 year (HR 2.3; 95% CI 1.2–4.5; $p = 0.009$) [54].

3.4 Coagulation factor VIII

Factor VIII mediates coagulation through the intrinsic pathway by binding its co-factor IXa, forming the tenase complex and leading to factor Xa activation and subsequent thrombin formation. Elevated factor VIII levels have been demonstrated in patients with multiple types of cancer, including breast cancer, colon cancer, and multiple myeloma, even in the absence of clinically apparent VTE [58–61]. An early prospective matched trial [62] demonstrated significantly elevated factor VIII levels in cancer patients with VTE but no relationship between baseline factor VIII levels and the incidence of VTE or survival.

Vormittag et al [58] performed a prospective observational study with patients from the CATS study who had solid and hematologic cancers, with and without disease progression, and followed them for 2 years. Baseline factor VIII levels were significantly higher among the 7.4% patients who experienced thromboembolic events than among event-free individuals ($p < 0.001$). This relationship was independent of gender, previous treatment, and

blood type (although patients with blood type O had significantly lower factor VIII activity than did those with other blood types). Interestingly, HRs decreased as age increased; a 20% increase in factor VIII resulted in a nearly 2-fold increase in risk of VTE among 40-year-old patients (HR 1.9; 95% CI 1.5–2.7; $p<0.0001$) but only a 1.4-fold increase among 60-year-old patients (HR 1.4; 95% CI 1.2–1.6; $p<0.0001$) [58].

3.5 Tissue factor and microvesicle/microparticles

TF is a cell surface glycoprotein normally constitutively expressed on subendothelial matrix cells and on the surface of monocytes, macrophages, neutrophils, and endothelial cells [63]. TF has been implicated in angiogenesis and tumor spread, and it correlates with vascular endothelial growth factor expression and microvessel density in pancreatic cancer [64].

Studies investigating TF and associated microparticles as predictive biomarkers for VTE in cancer patients have yielded mixed results. Part of the discrepancy may be attributable to a lack of standard assay for measuring TF or microparticles expression or activity. Current assays include immunohistochemical TF staining, measurement of TF antigen by enzyme-linked immunosorbent assay, measurement of TF microparticle activity, as well as flow cytometry-based methods [65]. Retrospective analyses have shown higher levels of TF activity in patients with breast and pancreatic cancer [64,66], as well as in patients with advanced colorectal cancer, than in patients without cancer [67]. Likewise, prospective reports indicate that TF may serve as a predictive biomarker for VTE in patients with cancer. In particular, elevated TF (measured by antigen levels and a procoagulant activity assay) was associated with VTE in 11 patients with locally advanced and metastatic pancreatic adenocarcinoma [68]. Manly and colleagues [69] measured microparticle TF activity in 66 patients with various tumors in a cross-sectional study and found that microparticle TF activity was significantly increased in patients with VTE ($p<0.05$). In a comparison of different cancer types, the highest microparticle TF activity levels were found in pancreatic cancer, followed by lung and colon cancer. This is consistent with established epidemiologic data showing the highest risk of VTE in patients with pancreatic cancer [70]. Importantly, among patients with VTE, those who have elevated microparticle TF activity had significantly worse survival ($p=0.01$), a finding confirmed by Thaler and colleagues [71] in patients with pancreatic cancer. Further investigation by Tesselaar and colleagues [72] showed that microparticle TF activity was significantly elevated in 51 patients with cancer and VTE compared with 49 patients with cancer but without VTE who were matched for age, sex, cancer type, stage, and treatment ($p=0.001$). However, the absolute number of microparticles did not differ between patients and healthy controls, suggesting that microparticles in patients with cancer are rich in TF.

Whereas Tesselaar and colleagues [66] used both microparticle TF activity and light scattering-based flow cytometry to measure TF-bearing microparticles, Zwicker and colleagues [73] developed an impedance-based flow cytometry assay to assess the predictive value of TF-bearing microparticle levels in case control and retrospective analyses of cancer-associated VTE. At baseline, TF-bearing microparticles were detected in most but not all cancer patients but in significantly fewer cancer-free controls. In the case control study, TF-bearing microparticles were elevated in a higher proportion of patients with VTE compared with matched controls (60% vs. 27%; $p=0.01$) [73]. A subsequent analysis determined TF microparticles levels in 60 patients with cancer but without VTE. These patients were followed over 2 years to detect thromboembolic disease. Those with baseline detectable TF-bearing microparticles had a 7-fold increased probability of developing VTE (odds ratio 7.00; 95% CI, 0.85–82.74). Elevated levels were associated with a sensitivity for VTE of 0.80 and specificity of 0.78, with a respective negative predictive value (NPV) and positive predictive value (PPV) of 0.97 and 0.25 [73]. Similar studies in patients with idiopathic VTE (not excluding inherited thrombophilias) revealed significantly higher rates

and levels of detectable TF-bearing microparticles in cancer-related VTE than in idiopathic VTE [73]. In a small subset of patients, TF-bearing microparticle levels measured after resection were significantly decreased, suggesting that these microparticles are tumor derived.

Acknowledging the different forms of TF, Tilley and colleagues [30] used a chromogenic assay to evaluate combined microparticle and platelet-associated TF activity. Samples from 20 patients undergoing chemotherapy for cancer and 23 healthy individuals were tested. The cancer group showed significantly increased TF activity ($p<0.001$) and were found to have higher sP-selectin and D-dimer levels ($p<0.001$), although these levels did not correlate with TF activity. The study was limited by its modest size, but the results raise questions regarding the pathological relevance of different versions of TF and whether an activity assay that combines microparticle and platelet sources of TF would be a more accurate biomarker of VTE risk.

Furthermore, recent prospective data has shed some doubt on the predictive potential of TF or microparticles in VTE. For example, Thaler and colleagues [71] did not find a significant association between microparticle TF activity (using a chromogenic assay) and VTE incidence in patients with colorectal, pancreatic, brain, or gastric cancer. In an examination of 728 patients from the Vienna CATS study, the number of circulating microparticles was significantly increased in cancer patients relative to healthy controls, yet microparticles levels were not predictive of VTE [74].

Lastly, antitumor therapy can increase TF expression. One in vitro study demonstrated increased TF activity after treatment of endothelial cells with chemotherapeutic and antiangiogenic agents [75], and another confirmed these findings and related the increased activity to phosphatidylserine exposure [76].

The sum of current evidence suggests that TF and associated microparticles may serve a role in predicting which patients with cancer, particularly pancreatic cancer, will develop VTE. However, a standard assay must first be established and validated in large prospective studies before this biomarker can be incorporated into composite predictive models [77].

3.6 Thrombocytosis

Studies have shown conflicting results regarding thrombocytosis as a risk factor for VTE. Zakai and colleagues [78] found that an elevated platelet count at the time of hospital admission was an independent predictive factor for VTE in medical inpatients. A later prospective population-based study [79] conducted over 11 years examined mean platelet volume (MPV; a surrogate of platelet reactivity) and platelet count in patients with provoked or unprovoked VTE. Whereas MPV was linked to a 1.5-fold increased risk of unprovoked VTE (95% CI 1.1–2.3; $p=0.03$), platelet count (cutoff value $300 \times 10^9/L$) did not show any significant association with provoked VTE, a finding corroborated by the Longitudinal Investigation of Thromboembolism Etiology (LITE) study [80].

There has been limited exploration of the predictive value of the platelet count in cancer-associated thrombosis. Khorana and colleagues [13] conducted an observational study in more than 3,000 patients (from the Awareness of Neutropenia in Chemotherapy Study Group Registry) who had received at least one cycle of chemotherapy over a median of 2.4 months to determine the relationship between platelet levels and VTE. Thromboembolic events occurred in 58 patients (1.93%). A pre-chemotherapy platelet count of $350 \times 10^9/L$ was associated with a significantly elevated risk of VTE. Moreover, multivariate analyses showed that cancer site (lung and upper gastrointestinal cancer), hemoglobin <10 g/dL or

use of erythropoietin, and use of granulocyte-stimulating factors were also significant independent predictors of VTE [13].

Investigators analyzing the CATS cohort also determined the utility of pre-chemotherapy platelet count for predicting VTE in patients with cancer [81]. For each $50 \times 10^9/L$ increase in platelet count, the HR for VTE increased by 1.28 (95% CI 1.12–1.46; $p=0.0003$) in univariate and multivariate analysis that adjusted for age, gender, tumor stage, surgery, prior chemotherapy and radiotherapy, sP-selectin, and leukocyte count. Platelet count above the 95th percentile of this cohort ($>443 \times 10^9/L$) was associated with a significantly higher risk of developing VTE in univariate (HR 5.07; CI 2.35–10.95; $p<0.0001$) and multivariate (HR 3.5; CI 1.52–8.06; $p=0.0032$) analyses. Univariate analysis revealed a 2-fold increased risk of VTE when using a platelet count cutoff of $350 \times 10^9/L$ (HR 2.42; 95% CI 1.22–4.80; $p<0.011$); however, this increase did not retain its significance in an adjusted multivariate model [81]. Previously, Khorana and colleagues [82] found this elevated platelet cut-off to be predictive of VTE in a 3-tiered classification scheme that stratifies risk among patients starting chemotherapy. Lastly, CATS data [81] showed that thrombopoietin levels were not associated with VTE or platelet counts. To what degree thrombopoietin regulates platelet count and risk of thrombosis for patients with cancer remains unclear.

3.7 Leukocytosis

An elevated white blood cell (WBC) count was shown to be prognostic and associated with increased mortality among patients with cancer [83–87]. The predictive value of leukocytosis in determining thromboembolic risk has also been evaluated. The Computerized Registry of Patients with Venous Thromboembolism (Registro Informatizado de Enfermedad TromboEmbólica) databank [88] was used to investigate the relationship of WBC at the time of incident cancer-associated VTE and recurrent VTE within 3 months. Among 3805 patients, those with a WBC count $>11 \times 10^9$ cells/L were found to have a significantly increased risk of VTE. In a multicenter observational study, Connolly and colleagues [83] analyzed data from more than 4,000 patients with solid tumors and lymphoma, stratifying leukocyte count into quartiles between 5.7 and 11×10^9 cells/L, measured before initiation of chemotherapy and before each chemotherapy cycle. Patients with the highest total leukocyte counts and, more specifically, patients with elevated absolute monocyte and neutrophil counts had a higher incidence of VTE ($p<0.0001$ for each). Although leukocytosis was significantly associated with a higher platelet count, elevated WBC counts remained an independent predictor of VTE after adjusting for thrombocytosis; pre-chemotherapy leukocytosis had a HR for VTE of 2.1 (95% CI 1.3–3.4, $p=0.003$) after adjusting for malignancy type, stage, baseline platelet and hemoglobin levels, BMI, and use of erythropoietin-stimulating agents. Kaplan-Meier analyses showed an increased 150-day mortality rate among patients with WBC $>11 \times 10^9$ cells/L compared with patients with normal leukocyte count (14.0% vs. 4.4%; $p<0.0001$). Leukocytosis was also associated with increased early mortality in a multivariate model that adjusted for acute VTE. These findings should be approached with caution, as mortality and VTE were not pre-specified primary outcomes of this cohort study and patients were not actively screened for VTE. Nonetheless, leukocytosis may be a risk factor for cancer-associated thrombosis and mortality [83].

4. Use of risk assessment models

4.1 Developing risk models

For patients with cancer who may be at risk for VTE, it is possible to determine if decision rules incorporating a defined set of risk factors and biomarkers can help clinicians decide on whether to initiate primary thromboprophylaxis. The goal of risk assessment models is to

facilitate better, more efficient clinical decision-making and optimize VTE risk reduction versus adverse events. Specifically, for patients with cancer, does use of a decision rule improve clinical outcomes (i.e., lower the incidence of VTE and death with a higher but acceptably higher risk of bleeding)? When a clinical risk assessment model is developed, each additional risk factor or biomarker should show statistically significant improvement in the performance of the decision rule. A useful risk factor should improve the discrimination and be generalizable. Discrimination refers to how well the new model predicts which patients will develop VTE versus those who will not. It is also important that a new risk model add sufficiently to the model considered the gold standard, such that it changes how patients are treated based on superior outcomes.

4.2 Risk models for VTE among patients with cancer

Several groups have developed risk assessment models that incorporate demographic, tumor, host, treatment, and laboratory data. Khorana and colleagues [82] stratified patients undergoing chemotherapy for cancer into three risk categories based on five factors: primary cancer site, pre-chemotherapy platelet count $< 350 \times 10^9/L$, hemoglobin $< 10 \text{ g/dL}$ or use of erythropoietin-stimulating agents, leukocyte count $> 11 \times 10^9/L$, and BMI $> 35 \text{ kg/m}^2$. Primary cancer sites were classified as “very high risk” (gastric and pancreatic cancer), “high risk” (lung, lymphoma, gynecologic, and genitourinary excluding prostate), and “other/average risk” (breast, colorectal, prostate) categories. Points were assigned to each risk factor based on the magnitude of the respective regression coefficients, and risk was divided into three groups: low (score 0), intermediate (score 1–2), and high (score ≥ 3). The authors reported that at a cutoff of three points for high risk, the NPV was 98.5% (score < 3), the PPV was 7.1%, sensitivity (if VTE present, score of ≥ 3) was 40.0%, and specificity (if no VTE present, score < 3) was 88.0% in the derivation cohort; respective values in the validation cohort were 98.5%, 6.7%, 35.7%, and 89.6%. Limitations to the generalizability of this model include the limited spectrum of cancer types included, the likely good performance status of this cohort that received outpatient chemotherapy, and the absence of VTE as a formal outcome measure [82]. Nevertheless, the risk factors that were identified—elevated platelet count, elevated leukocyte count, anemia or use of erythropoietin, and specific types of cancer—are acknowledged as important predictors of VTE among patients with cancer [14]. Furthermore, the Khorana model has demonstrated predictive power in studies including more than 10,000 cancer patients receiving chemotherapy [89]. For instance, in a series of 1,415 patients receiving both cytotoxic and targeted chemotherapy through phase I studies conducted by the Southern Europe New Drugs Organization (SENDO), the Khorana risk model predicted VTE incidence with HRs of 7.88 and 2.74 for those with high and intermediate risk, respectively, compared with patients with low risk [90]. In patients receiving cisplatin-based regimens for various cancers, one retrospective analysis from Memorial Sloan-Kettering showed the Khorana score as predictive for developing both venous and arterial thromboembolic events within 100 days of treatment initiation [91]. Other subgroup analyses have also shown the Khorana model to predict VTE incidence as well as benefit from thromboprophylaxis [92]. In the double-blind SAVE-ONCO trial exploring semuloparin thromboprophylaxis in patients receiving chemotherapy, semuloparin reduced the incidence of VTE or VTE-related death in all risk groups compared with placebo, although the benefit was relatively greater in patients with moderate and high risk (HR 0.37 and 0.27, respectively) than for those with low risk (HR 0.71) based on the Khorana model [93,94].

Expanding on the Khorana model using the CATS database, Ay and colleagues [14] integrated P-selectin and D-dimer (each assigned 1 point in the overall score) into their risk assessment model. This study included more types of cancer assigned to the high-risk cancer group, which included high-grade glioma, multiple myeloma and renal carcinoma, compared

with the original derivation cohort from Khorana and colleagues. Risk stratification was compared using the original (Khorana and colleagues [82]) and a modified model. The CATS report confirmed that the Khorana score showed a linear trend for an association with VTE, with a HR of 2.1 (95% CI 1.6–2.6; $p<0.001$) for every point increase in the risk score [14]. Stratification was strengthened using a modified model that included P-selectin and D-dimer. Specifically, the stepwise cumulative probabilities of developing VTE within 6 months for a score of 0 to >5 were 1.0%, 4.4%, 3.5%, 10.3%, 20.3%, and 35%, respectively ($p<0.001$). Furthermore, using a cutoff score of 5, the expanded model had a NPV of 94.4%, a PPV of 42.9%, and sensitivity and specificity of 19.1% and 98.2%, respectively. Patients with the highest risk (score ≥ 5) had an almost 26-fold higher rate of thromboembolic disease than did individuals with the lowest risk (score 0).

5. Future directions

Future analyses should explore the usefulness of incorporating other biomarkers, such as P-selectin, CRP, factor VIII, prothrombin F 1+2, and TF-bearing microparticle levels, into VTE prediction models and expand the risk models to include patients with a cancer diagnosis who are not receiving chemotherapy. Investigations should be performed to determine which chemotherapy regimens are associated with increased risk of VTE (e.g., thalidomide agents in multiple myeloma). Potential barriers to use of VTE prediction models are the absence of widespread clinical availability of tests for these biomarkers, lack of test standardization, and cost. However, cancer-associated VTE may be associated with increased health care expenditures [95], and effective prophylaxis targeted at high-risk groups might therefore reduce overall health care costs.

Current guidelines recommend primary VTE prophylaxis in all patients with cancer who require hospitalization and who require major surgery [96]. Primary prophylaxis in patients who have central venous catheters is not currently standard of care as only limited data show clinical benefit. Routine prophylaxis is currently recommended only in ambulatory cancer patients with additional risk factors (including previous deep venous thrombosis, immobilization, hormonal therapy, angiogenesis inhibitors) and those with multiple myeloma being treated with thalidomide or lenalidomide [97,98]. There is currently insufficient data to support instituting universal prophylaxis in ambulatory cancer patients, as this may result in an excess of bleeding complications [99] in addition to increased healthcare costs and impaired quality of life associated with daily injections.

The efficacy of thromboprophylactic therapy with the ultra-low-molecular-weight heparin (ULMWH) semuloparin was recently reported in a large series of cancer patients undergoing chemotherapy. SAVE-ONCO [94] was a double-blind study that randomized 3212 patients with metastatic or locally advanced lung, pancreatic, stomach, ovarian, colorectal, or bladder cancer to receive semuloparin or placebo during active chemotherapy. Rates of VTE were reduced by 64% in the semuloparin group compared with the placebo group (1.2% vs. 3.4%; HR 0.36; 95% CI 0.21–0.60; $p<0.001$), with no increase in major and clinically relevant nonmajor bleeding (2.8% vs. 2.0%; HR 1.4; 95% CI 0.89–2.21). There was no heterogeneity of effect across tumor types or risk groups for VTE. Treatment with semuloparin did not affect survival [94]. In an editorial [100] accompanying the SAVE-ONCO report, it was posited that ULMWH compared well with other types of adjuvant therapy for patients with cancer in terms of survival, adverse effects, and burden with the proviso that additional evidence is required to determine which patient groups would realize the greatest benefit.

Further development of risk stratification models similar to that of Khorana and colleagues might aid in identifying those newly diagnosed ambulatory cancer patients who are at

increased risk for developing VTE and who might benefit from primary thromboprophylaxis. Confirmatory studies are needed to determine whether the primary prophylaxis reduces the incidence of VTE, improves survival and overall quality of life, and reduces healthcare costs. Given the numerous challenges patients with cancer face, using the newer oral anticoagulants under development rather than subcutaneously injected heparins may change physician and patient perceptions about the relative benefits of primary thromboprophylaxis, although patients may be more accepting of chronic injectable agents than is commonly thought. However, these newer agents need to be studied specifically in cancer patients. Future research will focus on developing risk models for VTE that can be applied to the majority of patients with cancer and on clinical trials designed to determine if the newer oral anticoagulants improve important clinical outcomes for these patients.

6. Conclusions

Malignancy and its treatment lead to complex pathophysiologic changes that increase the risk of morbidity and mortality associated with VTE. Although hospitalized cancer patients and those who undergo surgery are targeted for thromboprophylaxis, widespread use of primary prevention in the outpatient setting is not yet justified. Risk models that incorporate important clinical and epidemiologic factors, coupled with the presence or absence of specific biomarkers, show some promise as predictive tools for assessing the likelihood of developing VTE. Of these, the Khorana score is the most widely validated. If future studies show that use of risk models leads to clinically effective and cost-effective thromboprophylaxis, the quality of life of patients with newly diagnosed cancer may improve.

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Biography

Dr. Ted Wun is Chief of the Division of Hematology and Oncology at UC Davis Cancer Center and Professor of Medicine and Professor of Pathology and Laboratory Medicine at UC Davis School of Medicine. Dr. Wun also is Medical Director of the Clinical and Translational Sciences Center's Clinical Research Center and Chief of the Section of Hematology and Oncology for the VA Northern California Health Care System. His current research interests include vascular complications of sickle cell disease and the association of cancer and thrombosis.

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